INTRATHECAL GLUCOCORTICOIDS FOR NEUROPATHIC PAIN

Mienke Rijsdijk

INTRATHECAL GLUCOCORTICOIDS FOR NEUROPATHIC PAIN

Intrathecale glucocorticoïden voor de behandeling van neuropathische pijn

met een samenvatting in het Nederlands

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Aan mijn ouders

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1

INTRODUCTION

CHAPTER 1
General introduction

INTRODUCTION

Pain, an unpleasant sensory and emotional experience associated with actual or potential tissue damage, is a phenomenon we all experience in life.[1] Fortunately, the state of acute pain lasts for a limited time until the underlying pathology resolves. If, however, pain persists for a longer period of time and/or at low levels of underlying pathology not explaining the presence and extent of the pain, it is indicated as chronic pain. In western society, chronic pain poses a major health burden. The prevalence of chronic pain ranges from 12 to 30% in the adult European population.[2;3] In the Netherlands approximately 3 million adults (18% of the population) suffer from chronic pain.[3] Pain can be of nociceptive, inflammatory, neuropathic or of mixed origin (described in more detail below). This thesis focuses on chronic neuropathic pain,[4] with a prevalence of 6.9% to 10% in the general population.[2;5]

NEUROPATHIC PAIN

Neuropathic pain is defined as pain caused by a lesion or disease of the somatosensory nervous system.[1] Examples of neuropathic pain are postherpetic neuralgia and lumbar radiculopathy. In comparison, nociceptive pain is pain that arises from actual or threatened damage to non-neural tissue, for example after fracture of a bone or a superficial knife cut, and is due to the activation of nociceptors.[1] All types of pain, nociceptive, inflammatory, neuropathic or of mixed origin, can become chronic. The exact mechanism of chronification of pain is still subject to research. To investigate pain mechanisms, several preclinical pain models have been developed. Some widely used pain models in rats (studied in this thesis) are a) spinal nerve ligation, inducing pain-like behavior by a direct nerve injury stimulus,[6] b) intraplantar carrageenan injections, inducing pain-like behavior with an inflammatory stimulus,[7] and c) intraplantar formalin injections, a predominantly neurotoxic stimulus.[8;9] Preclinical and clinical research have indicated several mechanisms contributing to the development of chronic pain: peripheral and central sensitization, phenotypic switching, ectopic excitability, structural reorganization, and decreased inhibition.[10] Below, a general description is given of the events leading to chronic pain in an intact somatosensory system.[10-13]

First noxious and/or inflammatory stimuli activate the peripheral terminals of nociceptors. Nociceptors have unmyelinated (C-fiber) or myelinated (Aδ fiber) axons which have transducer ion channels that open once activated. The activated ion channels allow sodium and calcium ions to flow into the nociceptor peripheral terminal, producing an inward current that depolarizes the membrane. If the depolarization is sufficient to open voltage-gated sodium channels, the membrane will depolarize further, initiating a burst of action potentials with a frequency and duration reflecting the intensity and duration of the noxious stimulus. These action potentials are

conducted along the sensory axons (slow, unmyelinated C-fibers and fast, myelinated Aδ-fibers) to the dorsal root ganglion (DRG) and into the spinal dorsal horn, where the central terminals make synaptic contact with spinal dorsal horn neurons. High-frequency action potentials result in the release of neuropeptides, such as substance P and glutamate from the nociceptor central terminals in the spinal cord. When a noxious stimulus is intense enough (high enough action potential frequency of a long enough duration), the N-methyl-d-aspartate (NMDA) receptor will be activated by the excitatory glutamate, further increasing the output from spinal dorsal horn neurons. In this process intracellular kinases are activated leading to phosphorylation of ion channels and receptors, inducing genes and changing the chemical character or phenotype of the neuron leading to central sensitization. Central sensitization can be defined as pain hypersensitivity arising from reduced activation thresholds and abnormal amplification of sensory signaling within the central nervous system. In the process of central sensitization, release of cytokines such as interleukin-1β activate a) microglia, a source of many more cytokines and chemokines acting on neurons, and b) their supporting glia to alter properties or patterns of gene transcription. Later changes in the central sensitization process include sprouting of low-threshold afferents into the zone normally occupied by nociceptor terminals, which is called structural reorganization and leads to allodynia, pain upon non-noxious sensory stimuli. Also a loss of inhibitory neurons, using the inhibitory neurotransmitters gamma-aminobutyric acid (GABA) and glycine, is present in the later phases of central sensitization and reduces the normal inhibitory mechanisms in the pain circuitry. Central sensitization can spread from spinal sites to supraspinal levels, where various brain structures influence spinal processing of nociceptive input in a top-down fashion with the key neurotransmitters noradrenalin and serotonin both in a inhibitory and excitatory way.

When there is a somatosensory lesion or disease (in neuropathic pain syndromes), the origin of the afferent traffic reflects ectopic activity arising from the nerve injury site and importantly from the DRG of the injured axon instead of from the peripheral terminals of nociceptors. Ectopic excitability, structural reorganization and decreased inhibition are more prevalent and profound in neuropathic pain syndromes.

DIAGNOSING NEUROPATHIC PAIN

To diagnose neuropathic pain, it is important to evaluate if the underlying disease or trauma has resulted in damage to the somatosensory system, is limited to non-neural tissue, or effects both. The experienced pain sensations differ between the pain modalities. Neuropathic pain is characterized by a constant, burning sensation with spontaneous sharp-shooting exacerbations referred to the peripheral distribution of the injured nerve and worsening upon normal sensory triggers (allodynia) or painful sensory triggers (hyperalgesia). In contrast, nociceptive pain is more often

described as sharp or dull, heavy and tiring. There are several questionnaires aimed at discriminating between neuropathic pain and other pain such as the (Self report)-Leeds Assessment of Neuropathic Symptoms and Signs (LANSS), Neuropathic Pain Questionnaire-(short form) (NPQ), PainDETECT, and the Neuropathic Pain Diagnostic Questionnaire (DN4).

General clinical examination and targeted examination guided by the character and localization of the symptoms should be performed to find signs of damage to the somatosensory system, and an effort undertaken to diagnose positive sensory phenomena such as allodynia and hyperalgesia. Quantitative sensory testing is of additional value in the diagnostic process and includes testing of touch, vibration, pinprick, cold and warmth. The impact of pain on daily life, including disability and effect on sleep and mood, provides essential information influencing the choice of treatment.[4] The effect of treatment should be assessed by repeated evaluation of these aspects of pain.[4]

TREATMENT OF NEUROPATHIC PAIN

Treatment of neuropathic pain is aimed at the pathophysiologic mechanism generating or maintaining pain in a specific patient[10;11] such as reducing afferent input (e.g. topical lidocaine, topical capsaicin, peripheral nerve/plexus/DRG/epidural blocks with local anesthetics, radiofrequency/pulsed radiofrequency treatment of DRG), reducing NMDA receptor activity (e.g. esketamine, methadone), decreasing calcium influx, thereby inhibiting the release of excitatory neurotransmitters such as glutamate (anti-epileptic drugs (gabapentin, pregabalin)), inhibition of norepinephrine and serotonin reuptake (tricyclic antidepressants, tramadol), μ-agonists (opioids, tramadol) and increasing inhibition (transcutaneous electrical nerve stimulation (TENS), spinal cord stimulation). Also mood disturbances and sleep deprivation as a result of pain should be treated and rehabilitation of the patient optimized to minimize disability.

Below, one neuropathic pain syndrome, postherpetic neuralgia, is discussed in more detail as it is the study domain in the randomized controlled trial described in Chapter 5.

POSTHERPETIC NEURALGIA

One of the four most prevalent neuropathic pain syndromes is herpes zoster induced pain, also called postherpetic neuralgia (PHN).[5;14] PHN is defined as pain that persists for more than 120 days following the rash caused by a herpes zoster infection.[15] Herpes zoster is the result of a reactivation of the varicella zoster virus, which resides in a latent form in sensory ganglia after the primary varicella zoster infection, known as chicken pox.[16] With increasing age, the specific immunity to the virus gradually reduces and the virus can overcome the host's defense. When the virus replicates, it

spreads within the DRG and along the corresponding peripheral nerves within one dermatome. This results in inflammatory damage to neuronal tissue, and can evolve to peripheral and central sensitization, leading to allodynia. Allodynia may also result from sprouting of Aβ-fibers in the superficial layers of the dorsal horn in response to partial loss of C-fiber input. Autopsy[17] and biopsy[18;19] studies found that PHN patients showed marked atrophy of the spinal dorsal horn and reduced innervation density in the affected dermatome on the ipsilateral compared to the contralateral side. Loss of sensory afferent fibers can also result in development of spontaneous discharge in deafferentiated central neurons. Data suggest that there may be subsets of PHN patients who have different underlying mechanisms responsible for the generation and maintenance of their pain.[10]

Pain from herpes zoster tends to resolve spontaneously with time in most patients, but can also persist for years. The risk of developing persisting pain, PHN, is reported to be between 10-25%, depending on the applied definition.[15;20;21] Patients suffering from PHN typically describe different types of pain sensations as the pathophysiologic process suggests, including continuous burning or throbbing pain, intermittent sharp or electric shock-like pain, and allodynia.[16] The neuropathic pain disorder is frequently severe and disabilitating.[15;22]

TREATMENT OF PHN

Treatment of PHN is difficult and often requires multiple modalities due to the different underlying mechanisms generating and maintaining the neuropathy. First-line treatments for PHN include tricyclic antidepressants (number needed to treat (nnt) of 2.8), the anti-epileptic drugs e.g. gabapentin and pregabalin (nnt 4.3 respectively 4.2), and the topical lidocaine 5% patch.[16;23] Opioids (nnt 2.6), tramadol (nnt 4.8), topical capsaicin (nnt 3.2) are recommended as either second- or third-line therapies.[24-26] These therapies often have disappointing long-term effects and side effects are frequently reported.[16;25;27]

Regarding the invasive treatment options, there is weak evidence that epidural or paravertebral local anesthetic and steroid nerve blocks provide relief of acute pain associated with herpes zoster.[28] The use of sympathetic nerve blocks in PHN has not proven to be benefical.[28] The development of PHN cannot be prevented with a single epidural injection of steroids and local anaesthetics.[29] In conclusion, successful medication and invasive treatment options for PHN are limited and there is a demand for new treatment possibilities.

In 2000, a glimmer of hope appeared after the publication of a randomized controlled trial (RCT) in the New England Journal of Medicine in which intrathecal administration of methylprednisolone acetate (MPA) was associated with high efficacy in PHN patients.[30] The rationale in the Kotani trial behind the anti-inflammatory MPA

treatment was that the pain in PHN patients was maintained by an ongoing inflammatory process. The authors referred to a) high concentrations of interleukin-8 in the cerebrospinal fluid of PHN patients observed in a study by Kikuchi et al,[31] and b) postmortem studies in PHN patients showing inflammation around the spinal cord.[17] After four intrathecal injections with MPA and lidocaine, 82 out of 89 PHN patients had 'good or excellent' pain relief at one year follow-up, against 5 out of 91 and 3 out of 90 patients after lidocaine or no treatment respectively. No side effects or complications were reported. Since PHN is a devastating and often therapy resistant neuropathic pain disorder, some clinicians accepted the risks of the invasive treatment and offered the treatment to their patients. However, despite the excellent results reported in this trial, intrathecal MPA never became part of standard care for intractable PHN.

'EXTRAORDINARY CLAIMS REQUIRE EXTRAORDINARY EVIDENCE' (CARL SAGAN)

It was surprising that after the excellent results reported in the Kotani trial,[30] intrathecal MPA did not become part of standard care for intractable PHN. Personal communications among clinicians questioned the efficacy of intrathecal MPA for PHN as reported by Kotani et al. Affiliated pain clinicians reported that they had offered the treatment to their PHN patients, but doubted the efficacy described by Kotani. We were convinced the trial needed to be replicated, which was the fundament for this thesis.

Up to that date there was no good alternative for the treatment of intractable PHN and patient suffering was tremendous.

A second reason to replicate the Kotani trial was to develop a safe successful alternative treatment for patients with intractable PHN.

Third, the pharmacokinetic profile of intrathecal MPA treatment was unclear. In theory, the administered insoluble MPA first needs to be hydrolyzed to the soluble methylprednisolone to become biologically active. We were not informed about the pharmacokinetic parameters such as clearance of MPA from the cerebrospinal fluid and half-life of MPA after intrathecal administration.

Fourth, there was doubt about the safety of intrathecal MPA, which contains a potentially harmful preservative, myristyl-gamma-picoliniumchloride.[25;32;33] Kotani et al.[30] did not describe if or how they reduced the potential neurotoxic preservatives in the MPA formulation since a preservative-free formulation is not commercially available. The most feared complication of intrathecal MPA is adhesive arachnoïditis, reported in patients with multiple sclerosis.[34] Kotani et al.[30] did not find biochemical changes in the cerebrospinal fluid during the treatment period (that usually precedes this complication) and no clinical complications were observed. Also their magnetic resonance imaging four weeks, one and two years after the injections did not show

abnormalities.[30] Nevertheless no preclinical safety study on intrathecal MPA treatment was performed studying dose related toxicity.

In this thesis we designed a practical solution to reduce the potential neurotoxic preservative in the commercial MPA formulation (referred to as the reformulated MPA), studied the pharmacokinetics of repeated intrathecal MPA injections during a pilot study, and conducted a replication trial to evaluate the efficacy of intrathecal MPA for PHN patients.

OUTLINE OF THE THESIS

Part I, Chapter 2 provides a general overview of the development of neuropathic pain, the characteristics of glucocorticoids and their mechanism of action, the effect of glucocorticoids on neuropathic pain in patients and preclinical studies, with an emphasis on the glucocorticoid MPA.

In **Part II, Chapter 3** we present the results of a pharmacokinetic study in six PHN patients, showing data on the methylprednisolone concentrations in the CSF and pharmacokinetic parameters such as distribution volume, clearance and half-life after intrathecal administration of the reformulated MPA. **Chapter 4**, describes the results of a preclinical safety study in dogs of the reformulated MPA.

As mentioned previously, the present thesis project started with the design of a replicate RCT, according to the protocol used by Kotani et al,[30] studying the efficacy of intrathecal MPA in PHN patients. Since no adverse events were reported in the 270 patients that participated in the Kotani trial, and we had designed a protocol substantially reducing the potentially neurotoxic preservatives from the commercial MPA formulation, the risks of our replicate RCT were considered acceptable and with approval of the medical ethics committee we proceeded with inclusion of the first ten pilot patients. However no preclinical safety data on the use of intrathecal reformulated MPA had been published. During the first inclusions of our trial, a discussion arose on the off-label use of drugs in the intrathecal space and preclinical safety studies were advised.[35;36] We therefore decided to simultaneously perform a preclinical safety study in a dog model.

Part III presents the results of the efficacy studies of glucocorticoids in patients and animal models. The results of the replication trial with PHN patients are described in **Chapter 5**. No beneficial effects of intrathecal MPA treatment were observed. Since preclinical studies on the efficacy of intrathecal glucocorticoids in pain models showed conflicting results, and we were interested if there was any analgesic effect of intrathecal MPA, we decided that more preclinical research was warranted to verify our results. A preclinical study was designed, researching the effect of the maximum tolerable dose of two methylprednisolone formulations, the soluble methylprednisolone sodium succinate (MP) and the particulate MPA on pain-like behavior and pain-associated markers in three well established rodent pain models, **Chapter 6**. Subsequently we explored the effects of intrathecal MPA on the glucocorticoid receptor, since most glucocorticoid actions are mediated through its receptor. The total glucocorticoid receptor levels and activation of the glucocorticoid receptor

within the spinal dorsal horn and DRG were studied in a spinal nerve ligation model in rats, **Chapter 7**.

In **Part IV, Chapter 8**, we summarize and critically review the results of the various preclinical and clinical studies and outline some possibilities for future research.

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CHAPTER 2

The effects of glucocorticoids on neuropathic pain: A review with emphasis on intrathecal methylprednisolone acetate delivery

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ABSTRACT

Methylprednisolone acetate (MPA) has a long history of use in the treatment of sciatic pain and other neuropathic pain syndromes. In several of these syndromes, MPA is administered in the epidural space. On a limited basis, MPA has also been injected intrathecally in patients suffering from postherpetic neuralgia and complex regional pain syndrome. The reports on efficacy of intrathecal administration of MPA in neuropathic pain patients are contradictory and safety is debated. In this review we broadly consider mechanisms whereby glucocorticoids exert their action on spinal cascades relevant to the pain arising after nerve injury and inflammation. We then focus on the characteristics of the actions of MPA in terms of pharmacokinetics, efficacy and safety when administered in the intrathecal space.

INTRODUCTION

In the late 1940's, the Nobel laureate Philip Hench described the anti-inflammatory effects of glucocorticoids.[1] In that same period, an inflammatory process was described to be an important component in the development of sciatic pain in patients with disc herniation.[2,3] The appreciation of this inflammatory component in the development of nerve compression pain states along with the insight that steroids served to diminish the inflammatory cascade, led to the use of epidural glucocorticoids in controlling the pain related to disc avulsion.[4] It was not clear however, whether the analgesic effect was related to the reduction in swelling of the injured tissue after disc protrusion, or to an effect on neural tissue itself.

By the late 1960s, a number of articles had been published reporting treatment of back pain with epidural delivery of glucocorticoids.[5] Searching PubMed for 'epidural glucocorticoids AND pain' results in > 1000 published articles.

In the early 1960s, less hydrophilic glucocorticoid formulations such as methylprednisolone acetate (MPA) began to be used in the management of spinally originating pain states.[6,7] The rationale was that low water solubility essentially creates a slowly eluted drug depot that permitted extended tissue exposure after a single application.[8] In this review, we will broadly consider the mechanisms whereby synthetic glucocorticoids might be expected to exert their action on the inflammatory and neuropathic pain cascade and critically review the literature on intrathecal use of the depot glucocorticoid formulation MPA.

MOLECULAR MECHANISMS OF ACTION OF GLUCOCORTICOIDS

Many components of an inflammatory process are modified by the local action of glucocorticoids.[9,10] Glucocorticoids exert their actions after entering a cell, where they may bind to their glucocorticoid receptor and form a complex (GR). The GR is a ligand-driven transcription factor. In the unbound form, it resides in the cytoplasm. Its binding with a glucocorticoid triggers translocation of the GR to the nucleus.[11] In the nucleus, the GR binds as a homodimer to DNA sequences called glucocorticoid response elements (GREs). GREs transcriptionally activate (transactivation) or repress (transrepression) genes.[12] In addition to transcriptional regulation, glucocorticoids also have fast non-genomic effects on the inflammatory process. These actions jointly have been shown to impact on the expression of > 6500 genes.[13]

Below, we describe in more detail these three mechanisms by which glucocorticoids alter cellular responses to inflammatory stimuli and discuss the potential effects on nociceptive processing (see also Figure 1).

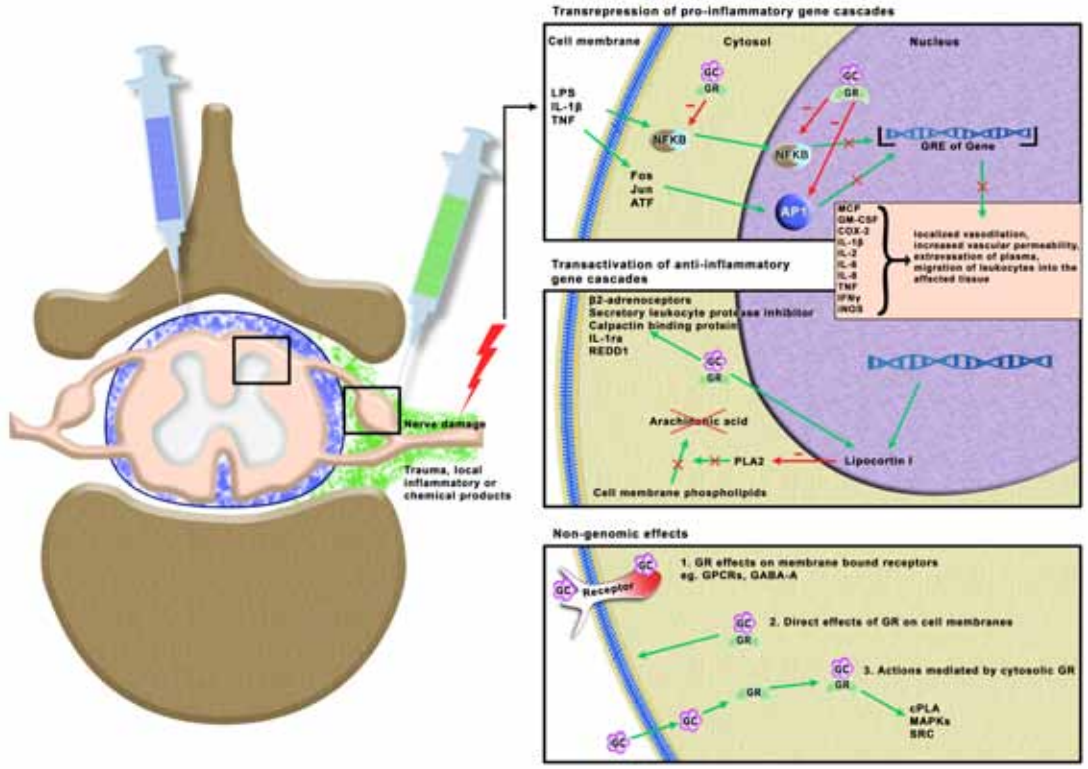


Figure 1 Molecular mechanism of action and targets of glucocorticoids. Glucocorticoids can act though three principle mechanisms. The three mechanisms by which glucocorticoids alter cellular responses in the targeted tissue can be divided into; a) Transrepression of pro-inflammatory gene cascades (upper right box), b) Transactivation of anti-inflammatory gene cascades (middle right box), and c) Nongenomic effects (lower right box). Green arrows represent activation and red arrows represent suppression. The explanation of the presented cellular processes may be found in the text. As reviewed in the text, many of these processes are believed to contribute to facilitated processing in the spinal cord dorsal horn and dorsal root ganglion (DRG) (both surrounded by a black square) leading to hyperpathic states. Treatment of the resulting pain syndrome with glucocorticoids through a transforaminal injection (green injectate) is considered to mainly target the nerve root and DRG (but not the spinal parenchyma), whereas intrathecal delivery (blue injectate) results in high glucocorticoid concentrations in the cerebrospinal fluid adjacent to the spinal dorsal horn.

TRANSCRIPTIONAL REGULATION

A) TRANSREPRESSION OF PRO-INFLAMMATORY GENE CASCADES

Transrepression is the best known mechanism of action of glucocorticoids. By binding of the GR to a GRE, transcription of inflammatory genes can be blocked. One of the important transcriptional regulators in this process is nuclear factor kappa B (NFκB). NFκB is activated by inflammatory signals such as lipopolysaccharide or pro-inflammatory cytokines such as interleukin (IL)-1β and tumor necrosis factor (TNF).[14] When activated, NFκB stimulates the transcription of a variety of products, including enzymes such as cyclooxygenase (COX)-2, IL-1β, IL-2, IL-6, IL-8, TNF, interferon γ (IFNγ), inducible nitric oxide synthase (iNOS), granulocyte-macrophage colony-stimulating factor (GM-CSF), and monocyte chemoattractant protein (MCP)-1.[14;15] These products all play a role in the inflammatory response leading to localized vasodilation, increased vascular permeability, extravasation of plasma (and humoral) proteins, and migration of leukocytes into the affected tissue. GR antagonize NFκB, thereby blocking transcription of inflammatory genes and synthesis of these pro-inflammatory products.

A second important transcriptional regulator is activator protein (AP)-1. It is a collection of related transcription factors belonging to the Fos (c-Fos, FosB, Fra1, Fra2), Jun (c-Jun, JunB, JunD) or activating transcription factor ATF (ATF2, ATF3) families, which dimerize in various combinations through a region known as a leucine zipper. AP-1 regulates various aspects of cell proliferation and differentiation. GR interacts directly and indirectly with AP-1. Interaction of GR with AP-1 and NFκB[16] leads to downregulation of inflammatory processes in the face of stimuli that would otherwise result in their upregulation and synthesis of inflammatory products.[12] Inflammatory products such as COX-2, IL-1β, IL-2, IL-6, IL-8, TNF, IFNγ, iNOS are well known and important components in neuropathic pain pathways, and reduction of these products has led to attenuation of the hyperpathic state.

B) TRANSACTIVATION OF ANTI-INFLAMMATORY GENE CASCADES

Gene products upregulated by glucocorticoids and playing a role in resolution of inflammation include lipocortin I and p11/calpactin binding protein. Lipocortin I (annexin-1A) is involved in the inhibition of phospholipase A2 (PLA2).[17] PLA2s hydrolyse phospholipid bonds, releasing arachidonic acid. Arachidonic acid plays a major role in the inflammatory cascade, and increased levels of arachidonic acid have been observed after disc avulsion as well as after nerve injury.[18] By activating lipocortin I, glucocorticoids inhibit PLA2s, thereby reducing the inflammatory response. In addition, components such as β2-adrenoceptors, secretory leucocyte protease

inhibitor, IL-1ra and REDD1 (Regulated in Development and DNA Damage-1; a stress-induced gene linked to repression of mammalian target of rapamycin (mTOR) signaling) are upregulated by glucocorticoids.[19-21] β2-agonists reduce neuropathic pain symptoms.[22] IL-1ra reduce inflammatory signs in degenerative joint disease.[23] mTOR activation plays a facilitatory role in pain states after peripheral tissue and nerve injury.[24-26]

NONGENOMIC EFFECTS

Glucocorticoids have been shown to have rapid (milliseconds to minutes) excitatory and inhibitory effects on a variety of neuronal systems. This rapidity emphasizes actions that are independent of gene transcription modulation. Evidence suggests that nongenomic effects of glucocorticoids can be classified in three ways: a) effects not mediated by a receptor, b) effects mediated by a membrane-bound receptor, and c) effects mediated by the cytosolic GR.[27]

a) Nonreceptor-mediated glucocorticoid signaling consists mainly of specific, direct effects of glucocorticoids on cell membranes. Alterations in physicochemical properties of the plasma membrane could change the activity of membrane-bound proteins (affecting calcium trafficking), or result in altered permeability of the membrane to ions.[28] These effects have been demonstrated *in vitro* using high dosages of glucocorticoids. Clinically, this mechanism of action is often discussed in the treatment of spinal traumas where high glucocorticoid doses might stabilize cell membranes in the injured area to such a degree that secondary damage is less compared with untreated patients.[29]
b) Actions mediated by membrane-bound receptors. Glucocorticoids exert rapid, transcription-independent actions through G-protein coupled receptors (GPCR) in various cells.[30] One example is the GPR30, a nongenomic estrogen receptor, discussed later in more detail. There is also some evidence that activity of the chloride ionophore γ amino butyric acid (GABA)-A-receptor is enhanced by glucocorticoid binding and may mediate some of the nongenomic effects of glucocorticoids, an effect that could modulate post nerve injury hyperpathia.
c) Actions mediated by cytosolic GR. Glucocorticoids interact with cytoplasmic proteins such as mitogen-activated protein kinases (MAPKs), phosholipases (cPLA) and protein kinases (SRC) influencing the inflammatory cascade. The SRCs and MAPKs have been widely shown to play a facilitatory role in changes in spinal function initiated by tissue injury and inflammation.[31,32]

PRO-INFLAMMATORY EFFECTS

Perhaps unexpectedly, pro-inflammatory effects by glucocorticoids have also been described. Dexamethasone increased adenosine triphosphate-induced IL-6 in epithelial cells,[33] an effect mediated through GR. It has been suggested that the effect of glucocorticoids, either an enhancement or suppression of the inflammatory response, is dependent on the timing and context of target cell exposure.[34] Glucocorticoids are clearly not uniformly immunosuppressive.

In conclusion, the overall effect of glucocorticoids is dominated by suppression of inflammatory cascades, either through a direct suppression of inflammatory processes or by enhancement of anti-inflammatory mechanisms. However, in several instances pro-inflammatory effects of glucocorticoids have been reported.[33-35] We note that such pro-inflammatory effects could be a result of the glucocorticoid action itself or due to other components of the glucocorticoid formulation. This issue is considered in more detail below.

ECTOPIC GENERATORS: TARGETS OF GLUCOCORTICOIDS IN NEUROPATHIC PAIN

After injury to primary afferents (by, for example trauma, local inflammatory or chemical products), a pain syndrome may arise often composed of a) sharp-shooting pain sensations referred to the peripheral distribution of the injured nerve and b) allodynia (abnormal sensations in response to light tactile (Aβ) stimulation of the skin). The origin of the afferent traffic associated with neuropathic pain reflects ectopic activity arising from the neuroma, for example, at the injury site and importantly, from the dorsal root ganglia (DRG) of the injured axon.[36,37] These observations emphasize that after a variety of nerve injuries, trophic changes occur in the DRG and proximal root. In contrast to the peripheral afferent axon, where mechanical compression can initiate transient depolarization in the axon, such compression in the DRG has been shown to be able to produce persistent discharges.[38-40] Also, local inflammatory products, such as TNF, IL-1β, and cytokine-induced neutrophil chemoattractant-1 that can arise from an avulsed disc, can initiate activity in DRG and injured roots.[41-43] These inflammatory products activate local DRG cell populations such as the satellite cells and pericellular glia, initiate the migration of inflammatory cells (macrophages and neutrophils) into the DRG,[42,44] and initiate downstream signaling though transcription factor activation.[45,46] The inflammatory cascade results in a further increase of the local expression of pro-inflammatory substances such as cytokines (TNF/IL-1β), chemokines,[41,45,47,48] and growth factors.[45] Blocking production or action of cytokines such as TNF and IL-1β reduces inflammation-induced pain states.[49-51]

It is important to note that activation of the DRG by these mechanical and chemical stimuli initiates a variety of downstream cascades that lead to major changes in the phenotype of the DRG. On an acute basis, for example, TNF enhances excitability of sodium channels through phosphorylation by p38 MAPK, leading to enhanced excitability.[52,53] Over a longer period of time, these DRG cascades can lead to prominent, time dependent, changes in transcription factors such as MAPKs and ATF3[54,55] and the activation of a myriad of genes through multiple downstream pathways such as those mediated by NFκB.[56,57] Specifically, there is an increase in the expression of voltage-sensitive sodium and calcium channels and a decrease in potassium channels.[58-60] This altered expression profile, often referred to as the neonatal phenotype, has the common property of enhancing membrane excitability. Although the injury is limited to the primary afferent and/or DRG, these interventions can lead to persistent changes in the spinal cord dorsal horn (SDH), including glial activation.[61,62] It is important to note that this ongoing activity, along with expression of the pro-inflammatory cytokines generated by injury in the nerve and DRG, leads to a facilitation of SDH responses[49,63,64] and hyperpathic states.[65]

The above commentary focuses on the events that transpire after frank injury or chronic compression of the peripheral nerve or the DRG. This sequence of events is also seen after other widely differing modes of nerve injury, studied in preclinical models. Chemotherapeutics[66,67] or viral inflammation (as in postherpetic neuralgia)[68-70] cause surprisingly similar changes in the SDH and DRG. Given the anti-inflammatory actions of glucocorticoids, these agents are thus expected to be able to intervene in the cascades that lead to those anomalous pain states.[62,71,72]

PRECLINICAL STUDIES CONSIDERING GLUCOCORTICOID EFFECTS ON MOLECULAR MECHANISMS CONTRIBUTING TO PAIN SECONDARY TO NERVE INJURY

Given the wealth of potential glucocorticoid targets, systematic preclinical assessments of the effects of spinal glucocorticoids on system function and surrogate markers (e.g. transcription) are unexpectedly rare.

PHYSIOLOGICAL RESPONSE

In various models of chronic pain, spontaneous nerve activity is one of the earliest abnormalities observed, and blocking this spontaneous activity is an effective way to block development of ongoing pain behaviors. Systemic triamcinolone acetonide reduced the incidence of bursting pattern ectopic discharge in DRG neurons.[73] Local

(perineural) application of either triamcinolone hexacetonide, triamcinolone diacetate or dexamethasone, substantially reduced the incidence of spontaneous ectopic discharge generated in experimental nerve end neuromas and prevented the later development of ectopic impulse discharge in freshly cut nerves.[74] There is also evidence that locally applied glucocorticoids inhibit signal transmission in nociceptive C fibers directly after application, but this did not seem to have a long-term effects on the electrical and structural properties of peripheral nerves.[75,76] Sympathetic sprouting and basket formation in the DRG was decreased after perineural application and after systemic administration of triamcinolone acetonide in the spinal nerve ligation (SNL) model.[73,77] In hippocampal slices, dexamethasone rapidly increased expression of proteins that regulate actin polymerization and produced increases in dendritic spines containing pERK but reduced other populations of spines.[78] At the spinal level, such regulation of spines would be considered to be important components contributing to synaptic plasticity underlying facilitated pain states.[79]

GLIAL CELL ACTIVATION

Activation of spinal glia after peripheral nerve injury is a common finding.[80,81] It has been reported that glucocorticoids attenuate the activation of glial cells. The activation of astrocytes was reduced in the SNL model and the spared nerve injury (SNI) model.[77,82,83] Microglia activation was attenuated after perineural treatment with triamcinolone acetonide in the SNL model, but not after intrathecal administration of dexamethasone in the SNI model.[77,84]

INFLAMMATORY FACTOR PRODUCTION

After SNI, intrathecal and perineural betamethasone reduced concentrations of pro-inflammatory cytokines TNFα and IL-1β involved in development and maintenance of central sensitization and neuropathic pain.[83] In the chronic constriction injury (CCI) and SNL models, cytokines (TNF, MCP-1, IL-6) were reduced after systemic triamcinolone acetonide.[73,85] In the development of neuropathic pain these cytokines and chemokines are regulated by the activation of transcription factor NFκB. An inhibition of NFκB function was observed after a single epidural administration of betamethasone at the time of nerve injury in a spinal nerve transsection model.[86] It partially inhibited development of hyperalgesia and attenuated elevated levels of pro-inflammatory cytokines (TNFα, IL-1β) in brain, while stimulating expression of the anti-inflammatory cytokine IL-10. Timing of these changes correlated with the development of neuropathic pain.[86]

After chronic compression of the DRG, a time-dependent upregulation of neuronal nitric oxide synthase and N-methyl-D-aspartate (NMDA) receptor 2B (NR2B) subunits of within the ipsilateral SDH was significantly diminished in a dose-dependent fashion by intrathecal prednisolone acetate. This effect was accompanied by inhibition of thermal hyperalgesia and tactile allodynia.[87]

PARADOXICAL EFFECTS OF GLUCOCORTICOIDS

Surprisingly, there is also evidence that glucocorticoids can increase neuropathic pain behavior. Preclinical evidence shows that after nerve injury there is an increased expression of glucocorticoid receptors ipsilateral to nerve injury in the SDH, with a time course parallel to that of the development of neuropathic pain behaviors.[83,88] The administration of intrathecal glucocorticoids is reported to further increase this GR expression.[83] In a subsequent preclinical study,[89] intrathecal dexamethasone exacerbated thermal hyperalgesia and mechanical allodynia in CCI rats, whereas intrathecal RU38486, a GR antagonist and potent progesterone receptor antagonist, reversed nociceptive behaviors.[89,90] In addition, an inhibition of central GR with RU38486 reduced upregulation of NMDA subunits (NR1 and NR2), which is the reverse of what has been previously noted [87,89] There is also a literature stating that the GR: a) downregulates expression of a spinal glutamate transporter (EAAC1), causing an increased level of extracellular glutamate (enhancing neuropathic pain), b) upregulates NFκB, the transcription factor that mediates production of pro-inflammatory cytokines such as TNFα, IL-1β, IL-6 etc., and c) is involved in upregulation of the cannabinoid-1 receptors (CB1R) within the SDH after CCI in rats.[91,92] There is no clear explanation for these results. Drug dosages appear comparable and all studies have used the neuropathic pain models SNL, CCI or SNI in male rats. Additional research is necessary to unravel the origin of these varying results and to further clarify the mechanisms by which glucocorticoids and their receptor influence the neuropathic pain cascade.

GLUCOCORTICOID RESISTANCE

From research in asthmatic patients we know that with increasing severity of the disease the beneficial response to glucocorticoids decreases.[93] Also in other inflammatory diseases such as acute respiratory distress syndrome, cystic fibrosis and severe rheumatoid arthritis, no clinical benefit of glucocorticoid treatment is observed. These disease entities have a severe state of inflammation in common. Studies have shown that this clinical phenomenon is caused by 'glucocorticoid resistance'.[14,93] The glucocorticoid resistance has several distinct molecular mechanisms and perhaps genetically related components, that may contribute to the decreased anti-inflammatory effects of glucocorticoids, including: a) defective GR binding and translocation to the nucleus and b) increased expression of GR β, known to influence the cell's sensitivity to glucocorticoids.[14,93-95] We hypothesize that glucocorticoid resistance might play a role in severe neuropathic pain states reducing the analgesic properties of glucocorticoids and perhaps contributing to the heterogeneous results in neuropathic pain studies.

Table 1 Effects of intrathecal glucocorticoids and their antagonists in preclinical nerve injury models

Study by	Species	Nerve injury model	Drug	Groups	Behavioral testing	Time points	Results behavior	Effect on surrogate markers
INTRATHECAL ADMINISTRATION OF GLUCOCORTICOIDS								
ANALGESIC EFFECTS								
Abram et al.[101]	Rat	Formalin	MP 400 µg IT 1h pretreatment, TCA 250 µg IT 24h pretreatment or TCA 4x 250 µg IT with 5d-interval pretreatment	G1 – Formalin + IT saline G2 – Formalin + IT MP -1h G3 – Formalin + IT TCA -24h G4 – Formalin + IT TCA -21d	Flinching	During first hour after formalin	Reduced flinching in G4 only	In SDH; No differences in the amount of dark stained neurons between the saline and glucocorticoid treated animals
Takeda et al.[82]	Rat	SNL	MP 80 µg/kg/d IT continuous POD 0 to 7 or 80 µg/kg/d IT POD 7 to 10	G1 – SNL + IT Saline G2 – SNL + IT MP POD 0 to 7 G3 – SNL + IT MP POD 7 to 10	MWT TWD	Pre, 4, 7, 10, 13, 17, 24, and 31 days, after surgery	Decreased pain behavior in all MP groups	In SDH; MP attenuated GFAP expression
Ma et al.[87]	Rat	CCD	PA every 3d IT 0.5 mg/kg, 1.0 mg/kg or 2.0 mg/kg until POD 7 or 15	G1 – Sham + IT ACSF G2 – CCD + IT ACSF G3 – CCD + IT PA 0.5mg/kg G4 – CCD + IT PA 1.0 mg/kg G5 – CCD + IT PA 2.0 mg/kg	MWT TWD	Pre, 1, 3, 5, 7, 9, 11, 13 and 15 days after surgery	Decreased pain in highest PA dose group only (G5)	In SDH; High dose PA only (G5) attenuated upregulation of NR2B and nNOS.
Gu et al.[96]	Rat	CCD	Dex 2 µg or 4 µg IT; 2x daily POD 2 to 4	G1 – CCD + vehicle G2 – CCD + IT Dex 2µg G3 – CCD + IT Dex 4µg	MWT TWD	Pre, 1, 4, 7, 10, 14, 17, 21 days after surgery	Decreased pain in both Dex groups	Not measured
INDIFFERENT OR PRO-ALGESIC EFFECTS								
Wang et al.[88]	Rat	CCI	Dex 2µg or 4µg IT 2x daily POD 1 to 6	G1 – CCI + vehicle G2 – CCI + IT Dex 2µg G3 – CCI + IT Dex 4µg	MWT TWD	Pre, 1, 2, 3, 4, 5, 6, 7 days after surgery	No effect of 2µg IT Dex on pain, exacerbation of pain with 4µg IT Dex	In SDH; Dex increased GR expression
Scholz et al.[84]	Rat	SNI	Dex 0.02 mg/kg/d IT continuous POD 0 to 14 or POD 7 to 21	G1 – SNI + vehicle G2 – SNI + IT Dex POD 0 to 14 G3 – SNI + IT Dex POD 7 to 21	MWT, cold allodynia	Pre, 4, 7, 11, 14 and 21 days after surgery	Dex caused an increase in pain behavior	In SDH; Dex did not attenuate CD11b expression
Wang et al.[83]	Rat	SNI	BM 4 µg IT administered 1x directly after nerve ligation	G1 – Sham G2 – SNI + IT saline G3 – SNI + IT BM	MWT TWD	Pre, 1, 3, 7 and 14 days after surgery	No effect of IT BM on pain behavior	In SDH; BM attenuated GFAP expression and TNFα & IL-1β levels but increased GR expression
INTRATHECAL ADMINISTRATION OF GLUCOCORTICOID ANTAGONISTS								
Takasaki et al.[90]	Mouse	SNL	GR antagonists RU486 and dexamethasone 21-mesylate IT 1x 2 to 3 wk after surgery	G1- IT Saline G2 – IT RU486 0.1 nmol G3 – IT RU486 1 nmol G4 – IT dexamethasone 21-mesylate 1 nmol	MWT TWD	2 to 3 weeks after surgery	Reversal of neuropathic pain behaviors by GR antagonists	Not measured after treatment
Wang et al.[89,91,92]	Rat	CCI	GR antagonist RU38486 or an antisense oligonucleotide against GRs IT 2x daily for POD 1 to 6	Multiple groups	MWT TWD	Pre and day 7 after surgery	Reversal of neuropathic pain behaviors by RU38486. Exacerbation of pain with 4 µg IT Dex 2x daily for 6 days	GR antagonist RU38486 and the GR antisense oligonucleotide; attenuated NMDA and cannabinoid-1 receptor expression; attenuated down-regulation of EAAC1

PRECLINICAL STUDIES ON INTRATHECAL DELIVERY OF GLUCOCORTICOIDS IN NEUROPATHIC PAIN MODELS

Of the six reports on *intrathecal* administration of glucocorticoids (methylprednisolone, prednisolone acetate, dexamethasone or betamethasone) in neuropathic pain models (SNL, CCI or SNI) in rats, three reports mention a reduction of thermal hyperalgesia and mechanical allodynia (Table 1).[82,-84,87,88,96] After local (perineural), epidural or systemic administration of glucocorticoids in neuropathic pain models (SNL, CCI or SNI) in rats, most report a reduction of nociceptive behavior.[73,77,83,97-100] Also in the formalin model suppression of phase 2 formalin flinching was observed after repeated (x4) but not single bolus dosing of intrathecal triamcinolone diacetate.[101]

SEX DIFFERENCES AND THE EFFECTS ON NEUROPATHIC PAIN

Current work has emphasized the potential contribution of sex differences in neuropathic pain cascades.[102] It has been shown that activation of the spinal innate immune receptor toll like receptor (TLR)-4 produces hyperalgesia in males, but not females[103;104] and that mutation of the TLR4 receptor in males, but not females reverse the allodynic effects of nerve injury.[103] The GPR30, a nongenomic estrogen receptor, was found to accumulate after selective spinal dorsal rhizotomy in the outer layer of the SDH after it was transported from the DRG to terminals. In ovariectomized female rats, GPR30 expression was downregulated in DRG neurons.[105]

> Table 1 SNL – Spinal nerve ligation, CCD – Chronic compression of the lumbar dorsal root ganglion, CCI – Chronic constriction nerve injury, SNI – Spared nerve injury, MP – Methylprednisolone sodium succinate, TCA – Triamcinolone diacetate, PA – Prednisolone acetate, Dex – Dexamethasone, BM – Betamethasone, GR – Glucocorticoid receptor, POD – Post operative day, IT – Intrathecal, G1 – Group 1, G2 – Group 2, G3 – Group 3, G4 – Group 4, G5 – Group 5, MWT – Mechanical withdrawal threshold, TWD – Thermal withdrawal duration, SDH – Spinal cord dorsal horn, GFAP – Glial fibrillary acidic protein (marker of astrocyte activation), NR2B – N-methyl-D-aspartate receptor 2B (subunit of NMDA receptor), nNOS – neuronal Nitric oxide synthase, CD11b – Cluster of differentiation molecule 11B (marker of microglial activation), TNFα – Tumor necrosis factor α, IL-1β – Interleukin 1β, NMDA – N-methyl-D-aspartate, EAAC1 – Excitatory amino-acid carrier 1 (glutamate transporter).

MECHANISTIC RATIONALE FOR THE INTRATHECAL DELIVERY OF GLUCOCORTICOIDS IN NEUROPATHIC PAIN STATES

Many of the above targets in the inflammatory cascade, leading to a pain state that are potentially affected by the genomic and nongenomic actions of glucocorticoids, are located within spinal cord and/or the DRG. At present, it is not feasible to parse the relative contributions of these two principal sites. Such theoretical considerations are however, of particular importance as it relates to the issue whether the glucocorticoid delivered in the intrathecal space has access to cascades in the DRG that are relevant to the injury-evoked processing. It is important to note that epidural methylprednisolone acetate is not believed to pass through the dura into the intrathecal space and/or spinal cord.[106] Hence, an intrathecal injection may be able to gain access to these intradural neuraxial systems and suggests the potential utility of the intrathecal route. If the target of glucocorticoid action is instead the DRG, there may be an issue of whether that target is reached more effectively by local application or the epidural versus the intrathecal route (Figure 1). Regarding the epidural route of delivery, if the mechanistic target is a DRG, an additional speculative consideration would be whether the interlaminar versus the transforaminal route of delivery might be more appropriate. The preclinical literature provides no specific insights into this question of either route of delivery.

CLINICAL USE OF INTRATHECAL STEROIDS

Glucocorticoids such as MPA, triamcinolone acetonide, and dexamethasone have been given in the intrathecal space in humans for different clinical pain syndromes.[7,107-109]

In the following sections, we focus on the use of intrathecal methylprednisolone, with an emphasis upon the acetate which is the depot formulation as compared to the succinate that is water soluble. MPA is of interest because it is the most commonly used glucocorticoid in the pain practice. It is primarily used for epidural injections, but as mentioned, it has also been used intrathecally in a limited fashion. Intrathecal use in pain patients has yielded contradictory results. Here we systematically review (Appendix 1 for method section) literature on the clinical efficacy of intrathecal administration of MPA in pain patients and shall elaborate on its safety, characteristics and pharmacokinetics to clarify the contradicting findings. A short note is made on the epidural use of MPA.

METHYLPREDNISOLONE ACETATE

INTRATHECAL CLINICAL REPORTS

More than 50 years ago, Sehgal and Gardner[6] described the use of epidural and intrathecal glucocorticoids for the relief of sciatica. The use of 80 mg intrathecal MPA in 75 sciatica patients was reported to result in 70 to 100% pain relief in 45 patients during four or more months.[110] Aside from an exaggeration of back or leg pain during the first 24 hours after the injection, no side effects were described. Use of intrathecal MPA was also described in other neurological disorders including arachnoiditis, multiple sclerosis and postherpetic neuralgia. In a timespan of four years over a thousand patients with various neurological disorders were treated. Positive effects were observed in patients with arachnoiditis and acute exacerbation of multiple sclerosis. No benefit was noted in postherpetic neuralgia patients, amyotrophic lateral sclerosis and trigeminal neuralgia.[7] It is important to note that none of these studies were randomized, controlled or blinded and as such should be considered as case series rather than clinical trials. Studies providing sufficient information on their study domain, treatment regimen and outcome in English are summarized in Table 2.[110-115]

Shortly after these initial reports, the use of intrathecal MPA increased and reports of serious adverse events including cerebral hemorrhage, meningitis, conus syndrome, progressive weakness, reversible bladder dysfunction and paresthesia began to appear.[116-121] Most of the side effects were reported in patients with multiple sclerosis who had received repeated administrations of MPA at short intervals. Owing to the severity of the adverse events and the frequency of their occurrence, the use of intrathecal MPA declined. Thirty years later, intrathecal glucocorticoid treatment regained interest when two randomized controlled trials (RCTs) were published in high impact journals, showing a markedly high efficacy in patients with intractable postherpetic neuralgia.[122,123] Of the 89 patients treated with four intrathecal injections of MPA combined with lidocaine, 82 (92%) had 'good or excellent' pain relief during one year follow-up, against 5 out of 91 (5%) treated with lidocaine only.[123] Several Letters to the Editor warned against this potentially harmful treatment, noting that the safety issues had not been adequately addressed.[124-128] Besides two positive case series, no other reports confirming the efficacy of MPA were published in the years after publication of these two trials.[113-114]

Recently, two negative RCTs were published.[129-130] One RCT in 21 patients with chronic complex regional pain syndrome was ended prematurely because of a lack of efficacy and a high rate of adverse events.[129] The other RCT was also stopped early because the six MPA-treated patients suffering from postherpetic neuralgia showed no clinical benefit from their MPA treatment (described in more detail in Part III, Chapter 5).[130] Taken together, the data suggests that the efficacy of intrathecal MPA

Table 2 Effects of intrathecal MPA on neuropathic pain syndromes in patients; observational, non-controlled, non-randomized studies

Study by	Type of study	Size of study population	Pain syndrome + duration of symptoms	Dosage and treatment regimen	Presence of preservative/ Additives	Results: efficacy	Results: safety
Gardner et al.[110]	Case series	75	Sciatica	1x 80mg MPA + 40mg procaine	Not mentioned	45 of 75 patients reported 70% to 100% pain relief for periods of >4 months	No adverse effects beside short term (<24 hours) increase of back or leg pain
Winnie et al.[115]	Case series	20	Sciatica	G1: 1x 80mg MPA ED G2: 1x 80mg MPA IT	Depo-medrol (PEG 29mg, MPC 0.195mg)	80% of patients in both groups experience complete pain relief	No adverse effects
Abram[111]	Case series	18	Sciatica	1x TCA 25 to 50mg or 1x MPA 40 to 80mg	Aristocort (PEG 3%, Benzyl alcohol 0.9%), Depo-medrol (PEG 29mg, MPC 0.195mg)	2 out of 18 patients; considerable decrease in pain, 6 of 18 increased pain	6 patients with increased pain during 1 to several days after injection, 1 patients with orthostatic headache > 2 wk
Benzon et al.112	Case report	1	PHN 2 y	3x 80mg MPA + 50mg lidocaine	Not mentioned	No effect	No adverse events
Candido et al.113	Case report	1	PHN > 2 y	3x 80mg MPA + 10mg lidocaine	Reduced concentration of PEG and MPC	Painrelief: 90% decrease in NRS score	No adverse events
Lu et al.114	Case series	8	PHN 1.5 months - 2 years	Four injections with 7-day intervals; 4x 60mg methylprednisolone + 0.9mL of 10% lidocaine and 0.6mL of 10% dextrose solution	Not mentioned	3 out of 8 patient had >50% decrease in pain score (of whom all had PHN for only 1.5 months)	No adverse effects mentioned

< Table 2 PHN – Postherpetic neuralgia, CRPS – Complex regional pain syndrome, G1 – Group 1, G2 – Group 2, G3 – Group 3, IT – Intrathecal, ED – Epidural, MPA – Methylprednisolone acetate, TCA – Triamcinolone diacetate, PEG – Polyethylene glycol, MPC – Myristyl-gamma picolinium chloride, PI-NRS - pain intensity numeric rating scale, VAS – Visual analogue scale.

administered according to the treatment regimens described in the trials is at best doubtful, and associated with a measurable risk.(RCTs are summarized in Table 3.)

Epidural MPA in neuropathic pain

Administration of MPA is a widely used therapy for radiculitis. The treatment is regarded as safe, though its efficacy remains debated.[131-133] In a systematic review, half of the RCTs showed benefit from epidural glucocorticoid injections and half did not.[131] In the RCTs that do show a positive effect of epidural MPA, the effect is often short lived.[132] Nevertheless, the popularity of the treatment is high among clinicians and paramedical personnel and it is used as standard practice in the treatment work-up of low back pain in most clinics. MPA is mainly used for lumbar epidural injections. For cervical epidural injections with a transforaminal approach, MPA is replaced by dexamethasone after reports of cerebrovascular events after inadvertent intravascular injection of MPA. Animal studies examining the effects of carotid artery injections demonstrate that MPA and its nonparticulate carrier and methylprednisolone succinate can produce significant injury to the blood-brain barrier.[134-135]

Recently, in the United States, there have been issues regarding contaminated vials of compounded MPA with the fungus Exserohilum rostratum that led to > 600 fungal infections of which > 300 were meningitis cases, leading to 40 deaths by January 2013.

Safety of neuraxial MPA

Despite the risk of inadvertent intravascular injections of MPA or of injecting contaminated MPA in the epidural space, the risk of epidural administration of MPA has been considered low after safety studies in preclinical models.[136] There are, however, several preclinical safety studies with intrathecal glucocorticoids reporting neurotoxicity (Table 4).[137-140] In all these studies, preservatives were not removed from the glucocorticoid formulation. Accordingly, one explanation for the observed neurotoxicity is the presence of preservatives. However, in dogs, repeated intrathecal delivery (x 4 at weekly intervals) using a reformulated MPA formulation with minimal preservatives, yielded dose-dependent histological indices of intrathecal toxicity (described in more detail in Part II, Chapter 4).[35] Although possible, we believe it is unlikely that the extent of the neurotoxic reaction is caused solely by the small concentrations of pre-

Table 3 Effects of intrathecal MPA on neuropathic pain syndromes in patients; randomized controlled blinded studies

Study by	N	Pain syndrome + duration of symptoms	Dosage and treatment regimen	Presence of preservative/ additives	Primary endpoint	Results: efficacy	Results: safety
Kikuchi et al.[122]	25	PHN >1 year	Four injections with 7-d intervals; G1- IT 4x60mg MPA + 60mg lidocaine 2% G2- ED 4x60mg MPA + 100mg lidocaine 2%	Yes; 60mg of MPA contained 43.5mg PEG and 0.3mg MPC	Global pain relief at end of treatment, 1 week, 24 weeks after treatment	VAS ¯ 50-100% 24 weeks; G1 - 12 out of 13 patients G2 - 2 out of 12 patients	No adverse events
Kotani et al.[123]	270	PHN >1 year	Four injections with 7-d intervals; G1 - IT 4x60mg MPA + 90mg lidocaine 3% G2 – IT 90mg lidocaine 3% G3 – No treatment	Not mentioned	Global pain relief at end of treatment, 4 weeks, 1 year and 2 years after treatment	VAS ¯ 50-100% 1 year; G1 – 82 out of 89 patients G2 – 5 out of 91 patients G3 – 3 out of 90 patients	No adverse events
Munts et al.[129]	21	CRPS >6 months	Single injection; G1 - 60mg MPA G2 - 1.5 ml sodium chloride 0.9%	Yes; use of commercial available Depo-Medrol containing PEG and MPC	Change in pain on the PI-NRS 6 weeks after treatment	No decrease in PI-NRS in G1 or G2	Adverse events in 16 out of 21 patients; 38% post-dural puncture headache, 43% backache. Study stopped prematurely because of lacking clinical benefit + adverse events
Rijsdijk et al.[130]	10	PHN >6 months	Four injections with 7-d intervals; G1 – IT 4x60mg MPA + 60mg lidocaine 2% G2 – IT 4x60mg lidocaine 2%	PEG and MPC reduced by ~95%	Global pain relief 8 weeks after treatment	VAS ¯ 50-100% 8 weeks; G1 – 0 out of 6 patients G2 – 2 out of 4 patients Trend of increased pain in the treatment group	No adverse events. Study stopped prematurely because of lacking clinical benefit

< Table 3 PHN – Postherpetic neuralgia, CRPS – Complex regional pain syndrome, G1 – Group 1, G2 – Group 2, G3 – Group 3, IT – Intrathecal, ED – Epidural, MPA – Methylprednisolone acetate, PEG – Polyethylene glycol, MPC – Myristyl-gamma picolinium chloride, PI-NRS – pain intensity numeric rating scale, VAS – Visual analogue scale.

servatives and have hypothesized, as have others,[134] that the particulate nature of the formulation or the glucocorticoid itself may play a role. In the following section, we shall discuss the properties and pharmacokinetics of the MPA formulation and their potential contributions to intrathecal drug efficacy and safety.

MPA DRUG FORMULATION

As noted, methylprednisolone formulations may be broadly divided amongst those that are soluble (methylprednisolone succinate) and those that are only modestly soluble (methylprednisolone acetate (MPA); 0.001% weight by volume). This lack of solubility leads to an extended release of the active product, methylprednisolone, when placed in a biological matrix (e.g., the intrathecal or epidural space), endowing the injectate with depot characteristics. The commercially available depot formulation of methylprednisolone, 1 ml of 40 mg/ml, Depo-medrol® (Pfizer, New York, USA) is a suspension of the active substance MPA as particles in a solution containing 29 mg/ml polyethylene glycol and 0.195 mg/ml myristyl-gamma-picolinium chloride. In theory, each of these components could be, individually or in combination, responsible for the observed neurotoxicity. We shall discuss each component of the drug formulation separately.

Particles

Particles in suspensions can cause a chemical irritation in tissue. An extensive literature search emphasizes that in systems such as the lung and prosthetic interfaces of joints, particulate materials such as carbon particles, particles from biomaterials and other environmental toxins including nanoparticles can initiate cytokine release that in turn leads to activation of a variety of cell adhesion factors, resulting in macrophage and neutrophil immigration.[141-143] In different study systems, particulates drive robust inflammatory reactions. The extent of the inflammatory reaction is inversely proportional to particle size and directly proportional to surface area.[141,143] In the MPA suspension, 30 to 40% are larger than 20 µm in diameter.[135,144] After dilution of MPA with saline, the proportion of large particulates increases[144] due to aggregation. The diameter of the particles does not exceed 60 µm in a lidocaine mixture.[130] The critical size range for wear particles from prosthetic joint to cause an inflammatory response has been estimated to be from 0.2 to 10 µm.[145] This suggests that most of

< Table 4 G1 – Group 1, G2 – Group 2, G3 – Group 3, G4 – Group 4.

the particles in the MPA suspension may be too large to cause a severe inflammatory response. Nevertheless, increased levels of IL-8 have been observed in the cerebrospinal fluid (CSF) after intrathecal administration of MPA in humans.[130] It is important to note that IL-8 has been hypothesized to be a pivotal mediator of particle-induced neutrophilic inflammation.[142,146] We hypothesize that the larger particles of MPA could contribute to mechanical irritation, when injected into the intrathecal space. In postmortem examinations in cows, 24 hours after administration of MPA into a joint, a significant quantity of white material believed to be MPA precipitated at the bottom of the synovial cavity embedded in a fibrin-like deposit.[147] Also after intrathecal MPA administration, white deposits were observed after one week.[35] Accordingly, it is probable that these white deposits remain present in the intrathecal space for an extended period of time. This could be a possible explaining cofactor for the inflammatory actions observed after intrathecal administration of MPA. An inflammatory response in the intrathecal space has not been observed with nonparticulate glucocorticoids such as methylprednisolone succinate.

Additives

Two additives are present in the formulation: polyethylene glycol (PEG) and myristyl-gamma-picolinium chloride (MPC).

a) PEG is used as an excipient in pharmaceutical products such as solvents in oral liquids and soft capsules, ointment bases and laxatives (e.g., movicolon, duclolax). PEG is one of the additives in MPA, and although it is often considered to be a preservative,[125,148] it is added to enhance the viscosity of the drug to increase its stability. Concerns about neurotoxicity of PEG have been voiced.[108] However, there is a substantial body of evidence indicating otherwise; PEG has been studied in spinal cord injury models because it is reported to promote restoration of functional and structural integrity of nerve tissue by direct application onto the spinal cord.[149,150] In addition, PEG is used for dural repair in humans.[151] In high concentrations, its prolonged focal application on the cord induces a conduction block.[152,153] No inflammatory responses or neurotoxicity have been observed in any of these neurosurgical applications. Although the long-term effects of intrathecal PEG have not been assessed specifically, these results suggest that PEG may not account for any evident signs of toxicity in the models thus far examined, though clearly specific studies are required to support this assertion.
b) MPC is added to enhance solubility of the MPA formulation. It maintains the stability of the particle size and reduces the likelihood of aggregation.[154] MPC is

Table 4 Safety of intrathecal glucocorticoids in preclinical animal models

Study by	Species and study size	Glucocorticoid	Groups, dose and treatment regime	Presence of preservative/ additives	Survival time	Clinical symptoms	Histopathology
Abram et al.101a	12 rats	Triamcinolone diacetate (TCA)	Four injections with 5-d intervals; G1 – 4x Saline (20µl) G2 – 4x 250 µg TCA (10µl +10µl saline flush)	Yes; 3% polyethylene glycol and 0.9% benzyl alcohol	21 days	None	G1- No signs of neurotoxicity G2- Saline and TCA treated animals showed comparable tissue compression and edematic changes near the intrathecal catheter
Latham et al.139	20 sheep	Betamethasone (BM)	G1 – 1x saline (4 to 16ml) G2 – 1x 5.7 mg BM (1ml) G3 – 1x 11.4 mg BM (2ml) G4 – 3x 5.7 mg BM (1ml)	Yes; not specified	6 weeks	None	G1 – normal, G2 – normal, G3 – 3/6 sheep; mild focal inflammation, 1/6; patchy subarachnoid fibrosis, G4 – 1/6 sheep; ischemic spinal cord damage
Kroin et al.138	28 rats	Dexamethasone (Dex)	Continuous drug infusion for 14 d; G1 – Saline G2 – 6.25 ng/h DM G3 – 12.5 ng/h DM G4 – 125 ng/h DM	Yes; 1% benzyl alcohol	14 days	None	G1/G2/G3- all animals had mild chronic inflammation and a fibrous capsule around the polyethylene catheter in the subarachnoid space. G4 – 2/6 rats; severe chronic inflammation (macrophages, lymphocytes) in the subarachnoid space, 1/6 rats; focal necrosis in the meninges at the catheter tip.
Barros et al.137	21 dogs	Betamethasone (BM)	G1 –1x saline (1ml) G2 –1x 1.75 mg BM (1ml) G3 – 1x 3 mg BM (1ml)	Yes	21 days	None	G1- normal, G2 & G3 – 4/14 dogs; fibrous thickening of the meningeal layer, presence of inflammatory cells, nerve tissue necrosis.
Lima et al.140	14 dogs	Methylprednisolone acetate (MPA)	G1 – 1x saline (1ml) G2 – 1x 1.15 mg/kg MPA (1ml)	Yes	21 days	None	G1 – normal, G2 – all 7 dogs; meningeal thickening and lymphocytic infiltration, 3 dogs; adherence among the pia, arachnoid, and dura matter, nerve roots completely encircled by fibrous tissue, 1 dog; necrosis of the dorsal spinal cord.
Rijsdijk et al.35	17 dogs	Methylprednisolone acetate (MPA)	Four injections with 7-d intervals; G1 – 4x lidocaine 2% (0.5ml + 0.5ml saline flush) G2 – 4x 10 mg MPA + lidocaine 2% (0.5ml + 0.5ml saline flush) G3 - 4x 40 mg MPA + lidocaine 2% (0.5ml + 0.5ml saline flush)	No	1 and 6 weeks	None	G1 – minimal histologic changes G2 – diffuse inflammatory reaction G3 - severe inflammatory response, with large inflammatory masses. No neuronal injury, demyelination, or gliosis was seen in any animal.

a cationic surfactant with emulsifying properties. It also has antibacterial activity. There have been no specific studies on the intrathecal safety of this agent. However, there are data on intravitreal delivery of MPC. Intravitreal delivery gives direct drug exposure to local neuronal systems (e.g., retina) in a low flow situation. In this regard, it has been shown that the intravitreal delivery of small volumes of MPC, in concentrations similar to those employed in the MPA formulation, resulted in loss of photoreceptors, thinning of the retina close to the injection site and irreversible effects upon electroretinograms in rabbits.[155] Comparable retinal effects are also seen with other cationic detergent surfactants such as benzalkonium chloride.[156] Such observations raise strong concerns about potential neuraxial toxicity. The spinal and neural delivery of other detergents has been reported to result in demyelination and ultrastructural changes.[157,158] Electrophysiologic studies have indicated that the local delivery of small amounts of lysophospholipids, the major component of detergents, into the dorsal part of the spinal column produces significant signs of increased electric activity, axonal cross talk, and mechanical sensitivity of the dorsal columns, presumably reflecting the underlying histopathologic changes.[159] These results suggest a potential deleterious effect of detergent-like molecules, such as MPC on neural tissues at high concentrations and their safety should be called into question until specifically shown otherwise at relevant concentrations.

SEPARATIVE TECHNIQUES

Given the above commentary, it is important to note that there are no preservative-free, commercial formulations of MPA.[148,160] Accordingly, several groups have attempted to reduce the adjuvant burden of the commercially available formulation using various 'bedside' separation protocols. Two practical methods have been described to decrease the concentrations of PEG and MPC in the commercial MPA formulation;

a) Centrifugation of the vial and aspirating and discarding the supernatant. The residual pellet, the MPA, can then be resuspended with, for example, saline and/or lidocaine.[35]
b) Inverting the vial for two hours to let the MPA sink against the lid and then carefully aspirating the precipitate (glucocorticoid) component.[148]

The concentration of MPC was markedly reduced to 0.01 mg/ml (from 0.2 mg/ml in the commercial formulation) using method a.[35] Unfortunately, the concentration of PEG was not measured in the reformulated dose form. PEG however is completely soluble in water and because the concentration of MPC was reduced by nearly 20-fold, the concentration of PEG is estimated to be reduced accordingly to <2 mg/ml. Using method b, the concentration of PEG decreased to 4.30 ± 1.07 mg/ml.[148] The concentration of the preservative MPC was not measured. The concentration of PEG

in both formulations is considered to be too low to induce a nerve induction block (seen at PEG concentrations above 40%)[152,153] or cause inflammation.[149] We cannot completely exclude that the MPC concentration is low enough to prevent any toxicity from occurring.

PHARMACOKINETICS OF MPA

An extremely important component of the rationale for intrathecal versus epidural delivery is the observation that in normal neuraxial kinetics, MPA injected systemically or in the epidural space does not lead to measurable methylprednisolone levels in the CSF.[8,106] It has been suggested that this phenomenon is caused by the effective exclusion at the blood-brain barrier by P-glycoprotein (*mdr1* gene product), an efflux transporter for which methylprednisolone has been identified as a substrate.[106] Thus, it is hypothesized that the poor bioavailability of glucocorticoids after intravenous administration results from active exclusion of the drug from the spinal cord by P-glycoprotein.

When MPA is injected in the intrathecal space, it is hydrolyzed by cholinesterases to become soluble and thus clinically active.[161] Although relative to plasma, the concentrations of cholinesterases in CSF are very low, it is not clear whether these CSF concentrations present a limiting factor in bioavailability resulting in the depot properties.[162,163] Free methylprednisolone enters cells, gets transported to the systemic circulation, and/or gets metabolized. There is a considerable individual variation in the rate of absorption of the glucocorticoid from the spinal fluid into the blood after intrathecal MPA administration.[8] Peak methylprednisolone plasma concentrations were observed between three and six hours after intrathecal MPA injection in dogs.[35] Although plasma levels decreased after six hours, methylprednisolone was still measurable after seven days, but went below the detection threshold three weeks after the last injection.[35] In humans, a similar timeframe was described; after 80 mg MPA, intrathecal peak plasma and CSF levels were observed after one day and were measurable until 21 days.[8,130] After intra-articular injection of MPA, methylprednisolone plasma levels peak at a similar point in time, 8 hours after administration.[164] However, the plasma levels decreased below the detection level much earlier, after 24 to 192 hours.[164] In a study on intra-articular injections in a bovine model, this timeframe was observed, but when measuring the methylprednisolone concentration in the synovial fluid itself, pharmacologically significant concentrations were observed for > 3 months after administration and a significant quantity of white material believed to be MPA present at the bottom of the synovial cavity.[147] Besides methylprednisolone plasma and CSF levels, other variables, such as blood glucose levels, total white blood cells and decreases in endogenous hydrocortisone have been used to determine the

length of effect after intrathecal MPA administration.[165] The effects on the variables were dose dependent. With intrathecal MPA doses above 80 mg, an increase in CSF protein levels occurred.[8] After intra-articular administration, untoward reactions and decreased plasma concentrations of endogenous glucocorticoids for a period of 3 days in horses, as long as 1 week in humans, and 12 weeks in cows have been reported.[147,166]

Summarizing the MPA formulation characteristics regarding their effect on safety and efficacy, we believe that the spinal/meningeal reactions observed after intrathecal administration have a multifactorial etiology. Both the particulate nature of the formulation, the presence of a minimal amount of the preservative MPC and the extremely long exposure of spinal tissue to methylprednisolone due to its pharmacokinetics properties could all contribute to the observed neuroinflammatory phenotype. Regarding efficacy, because we do not have a clear picture of the effects of glucocorticoids on neuropathic pain pathways and the positive effects could be nullified by the potential neurotoxic effects, the efficacy of intrathecal MPA remains debated.

CONCLUSIONS

The reports on the efficacy of intrathecal administration of MPA in patients with neuropathic pain are contradicting. Considering the doubtful clinical benefits and the potential risks of the treatment, intrathecal administration of MPA in neuropathic pain patients is not recommended. However we do not disregard all glucocorticoids for the treatment of chronic pain. There is evidence that at least a short term beneficial effect may be expected after epidural administration in patients with radiculitis.

We find it strange, however, that in spite of; a) the vast amount of work that has been accomplished with neuraxial glucocorticoids, b) the evident role of biological targets sensitive to glucocorticoid modification and c) the well defined role played by these neuraxial targets in tissue and nerve injury pain processing, we continue to be uncertain of either the likely mechanism of action of glucocorticoids in the neuropathic pain cascade or whether at therapeutic doses in clinical or preclinical models we can demonstrate surrogate marker changes (e.g. cytokine release, genomic activation in the DRG, and change in glial activation in the SDH). A better understanding of the mechanism of action of these drugs in the neuropathic pain cascade could possibly improve the clinical effect that they should theoretically have by modifying their use in current clinical practice. Finally, this review further emphasizes the critical important of appropriate preclinical safety assessments in the development of drugs and formulations for clinical delivery and the emphasis placed by the peer reviewed literature on such robust assessments.[167-169]

APPENDIX 1 SEARCH STRATEGY

The databases Pubmed, Embase and the Cochrane library were systematically searched to answer the research question; Does intrathecal administration of glucocorticoids decrease neuropathic pain? The search syntax contained the domain, all possible synonyms for neuropathic pain, and the determinant, synonyms for intrathecal glucocorticoids. We decided not to limit the determinant to the administration of methylprednisolone acetate only based on the small amount of studies retrieved during our first systematic search.

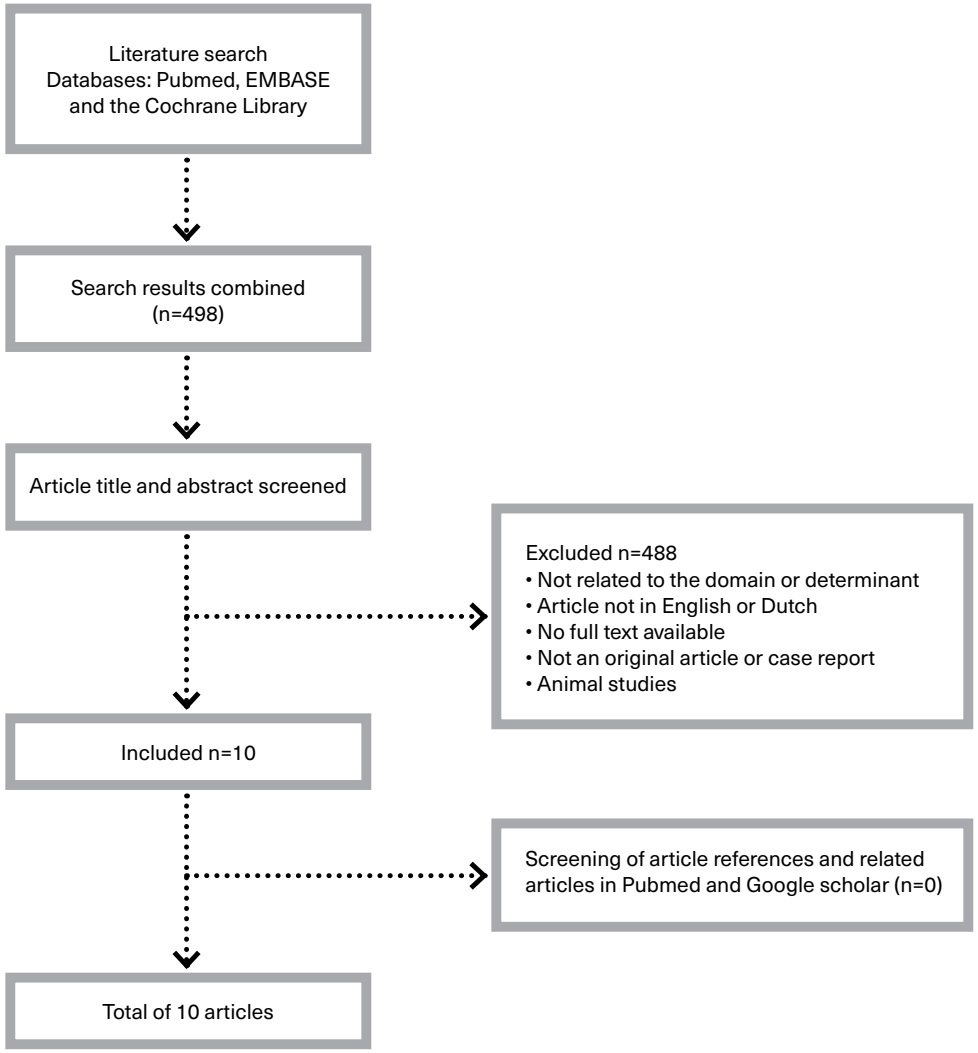
SEARCH SYNTAX

((“neuropathic pain” OR neuralgia OR allodynia OR allodynic OR hyperalgesia OR hyperalgesic OR neurogenic OR neuralgias OR “nerve pain” OR “nerve pains” OR neurodynia OR neurodynias OR phn OR crps OR “complex regional pain syndrome” OR sciatica OR “low back pain” OR radiculopathy) AND (neuraxial OR intrathecal OR spinal OR subdural OR arachnoidal OR intraspinal OR intradural) AND (steroids OR steroid OR corticosteroid OR corticosteroids OR glucocorticoid OR glucocorticoids OR methylprednisolone OR “depo medrol” OR “solu medrol” OR “depomedrol” OR triamcinolone OR dexamethasone OR hydrocortisone OR prednisolone OR prednisone OR cortisone OR betamethasone))

In all databases, we only searched for the above described terms in the study title and/or the abstract; In Pubmed [Title/Abstract] was added, in EMBASE & Cochrane Library ab,ti.

In total, 498 articles were retrieved of whom 6 were selected for Table 2 and 4 for Table 3. The references and the related articles (first 20 hits) of the selected articles were screened for missing articles in Pubmed en Google scholar. This revealed no extra articles.

Table 5 Flowchart literature search



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2

PHARMACOLOGY AND SAFETY OF INTRATHECAL METHYLPREDNISOLONE

CHAPTER 3

Pharmacokinetics of methylprednisolone acetate suspension administered in the intrathecal space for neuropathic pain

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Submitted

ABSTRACT

Aim – Methylprednisolone acetate (MPA), a glucocorticoid suspension that converts to the biologically active methylprednisolone (MP), has been administered in the intrathecal space to treat neuropathic pain syndromes. The objective of this study was to determine the pharmacokinetics of MP after intrathecal MPA administration.

Methods – In a randomized double-blinded trial, patients with intractable postherpetic neuralgia were treated with either four intrathecal injections of 60 mg MPA combined with 60 mg lidocaine or 60 mg lidocaine only (control group) for four consecutive weeks with 7-day intervals. Cerebrospinal fluid samples were obtained just before each injection (trough level) and 1, 4 and 8 weeks after the last injection to measure MP and MPA concentrations. Using NONMEM a one compartment population model could be established for MP.

Results – Six patients received intrathecal MPA. Up to 8 weeks after injection, MP and MPA were detectable in the cerebrospinal fluid. A distribution volume of MP of 27.5 L (relative standard error (RSE) 23.5%), a clearance of 90.4 mL/h (RSE 11.8%) and an elimination half-life of 8.8 days were estimated.

Conclusion – After intrathecal administration, MPA acts as a slow-release depot for MP. The result is prolonged exposure of neuraxial tissue to both MPA and MP.

INTRODUCTION

Intrathecal administration of methylprednisolone acetate (MPA) is considered an experimental treatment. In the 1960s, it was first used in patients suffering from sciatica and multiple sclerosis[1] and more recently in patients with chronic complex regional pain syndrome[2] and postherpetic neuralgia (PHN).[3-5] In a randomized controlled trial (RCT) published in the New England Journal of Medicine[4], 89 of 270 patients suffering from PHN received intrathecal treatment with MPA 60 mg, resulting in a profound reduction in pain (92% of patients had good or excellent pain relief up to two years follow up) without side effects or complications. This publication increased the interest for the treatment. Some clinicians initially offered the therapy to their patients suffering from intractable PHN, accepting the risks of the invasive approach. However, intrathecal MPA never became part of standard care for intractable PHN. The safety and efficacy results described by Kotani et al.[4] were considered 'too good to be true[6-11] and calls were made for at least one replication trial.[12] We designed a replication RCT using a similar treatment regime as in the previously mentioned trial. Because the commercially available MPA contains a potentially harmful preservative, myristyl-gamma-picolinium chloride, we reformulated it reducing the preservative concentration, potentially increasing the safety of the treatment. Cerebrospinal fluid (CSF) samples were collected to perform present pharmacokinetic study. Our RCT was stopped early by the Data Safety and Monitoring Board after inclusion of only ten patients, because a) all patients treated with intrathecal MPA reported increased pain, b) there was statistical evidence of futility and c) there were new doubts about the safety of the treatment.[5] A recent preclinical safety study reported that intrathecal MPA caused dose-dependent meningeal inflammation in dogs, a potentially severe neurotoxic side effect.[13] Intrathecal administration of MPA is therefore no longer a recommended treatment. To better understand the findings of increased pain and neurotoxicity – which could also be the result of the particulate suspension – we studied the kinetics of MPA and MP after intrathecal MPA administration in the patients randomized to MPA.

METHODS

Data were collected in a double-blinded RCT in which PHN patients were treated with either intrathecal MPA combined with lidocaine or with lidocaine only.[5] A detailed description of the methods is presented elsewhere[5], a summary can be found below. The protocol of the study[5] was approved by the Ethics Committee of the University Medical Center Utrecht, the Netherlands. The trial was registered in ISRCTN under the trial number TN88145753. Written informed consent was obtained from all subjects.

STUDY DESIGN: SUMMARY OF THE RCT

Patients referred to the pain clinic of the University Medical Center Utrecht, the Netherlands, who had intractable PHN were included in the study. Patients were randomly assigned to receive four intrathecal injections with either 60 mg MPA combined with 60 mg lidocaine (treatment group) or 60 mg lidocaine alone (control group). Intrathecal injections were performed at the L2-L3 intervertebral space and the study medication was administered with 7-day intervals. Lidocaine was added in the Kotani trial[4] and in our RCT[5] to confirm intrathecal injection with a motor block and delivery at the neuropathic dermatome by a sensory block.

DRUG PREPARATION AND ANALYSIS

The MPA study medication was reformulated from the commercially available MPA formulation, 1 ml of 40 mg/ml MPA (Depo-medrol®, Pfizer, New York, USA), to reduce the concentrations of the preservative myristyl-gamma-picolinium chloride 0.195 mg/mL and the adjuvant polyethylene glycol 29 mg/ml. The commercially available MPA formulation was centrifuged and the supernatant was discarded following aseptic precautions according to a previously published procedure.[14] The residual pellet of 60 mg MPA (1.5 ml of the commercially available MPA formulation) was resuspended in 3.0 ml of lidocaine 2% and 0.75 ml glucose 50% to a total volume of approximately 3.9 ml.

Three pilot batches of the study medication were prepared to study resuspendability, pH and relative density. The reformulated MPA, was resuspendable, with a pH between 6.2 to 6.6 and a relative density between 1.03 to 1.05 g/ml, comparable to the formulation mentioned in the Kotani trial (pH 6.2 to 6.7, relative density 1.040).[4]

To analyze particle size, two samples were taken from a batch of reformulated MPA on eight consecutive days and studied using a Olympus Optical microscope. In the reformulated MPA, particle size was in 100% smaller than 60 µm, 95% smaller than 40 µm, 88% than 20 µm and 85% than 10 µm, compared to the commercially available MPA formulation in which particle size was 100% smaller than 40 µm, 98% smaller than 20 µm and 71% smaller than 10 µm. Myristyl-gamma-picolinium chloride and MPA concentrations were determined using respectively an ultraviolet detector with a lower detection limit of 2 ng/m and high-performance liquid chromatography (HPLC-MS-MS). In the reformulated MPA, the measured MPA levels was 97% (relative standard deviation (RSD) 9%) of the targeted 60 mg. The concentration of myristyl-gamma-picolinium chloride in the commercial formulation was 0.20 mg/ml (as stated on the information leaflet) and was reduced to < 0.02 mg/ml in the reformulated MPA. The concentration of the adjuvant polyethylene glycol was not measured in the study medication. Due to the relative high solubility in water, polyethylene glycol is largely removed by aspirating the aqueous supernatant from the commercial formulation.

BIOANALYTICAL ASSAY

Before every intrathecal injection of the study medication and one, four and eight weeks after the last injection, 5 ml of CSF was sampled for measurement of methylprednisolone (MP) and MPA levels. Samples were immediately placed on ice and stored in a -80°C freezer. They were sent in three batches to the Doping Control Laboratory, Department of Clinical Chemistry, Microbiology and Immunology in Gent, Belgium. CSF MP and MPA levels were measured using High-Performance Liquid Chromatography with Mass Spectrometry with a lower detection limit of 1 ng/ml for MP and 2 ng/ml for MPA. The analysis was known to have an accuracy of 95% and precision of 98.7%.

PHARMACOKINETIC ANALYSIS

A pharmacokinetic analysis was performed using non-linear mixed effect modeling in NONMEM (version VI level 2, ICON, Ellicott City, MD, USA) with R (version 2.13.2), Xpose (version 4) for data visualization and Piraña for run management.[15;16] Since only trough concentrations were measured and no physical-chemical data was available on dissolution and hydrolyzation of MPA in the CSF, the assumption was made that all MPA hydrolyzed immediately into MP after intrathecal administration.

A one-compartmental model was developed for MP with first-order absorption. The first-order conditional estimation method with η-ε interaction was used (FOCEI). The log-likelihood ratio test was used to discriminate between hierarchical models, based on the objective function value (OFV), where $p<0.005$ (decrease in OFV of at least 7.83 points for one degree of freedom, χ^2 distribution) was considered to be statistically significant. Based on typical value parameter estimates of V and CL, MP half-life in the CSF was calculated using (ln2 * V)/CL.

RESULTS

PATIENT CHARACTERISTICS

Six patient were included in the study. Of the six included patients, four were female and two male with a median age of 76 years (range 67 – 88 years), median body mass index of 24.7 kg/m2 (range 19.4 – 32.0 kg/m2) and without known liver or kidney disease. PHN persisted with a median of 24 months (range 13 – 66 months), resulting in a median visual analogue scale score of 7.5 (range 5.6 – 9.3) in dermatomes ranging from Th4 - L3. Patients were treated with acetaminophen and/or nonsteroidal anti-inflammatory drugs only (two patients) or a combination of the following drugs; anti-epileptics, tricyclic antidepressants, topical lidocaine and/or fentanyl, and/or methadone (four patients).

MP AND MPA LEVELS IN CSF AFTER INTRATHECAL MPA DELIVERY

Observed MP concentrations in the CSF are shown in Figure 1. The highest MP concentrations were measured one week after the fourth intrathecal MPA administration in all patients with a median of 2488 ng/ml (range 1418 to 4626 ng/ml). The measured MPA concentration at that time was 2.1 to 9.9% of the MP concentration with a median of 141 ng/ml (range 41 to 423 ng/ml).

MP concentrations were detectable in the CSF until the last measurement, eight weeks after the fourth intrathecal MPA administration in all patients with a median concentration of 188 ng/ml (range 4.0 to 330 ng/ml). MPA concentrations were detectable until eight weeks follow-up in 3 out of 5 patients with a median concentration of 2.4 ng/ml (range 0 to 23.4 ng/ml).

We have excluded five measurements from the dataset before making the final model. Once a high MPA concentration of 7993 ng/ml was measured. Twice an unexpected low MPA and corresponding MP concentration was measured. A possible explanation for the low concentrations could be that the MPA injection ended partly in the epidural space. The high MPA concentration could be caused by CSF sampling near the precipitated MPA depot in the intrathecal space.

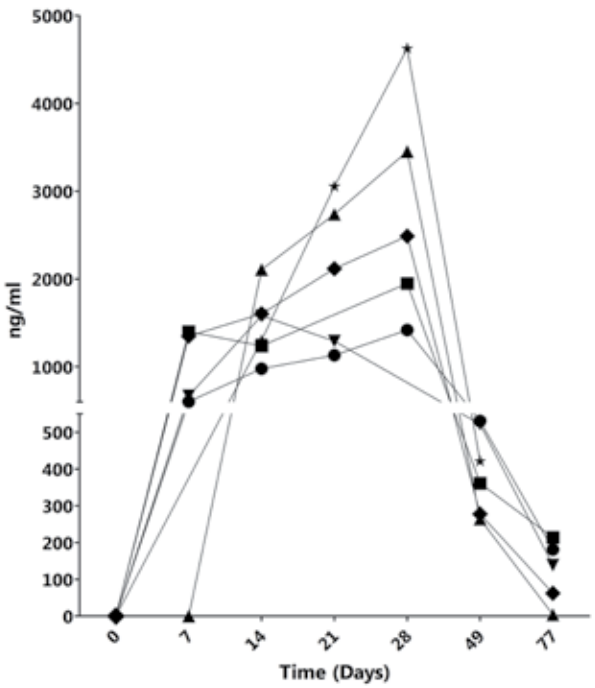

Figure 1 Observed individual methylprednisolone (MP) (black) trough concentrations. x-axis; time in days after first administration, y-axis; MP concentration in ng/ml. Squares = patient 1, diamonds = patients 2, circle = patient 3, upright triangle = patient 4, star = patient 5, upside down triangle = patient 6.

MODELING

A one-compartmental model was developed which resulted in a mean volume of distribution (V) of 27.5 L (RSE 23.5%) and mean clearance (CL) of 90.4 mL/hour (RSE 11.8%), presented in Table 1. MP half-life in the CSF was calculated to be 8.8 days. The estimated population concentration-time curve for MP is shown in Figure 2. Individual parameter estimates are displayed in Table 2. Estimated inter-individual variability was 28.8% on clearance and 51.2% on distribution volume.

Table 1 Parameter estimates of full MP dataset

RSE = relative standard error

	Estimate	RSE(%)
STRUCTURAL MODEL		
Clearance (mL/h)	90.4	11.8
Distribution volume (L)	27.5	23.5
INTERINDIVIDUAL VARIABILITY		
CL (%)	28.8	20.2
V (%)	51.2	31.9
RESIDUAL VARIABILITY		
Additive error (µg/L)	97.3	26
Proportional error (%)	19.4	26

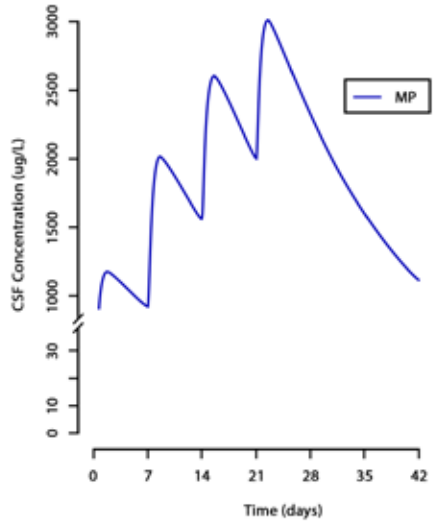

Figure 2 Estimated mean MP concentration curve. x-axis; time in days after first administration, y-axis; MP concentration in µg/L. Blue line represents MP concentration curve.

Table 2 Parameter estimates of individual patients

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6
Predicted Clearance (mL/h)	89.0	90.9	129.9	72.0	59.2	117.8
Predicted Distribution volume (L)	26.8	23.5	68.2	17.5	16.5	33.9
Calculated half-life (days)	8.7	7.5	15.2	7.0	8.0	8.3

DISCUSSION

After four repeated weekly intrathecal injections of MPA, neuraxial tissue is exposed for over eight weeks to both soluble MP and particulate MPA. The MPA suspension is slowly converted to MP and thus most likely acts as a slow-release depot for MP.

PHARMACOKINETICS

After intrathecal administration, the MPA formulation will be diluted by the CSF and spread along the spinal column. MPA particles are expected to sink due to gravitational forces as is observed ex vivo.[17] When injected in vivo, MPA will be slowly hydrolyzed to the active component MP. The freed MP will enter cells, get actively transported or passively diffuse across membranes into the systemic circulation and get metabolized. From preclinical research in rats and dogs, it is known that peak MP plasma levels are observed after respectively one and three hours (t_{max}) after intrathecal MPA injection.[13;18] After intra-articular injection of MPA, peak MP plasma levels were observed four to eight hours after injection.[19-21] MP plasma levels decreased fast between 24 to 72 hours[13;18;19] both after intrathecal and intra-articular injection, but were detectable up to 21 days after injection.[13;20;22] We hypothesize that the early peak MP plasma levels are mainly caused by the free MP fraction that is already present in the reformulated MPA preparation when injecting the drug, since hydrolyzation of MPA to MP is expected to occur slower than the observed t_{max}. This is confirmed in a study in dogs, in which at necropsy, white deposits believed to be MPA were observed in the intrathecal space near the injection site, one to six weeks after the last intrathecal MPA injection.[13] In addition, in cows white deposits were observed eight days after intra-articular injection of MPA, at the bottom and in the cul-de-sacs of the synovial cavity.[21] Analysis of this material revealed only the presence of MPA at a very high concentration.[21] Even though at that point, plasma MP levels were low, MP levels in the intrathecal and synovial space remained high (elevated up to 5.5 weeks in horses[20], up to 7 to 14 weeks in cows[21]). This corresponds with the results in the current study, where MP levels in the intrathecal space were detectable at eight weeks follow-up in the CSF.

In an in vitro study, butyrylcholinesterase (also known as plasma cholinesterase) was identified as one of the enzymes involved in hydrolysis of MPA to MP, increasing the speed of hydrolyzation.[23] A possible explanation for the slow clearance of MPA from the intrathecal space is that, relative to plasma, the concentration of butyrylcholinesterase in CSF is low (plasma: CSF = 700: 1).[24] Particle aggregation might also reduce MPA clearance from the CSF. In the reformulated MPA, particles were on average larger than in the commercially available MPA formulation (12% particles between 20 and 60 µm diameter in the reformulated versus 2% of particles between 20 and 40 µm and none >40 µm in the commercial formulation). Increased particle size leads to slower dissolution/hydrolyzation due to the fact that the total particle surface area is decreased, resulting in slower clearance. There is no presumed effect of the co-administration of lidocaine and/or glucose on the pharmacokinetic properties of the MPA formulation. The involvement of the active p-glycoprotein (mdr1gene product) transporter, a transmembrane protein located in the apical membrane of endothelial cells of brain capillaries, in the slow clearance of MPA from the CSF is also unlikely. P-glycoprotein is an efflux transporter in the blood brain barrier for which methylprednisolone has been identified as a substrate.[25] When methylprednisolone is injected systemically or in the epidural space it actively pumps methylprednisolone out of the cell and that results in no measurable methylprednisolone levels in the CSF.[22;25] Peak MP plasma levels after intrathecal MPA administration have, however, been observed within hours, making the role of p-glycoprotein as a barrier for rapid clearance of MP from the CSF unlikely.[26]

The median MP concentration in the CSF measured one week after the fourth intrathecal MPA injection (60 mg) in present study was 2488 ng/ml. This was a trough level, so higher MP CSF concentrations are expected earlier in time. Comparing our MP CSF concentration with peak MP plasma concentrations in literature, lower concentrations are observed after 80 mg oral and 80 mg intravenous MP of respectively 800 ng/ml and 650 to 1000 ng/ml.[27-29] In plasma, methylprednisolone binds to albumin.[28] The concentration of protein in CSF is only 0.2-0.5% of that in blood. Therefore higher free MP concentration shall be present in the CSF compared to plasma. A dose reduction for intrathecal administration might therefore be advised.

MODELING

In the present study the assumption was made that all MPA would be hydrolyzed to MP, a result of the fact that only trough concentrations were measured and no dissolution and hydrolyzation data were available. A mean volume of distribution of 16.7 to 68.8 liter was calculated which is rather large for the CSF, estimated to be 150 ml. We know from our CSF samples that it take at least over eight weeks before all MPA is converted to MP. The slow conversion of MPA to MP is not integrated in our one-

compartment model of MP. Because MPA slowly converts to MP, the measured MP CSF concentrations are relatively low compared to a situation in which all MPA was converted to MP. Therefore a large mean volume of distribution is estimated by the model. Our data shows that a dosing regimen of four intrathecal injections of MPA 60 mg with weekly intervals leads to accumulation of MPA and MP in the CSF and prolonged exposure of neuraxial tissue to the drug. A half-life of MP of 8.8 days was calculated with the V and CL estimated by our model. A dosing interval of 5*8.8 = 44 days would not lead to accumulation. A reduction of the MPA dose and/or an increase of the dosing interval would be indicated to reduce the exposure of neuraxial tissue to prolonged high dose MP and MPA.

The MPA and MP CSF concentrations varied within and between patients. Inter-individual variability has been previously observed.[21;22] In humans after intrathecal MPA administration, considerable variation in MP plasma levels was described, explained by the authors by variation in the rate of absorption of the glucocorticoid from the spinal fluid into the systemic circulation after intrathecal MPA administration.[22] Other explanations could be the interindividual difference in conversion of MPA to MP by differences between CSF butyrylcholinesterase concentrations in patients, and/or varying aggregation of MPA particles and pellet formation in the caudal sac. Also the sampling of CSF might have introduced variability by unintended CSF withdrawal above or below L2-3, contamination of the sample with blood and/or aspiration of aggregated MPA particles.

High predicted volumes of distribution were estimated for patient 3 and 6 of respectively 68 and 34 liters. Their patient characteristics could not explain why both patients had a higher volume of distribution compared to the other four patients (patient 3 was male, aged 89 with a BMI of 19.4, patient 6 was female, aged 79, with a BMI of 32). Describing their MP concentration curve, they both have lowest MP peak levels and highest MP levels at 4 and 8 weeks follow-up. This suggests that the conversion of MPA into MP is slower in both patient 3 and 6. Unfortunately we have not measured butyrylcholinesterase levels in our patients and cannot test our hypothesis that a reduced butyrylcholinesterase level is responsible for the observation.

SAFETY

The characteristics of reformulated MPA are isobaricity, a pH in the normal range, presence of particles smaller than 60 µm and a small restituent of a neurotoxic agent (< 0.02 mg/ml myristyl gamma picolinium chloride). The particulate nature of the formulation, the presence of a minimal amount of the preservative myristyl-gamma picolinium chloride and the prolonged exposure of neuraxial tissue to methylprednisolone owing to its pharmacokinetics properties, could all contribute to the observed

neuro-inflammatory phenotype.[13;30] In other biosystems such as lung and joints, particulate materials such as carbon particles as well as other environmental toxins can initiate robust inflammatory reactions.[31] The role of particles in inflammatory reactions in the intrathecal space has yet to be systematically studied. Further studies on this intriguing hypothesis are clearly warranted as they raise the question of whether particulate formulations in general may be contraindicated in the intrathecal space. To the best of our knowledge no other suspension have been researched after intrathecal administration.

LIMITATIONS

The RCT, during which the CSF samples were collected, was designed to study the efficacy of intrathecal MPA in patients suffering from postherpetic neuralgia, not to study the pharmacokinetics of MPA after intrathecal administration. Therefore some variables such as the concentration of free MP in the reformulated MPA and physical-chemical data on dissolution and hydrolyzation of MPA in the CSF were not determined. We also did not collect MP plasma levels after intrathecal MPA administration in our patients. The advantages of the collection of plasma samples were discussed, but our ethical committee regarded this as a too large burden for our patients since they already underwent four intrathecal CSF withdrawals combined with injections and blood withdrawal.

In conclusion, after intrathecal administration of MPA, probably a slow-release intrathecal depot of MPA is formed. We hypothesize that the dissolution of MPA and the conversion to MP is the rate-limiting-step for the appearance of MP in the CSF. Prolonged exposure up to eight weeks of neuraxial tissue to MPA and MP has been observed in current study. The combination of the long tissue exposure to methylprednisolone, the particulate nature of the drug, and the presence of a minimal amount of preservative in the MPA formulation could all contribute to neurotoxicity. The safety of a suspension in the intrathecal space should be studied separately.

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CHAPTER 4

Safety assessment and pharmacokinetics of intrathecal methylprednisolone acetate in dogs

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ABSTRACT

Background – Intrathecal methylprednisolone acetate (MPA) has been used in patients with chronic pain syndromes. Its safety has been debated after reports of adverse events. No systematic preclinical evaluation of MPA has been reported. In the current study, the acute and long-term effects of intrathecal MPA on dog spinal tissue was studied with the injectate reformulated to include minimal adjuvants.

Methods – Seventeen dogs were implanted with intrathecal catheters and randomized to three groups: vehicle (lidocaine; 4 dogs), MPA 20 mg/ml (human dose; 7 dogs), and MPA 80 mg/ml (maximum deliverable dose; 6 dogs). In parallel with the human protocols, dogs received four injections at 7-day intervals. Clinical observations and plasma methylprednisolone measurements were done before and at intervals after intrathecal delivery. One week (acute) or six weeks (long-term) after the last injection, animals were sacrificed and spinal tissues harvested for histopathology.

Results – Other than a brief motor block, no adverse clinical event occurred in any animal. Group A (vehicle) showed minimal histologic changes (median histology-score; acute: 1.3, long-term: 1.0). Group B (MPA 20 mg/ml) had a diffuse inflammatory reaction (acute: 2.0, long-term: 3.0), group C (MPA 80 mg/ml) a severe inflammatory response, with large inflammatory masses (acute: 4.0, long-term: 7.0) The severity of the inflammatory reaction increased significantly with increasing dose at long-term sacrifice (acute p = 0.167, long-term p = 0.014). No neuronal injury, demyelination, or gliosis was seen in any animal.

Conclusion – These results, showing dose-dependent intrathecal inflammatory reactions at MPA doses and injectate concentrations comparable to those employed in humans, indicate that the continued use of this modality in humans is not recommended.

INTRODUCTION

The use of intrathecal corticosteroids began in the 1960s as treatment for patients with sciatica, multiple sclerosis, or arachnoïditis.[1] The rationale to administer steroids via the intrathecal route was the direct action of the drug on inflamed nervous tissue, presumably decreasing inflammation and edema, and thereby alleviating pain and neurologic symptoms. Other advantages of intrathecal administration of steroids were considered to be the lower therapeutic dose required as compared to systemic administration, leading to a decrease in adverse effects. In addition, compared with epidural administration, the intrathecal route was thought to yield a more predictable spinal spread of the drug, a longer duration of action, and a lower risk of neuraxial hematoma or drug overdose.

A randomized controlled trial (RCT) performed in Japan in 2000 reported extraordinary efficacy of intrathecal methylprednisolone acetate (MPA) in patients with severe postherpetic neuralgia (PHN).[2] After four intrathecal injections with MPA and lidocaine, 82 out of 89 PHN patients had good or excellent pain relief, compared with only 5 of 91 and 3 of 90 patients after lidocaine or no treatment, respectively. No side effects or complications were reported. However, despite these excellent reported results intrathecal MPA injection has never become part of standard care for intractable PHN. Concerns about safety of intrathecal MPA and/or a lack of confirmation of the results may have played a role.[3-6] Indeed, a variety of adverse events have been reported with intrathecal corticosteroids, including chemical meningitis, transverse myelitis, cauda equina syndrome, lumbar radiculitis, intractable headache, urinary retention and adhesive arachnoiditis.[1,7] Several explanations may be offered for these adverse events, including the presence of formulation adjuvants (benzyl alcohol, benzalkonium chloride or myristyl-gamma-picolinium chloride) in corticosteroid injection fluids; the use of particulate material (as with MPA) that may represent a physical stimulus; or an unknown mechanism resulting from the high concentrations of the steroid itself. Preclinical safety studies with other intrathecal steroids such as betamethasone have been associated with development of arachnoïditis.[8,9] However, in these studies, preservatives were again present in the studied formulation and may represent a possible alternative explanation for the inflammatory response observed. To the best of our knowledge, there has been no systematic preclinical safety study on the use of repeated intrathecal dosing of MPA following the paradigms and dosing ranges used in previous human studies. To minimize the concerns related to the potential effects of preservatives, such an evaluation should be carried out in their absence. Accordingly, the aim of the current study was to provide information on the safety and kinetics of the effects of multiple intrathecal injections of methylprednisolone acetate after minimization of the preservatives in which it is commercially formulated (Depo-medrol®, Pfizer), using a well-defined canine model.

MATERIALS AND METHODS

The protocol of the study has been approved by the International Association for Assessment and Accreditation of Laboratory Animal Care accredited Institutional Animal Care and Use Committee of the University of California, San Diego.

DRUG PREPARATION

The test article was prepared from the commercially available MPA formulation (Depo-medrol®, 40 mg/ml MPA, Pfizer). This suspension of MPA also contains the preservative myristyl-gamma-picolinium chloride and the adjuvant polyethylene glycol. To minimize the presence of these soluble adjuvants from the commercial formulation, we took the following practical steps. The contents of each vial were centrifuged in a minicentrifuge (Fisher Scientific, Hampton, NH, Cat no 05-090-128, 14.000 rpm) for 10 minutes. The supernatant was aspirated following rigid aseptic precautions with a needle and syringe. The residual pellet of 10 mg, 20 mg or 40 mg (depending on the dose) MPA was resuspended in a mixture with 0.4 ml lidocaine 2% and 0.1 ml glucose 50% (=vehicle) to a total volume of 0.5 ml. Lidocaine was added to confirm that the needle tip was indeed located within the intrathecal space by eliciting a brief sensory and motor block. To summarize, the dose of MPA 20 mg/ml contains 10 mg of MPA in a volume of 0.5 ml with a lidocaine concentration of 1.6% and glucose 10% and the dose of 80 mg/ml 40 mg of MPA, also in a volume of 0.5 ml with similar lidocaine and glucose concentrations. The vehicle had the same constituents in which the formulation was resuspended including lidocaine. This preparation was made immediately before use. The test article had a pH of 6.5 and a measured relative density of 1.04 kg/L. Methylprednisolone, MPA, and myristyl-gamma-picolinium chloride concentrations were determined using high-performance liquid chromatography and an ultraviolet detector. The measured concentration of myristyl-gamma-picolinium chloride in the unseparated supernatant of the commercial formulation was 0.36 mg/ml and after reformulation that was significantly reduced to 0.023 mg/ml in the MPA 80 mg/ml dosing formulation and below detection limits in the MPA 20 mg/ml formulation. The concentration of the preservative polyethylene glycol was not measured in the test article. However, polyethylene glycol is completely soluble in water, and by removing the supernatant of the commercial formulation most of the polyethylene glycol was removed from the MPA; based on the myristyl-gamma-picolinium chloride data the concentration of polyethylene glycol in the reformulated material would be estimated to be approximately 0.1 times that of the commercial formulation.

ANIMALS

Destination-bred beagle dogs (Marshall BioResources, North Rose, NY), twelve males (9-14 kg) and six females (6-8 kg, age 11-14 months), were individually housed and given *ad libitum* access to food and water. The lighting in the kennel was set on a 12-h daily light-dark cycle. Animals were acclimated for a minimum of eight days and adapted to the testing protocols for five days before surgery. All dogs were preoperatively screened for normal blood chemistry (IDEXX, Laboratories, West Sacramento, CA) absence of infectious diseases, and normal neurologic status.

SURGICAL PROCEDURE

A chronic intrathecal catheter was placed for repeated intrathecal bolus injections of MPA. Surgical placement of the intrathecal catheter was accomplished 9 to 11 days before the first dose. Prophylactic antibiotic treatment (sulfamethoxazole trimethoprim tablet 15-20 mg/kg oral twice daily) was given 24 hours before surgery continuing until 48 hours after surgery. After administration of atropine (0.04 mg/kg intramuscular) and xylazine (1.5 mg/kg intramuscular) animals received 4-5% isoflurane in 50% oxygen and 50% air and then the trachea was intubated. Anesthesia was maintained under spontaneous ventilation with 1.0 – 2.0% isoflurane in 40% oxygen and 60% nitrous oxide. Surgical areas were shaved and prepared with chlorhexidine scrub and solution. Using sterile technique the cisternal membrane was exposed. A cisternal cerebrospinal fluid (CSF) sample of 3 ml was taken for laboratory analysis and the intrathecal catheter (polyurethane 0.012" ID x 0.025" OD, previously sterilized by ethylene oxide gas) was inserted and passed caudally at a distance of approximately 38 to 45 cm to a level corresponding to the L3-L6 vertebral segment. The external part of the catheter was then tunneled subcutaneously to exit on the upper back and plugged. The incision was closed in layers with 3-0 Vicryl suture (Ethicon Inc., Somerville, NJ). Upon closure, the isoflurane and nitrous oxide were discontinued and a subcutaneous injection with 4.5 mg/kg carprofen was administered for postoperative pain control. The dogs were observed during recovery and buprenorphine (0.02 mg/kg) was given intramuscularly if necessary to relieve any remaining postoperative discomfort. In six dogs a saline pump (Medtronic MiniMed 508; Medtronic Inc., Minneapolis, MN) was used to deliver a low volume of saline to ensure intrathecal catheter patency. This was later found to be unnecessary and the use of the pumps was discontinued.

CLINICAL OBSERVATIONS

After surgery, before the first dose, 7 days after the final dose of MPA and before sacrifice, all dogs underwent a detailed neurological assessment consisting of general attitude observations, preferred cage position, gait observations, muscle tone, sensation and pain observation, spinal reflexes (quadriceps, extensor carpi, flexor, perineal,

extensor thrust, crossed extensor), proprioceptive reflexes (wheelbarrow, extensor postural thrust, proprioceptive positioning, hemistand and hemiwalk, placing response visual and tactile, righting reflex) and cranial nerve reflexes (nerves I to IX and XII).

Daily observations including body temperature and general behavior (arousal, muscle tone and coordination) were assessed in all dogs. The clinical observations made previous to and at certain time points (1, 3 and 6 hours, and 1, 3 and 6 days) after administration of intrathecal MPA included indices of arousal, muscle tone, coordination, body weight, body temperature, heart rate and blood pressure (measured at the base of the tail), thermal latencies measured with the Canine Thermal Testing System[10] and blood glucose values (One Touch Ultra, LifeScan Inc., Main, CA).

STUDY DESIGN

First, the acutely tolerable dose of methylprednisolone was established using three male dogs; the first dog received a dose of MPA 20 mg/ml, the second dog, 40 mg/ml, and the third, 80 mg/ml. Clinical observations were made as described previously, before injection and 1, 3, and 6 hours and 1, 3, and 6 days after the injection. At each time point, blood was withdrawn to measure plasma levels of methylprednisolone and blood glucose. The highest tolerable dose (80 mg/ml) was repeated in the same three dogs with a maximum of a total of four intrathecal injections with 7-day intervals between injections. One week after the last injection, the dogs were sacrificed and tissue harvested as described in the Necropsy section.

A second pilot group of three male dogs received four intrathecal injections of MPA 20 mg/ml, the therapeutic dose administered in humans, with 7-day intervals following the same schedule for clinical observations, methylprednisolone plasma levels, and sacrifice.

In the final phase of the preclinical study we randomized twelve dogs, six males and six females to three groups. The first group of four dogs (two male, two female) received vehicle, lidocaine with glucose (+ 0.5 ml saline 0.9% flush), the second group (two male, two female) received a low dose MPA 20 mg/ml (+flush) and the third group a high dose 80 mg/ml (+flush) during four weeks with a 7-day interval between the injections. Clinical observations were made as described in the Clinical Observation section, before injection and 10 minutes, 1, 3, and 6 hours and 1, 3, and 6 days after the injection. In addition, blood was withdrawn at each time point to measure plasma levels of methylprednisolone and blood glucose. Seven days after the last injection, one female and one male dog from the vehicle group, one female from the MPA 20mg/ml and one female from the MPA 80mg/ml group were sacrificed as described in the Necropsy section. In the remaining eight dogs the external part of the intrathecal catheter was internalized by cutting the external catheter short, plugging the end and letting the remaining external section slip subcutaneously. Six weeks after the last injection, the eight dogs were sacrificed and tissue harvested as described in the Necropsy section.

NECROPSY

For euthanasia, dogs were deeply sedated with acepromazine (10 mg/ml intramuscularly). Blood samples were taken for chemistry, complete blood count, and methylprednisolone levels. Dogs, then were deeply anesthetized (sodium pentobarbital, 30 mg/kg, intravascularly) and cisternal CSF samples for laboratory analysis and methylprednisolone levels and urine, obtained by cystocentesis, for clinical analysis and creatinine/cortisol ratio were taken. The animals were exsanguinated by perfusion with saline 0.9% followed by 10% neutral-buffered formalin. The spinal cord and brain were exposed and examined and the presence and localization of MPA plaques noted. The position and integrity of the intrathecal catheter was established by injection of methylene blue dye at necropsy. The spinal cord, including meninges were resected in sections at specific regions (coded A: Cervical, B: Thoracic, C: Lumbar at catheter tip, D: Low lumbar including cauda equina) and placed in fixative (10% neutral-buffered formalin). The brain was resected by cutting the brainstem, cranial nerves and vessels, and also placed in fixative.

HISTOPATHOLOGY

Paraffin sections of thoracic, lumbar and sacral spinal cord with surrounding meninges were stained with hematoxylin and eosin. All sections were examined by a neuropathologist (MG) who was unaware of the treatment group assignments until the complete review was accomplished. Sections were examined for the presence, location and type of inflammatory reaction, including inflammatory cell infiltrates, granulation tissue, and fibrosis. Lumbar and sacral sections were scored on a scale of 0 to 4, with 0 being no inflammatory response and 4 being the maximal response observed in this cohort. Separate scores were given for dura and arachnoid. Further evaluation of spinal cord injury was performed on sections stained with FluoroJade C (degenerating neurons) and Luxol Fast Blue/cresyl violet (myelin), and with immunohistochemical stains for Neuronal nuclear antigen (neurons), Glial fibrillary acidic protein (astrocytes), and Ionized calcium binding adaptor molecule 1 (microglia/macrophages). Spinal cord parenchymal pathology was given a score of 0 to 4 (normal to severe injury) based on evaluation of all stains. Total histology score was the sum of the scores for dura, arachnoid, and spinal cord (possible score of 0 to 12).

METHYLPREDNISOLONE PLASMA AND CSF SAMPLING

In dogs, plasma levels of methylprednisolone were measured 1, 3, 6, 24, 72, 144 and 168 hours after the first intrathecal injection of MPA. A plasma sample was also taken before the second, third, and fourth intrathecal injections and at sacrifice. In the dogs with a long-term recovery, periodic plasma samples were taken at 7-day intervals after the last intrathecal injection until sacrifice. The methylprednisolone concentrations in blood plasma and CSF samples were measured with an enzyme-

linked immunosorbent assay kit (Neogen Corporation, Lexington, KY) with external standards diluted for quantification.

STATISTICAL ANALYSIS

The three groups were compared for the clinical parameters using a two-way ANOVA model. For the plasma methylprednisolone values, areas under the curve were calculated for every animal. Differences in area under the curve between the two dosing groups were calculated using a Student t-test. Differences between the median histology scores were calculated with a Kruskal Wallis test. Two-sided p-values ≤ 0.05 were considered significant (PASW Statistics version 17.0, Chicago, IL).

RESULTS

Eighteen dogs were included in the study. During dose ranging, a catheter in one of the male dogs receiving his third MPA 80 mg/ml dose became blocked. This animal, although examined, was excluded from further analysis.

The clinical observations (body temperature, heart rate, blood pressure, thermal latencies), methylprednisolone plasma levels and laboratory results were based on the data collected in the final phase of the preclinical study using 12 dogs. The pathology and histopathology results are based on all 17 included dogs.

CLINICAL OBSERVATIONS

All dogs displayed a brief motor block (10-40 minutes) after dosing, confirming correct intrathecal delivery of the lidocaine containing vehicle or test article. Other than the brief decrease in muscle tone and coordination, no behavioral changes were observed in any of the dogs during or after intrathecal injection. There were no significant differences in body temperature, heart rate, blood pressure, plasma glucose levels and thermal latencies between the treatment groups and control group at the time points after drug delivery (Figure 1). In addition, no significant differences were observed after correction for sex or between the first and last injection. Body weight increased to a larger extent in animals treated with high dose MPA ($p = 0.04$). The percentage body weight increase from baseline until after the last intrathecal drug delivery was 8.9% in the high-dose MPA versus 1.9% in the vehicle treated group, suggesting a systemic effect of the intrathecal MPA (Figure 2).

Figure 1
A) Time course of mean paw withdrawal latencies after first intrathecal injection. B) Time course of mean paw withdrawal latencies at 7-day intervals after intrathecal injections. IT = intrathecal, MPA = methylprednisolone acetate. Data are plotted as mean, error bars are the standard deviation of the mean.

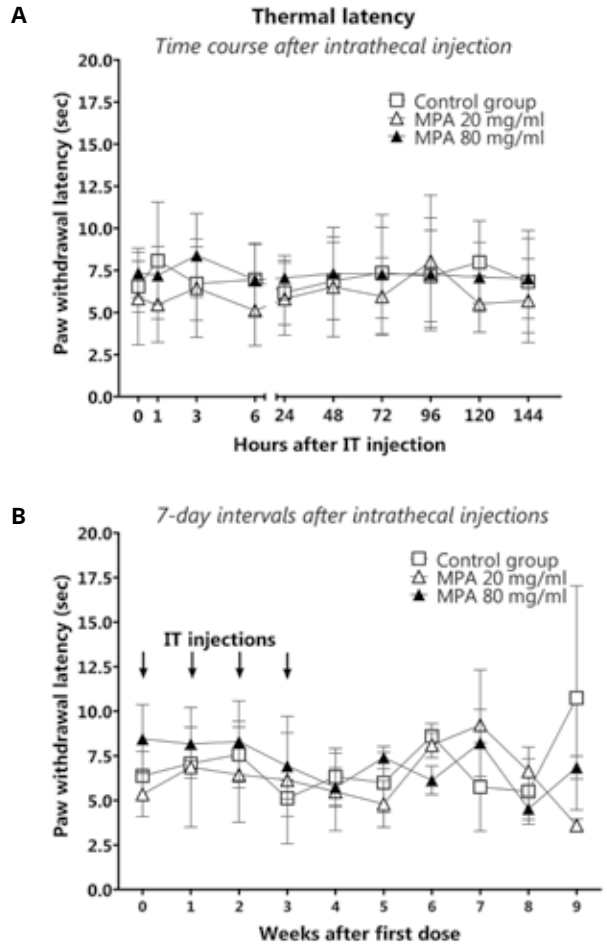
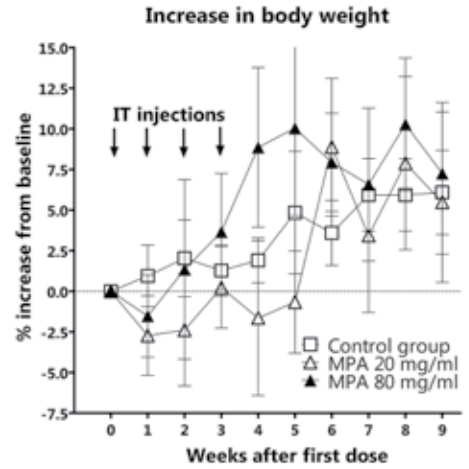


Figure 2
Percentage increase in body weight from baseline for the three groups. IT = intrathecal, MPA = methylprednisolone acetate. Data are plotted as mean, error bars are the standard deviation of the mean.



METHYLPREDNISOLONE PLASMA LEVELS

In all drug-treated animals, peak plasma levels of methylprednisolone were observed three to six hours after the first drug delivery (low-dose MPA (20 mg/ml); 35.6 and 33.5 ng/ml at 3 and 6 hours, versus high dose MPA (80 mg/ml); 61.3 and 57.3 ng/ml respectively. A steep decrease occurred after 24 hours, and, seven days after the first drug delivery, before the second injection, methylprednisolone levels were very low but detectable (low dose MPA; 5.5 ng/ml and high dose MPA; 12.3 ng/ml). The area under the curve for MPA 20 mg/ml was 1790, for MPA 80 mg/ml 5227 ($p<0.001$; Figure 3A). Methylprednisolone levels remained detectable up to two weeks after the last drug delivery (Figure 3B).

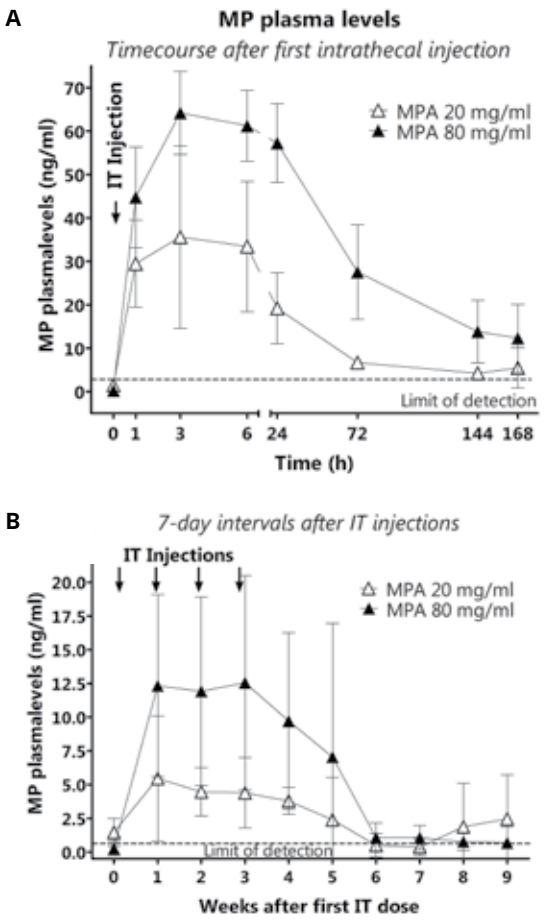


Figure 3 Time course of methylprednisolone plasma levels A) after the first intrathecal MPA 20 mg/ml or 80 mg/ml injection, B) before every intrathecal drug administration, at 7-day intervals. IT = intrathecal, MPA = methylprednisolone acetate, MP = methylprednisolone. Data are plotted as mean, error bars are the standard deviation of the mean.

LABORATORY RESULTS

All dogs showed normal plasma and urine laboratory results (complete blood count, kidney and liver function, creatinine/cortisol ratio) before surgery. At acute and long-term sacrifice, normal plasma and urine laboratory results were again observed in almost all dogs. Only alkaline phosphatase increased to 328 U/l (normal values: 10-150 U/l) in one acutely sacrificed dog, and leukocyte number was decreased in two acutely sacrificed dogs to 5.6 and 4.9 (normal value; 6.0 – 17.0 10^3/µl), all treated with the high-dose MPA.

The cisternal CSF samples showed abnormalities in all dogs, but the severity of the deviation was clearly dose-dependent. Increased nuclear cell count and protein levels were observed, with the highest values in dogs treated with high-dose MPA. At acute sacrifice the elevations in nuclear cell count (normal value; 0 – 5 cells/µl, vehicle: 27 and 180 cells/µl, low-dose MPA: 310 cells/µl, and high-dose MPA: 1210 cells/µl) were more prominent than at long-term sacrifice (vehicle: 2 and 10 cells/µl, low-dose MPA: 8, 21 and 22 cells/µl, and high-dose MPA: 193, 299 and 308 cells/µl).

CSF protein levels were also highest at acute sacrifice in dogs treated with high-dose MPA (vehicle: 34.4 and 47.4 mg/dl, low-dose MPA: 41.5, and high-dose MPA: 142.7 mg/dl) compared with levels at long-term sacrifice (vehicle: 20.3 and 21.8 mg/dl, low-dose MPA: 24.3, 38.9 and 44.5 mg/dl, and high-dose MPA: 52.1, 54.2 and 68.9 mg/dl).

CSF glucose levels decreased with increasing dose and were lowest at long-term sacrifice (acute sacrifice; vehicle: 64 and 65 mg/dl, low-dose MPA: 66, and high-dose MPA: 59 versus long-term sacrifice vehicle: 65 and 72 mg/dl, low-dose MPA: 64, 68 and 68, and high-dose MPA: 54, 55 and 58) suggesting the presence of an intrathecal inflammatory process.

PATHOLOGY

At necropsy, acutely sacrificed animals reliably displayed a white, acellular, solid deposit in the intrathecal space at or below the catheter tip on the spinal cord in all drug-treated animals. This deposit typically extended a distance of 1-3 cm in length and was believed to be MPA (Figure 4). At long-term sacrifice, such deposits were observed in four of six dogs. No other visibly evident pathological signs were observed in any animal at acute or long-term necropsy.

HISTOPATHOLOGY

Vehicle-treated animals showed minimal changes in dura and arachnoid, with mild infiltrates of lymphocytes, plasma cells, and macrophages at acute sacrifice and focal fibrosis and thickening of dura with minimal arachnoid inflammation at long-term sacrifice. No spinal cord changes were seen in vehicle-treated animals. The median total histology score for the vehicle-treated animals was 1.3 at acute and 1.0 at long-

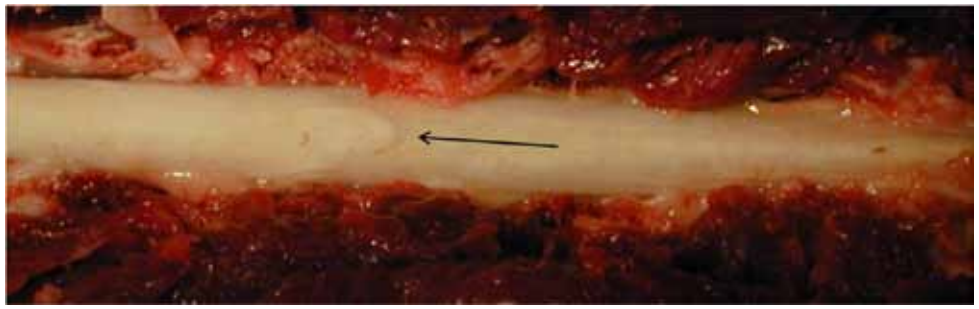


Figure 4 A white solid deposit of 2 cm on the dorsal side of the spinal cord (arrow).

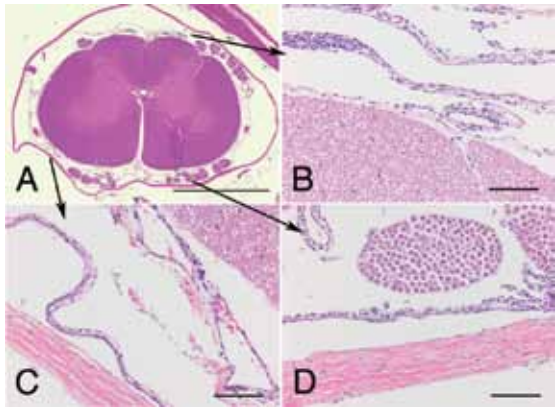


Figure 5 Vehicle, acute sacrifice. A) Minimal inflammatory infiltrates in arachnoid (bar = 3 mm). B-D) Arrows indicate regions shown at higher power. B) Mild arachnoid and perivascular inflammatory infiltrates (bar = 100 µm). C and D) Areas with no to minimal inflammatory infiltrates (bar = 100 µm).

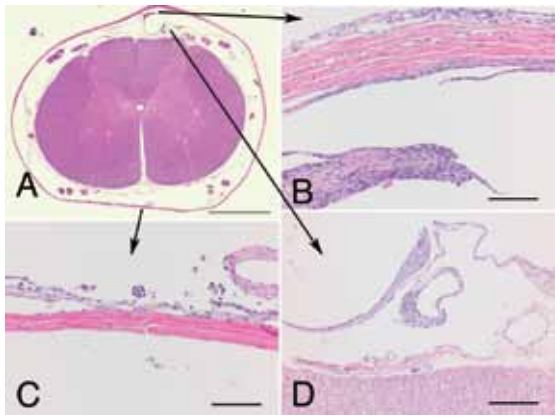


Figure 6 Vehicle, long-term sacrifice. A) Mild dural and arachnoid inflammation, with catheter site in dorsal arachnoid (bar = 3 mm). Arrows indicate regions shown at higher power. B) Mild chronic inflammation and fibrosis on outer and inner surfaces of dura adjacent to catheter site, with focal fibrosis and chronic inflammation around catheter (bar = 100 µm). C) Minimal arachnoidal chronic inflammation, consisting primarily of macrophages (bar = 100 µm). D) Chronic perivascular inflammation adjacent to catheter site (bar = 200 µm).

term sacrifice (Figure 5 & 6). Low-dose MPA-treated animals at both acute and long-term sacrifice had a dural reaction that consisted of a diffuse infiltrate of macrophages along the inner surface of the dura, with variable numbers of lymphocytes, plasma cells and neutrophils in dura and arachnoid. The median total histology score was 2.0 at acute and 3.0 at long-term sacrifice (Figure 7 & 8). High-dose MPA-treated animals had more severe inflammatory responses, with large inflammatory masses (one with neutrophil aggregates in the center, suggesting abscess formation) on the inner surface of the dura and in arachnoid in two of three animals at each survival time. The median total histology score was 4.0 at acute and 7.0 at long-term sacrifice (Figure 9 & 10). The severity of the inflammatory reaction expressed as the total histology score, increased significantly with increasing dose at long-term sacrifice (acute $p = 0.167$, long-term $p = 0.014$).

Spinal cord pathology consisted of focal aggregates of activated microglia (in one of four animals of the low-dose MPA group at acute sacrifice, one of three animals in the high-dose MPA group at acute sacrifice and all three animals of the high-dose

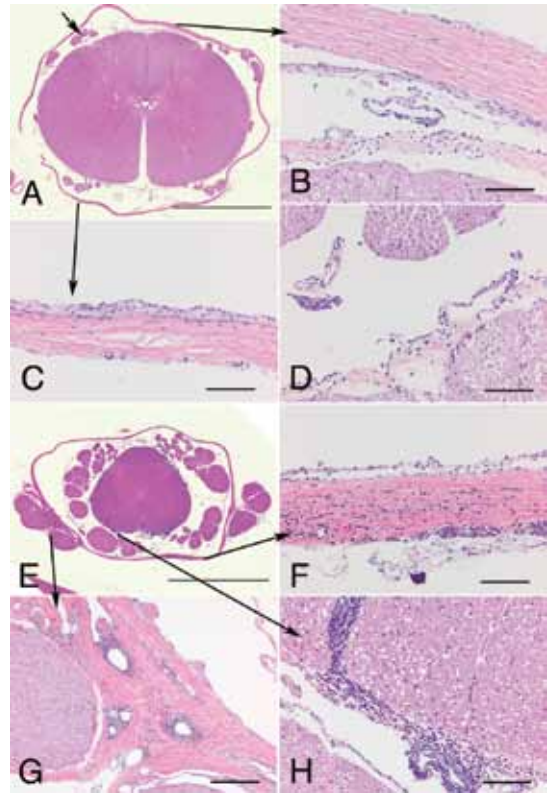


Figure 7 Methylprednisolone acetate 20 mg/ml, acute sacrifice. A) Mild leptomeningeal inflammation (bar = 3 mm). Long arrows indicate regions shown at higher power in B and C. Short arrow indicates region shown at higher power in D. B) Mild perivascular/arachnoid inflammation. C) Macrophages on inner surface of dura. D) Mild leptomeningeal inflammation and focal cluster of macrophages. B-D, bar = 100 µm. E) Moderate inflammatory infiltrates in dura, arachnoid, and Virchow-Robin spaces (bar = 3 mm). Arrows indicate regions shown at higher power in F-H. F) Inflammatory infiltrates through full thickness of dura (bar = 100 µm). G) Perivascular inflammatory infiltrates in dura (bar = 200 µm). H) Inflammatory infiltrates in arachnoid, extending into Virchow-Robin spaces in spinal cord (bar = 100 µm).

MPA group at long-term sacrifice) and focal, mild inflammation (in one of three animals of the low-dose MPA group at long-term sacrifice; Table 1). No evidence of neuronal injury (by hematoxylin and eosin or FluoroJade C stains), demyelination or gliosis was seen in any animal. Specific examination of the adjacent nerve roots revealed no signs of Schwann cell injury or demyelination.

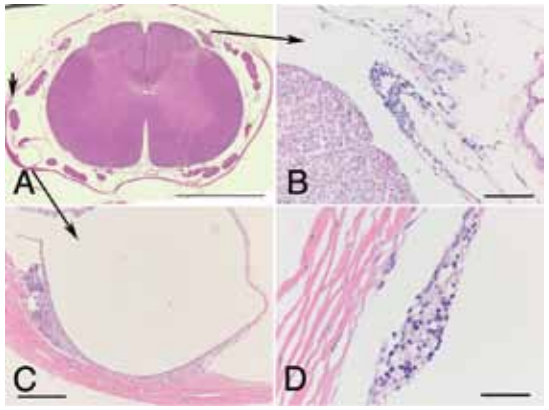


Figure 8 Methylprednisolone acetate 20 mg/ml, long-term sacrifice. A) Minimal inflammatory infiltrates and fibrosis in arachnoid and surrounding catheter site (bar = 3 mm). Long arrows indicate regions shown at higher power in B and C. Short arrow indicates region shown at higher power in D. B) Mild arachnoid inflammation away from catheter site (bar = 100 µm). C) Mild fibrosis and chronic inflammation around catheter (bar = 200 µm). D) Focal aggregate of macrophages on inner surface of dura (bar = 50 µm).

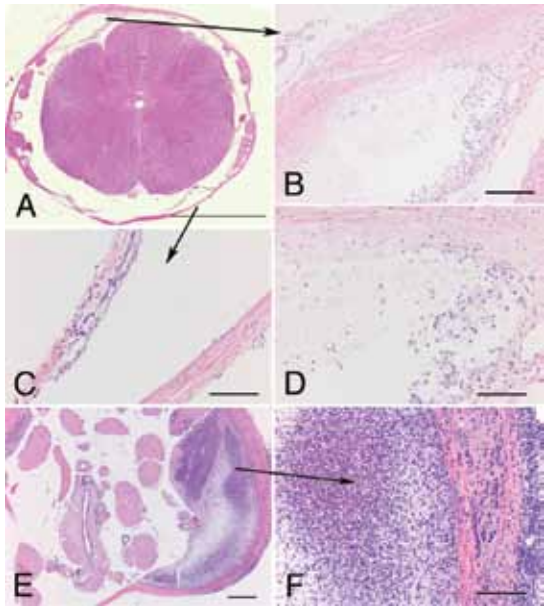


Figure 9 Methylprednisolone acetate 80 mg/ml, acute sacrifice. A) Focal subdural aggregates of macrophages surrounding foreign material (bar = 3 mm). Arrows indicate regions shown at higher power in B and C. B) Subdural aggregates of macrophages surrounding foreign material (bar = 200 µm). C) Mild arachnoid inflammatory infiltrates (bar = 100 µm). D) Higher power of region shown in B (bar = 100 µm). E) Caudal sacral section demonstrating severe inflammation in dura and arachnoid, with large inflammatory mass (bar = 300 µm). Arrow indicates region shown at higher power in F. F) Higher power of inflammatory mass, with inflammation in dura and arachnoid; necrotic center with neutrophils resembling an abscess (bar = 100 µm).

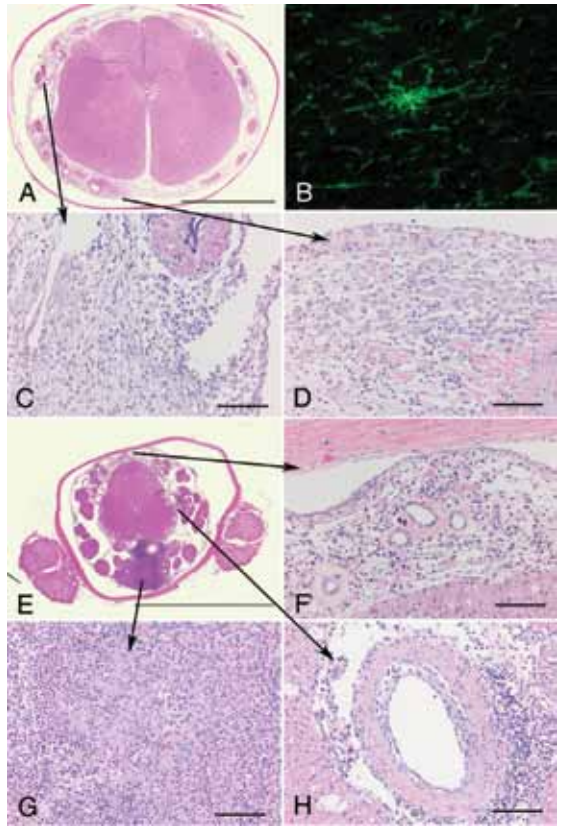


Figure 10 Methylprednisolone acetate 80 mg/ml, long-term sacrifice. A) Diffuse inflammatory infiltrates and fibrosis in arachnoid. Arrows indicate regions shown at higher power in C and D (bar = 3mm). B) Immunofluorescent staining for Iba1 (green). The large cell in the center is an activated microglia. There are multiple smaller resting microglia in the surrounding tissue. C) Fibrosis and macrophage infiltration in arachnoid and inflammatory cell infiltration of a nerve root (bar = 100 µm). D) Focal aggregate of pigment-laden macrophages in arachnoid (bar = 100 µm). E) Large arachnoidal inflammatory mass. Arrows indicate regions shown at higher power in F-H (bar = 3 mm). F) Mixed inflammatory infiltrate away from inflammatory mass. G) Granuloma formation with epithelioid macrophages within inflammatory mass. H) Perivascular inflammation and vasculitis. B-D, bar = 100 µm.

Table 1 Overview histopathology results

Treatment	Survival	N	Dura *scores*	Arachnoid *scores*	Spinal cord *scores*	Total histology score
Vehicle	1 wk	2	0 / 0	0.5 / 2	0 / 0	0.5 / 2
Vehicle	6 wk	2	0.5 / 1	0 / 0.5	0 / 0	0.5 / 1.5
MPA 20mg/ml	1 wk	4	1 / 4 / 1 / 1	0.5 / 3 / 1 / 1	0 / 1 / 0 / 0	1.5 / 8 / 2 / 2
MPA 20mg/ml	6 wk	3	1 / 1 / 2	0.5 / 1 / 1	0 / 1 / 0	1.5 / 3 / 3
MPA 80mg/ml	1 wk	3	2 / 1 / 3	2 / 2 / 4	0 / 0 / 1	4 / 3 / 8
MPA 80mg/ml	6 wk	3	2 / 4 / 2	4 / 4 / 2	1 / 1 / 1	7 / 9 / 5

The histopathology scores are based on the lumbar and sacral sections. Dura, arachnoid and spinal cord are examined for the presence, location and type of inflammatory reaction, including inflammatory cell infiltrates, granulation tissue, and fibrosis. Scoring system from 0 to 4: 0 being no inflammatory response and 4 being the maximal response observed in this cohort. Total histology score was the sum of the scores for dura, arachnoid, and spinal cord (possible score of 0-12). MPA = methylprednisolone acetate, N = Number of dogs, wk = weeks after the last intrathecal MPA dose.

DISCUSSION

This study aimed to provide a systematic assessment of the safety of repeated intrathecal administration of reformulated MPA, delivered in doses comparable to those reported to be used in humans. The model, the chronically catheterized canine, has been widely used for defining the potential spinal toxicity and kinetics of a large number of spinal agents.[11-14] In short, in the current studies using this model, we observed a dose-dependent inflammatory reaction proximal to the lumbar catheter delivery site. Issues pertinent to the interpretation of this observation are considered in the next paragraphs.

ACUTE TOLERABILITY OF INTRATHECAL MPA

Repeated intrathecal injections of vehicle or MPA solutions in the dog were well tolerated and did not have any deleterious effects upon neurological function. The absence of effect upon an acute thermal threshold other than the acute block associated with the action of the lidocaine is not unexpected, because no anesthetic or acute analgesic properties have been ascribed to steroids after intrathecal delivery. Similarly, the absence of any reaction upon injection in the unanesthetized animal or any change in the thermal escape threshold suggested no proalgesic action.

PHARMACOKINETICS OF INTRATHECAL MPA

In dogs, peak methylprednisolone plasma concentrations were observed between three and six hours after intrathecal MPA injection. Although plasma levels decreased after six hours, methylprednisolone was still measurable after seven days but went below the detection threshold three weeks after the last injection.

In humans, a similar timeframe was described; after 80 mg MPA, intrathecal peak plasma and CSF levels were observed after one day and were measurable for at least two weeks.[15] After intraarticular injection of MPA, methylprednisolone plasma levels decreased below the detection level after 24 hours, much shorter than observed after intrathecal administration and likely suggesting more rapid clearance though lymphatic drainage.[16]

Twenty-four hours after intraarticular administration, when methylprednisolone plasma levels were undetectable, postmortem examinations showed a significant quantity of white material believed to be MPA precipitated at the bottom of the synovial cavity. In the current study, similar white deposits were observed one week after the last intrathecal delivery, when methylprednisolone plasma levels were still measurable. Although the deposits were not chemically identified, it seems likely based on the comparable results observed in joints that these deposits were methylprednisolone. Accordingly, it is probable that these white deposits were present in the intrathecal space for a longer period of time, a possible explaining cofactor for the inflammatory process observed at 6 weeks after the last intrathecal injection.

HISTOPATHOLOGIC EFFECTS OF INTRATHECAL MPA

Despite the absence of clinical symptoms, there were evident histologic signs of inflammation in all drug-treated animals. An inflammatory meningeal reaction was seen, one accompanied with inflammatory masses suggesting abscess formation, the other with granuloma formation. In the spinal cord, focal aggregates of activated microglia were observed, but there was no evidence of neuronal injury or demyelination in adjacent nerve roots. These physical observations were in addition accompanied by increased protein in the CSF that appeared to be most evident in animals sacrificed within 7 days of the last injection.

This work thus reveals an inflammatory response in the intrathecal space after repeated administration of an anti-inflammatory drug after the removal of its preservatives. Importantly, this effect was not seen in the vehicle-treated animals, emphasizing that the results were not secondary to either the chronic polyurethane catheter or to the vehicle (2% lidocaine). Although lidocaine has been previously reported to produce signs of demyelination, no evidence of such untoward signs were observed in the current study with the brief exposures and low concentrations used.[17,18]

The appearance of inflammatory responses described as chemical meningitis, transverse myelitis and adhesive arachnoïditis have been observed in patients after intrathecal administration of MPA.[1,7] Unlike in the current study, however, these previous reports have used a MPA formulation that included the preservative myristyl-gamma-picolinium chloride upon which the untoward reaction was attributed. In addition, in animal studies intrathecal MPA and other steroids have been shown to cause an inflammatory response.[8,9,19,20] Accordingly, we believe the current study points out that the intrathecal delivery of MPA, a particulate suspension, has a role itself in the inflammatory response.

THE MPA STUDY FORMULATION

In the current study we attempted to obtain a preservative-free MPA formulation by centrifuging and resuspending the general formulation of MPA. To be able to truly assess the test article used by Kotani *et al.*[2] and which was to be used in a replication RCT in the Netherlands, we decided not to construct the test article from pure compound. Our drug analysis showed that less than 0.025 mg/ml myristyl-gamma-picolinium chloride (0.36 mg/ml in general formulation) remained in the test article. Myristyl-gamma-picolinium chloride, a bacteriostatic agent, is known to be neurotoxic.[21] In concentrations of approximately 0.4 mg/ml it has been shown to cause retinal damage in a rabbit eye.[21] The concentration measured in our drug assays was less than 0.025 mg/mL, nearly 20-fold lower. To the best of our knowledge there are no studies available testing the neurotoxicity of myristyl-gamma-picolinium chloride in concentrations less than 0.025mg/ml. Although we cannot completely rule out that this low concentration contributed to the toxicity findings in the current study,

we believe that the extent of the toxicity findings are not likely to be caused solely by the small concentrations of myristyl-gamma-picolinium chloride.

Polyethylene glycol has recently been studied in spinal cord injury models because it is reported to promote the restoration of functional and structural integrity of nerve tissue by direct application on the spinal cord.[22] In high concentrations, its prolonged focal application may induce a conduction block[23] but no inflammatory responses or neurotoxicity have been observed. In addition, polyethylene glycol is used as a spinal sealant after dural repair during neurosurgery in humans. No neurotoxicity after use of the polyethylene glycol sealant was reported.[24] Thus, although we are aware of no specific assessments of the long-term effects of intrathecal polyethylene glycol, these results suggest that polyethylene glycol does not possess evident signs of toxicity in the models thus far examined at the concentrations used in these studies.

ORIGINS OF OBSERVED TOXICITY

The observation of the intrathecal inflammatory reactions was surprising given the touted role of steroids as anti-inflammatory agents. Specific studies on the mechanisms of this observed toxicity were not undertaken. In the current study, we would raise the possibility that the particulate nature of the formulation itself might account for some component of the inflammatory response. Two issues may be considered regarding mechanisms underlying this inflammatory reaction.

First, steroids acting though intracellular linkages have historically been appreciated to suppress the inflammatory response through an effect on several signaling cascades such as that for nuclear factor κ B and to accordingly diminish the release of proinflammatory and proalgesic cytokines, such as tumor necrosis factor and interleukin-8.[25] However, there are data suggesting that steroids may, under certain proinflammatory conditions, enhance the release of chemoattractants such as interleukin-8 and produce an increased transcription of adhesion molecules such as intercellular adhesion molecule-1 and vascular cell adhesion molecule-1, agents known to mediate inflammatory cell migration.[26] The observations here suggest that the depot formulation has that proinflammatory action. This possibility is supported by the appearance of inflammatory cells in the mass perimeter. Given that soluble steroids do not initiate such migratory activity[27] or cytokine release, the physical nature of the MPA particle may contribute to this effect.

This leads to the second possibility, which is that the inflammatory reaction reflects the presence of the MPA particles. Systematic examination of MPA revealed that between 30-40% of the particles observed in MPA solution were larger than 20 μm in diameter.[28,29] An extensive literature search emphasizes that in other systems such as lung, particulate materials, such as carbon particles as well as other environmental toxins including nanoparticles can initiate an activation of cytokine release that in turn leads to an activation of a variety of cell adhesion factors leading to macrophage

and neutrophil migration.[30-32] Thus, in a number of different study systems, particulates have been shown to drive robust inflammatory reactions.[33-36] Whether this phenomenon is observed with nonparticulate steroids is not known and may reflect a peculiarity of the steroid effects with the stimulus provided by the particulate aggregation.

The role of such spinal particulates has yet to be systematically studied, but the current results suggest the speculative hypothesis that particulates in the intrathecal space may promote inflammatory cell migration. Further studies on this intriguing hypothesis are clearly warranted as they raise the question of whether particulate formulations in general may be contraindicated in the intrathecal space.

CONCLUSIONS

This preclinical study was performed to provide supporting information on a parallel pilot study of a RCT studying the efficacy of intrathecal MPA in patients suffering from postherpetic neuralgia.[37] That study was designed to verify the results of a previous large RCT.[2] Importantly, however, the current results suggest an untoward effect of intrathecal MPA and do not support performing additional human studies with even this reformulated material having minimal additives. This study shows again that the intrathecal implementation of a novel therapy requires robust preclinical safety assessment in well-validated models.[38,39]

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3

EFFICACY OF INTRATHECAL METHYLPREDNISOLONE

CHAPTER 5

No beneficial effect of intrathecal methylprednisolone acetate in postherpetic neuralgia

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ABSTRACT

Background – High efficacy of intrathecal methylprednisolone acetate (MPA) with lidocaine has been reported in a large patient group suffering from intractable postherpetic neuralgia (PHN). Because the treatment effect was never independently confirmed and there are ongoing safety concerns, intrathecal MPA did not become standard care for intractable PHN. We report the results of a replication trial assessing pain relief and spinal cytokine/chemokine levels in PHN patients.

Methods – The number of patients to be included was determined by using sequential analysis to limit patient exposure to the invasive experimental treatment. Patients were randomized to the treatment group receiving MPA 60 mg + lidocaine 60 mg or control group receiving lidocaine 60 mg only. Four injections at 7-day intervals were administered after cerebrospinal fluid (CSF) collection to measure cytokine/chemokine levels. Visual analogue scores for pain and the square allodynic area were collected during follow-up, with the primary endpoint set at 8 weeks follow-up.

Results – In total, 10 patients were included, of whom six were randomized to the treatment group. All six MPA-treated patients experienced a pain increase at 8 weeks, versus one of four patients in the control group. The square allodynic area increased in four of six MPA-treated patients versus one of four control patients. CSF interleukin-8 levels remained stable in the control group, but increased significantly after the first intrathecal MPA injection. The trial was stopped because of safety concerns and futility.

Conclusion – Considering the absence of clinical benefits and the potential risks of the treatment, intrathecal administration of MPA is not recommended.

INTRODUCTION

Intrathecal administration of methylprednisolone acetate (MPA) is a treatment described since the 1960s.[1] Initially, it was used in patients suffering from sciatica and multiple sclerosis.[1] Shortly after the publication of several clinical efficacy studies, reports on severe complications appeared and the use of intrathecal MPA declined.[2] Its use had a revival when the results were published of a randomized controlled trial (RCT) on the efficacy of intrathecal MPA in postherpetic neuralgia (PHN) patients.[3] After four intrathecal injections with MPA and lidocaine, 82 out of 89 PHN patients had 'good or excellent' pain relief, against five out of 91 and three out of 90 patients after lidocaine or no treatment respectively at 1-year follow-up. No side effects or complications were reported. Since PHN is a devastating and often therapy-resistant neuropathic pain disorder,[4;5] some clinicians accepted the risks of the invasive treatment. However, despite the excellent results in the trial, intrathecal MPA never became part of standard care for intractable PHN. Concerns about safety of intrathecal MPA remained and also lack of independent confirmation of the results may have played a role.[6-9]

We considered that the promising results of intrathecal MPA with lidocaine in PHN patients warranted confirmation. Therefore, we designed a replication RCT comparable with that previously published.[3] Patients suffering from intractable PHN received four intrathecal injections with 7-day intervals with either MPA combined with lidocaine or with lidocaine only. The outcome measures before the treatment, at the end of treatment, at 4-week and 1-year follow-ups were similar to the Kotani trial. Key differences between our trial and the Kotani trial were a) the duration of PHN at inclusion (6 months in our trial versus 1 year in the Kotani trial), b) presence of a third group receiving no treatment in the Kotani trial, c) the use of 3 ml of lidocaine 2% in the drug formulation in our trial versus 3 ml of lidocaine 3% in the Kotani trial and d) reformulation of the commercial MPA in our trial to reduce the amount of preservatives and use of the commercial MPA in the Kotani trial not mentioning any reformulation reducing preservatives.

MATERIALS AND METHODS

The protocol of the RCT was approved by the Ethics Committee of the University Medical Centre Utrecht, the Netherlands. The trial was registered in ISRCTN under the trial number TN88145753.

PATIENTS

Patients referred to the Pain Clinic of the University Medical Centre Utrecht, the Netherlands, who had intractable postherpetic neuralgia were eligible for inclusion. Patients were included if they had a) a history of PHN for at least 6 months after the onset of the herpes zoster skin rash, b) did not respond to at least one anti-epileptic drug and one tricyclic antidepressant, c) reported a pain intensity due to PHN of at least 4 cm on the 10-cm visual analogue scale (VAS), and d) did not use medication for PHN or maintained medication on a stable dose for at least 4 weeks prior to randomisation. Exclusion criteria were a) PHN in regions innervated by the trigeminal nerve, b) other pain sensations or skin conditions, which could confound the assessment of the neuropathic pain due to PHN, c) polyneuropathy or severe other neurologic diseases, d) an immunocompromised state, e) previous neurolytic, neurosurgical or intrathecal treatment for PHN, f) contraindications for spinal anaesthesia, g) clinically significant psychiatric diagnoses, h) a history of poor compliance to clinical studies or treatment regiments, and i) difficulty in communication.

DRUG PREPARATION AND ANALYSIS

With respect to the formulation of MPA, we wanted to replicate the RCT reported by Kotani et al.[3] Importantly, we wanted to administer preservative-free MPA, since several preservatives have been shown to be neurotoxic when administered in the intrathecal space. However, in the previous RCT the exact MPA formulation used was not reported. The information provided was that 60 mg of MPA (specific gravity, 1.040; pH, 6.2 to 6.7) combined with 3 ml of lidocaine 3% was administered in the intrathecal space. Until today, no commercially available formulation of MPA without preservatives is available.[10]

We prepared the study medication in the hospital pharmacy from the commercially available MPA formulation, 1 ml of 40 mg/ml MPA (Depo-medrol®, Pfizer, NY, USA). This suspension also contains the preservative myristyl-gamma-picolinium chloride and the adjuvant polyethylene glycol. To minimize the presence of these soluble adjuvants in the commercial formulation, two vials of 1 ml MPA were centrifuged in a Hettich centrifuge at 4000 rpm for 10 minutes. The supernatant was aspirated following aseptic precautions with a needle and syringe and discarded. The residual pellets of 40 mg MPA were resuspended with 0.9 ml lidocaine 2%. In total 1.5 ml was taken (=60 mg MPA) and 1.65 ml of lidocaine 2% and 0.75 ml glucose 50% were added to a total volume of 3.9 ml. We decided to decrease the lidocaine dose to 60 mg to reduce the risk of complications from spinal blockade such as hypotension.[11;12] The glucose was added to increase the specific gravity to the level mentioned in the Kotani trial. To summarize, the study medication contained 60 mg of MPA (=15.4 mg/ml), 60 mg of lidocaine (=1.5%) and 375 mg glucose (=10%) in a volume of 3.9 ml. The control medication contained 3 ml lidocaine 2% and 0.75 ml glucose 50%.

Three pilot batches of the study medication were prepared to examine resuspendability, pH and relative density. The final procedure resulted in a well resuspendable product with pH = 6.5 and relative density of 1.04 kg/L. MPA and myristyl-gamma-picolinium chloride concentrations were determined by using high-performance liquid chromatography and an ultraviolet detector. The concentration of myristyl-gamma-picolinium chloride in the commercial formulation was 0.20 mg/ml and after reformulation that was markedly reduced to 0.01 mg/ml. The concentration of the adjuvant polyethylene glycol was not measured in the study medication. However, polyethylene glycol is completely soluble in water and by removing the supernatant of the commercial formulation the majority of the polyethylene glycol was removed from the MPA and based on the myristyl-gamma-picolinium chloride data the concentration of polyethylene glycol in the reformulated material would be estimated to be approximately one-tenth of that in the commercial formulation.

STUDY DESIGN

Patients were randomly assigned (using computerized block-randomisation by the Department of Pharmacy) to receive four intrathecal injections with 60 mg MPA + 60 mg lidocaine (treatment group) or 60 mg lidocaine alone (control group).

During a pre-study period of two weeks, patients were maintained on a stable dose of their medication for PHN (e.g., anti-epileptic drugs, antidepressants, opioids, capsaicin). Patients in both groups were permitted to take acetaminophen and/or non-steroidal anti-inflammatory drugs (NSAIDs) (if indicated combined with a H+ receptor antagonist) at any time during the pre-study and study period. Patients were not allowed to start using other pain relief medication than the one(s) they were using at time of randomisation or change their dose. No intrathecal, epidural or neurolytic nerve blocks were allowed during the entire follow-up period.

After the pre-study period, the study medication was administered by four intrathecal injections with 7-day intervals. The intrathecal injections were performed at the L2-L3 intervertebral space. Before every intrathecal injection of the study medication and one, four, and eight weeks after the last injection, 5 ml of cerebrospinal fluid (CSF) was sampled for measurement of white and red cell count, C-reactive protein levels, glucose, total protein, interleukin (IL)-1α, IL-1β, IL-6, IL-8, IL-10, monocyte chemotactic protein (MCP)-1, macrophage inflammatory protein (MIP)-1α, MIP-1β, fractalkine and tumor necrosis factor (TNF)α concentrations, varicella zoster and herpes simplex viral activity and methylprednisolone (MP) and MPA levels. All intrathecal injections were administered by an experienced anaesthesiologist not involved in the study. The patients were blinded to the study medication. Immediately after the intrathecal injection the patients were positioned in the lateral decubitus position lying on the affected side for 30 minutes. For patients whose pain was located above thoracic level 10, the operating table was tilted into Trendelenburg

position immediately after the intrathecal injection to allow the injected material to spread to the involved dermatomes. Patients with pain below thoracic level 10 were kept in a horizontal (lateral decubitus) position. In all patients the height of the sensory block was measured by stroking the skin with an icepack and asking the patient for cold sensations.

CLINICAL OBSERVATIONS

Pain was evaluated just before the first and fourth intrathecal injection and at 4, 8 weeks, 6 months, and 1 year after the last intrathecal injection. Use of acetaminophen, NSAIDs or opioids was not permitted during the 24 hours before each evaluation. Global pain was evaluated using a 10-cm VAS on which 0 cm represented no pain and 10 cm the worst imaginable pain. The results were classified as excellent (>75% reduction in VAS), good (50 to 75% reduction), fair (25 to 50% reduction) or poor (<25% reduction). The severity of burning and lancinating pain and allodynia were also evaluated using VAS. The area of allodynia was determined by gentle stroking of the skin with a brush. As the skin was stroked in a direction toward the area of neuralgia, the patient was asked about changes in sensation. The border of the area where sensation changed was defined and marked on the skin and the size of the marked area calculated. Quality of life (QOL) was assessed by using EQ5D.[13] All evaluations of pain were conducted by physicians or study nurses who were blinded for the patients' treatment assignment. The anaesthesiologist who had administered the study medication was not involved in any way in the outcome evaluation of patients.

The primary end-point of the study was global pain relief eight weeks after the last intrathecal injection. Secondary end-points were a) global pain relief at the other time points, b) relief of burning and lancinating pain, and allodynia, c) allodynic area, d) mean number of acetaminophen and NSAID tablets consumed per week, and e) QOL.

LABORATORY ANALYSES

The CSF was centrifuged at room temperature with 700 g for ten minutes. First, white and red cell count, C reactive protein levels, glucose and total protein were measured. Second, CSF concentrations of IL-1α, IL-1β, IL-6, IL-8, IL-10, MCP-1, MIP-1α, MIP-1β, fractalkine and TNFα in the CSF were analyzed by using a multiplex bead array (Millipore MILLIPLEX MAG Human Cytokine/Chemokine kit (Millipore corp., St. Charles, MO, USA)) according to manufacturer's instructions. The lower detection limits of the kit were respectively 0.58 pg/ml, 0.63 pg/ml, 0.59 pg/ml, 0.63 pg/ml, 4.91 pg/ml, 4.01 pg/ml, 5.09 pg/ml, 4.13 pg/ml, 10.15 pg/ml and 0.68 pg/ml.

Using polymerase chain reaction, CSF was analyzed for varicella zoster and herpes simplex viral reactivation.

The CSF MP and MPA levels were measured at the Doping Control Laboratory, Department of Clinical Chemistry, Microbiology and Immunology in Gent, Belgium using High-Performance Liquid Chromatography with Mass Spectrometry with a lower detection limit of 1ng/ml for MP and 2 ng/ml for MPA.

STATISTICAL ANALYSIS

The study was designed using continuous sequential analysis to limit patient exposure to the invasive experimental treatment.[14;15] First, a sample size calculation was performed assuming that at least half of the effect as described previously[3] would be observed in the present trial (i.e. 50% of the patients in the treatment group experiencing good or excellent pain relief after one year). Also, we assumed that the pain relief in the lidocaine-only group would double to 10% compared to the effect described[3], leading to a conservative estimate of the sample size. To detect a 40% difference between the treatment and control group, 19 patients in each of the two groups were required, with a two-sided 10% type I error and a power of 90%. Secondly, these hypotheses were used to construct the triangular boundary used in the sequential analysis. Finally the sequential analysis was performed. After every patient starting after a minimum of ten included patients an analysis was performed. To be able to stop inclusion of new patients in time, the primary endpoint of global pain reduction was set as early as reasonably possible at 8 weeks follow-up. The sequential analysis was based on the dichotomous data; presence or absence of good or excellent pain relief and analyzed using a Chi-square test with conditions mentioned earlier.

Differences between the treatment and the control group in VAS scores regarding pain intensity over time and cytokine/chemokine levels over time were analysed by using a mixed model analysis with random intercepts (PASW Statistics version 17.0). The pre-randomisation measurements were not included in the mixed model analysis. Because of the small sample size, descriptive statistics were given for the other end-points, allodynic square area and EQ5D scores.

RESULTS

Twenty-five patients, referred to the pain clinic of the University Medical Center Utrecht between October 2008 and March 2010, met the inclusion criteria of our study. Fifteen patients decided not to participate in the trial because of the invasive nature of the treatment. Ten patients were included in the clinical study of whom six were randomized to the treatment group. Patient characteristics are shown in Table 1.

Table 1 Baseline characteristics

	TREATMENT GROUP MPA + Lidocaine n=6	**CONTROL GROUP** Lidocaine n=4
Sex – number of males (%)	2 (33.3)	2 (50.0)
Age – years	76 (70 – 88)	70 (60 – 72)
Duration of PHN – months	24.0 (17.5 – 50.5)	21.5 (9.3 – 87.0)
Affected dermatomes – (min. to max. range)	Th 4 – L 3	Th 6 – Th 12
VAS score – cm (mean with SD)	7.5 (1.4)	6.7 (1.3)
Quality of life – EQ5D score	0.23 (0.06 – 0.56)	0.66 (0.14 – 0.93)

Values given are medians with interquartile ranges for all comparisons except when indicated otherwise. MPA, methylprednisolone acetate; PHN, postherpetic neuralgia; n, number of patients; SD, standard deviation; VAS, visual analogue scale.

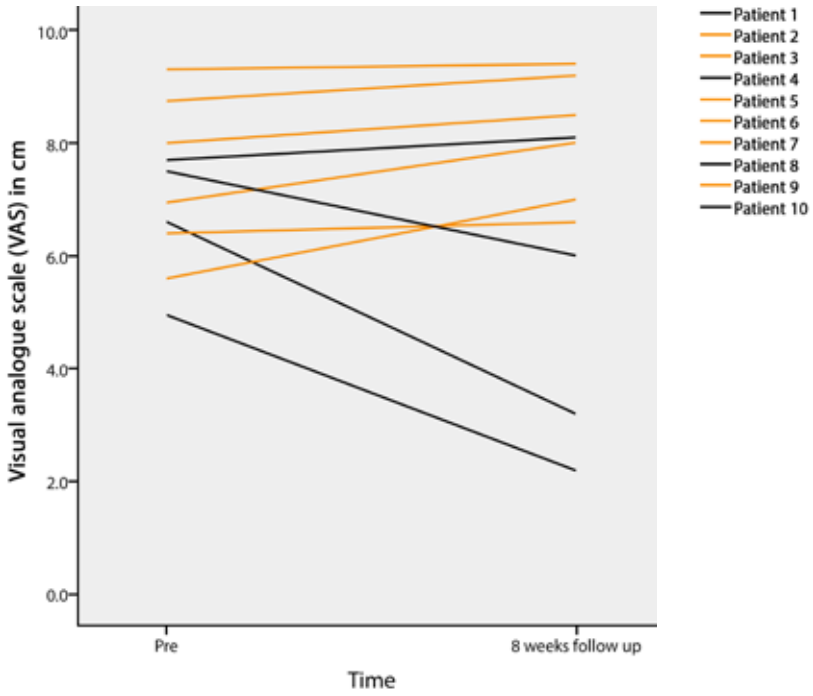

Figure 1 Global visual analogue scale score previous to the first intrathecal injections and at the primary endpoint; 8 weeks follow-up. The data is graphed per patient. Black lines: control patients; yellow lines: methylprednisolone acetate-treated patients

CLINICAL OUTCOMES

All patients experienced a combined sensory and motor block after the intrathecal injection. During the intrathecal injection none of the patients reported pain other than that of the needle puncture. The height of the sensory block included the PHN dermatome in two out of four patients in the control group and in five out of six patients in the treatment group.

One patient died 6 months after the last injection from the complications of metastasized colon cancer. One patient experienced weakness of one leg at 1-year follow-up. A MRI scan showed a herniated lumbar disc corresponding with the clinical symptoms as assessed by a neurologist not involved in the RCT. Both patients had been randomized to the treatment group.

At the primary endpoint, 8 weeks after the last injection, all patients in the treatment group experienced an increase in global pain (mean VAS from 7.5 to 8.1 (Δ = 0.6; 95% confidence interval (CI) 0.1 to 1.2)). In the control group two patients had good/excellent pain relief, one poor/fair pain relief and one experienced an increase in pain (mean VAS from 6.7 to 4.9 (Δ = -1.8; 95% CI -4.5 to -0.9); Figure 1). Including all follow-up data, mixed model analysis showed a significantly higher global VAS score of 2.8 (95% CI 1.4 to 4.2, p = 0.002) in the treatment group.

The mean VAS scores in the treatment group decreased for burning pain (from 6.3 to 5.4 (Δ = -0.9; 95% CI -6.9 to 5.2), increased for lancinating pain (from 6.3 to 8.1 (Δ = 1.8; 95% CI -1.7 to 5.2) and remained the same for allodynia (from 7.1 to 7.2 (Δ = 0.1; 95% CI -2.0 to 2.1)) at 8 weeks follow-up (Figure 2). In the control group a decrease in VAS was observed for all three, burning, lancinating pain and allodynia (resp. 6.5 to 3.2 (Δ = -3.3; 95% CI -7.6 to 1.0), 7.0 to 4.7 (Δ = -2.3; 95% CI -7.3 to 2.8) and 7.6 to 4.7 (Δ = -2.9; 95% CI -7.1 to 1.2). Including all follow-up data, mixed model analysis showed a trend towards a higher VAS score for burning pain in the treatment group of 2.5 (95% CI -0.03 to 5.0, p = 0.08), a significantly higher VAS score for lancinating pain of 3.4 (95% CI 1.8 to 5.0, p = 0.002) in the treatment group, and no significant difference in VAS score for allodynia between the two groups (increase of 0.1; 95% CI -0.04 to 0.2, p = 0.07).

The allodynic area increased in four out of six patients in the treatment group (median ratio area follow-up/area previous-to-treatment of 2.5 (interquartile range (IQR) 0.5 to 8.0)) 8 weeks after the last injection; the area increased in one out of four patients in the control group (median ratio of 0.7 (IQR 0.1 to 1.4); Figure 3).

The EQ5D[13] score increased in both groups at 8 weeks follow-up; in the treatment group with a median Δ = 0.11 (IQR -0.01 to 0.60) and in the control group a median Δ = 0.14 (IQR -0.44 to 0.54).

Patients, both in the treatment and in the control group did not reduce the number of doses of NSAIDs or acetaminophen during the study period. Three patients, two of whom were in the treatment group, even reported an unbearable increase of

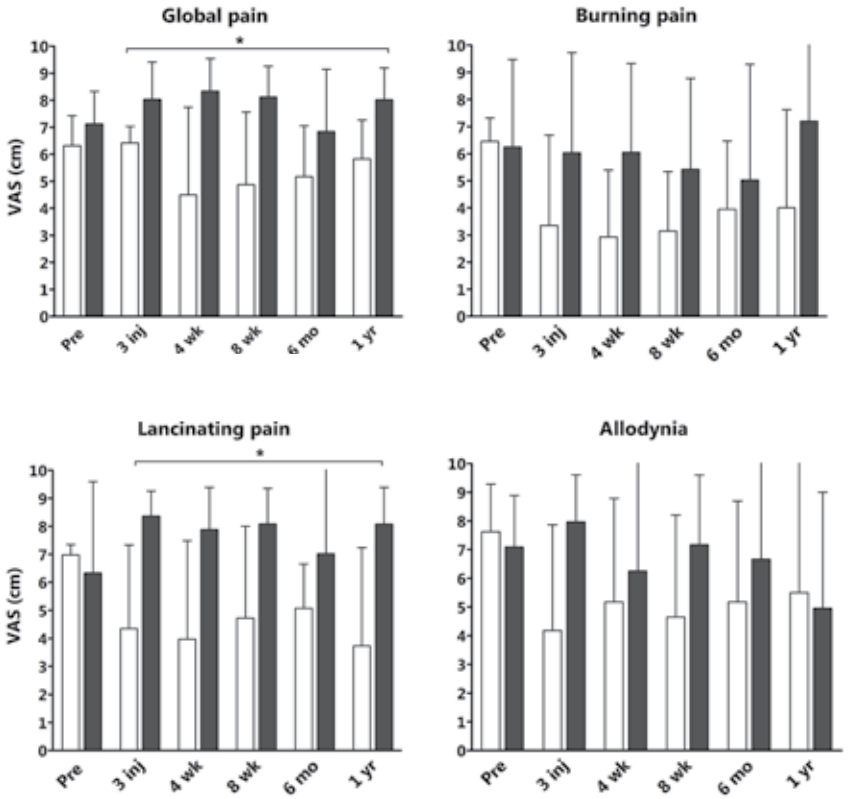

Figure 2 Visual analogue scale (VAS) pain scores and allodynia for both study groups until 1-year follow-up. Error bars represent the standard deviation of the mean. The asterisk (*) indicates the significant differences.

their pain and requested a nerve blockade to obtain pain relief. One patient from the treatment group stated: 'The more injections I get, the more the pain increases.'

After the first interim analysis we observed that none of six patients in the treatment group reported good or excellent pain relief versus two out of four patients in the control group. The lower boundary of the sequential analysis was crossed (two-sided p = 0.053, estimated Risk Difference (% failures in MPA – % failures in Control) = 50% with an approximate 90% C.I. (0 to 82%); Figure 4).[16] The trial was stopped for reasons of futility. An additional reason for discontinuing patient inclusion was new evidence from a preclinical study indicating that intrathecal preservative-free MPA caused local inflammatory reactions in dogs.[17]

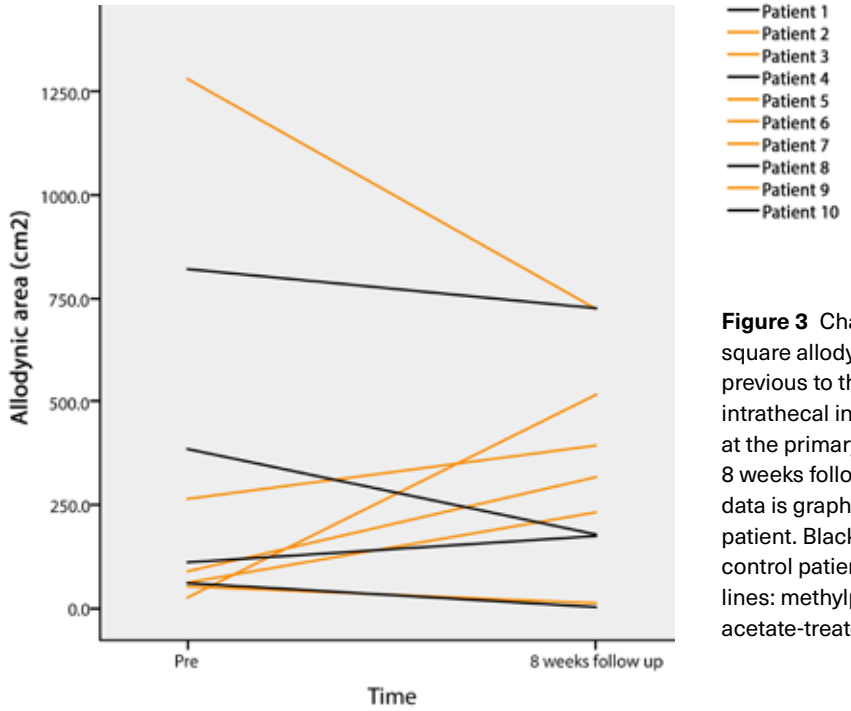

Figure 3 Changes in square allodynic area previous to the first intrathecal injections and at the primary endpoint; 8 weeks follow-up. The data is graphed per patient. Black lines: control patients; yellow lines: methylprednisolone acetate-treated patients.

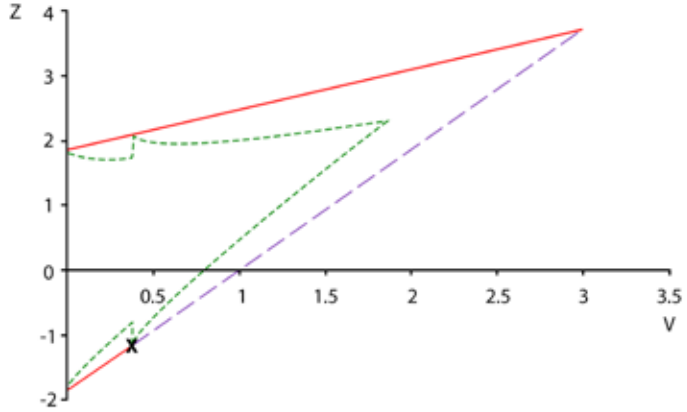

Figure 4 The Z on the y-axis represents the efficacy score. V on the x-axis represents the amount of information available. The upper and lower red lines show the borders of significance; crossing the upper red line means that more than 50% of patients in the treatment group experienced good to excellent pain relief, compared to less than 10% in the control group. The green 'Christmas Tree' boundaries are an adjustment to accommodate discrete monitoring in a design for continuous monitoring. In this figure the lower boundary of the sequential analysis was crossed (black cross), which means that the MPA treated patients experience significantly less pain relief that the control patients.

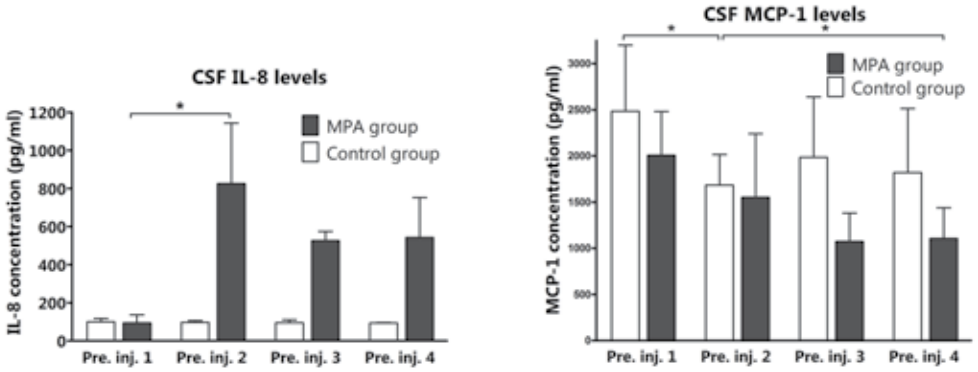

Figure 5 Cerebrospinal fluid (CSF) Interleukin (IL)-8 and monocyte chemotactic protein (MCP)-1 levels for both study groups before every intrathecal injection. Error bars represent the standard deviation of the mean. The asterisk (*) indicates the significant differences.

LABORATORY RESULTS

CSF red and white blood cell counts, glucose and C-reactive protein levels were within normal ranges in all patients during the entire study period. Total CSF protein levels were above the normal range in 9 out of 10 patients at baseline; treatment group min-max range: 0.39 – 0.56 g/l and control group: 0.49 – 0.76 g/l (normal CSF protein concentration: 0 – 0.40 g/l) and did not change significantly during the study period.

Levels of the cyto-/chemokines IL-1α, IL1β, IL-10, TNFα, MIP-1α, and fractalkine were below the detection limit in more than 70% of the samples. IL-6 and MIP-1β were below detection limit in respectively 19% and 48% of the samples; no significant differences were observed between groups or over time. IL-8 and MCP-1 levels were detected in all samples.

The mean IL-8 levels in the control group were comparable to the treatment group before the first injection (respectively 99.41 pg/ml (95% CI 70.78 to 128.04) and 94.01 pg/ml (95% CI 41.74 to 146.28). Mean IL-8 levels did not change during the study period in the control group, but increased in the treatment group to 849.04 pg/ml (95% CI 501.87 to 1196.21, group x time interaction: p-value = 0.077) and remained high (420.53 pg/ml; 95% CI 5.75 to 835.31) until the last measurement 4 weeks after the last intrathecal MPA injection (Figure 4). Mean MCP-1 values decreased after the intrathecal injections in the treatment group during the study period (group x time interaction: p-value = 0.038; Figure 5).

No viral activity of the varicella zoster or herpes simplex virus was observed either before or after treatment in both patient groups.

CSF MP levels were highest one week after the last intrathecal MPA injection (mean MP level of 2786.43 ng/ml; 95% CI 1669.86 to 3903.00) and were still measurable 8 weeks after the last injection in all MPA treated patients (mean MP level of 158.23 ng/ml; 95% CI 45.40 to 271.06). MPA, the particulate material, was still detected in 2 out of 6 patients at 8 weeks after the last injection.

DISCUSSION

In the present RCT, the efficacy of multiple doses of intrathecal reformulated MPA with minimal adjuvants was studied in a small number of patients with PHN. Because of increased pain reports in patients treated with intrathecal MPA and the statistical evidence of futility or perhaps even unsafety, and also because of new evidence suggesting neurotoxicity of intrathecal MPA in dogs,[17] patient inclusion was halted and the trial terminated.

Before considering issues pertinent to the interpretation of these data, we briefly point out some influencing events surrounding the trial. The present RCT was designed according to the protocol used in an earlier RCT.[3] Since there had not been any adverse events in the 270 patients included in that trial and the potentially neurotoxic preservatives were removed from the MPA, the risks of the present study were considered acceptable and with approval of the medical ethics committee we proceeded with the inclusion of patients. However, no preclinical safety data on the use of intrathecal reformulated MPA had been published. During the first inclusions of the present trial, a discussion had risen on the off-label use of drugs in the intrathecal space and preclinical safety studies were advised.[18;19] Therefore, we decided to perform a parallel preclinical safety study in a dog model. The detailed results of this study were obtained in 2010 and have been published in a separate paper.[17] The preclinical safety study was completed at the first planned interim analysis after ten included patients. In dogs a dose-dependent inflammatory meningeal reaction, accompanied with inflammatory masses suggesting abscess formation was seen after administration of both 10 mg and 40 mg MPA with lidocaine. These results in combination with evidence for clinical deterioration of PHN in all patients treated with MPA, led to the decision to end the trial after inclusion of the first ten patients.

Patients reported an increase of pain in the neuralgic skin area starting the first day after intrathecal MPA treatment. During the intrathecal injection none of the patients experienced pain. There are no other reports on pain enhancement in the neuralgic skin areas after intrathecal MPA administration, but there are reports on pain enhancements in the days after epidural administration of MPA.[20] In a review on the complications after epidural steroid administration, an increase of pain was the most common complication.[20] Also adverse events have been reported after the

administration of intrathecal MPA with preservatives.[21-25] Adverse events after intrathecal MPA included transverse myelitis, radiculitis and cauda equina syndrome causing severe radiating pains in limbs.[1] In our study, one patient in the treatment group reported similar symptoms at 1-year follow-up, but was later diagnosed with a herniated lumbar disc. None of the other MPA-treated patients with increased PHN had radiating limb pains, other adverse neurological symptoms or signs of a neuraxial inflammatory process.

A possible explanation for the increase in pain after intrathecal MPA administration is that MPA initiates an inflammatory process as observed in preclinical studies.[2;17;21;25;26] However, the inflammatory responses in these preclinical studies were accompanied by an increase in CSF protein. We did not observe significant increases in CSF protein in our patients during or after the treatment period. In addition, the CSF concentrations of the pro-inflammatory mediators IL-1α, IL-1β, IL-6 and TNFα were below detection levels in most samples before and after intrathecal MPA administration, suggesting no marked inflammatory activity.[27] However, in the treatment group CSF IL-8 levels were significantly increased after the first intrathecal MPA administration and remained high until the last measurement. Similar results, low protein, IL-1β, IL-6, and TNFα but increased IL-8 concentrations, were reported in a study in patients with disc herniation.[28] In that study, increased IL-8 levels were associated with more pronounced disc herniation which could cause more mechanical irritation or exposure to nucleus pulposis material. IL-8 is a pro-inflammatory cytokine capable of attracting neutrophils as mediators of acute inflammation. There are data in the literature indicating that steroids may under certain pro-inflammatory conditions enhance the release of chemoattractants such as IL8.[29] The observations here indicate that intrathecal MPA has a pro-inflammatory action, but in view of the finding that white blood cell counts were not elevated after MPA treatment, we do not have evidence for sustained meningitis in our patients.

The CSF levels of MCP-1, a lymphocyte and inflammatory chemokine associated with persistent inflammatory states, decreased in patients treated with MPA. This finding is in line with the immunosuppressive effects assigned to steroids. The finding that MCP-1 levels decreased in patients treated with MPA, whereas IL-8 levels increased may be explained by differences in the cellular source of these two pro-inflammatory mediators.

It has been hypothesized that intrathecal administration of steroids in PHN patients could lead to reactivation of the varicella zoster virus,[8] or that PHN is associated with persistent viral activity.[30] However in the present study no viral reactivation was observed after treatment with MPA, thus not providing an explanation for the observed increase in pain.

The decision to stop our RCT was based on the first sequential analysis of ten patients; a small sample size. Nonetheless, since all MPA treated patients experienced

an increase of pain and we assumed a pain decrease of 50% for our sequential analysis (based on the 92% of patients in the Kotani trial experiencing a decrease of pain[3]), statistically significant results were obtained. When patient inclusion would have continued, the probability of finding a decrease of global pain in 50% of patients was negligible (i.e. < 0.00003).

The differences in design between both RCT's were small,[3] the results markedly different. In the Kotani trial nearly all MPA-treated patients experienced pain relief and had decreased CSF IL-8 levels,[3;31]whereas in our study all MPA treated patients reported an increase in pain and had increased CSF IL-8 levels. We find it difficult to explain these differences. The most important difference between the studies is the study formulation. In our trial we used lidocaine 2%, 60 mg compared to lidocaine 3%, 90 mg used in the Kotani trial. We decided to reduce the concentration and dose of lidocaine to decrease the risk of complications such as toxicity and hypotension.[11;12;32] However, we do not think that the 30 mg lower dose of lidocaine can explain the large differences in study results.

In the present trial we aimed at reducing the concentration of the preservative myristyl-gamma-picolinium chloride to promote the safety of the formulation. The exact details of the formulation used in the Kotani trial are unclear. In their discussion section, the authors mention that they have used a formulation containing 3% propylene glycol corresponding with the polyethylene glycol concentration in the commercially available formulation containing preservatives. In a letter to the editor in the New England Journal of Medicine it is stated that 'Kotani et al. misidentified polyethylene glycol as propylene glycol'.[9]

The remaining 0.01 mg/ml myristyl-gamma-picolinium chloride in our study formulation could have been a factor causing an increase in pain. In concentrations of approximately 0.4 mg/ml it has been shown to cause retinal damage in the rabbit eye.[33] The concentration measured in the study formulation is substantially lower. Although we cannot completely rule out that this low concentration contributed to the increase of pain, we believe that the differences between the results of the two studies are not likely to be caused solely by the small concentration of myristyl-gamma-picolinium chloride.

Potentially racial differences between Japanese and Western-European PHN patients could have played a role, however also not fully explaining the large differences between the studies.

Because of the small sample size there were clear differences in baseline characteristics between the two groups. Patients in the treatment group were older and their VAS at admission was higher. In addition, at inclusion the QOL of patients in the treatment group was lower than in the control group. These baseline differences might have influenced the results, but are unlikely to be responsible for the complete lack of positive effects in the treatment group.

The domain of the study included patients with intractable PHN; none of the available treatment options had any positive effect on their pain. These patients however would be the main candidates for such an invasive treatment option in clinical practice. Therefore the lack of efficacy in this patient group makes its use in clinical practice unlikely.

In conclusion, there was no evidence of any clinical benefit of intrathecal MPA for patients suffering from intractable PHN. Considering this absence of clinical benefits and the potential risks of the treatment,[17] intrathecal administration of MPA for PHN is not recommended.

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CHAPTER 6

Analgesic properties of intrathecal glucocorticoids in three well established preclinical pain models

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ABSTRACT

Background and aims – Glucocorticoids, a group of anti-inflammatory agents, are frequently administered in pain medicine. Of interest is the reported activity after intrathecal delivery in patients with neuropathic pain syndromes such as postherpetic neuralgia, though its efficacy is controversial. After the publication of two randomized clinical trials in postherpetic neuralgia patients treated with similar intrathecal methylprednisolone acetate (MPA) dosing regimes with conflicting results; one showing significant pain reduction,[1] the other increased pain sensations,[2] we decided additional research was warranted. Present study sought to determine effects of intrathecally delivered methylprednisolone on pain-like behavior and pain-associated markers in three well established rodent pain models; a) intraplantar carrageenan, b) intraplantar formalin, and c) ligation of L5/L6 spinal nerves (SNL model).

Methods – Male rats with intrathecal catheters were examined for a) tactile allodynia after unilateral hindpaw intraplantar carrageenan

injection (2%), b) flinching and subsequent long term tactile allodynia after unilateral hindpaw intraplantar formalin injection (2.5%) or c) tactile allodynia after unilateral ligation of the L5 and L6 spinal nerves. Rats were treated with the maximum tolerable intrathecal dose of the soluble methylprednisolone sodium succinate (MP) or the particulate methylprednisolone acetate (MPA). Dorsal root ganglia and spinal cords were harvested for immunohistochemistry to assess markers of neuronal damage (ATF3) and glial activation (GFAP, Iba1).

Results – During dose finding, severe generalized allodynia was observed with high intrathecal doses of both MPA and MP in naive rats. MPA had no effect upon tactile allodynia after carrageenan. MP and MPA did not reverse tactile allodynia in the SNL model, and did not reduce flinching in the formalin model. MP and MPA prevented the delayed (7-day) tactile allodynia otherwise

observed in the formalin-injected paw. Systemic MP or perineural MP or MPA did not reduce pain-like behavior in the SNL model. No reduction of neuronal injury (ATF3) in the dorsal root ganglion or astrocyte activation (GFAP) in the spinal dorsal horn with intrathecal MP or MPA was observed. There was a decrease in microglial activation (Iba1) in the spinal dorsal horn with MPA after SNL.

Conclusion – Severe generalized allodynia was observed after high intrathecal doses of MP and MPA in naive rats. No acute analgesic effects with intrathecal glucocorticoids were observed in three well established pain models. Only a late antiallodynic effect was present in the formalin model, 7 days after formalin injection and drug treatment.

Implications – Our results do not support use of intrathecal methylprednisolone in the treatment of pain.

INTRODUCTION

Glucocorticoids, a group of anti-inflammatory agents, are frequently administered in pain medicine. Of interest is the reported activity after neuraxial (epidural and intrathecal) delivery in patients with low back pain and in patients with neuropathic pain syndromes such as postherpetic neuralgia and complex regional pain syndrome,[1-4] though their efficacy is controversial.[5-8] This controversy is surprising, given that glucocorticoids act upon a variety of crucial biological links in the neuraxial pathways after inflammation and nerve injury leading to hyperpathic states.[9] Glucocorticoids can act through transrepression of pro-inflammatory genes (interacting with activator protein-1 and nuclear factor kappa B (NFκB)), transactivation of anti-inflammatory genes (lipocortin I, p11/calpactin-binding protein) and nongenomic effects (interacting with G-protein coupled receptors, mitogen-activated protein (MAP) kinases, phosholipases and protein kinases (SRC)), to down regulate inflammatory processes,[10] leading to a reduced production and secretion of inflammatory products such as cyclooxygenase-2, interleukin (IL)-1β, IL-2, IL-6, IL-8, tumor necrosis factor (TNF), interferon-gamma, and inducible nitric oxide synthase.[11;12] These effector products are considered to be important components in neuropathic pain signaling. If these neuroinflammatory products are indeed reduced, it is not clear why the analgesic effects of glucocorticoids are varying and often disappointing.

Our research team encountered disappointing analgesic effects with glucocorticoids in a randomized controlled clinical trial conducted in patients suffering from postherpetic neuralgia.[4] Four intrathecal injections with methylprednisolone acetate (MPA) with 7 day intervals were administered in patients with intractable neuropathic pain. Patients treated with intrathecal MPA reported increased pain and with statistical evidence of futility, the trial was ended early. Our results were in sharp contrast with results of an earlier trial with a similar drug and dosing regime, showing pain reduction in 92% of patients in the intrathecal MPA treated group.[13] Since we did not understand the differences in results between the two trials, we decided to conduct a preclinical study using a similar MPA formulation. Additional to a) the MPA formulation, a suspension with depot characteristics and reduced preservative concentrations, we also studied b) methylprednisolone sodium succinate (MP), a solution without preservatives, both frequently used in pain medicine.

We argued that since the etiology of postherpetic neuralgia is not clear and we were interested to see if intrathecally delivered MPA had any effect on pain like behavior and surrogate markers in a severe pain state, we should study the efficacy of glucocorticoids in multiple models inducing pain like behavior with; a) an inflammatory, b) a neurotoxic, and c) a direct nerve injury stimulus.

Three well established preclinical pain models in rats were selected; a) Intraplantar carrageenan leading to a robust inflammation and tactile allodynia of the injected paw,[14] b) Intraplantar formalin leading to a biphasic flinching behavior acutely after injection and an evolving tactile allodynia that develops over the ensuing 7 days,[15;16] c) Unilateral ligation of the L5/6 spinal nerves (SNL model) yielding a unilateral mononeuropathy characterized by a robust tactile allodynia.[17] We further examined the effects of glucocorticoids on the expression of well characterized markers including activation transcription factor 3 (ATF3) in the dorsal root ganglion (DRG) and induced astrocyte (glial fibrillary acidic protein (GFAP)) and microglia activation (ionized calcium-binding adapter molecule 1 (Iba1)) in the spinal dorsal horn. ATF3 is upregulated in injured DRG neurons after peripheral nerve injury and has a survival function driving neurite outgrowth.[18] Glial cells are activated by cytokines such as IL-1β, IL-6, TNFα and monocyte chemoattractant protein (MCP)-1 produced through MAP kinases (p38 and SRC-family kinases) phosphorylation and NFκB activation. We hypothesized that intrathecal glucocorticoids would reverse the hyperpathic states and the indices of DRG and dorsal horn neuroinflammation.

METHODS

The protocol of the present study has been approved by the AAALAC accredited (International, Association for Assessment and Accreditation of Laboratory Animal Care) Institutional Animal Care and Use Committee (IACUC) of the University of California, San Diego, USA.

ANIMALS

Male Harlan Sprague-Dawley rats (200-225 gram for the carrageenan and 80-100 gram for the SNL model) and male Holtzman rats (200-225 gram for the formalin model) (Indianapolis, IN, USA) were maintained 2 per cage in standard cages at room temperature on a 12:12 hour light/dark cycle with free access to food and water. After arrival at the housing facility, they were allowed at least 2-3 days of acclimation before use.

DRUG ADMINISTRATION

In all three pain models rats received intrathecal drug treatment. Only in the SNL model, additional groups of rats received intraperitoneal or perineural drug treatment;

a) For intrathecal drug injections, rats were surgically implanted with intrathecal catheters as described previously under general anesthesia (inhalation of isoflurane 2.4% in a room air/oxygen mixture).[19] Intrathecal catheters were externalized for

injection. Rats were given post-operative subcutaneous fluids including analgesics (lactated Ringers + 5 mg/kg Carprofen) and then housed individually for post-operative recovery. Following implantation, catheters were flushed with saline and rats were monitored daily for viability, allowing at least 5 days of recovery before testing. Animals showing any evidence of motor dysfunction or distress after catheter placement were immediately euthanized in a carbon dioxide chamber.

b) Intraperitoneal drug injections were performed in awake rats. The injection was given in the lower left abdominal quadrant. Injection of the drug was preceded by careful aspiration to determine correct placement of the needle tip.

c) Perineural drug administration was performed under general anesthesia with inhalation of isoflurane 2.4% in a room air/oxygen mixture during the nerve ligation procedure (described below). Directly after nerve ligation, a 32 gauge blunt needle was positioned in parallel to the spinal nerve, proximal to the ligation, aiming toward the foramen and cautiously blindly advanced until a resistance was felt. There the drug was administered. This was performed directly after the L5 and L6 spinal nerve ligation.

DRUG PREPARATION AND DOSING

The drugs used in the present study, are methylprednisolone acetate, MPA (depomedrol® from Pfizer) a slow release formulation, and the solution methylprednisolone sodium succinate (MP). The following preparations were employed:

a) MPA contains the preservatives polyethylene glycol and myristyl-gamma-picolinium-chloride, which are potentially neurotoxic. To remove these preservatives 1 ml of the general formulation of MPA 40 mg/ml (Pfizer) was centrifuged in a mini centrifuge (Fisher Scientific, Cat no 05-090-128, 14.000 rpm) for 10 minutes. The supernatant was aspirated with a 20 G needle and syringe and discarded. The residual pellet of 40 mg MPA was resuspended in 1 ml saline to give a stock solution of 20 mg/ml.

b) MP, a powder, was dissolved in saline to a concentration of 40 mg/ml. Since this solution has a limited stability, this was done less than 10 minutes before administration.

Based on preliminary studies, the highest tolerable dose of either drug was administered in the present study. The highest tolerable intrathecal dose of MPA was 400 µg. With higher doses (800 µg) severe generalized allodynia developed, causing the animals to vocalize and adapt aggressive guarding behavior when stroking their fur. The 400 µg MPA dose easily passed through the intrathecal catheters. Initially a comparable dose of MP was chosen, 400 µg. The animals did not tolerate this dose and showed a similar generalized allodynia as was seen with the 800 µg MPA dose. The highest tolerable dose of MP was found to be 40 µg. Since the highest tolerable

intrathecal dose of MP was 40 µg, we decided to give a similar perineural dose per treated spinal nerve root, so a total of 80 µg. The perineural MPA dose was adjusted to a total dose of 80 µg.

DRUG PHARMACOKINETICS

To be able to time the clearance of MPA during the initial phase after the pain stimulus, we started our experiments with a pharmacokinetics study measuring methylprednisolone plasma levels. Rats were given a 400 µg intrathecal MPA dose and 1, 3, 6, 24, 48, 72 and 144 hours after administration, a cardiac blood sample was obtained under general inhalational anesthesia with isoflurane 2.4% in a room air/oxygen mixture. Also four blood samples from rats with intrathecal catheters were included in which no drug treatment was administered. All rats, still under general anesthesia, were immediately euthanized in a carbon dioxide chamber after the blood sample was drawn. Blood samples were centrifuged at low g-force for 5 minutes and plasma was aspirated from the sample and stored in a -80 °C freezer. The methylprednisolone concentrations in blood plasma were measured with an enzyme-linked immunosorbent assay (ELISA) kit (Neogen corporation, Lexington, KY, USA).

ANIMAL MODELS

Rats were randomly assigned to one of three pain models; 1) carrageenan model to study inflammatory induced pain, 2) formalin model to study chemically induced pain and 3) spinal nerve ligation model (SNL model) to study neuropathic pain after direct nerve injury.

Carrageenan model

After one hour drug pretreatment with either intrathecal saline or intrathecal MPA, the paw thickness of both hind paws were measured using calipers after which rats received a plantar injection with a 30 G needle in the left hind paw with 0.1 ml of 2% carrageenan under general inhalational anesthesia with isoflurane 2.4% in a room air/oxygen mixture. Directly after the injection, rats were allowed to recover and tested for the development of thermal hyperalgesia and mechanical allodynia at 1, 2, 4 and 8 hours. Eight hours after the carrageenan injection, paws thickness was measured again under anesthesia and rats were sacrificed in a carbon dioxide chamber.

Formalin model

After one hour pretreatment of intrathecal saline, intrathecal MP or MPA, measurement of the baseline paw thickness and acclimation to the model in individual Plexiglas chambers for at least 30 min, rats received an injection with a 30 G needle in the dorsal side of the left hind paw of 0.05 ml of 2.5% formalin. Formalin induced flinching behavior was recorded during the hour after injection using an automated detection system.[20] Flinches were counted in 1-min intervals for 60 min. The data are expressed as total number of flinches observed during phase 1 (0–9 min)

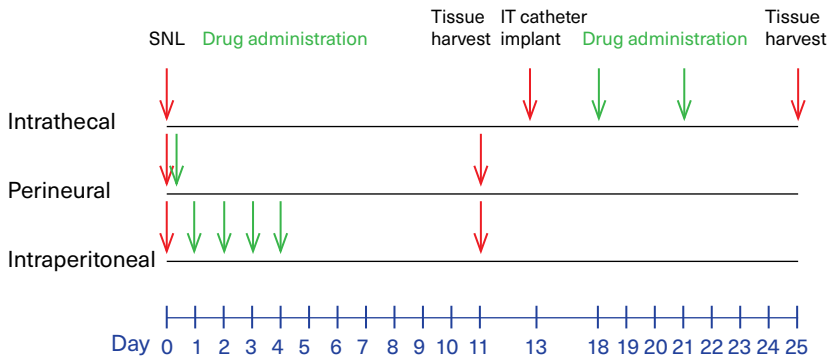


Figure 1 Different dosing regiments in the spinal nerve ligation (SNL) model. Intrathecal dosing: Thirteen days after SNL, an intrathecal (IT) catheter was implanted. Intrathecal drug treatment started on post-operative day 18 and was repeated on day 21. Tissue (spinal dorsal horn and dorsal root ganglia (DRG)) was collected 7 days after the first drug dose. Perineural dosing: perineural injections were given during the SNL and tissue was collected 11 days later. Intraperitoneal dosing: Drugs were given daily for four consecutive days starting on the first postoperative day. Tissue was collected 11 days after SNL.

and phase 2 (10–60 min).[21;22] Rats were allowed to recover for seven days after which their mechanical thresholds were measured and rats were sacrificed (procedure described below) for tissue collection for immunohistochemistry. Previous work has shown the development of a late phase tactile allodynia.[15]

SNL model

Spinal nerve injury was induced by the procedure described by Kim and Chung.[17] Briefly, the left L5/L6 lumbar spinal nerves were exposed in isoflurane/oxygen-anesthetized rats and tightly ligated with 6.0 silk suture at a point distal to their DRGs and proximal to their conjunction to form the sciatic nerve. An intrathecal catheter was implanted 13 days after SNL. Intrathecal injections (Saline/MP/MPA), starting 18 days after SNL, were given twice with a 3-day interval. Intraperitoneal injections (Saline/ MP) were given on the first postoperative day after SNL for four consecutive days, and perineural injections (Saline/MP/MPA) were given during SNL (Figure 1). Spinal cord and DRG tissue were collected for immunohistochemistry seven days after drug treatment in all rats.

BEHAVIORAL MEASUREMENTS

All behavioral measurements were made by observer (MR) blinded to the treatment groups and were conducted at fixed times (9:00 a.m.–5:00 p.m.). To measure mechanical allodynia, thresholds were measured with a series of calibrated von Frey

filaments (Stoelting, Wood Dale, IL, USA), ranging from 3.16 to 5.18 (0.41-15.0 g). The animals were acclimated for 45 min in the test chamber on mesh flooring suspended above the observer, and von Frey filaments were applied perpendicularly against the plantar surface of the paw. The 'up-down' method of Dixon as described by Chaplan et al.[23] was used to determine the value at which paw withdrawal occurred 50% of the time, interpreted as the mechanical threshold. Severe generalized allodynia was defined as vocalization within the first hour after intrathecal drug administration in combination with aggressive guarding behavior when stroking the animal's fur.

IMMUNOHISTOCHEMISTRY

Animals were anesthetized with isoflurane 4.0% in a room air/oxygen mixture and transcardially perfused with saline 1 ml/gram bodyweight followed by freshly prepared 4% paraformaldehyde in 0.1 M phosphate buffered saline (PBS) 1 ml/gram bodyweight. Spinal cord and DRGs of L5 and L6 roots were removed, post-fixed overnight in the same fixative and moved to 30% sucrose for at least 72 hours. Free floating transverse sections (30 µm) were taken from the spinal cord using a microtome. DRGs were embedded in Tissue-Tek® (O.C.T. Compound, Sakura® Finetek, PA, USA) frozen and cut (10 µm) on a Leica CM1800 Cryostat (IMEB, CA, USA) and directly mounted on glass slides. Both free floating sections as mounted DRG tissue were permeabilized with 0.3% Triton X-100 (Sigma, St. Louis, MO, USA), blocked with 5% goat serum in PBS and incubated with the following antibodies; a marker for neuronal damage, ATF3 (rabbit, 1:500, cat. no. sc188, Santa Cruz Biotechnology INC, CA, USA), markers for astrocytes GFAP (mouse, 1:4000, cat. no. #MAB360, Chemicon, USA), microglia Iba1 (rabbit, 1:2000, cat. no. #019-19741, WAKO, VA, USA), and neurons NeuN (mouse, 1:1000, cat. no. MAB377, EMD Millipore Corporation, MA, USA) overnight at room temperature. Binding sites were visualized with secondary antibodies conjugated with fluoro-Alexa-594 (1:1000, cat. no. A11032, Invitrogen, NY, USA), fluoro-Alexa-488 (1:1000, cat. no. A11058, Invitrogen, NY, USA) or streptavidin conjugated fluoro-Alexa-488 (1:1000, cat no. S-32354, Life Technologies, CA, USA). A streptavidin/biotin blocking kit (Vector Labs, CA, USA) was utilized as appropriate before biotinylated ATF3. To confirm that the marker for neuronal damage, ATF3, was only observed in neurons, sections were double labeled with the NeuN antibody. Images were captured using a fluorescence microscope (Nikon TE300 fluorescence microscope (Nikon Corp, Tokyo, Japan)) and overlay performed with Adobe Photoshop Creative suite (CS6; Adobe Systems Incorporated). The investigators (MR and EdG) were blinded for the experimental conditions during quantification of ATF3, Iba1 and GFAP staining. Quantification of ATF3 in DRGs (by MR and EdG) was assessed by counting the number of cells with ATF3 positive nuclei over the total number of NeuN positive

cells. L5 and L6 DRGs of the ipsi- and contralateral sides (4-6 sections per side) were group mounted for individual animals onto slides. All sections on a slide were separated by at least 60 µm. Three sections per DRG per side (ipsi- & contralateral) per animal were counted (total of 12 sections per animal). The quantification of Iba1 and GFAP in the spinal cord (MR) was performed by measuring the total integrated signal intensity of pixels in lamina I-II of the dorsal horn after subtraction of the background signal intensity in this area using ImageJ 1.47 software. The total signal intensity was measured in at least 6 sections of the L4 to 6 areas of the ipsi- and contralateral dorsal horn (total of 12 sections per animal). Per section, 3 background signal intensity measurements were performed in the lamina I-II area and pooled to a mean for subtraction. An increase in the signal intensity for Iba1 and GFAP staining was interpreted to signify microglia and astrocyte reactivity, respectively.

DATA ANALYSIS

Data are presented as means with 95% confidence intervals (CI). Significance was ascribed for $p < 0.05$. Behavioral time-course data in the carrageenan and SNL model (tactile thresholds) was analyzed using two way ANOVA with repeated measures across time. If statistical main effects were observed, the analysis was followed by Bonferroni post hoc tests (e.g., unpaired t-tests with Bonferroni corrections) at each time point unless otherwise stated. Differences in paw thickness between groups in the carrageenan model were analyzed with an unpaired two tailed t-test. For behavioral time course data in the formalin model (flinching behavior) we calculated the area under the curve (AUC). Differences between group AUCs were calculated with an unpaired two tailed t-tests. Differences in tactile thresholds previous to formalin compared to 7 days after formalin within one group were analyzed with a paired two tailed t-test. Differences in signal intensity (immunohistochemistry; GFAP, Iba1) and ATF3 count between treatment groups were calculated using two way ANOVA with repeated measures per side (ipsi versus contralateral). If statistical main effects were observed, the analysis was followed by Bonferroni post hoc tests for each side. All analyses were carried out using Graphpad Prism version 5.

RESULTS

PHARMACOKINETICS OF INTRATHECAL MPA

The pharmacokinetics of intrathecal administered MPA, show that there is a rapid diffusion out of the intrathecal space into the blood plasma (Figure 2). Peak plasma values are reached 1 to 3 hours after intrathecal injection. Methylprednisolone plasma levels go below detection limits by 72 hours after injection.

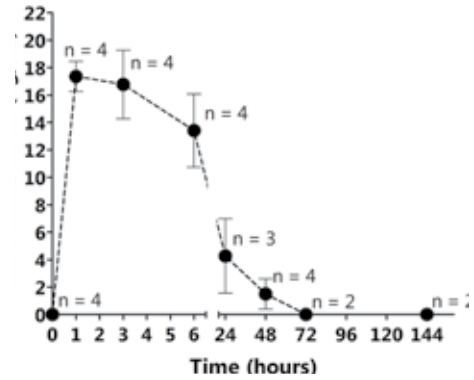


Figure 2 Pharmacokinetics after intrathecal methylprednisolone acetate (MPA) injection. Time in hours (h) on the x-axis. Methylprednisolone (MP) plasma levels in ng/ml on the y-axis. The n indicates the number of rats that were sampled from at the corresponding time point.

HIGH DOSES OF INTRATHECAL MP AND MPA CAUSE SEVERE GENERALIZED ALLODYNIA IN RATS

While searching for the highest tolerable intrathecal dose of both MP (40 µg) and MPA (400 µg), we encountered severe generalized allodynia (MPA dose of 800 µg, MP of 400 µg, 200 µg) in rats, which lasted for 30 minutes up to an hour. Since both the MP solution and the MPA suspension had this allodynic effect, it is unlikely that particles, found in the MPA suspension, caused this phenomenon. The presence of preservatives was minimized in the MPA formulation[24] and absent in the MP formulation, and therefore not expected to play a role in the development of severe allodynia. Saline, MP and MPA were analyzed for their pH and osmolarity (Table 1). None of these variables are known to cause allodynia. To rule out an effect of the higher osmolarity values observed in the higher MP doses, we injected mannitol with a similar osmolarity (measured with a 5002 Osmette) in the intrathecal space. No adverse reaction was observed with equivalent mOsm mannitol doses, so we conclude that the severe generalized allodynia is caused by the glucocorticoid itself (Table 2).

Table 1 MP and MPA drug characteristics; pH and mOsm

Drug	pH	mOsm
Saline	6.0	272
MPA 400 µg (40 mg/ml)	6.2	226
MP 400 µg (40 mg/ml)	7.6	399
MP 40 µg (4 mg/ml)	7.2	287
MP 4 µg (0.4 mg/ml)	6.4	274

MP = methylprednisolone sodium succinate, MPA = methylprednisolone acetate.

Table 2 Mannitol doses with equivalent mOsm values to MP doses

Drug	N	mOsm	Behavior
Saline	>50	272	No adverse behavior
Mannitol 5%	2	276	No adverse behavior
Mannitol 7.2%	2	397	No adverse behavior
MP 4 µg (0.4 mg/ml)	>50	274	No adverse behavior
MP 40 µg (4 mg/ml)	5	287	2 out of 5 rats had severe generalized allodynia
MP 400 µg (40mg/ml)	2	399	Both rats had severe generalized allodynia

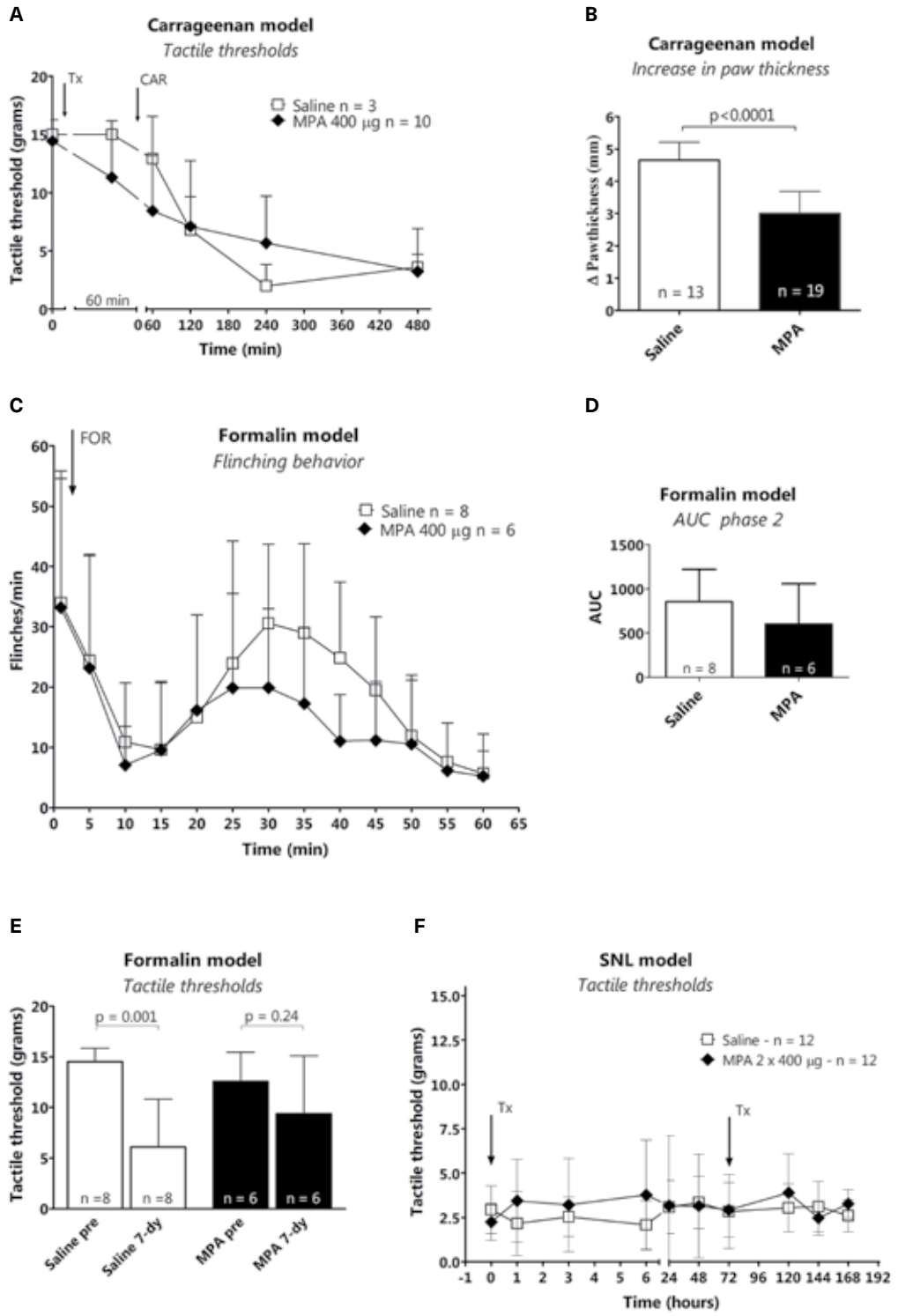
MP = methylprednisolone sodium succinate, N = number of rats

THERE IS NO ACUTE ANALGESIC EFFECT OF INTRATHECAL MPA IN ANY OF THE THREE PAIN MODELS AND ONLY A LATE MODERATE ANTIALLODYNIC EFFECT IN THE FORMALIN MODEL

Intraplantar carrageenan: In the inflammatory carrageenan model, rats displayed a significant inflammation of the injected paw (as measured by paw thickness) and tactile allodynia which developed over 2-3 hours. Pretreatment with intrathecal MPA had no significant effect on tactile thresholds compared to saline controls during the eight hour follow up (two-way rm-ANOVA; time p = < 0.0001, drug p = 0.50, interaction p = 0.21; Figure 3A). However the increase in paw thickness was significantly

> Figure 3 Effect of intrathecal methylprednisolone acetate (MPA) treatment on pain-like behavior in the carrageenan, formalin and spinal nerve ligation (SNL) model. A) Carrageenan model: Tactile thresholds in grams (y-axis) over time in minutes. Tx indicates drug treatment one hour before the injection of carrageenan (CAR) in the hind paw. Baseline thresholds have been assessed twice; once before drug treatment and once 45 minutes after drug treatment. B) Increase in paw thickness in mm 8 hours after carrageenan injection. C) Formalin model: Mean number of flinches per minute (y-axis) averaged over 5 minutes (x-axis) after formalin injection (FOR). D) The area under the curve (AUC) of phase 2 flinching (11-60 minutes after formalin injection). E) Formalin model: Tactile thresholds in grams (y-axis) per drug treatment; saline (white), MPA (black). Saline/MPA pre represent the baseline thresholds before formalin injection after intrathecal saline/MPA injection. Saline/MPA 7-dy represent the thresholds 7 days after formalin injection and drug treatment. F) SNL model: Tactile thresholds in grams (y-axis) over time in hours (x-axis). Tx indicates drug treatment on day 0 (= 18 days after nerve ligation) and day 3 (= 21 days after nerve ligation). All graphs: White square = saline controls, Black diamond = MPA treatment, n = number of animals, data are plotted as mean, error bars are the standard deviation of the mean.

Figure 3



smaller in the intrathecal MPA pretreated animals Δ = 3.0 mm versus saline controls Δ = 4.7 mm (difference 1.7 mm; 95% CI 1.2 to 2.1; p<0.0001; Figure 3B).

Intraplantar formalin: Intraplantar formalin injection resulted in a robust biphasic flinching of the injected hind paw. Pretreatment with intrathecal MPA had no effect upon phase 1 (0-10 min after injection; mean AUC saline = 205 vs AUC MPA = 188; Δ = 17, 95% CI -132 to 166) or phase 2 flinching (11-60 min after injection; mean AUC saline = 854 vs AUC MPA = 599; Δ = 255; 95% CI -222 to 733) compared to saline controls (Figure 3C+D). When measuring tactile thresholds 7 days after the formalin injection in saline controls, a significant decline in tactile thresholds was observed indicating development of tactile allodynia (previous to formalin injection; 14.5 grams vs 7-day follow up 6.1 grams; Δ = 8.4, 95% CI 4.7 to 12.2, p = 0.001). In contrast, intrathecal MPA pretreated rats (pre; 12.6 grams vs post; 9.4 grams; Δ = 3.2, 95% CI -2.9 to 9.2, p = 0.24) did not develop such allodynia (Figure 3E).

SNL mononeuropathy: Following nerve ligation, all rats developed a robust tactile allodynia that was maximum by postoperative day 7. Intrathecal MPA delivered on postoperative day 18 and 21 (corresponding with, respectively, 0 hours and 72 hours in the graph), had no effect upon the tactile thresholds observed in the first hours or the following days after treatment compared to saline controls (Figure 3F).

INTRATHECAL INJECTION OF THE SOLUBLE MP DID NOT DECREASE PAIN-LIKE BEHAVIOR IN THE FORMALIN OR SNL MODEL

Concurrently with the MPA experiments, we ran experiments with the soluble MP to determine if the drug characteristics had any influence on pain-like behavior in the formalin and SNL model. With the maximum tolerable dose of soluble MP (40 µg), no difference in flinching behavior during either phase 1 (0-10 min after injection; mean AUC saline = 205 vs AUC MP = 296; Δ = 91, 95% CI -15 to 197) or phase 2 (11-60 min after injection; mean AUC saline = 854 vs AUC MP = 881; Δ = 27; 95% CI -328 to 383) was observed as compared to saline controls (Figure 4A+B). The development of mechanical allodynia 7-days after formalin injection was, like in the MPA pretreated rats, also attenuated in MP pretreated rats (pre; 12.8 grams vs post; 10.3 grams; Δ = 2.5, 95% CI , p = 0.39; Figure 4C). No decrease in pain-like behavior was observed in the SNL model (Figure 4D).

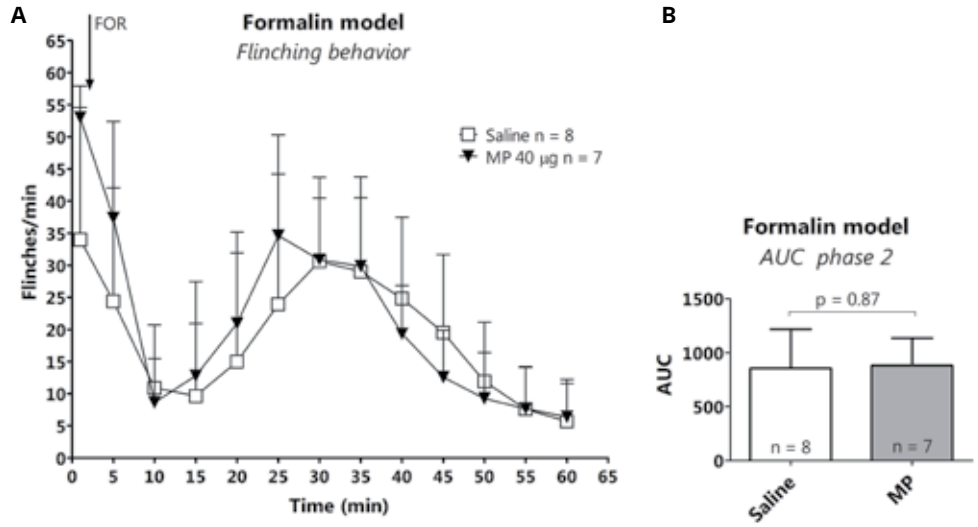


Figure 4 Effect of intrathecal methylprednisolone sodium succinate (MP) treatment on pain-like behavior in the formalin and spinal nerve ligation (SNL) model. A) Formalin model: Mean number of flinches per minute (y-axis) averaged over 5 minutes (x-axis) after formalin injection (FOR). B) The area under the curve (AUC) of phase 2 flinching (11-60 minutes after formalin injection). C) Formalin model: Tactile thresholds in grams (y-axis) per drug treatment; saline (white), MP (grey). Saline/MP pre represent the baseline thresholds before formalin injection after intrathecal saline/MPA injection. Saline/MP 7-dy represent the thresholds 7 days after formalin injection and drug treatment. D) SNL model: Tactile thresholds in grams (y-axis) over time in hours (x-axis). Tx indicates drug treatment on day 0 (= 18 days after nerve ligation) and day 3 (= 21 days after nerve ligation). All graphs: White square = saline controls, Black triangles = MP treatment, n = number of animals, data are plotted as mean, error bars are the standard deviation of the mean.

SYSTEMIC ADMINISTRATION OF MP OR PERINEURAL ADMINISTRATION OF MP OR MPA DID NOT REDUCE PAIN-LIKE BEHAVIOR IN THE SNL MODEL

We conducted additional experiments to examine the effects of systemic and perineural glucocorticoids. There was no analgesic effect of systemic MP (two-way rm-ANOVA; time $p = 0.77$, drug $p = 0.73$, interaction $p = 0.08$) or perineural MP or MPA (two-way rm-ANOVA; time $p < 0.0001$, drug $p = 0.74$, interaction $p = 0.69$) compared to saline controls in the SNL model (Figure 5A+B).

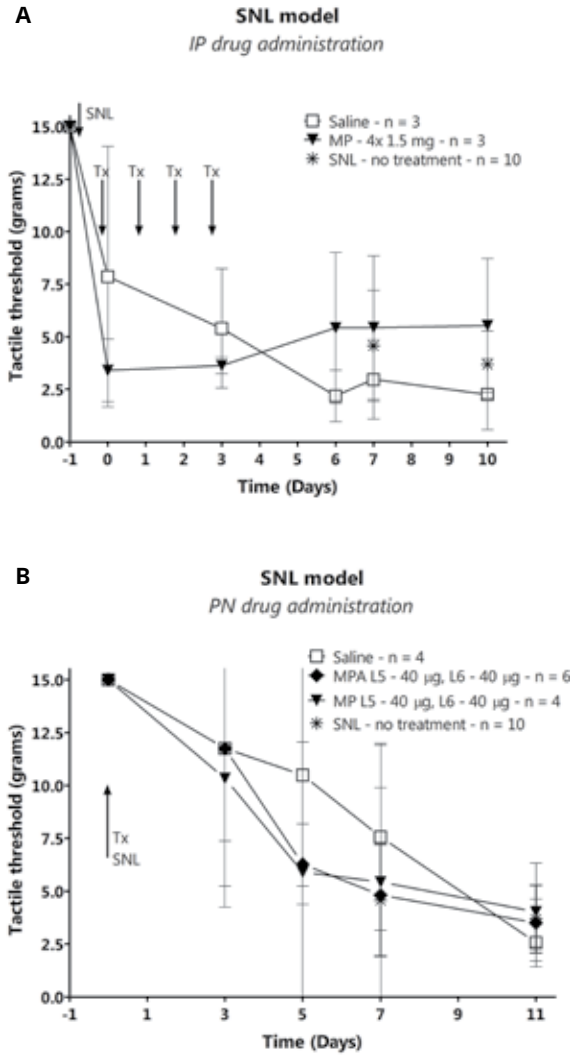


Figure 5 Effect of A) intraperitoneal (IP) methylprednisolone sodium succinate (MP) treatment and B) perineural (PN) MP and methylprednisolone acetate (MPA) treatment on pain-like behavior in the spinal nerve ligation (SNL) model. Tactile thresholds in grams (y-axis) over time in days (x-axis). 'SNL' indicates the moment where SNL was performed. Tx indicates drug treatment. White square = saline controls, black triangles = MP, black diamonds = MPA, black star = rats with SNL without drug treatment. Data are plotted as mean, error bars are the standard deviation of the mean.

THERE WAS NO EFFECT ON DRG ATF3 EXPRESSION AFTER INTRATHECAL MP OR MPA TREATMENT IN THE FORMALIN OR SNL MODEL

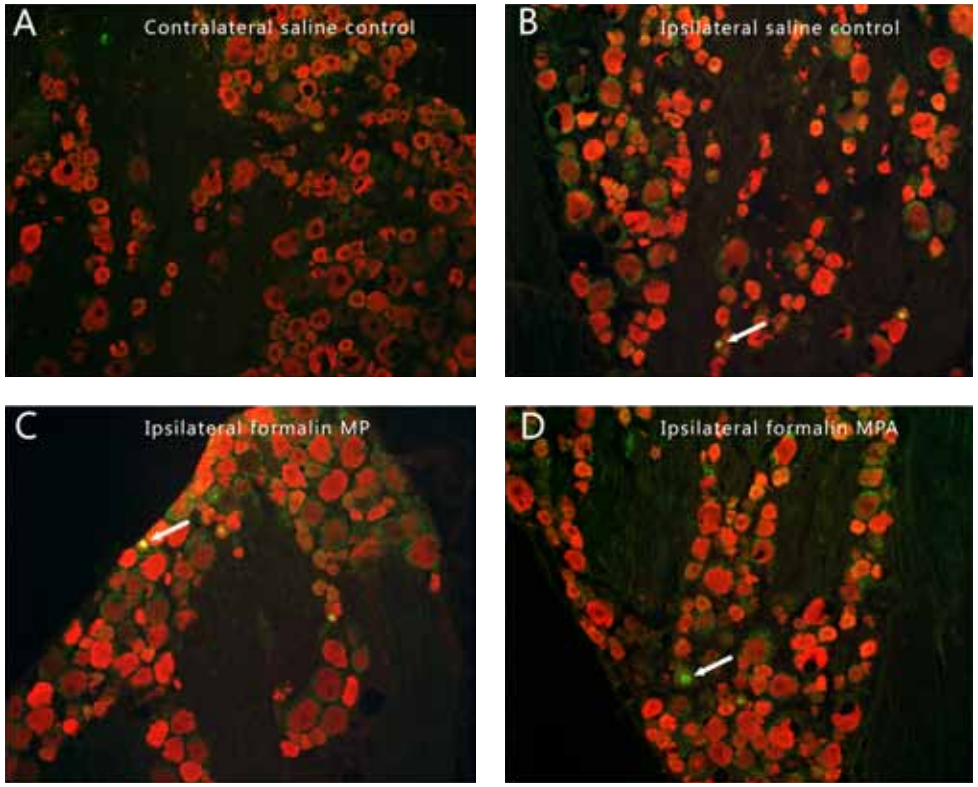
Intraplantar formalin: After intraplantar formalin injection, a few ATF3 containing neurons were observed in the ipsilateral DRG in all three groups (percentage of ATF3 stained neurons: intrathecal saline group 2.1%, MP 1.7%, and MPA 1.5%). No ATF3 containing neurons were observed in the contralateral DRG in all three groups. The incidence of ATF3 containing neurons was too small to make a statistically meaningful comparison between groups (Figure 6).

SNL mononeuropathy: After SNL the percentage of ATF3 (+) neuronal nucleï was significantly higher in the ipsilateral DRGs compared to the contralateral DRGs in all groups (saline ipsi mean 61% vs contra 0.04%, MP ipsi 58% vs contra 0.1%, MPA ipsi 62% vs contra 0%; two-way rm-ANOVA; side $p = 0.0002$, drug $p = 0.97$, interaction $p = 0.96$). In the contralateral DRG, ATF3 (+) neuronal nucleï were observed in saline and MP treated animals, but not in intrathecal MPA treated animals. There were no differences in ATF3 (+) containing neuronal nucleï in the ipsilateral DRGs between groups ($p = 0.97$; Figure 7).

INTRATHECAL MPA TREATMENT REDUCED MICROGLIAL ACTIVATION IN THE SNL MODEL, BUT THERE WAS NO EFFECT OF INTRATHECAL MP OR MPA TREATMENT ON ASTROCYTE ACTIVATION

Intraplantar formalin: Seven days after intraplantar formalin injection, GFAP and Iba1 immuno reactivity in the spinal dorsal horn did not increase on the ipsilateral side as compared to the contralateral side in either saline treated animals or in glucocorticoid treated animals.

SNL mononeuropathy: After SNL, GFAP protein levels in the spinal dorsal horn were significantly elevated on the ipsilateral side compared to the contralateral side as measured at 25 days after injury (saline mean ipsi 11.4 vs contra 9.0, MP ipsi 11.7 vs contra 10.2, MPA ipsi 12.1 vs contra 8.6; two-way rm-ANOVA; side $p = 0.009$, drug $p = 0.88$, interaction $p = 0.47$), but there were no differences between groups (Figure 8). Iba1 protein levels were also significantly increased on the ipsilateral side (saline mean ipsi 3.8 vs contra 1.6, MP ipsi 1.8 vs contra 0.7, MPA ipsi 1.3 vs contra 0.6; two-way rm-ANOVA; side $p = 0.02$, drug $p = 0.13$, interaction $p = 0.28$) and Iba1 protein levels were significantly lower on the ipsilateral side after intrathecal MPA treatment compared to saline controls (Bonferroni $p < 0.05$; Figure 8).



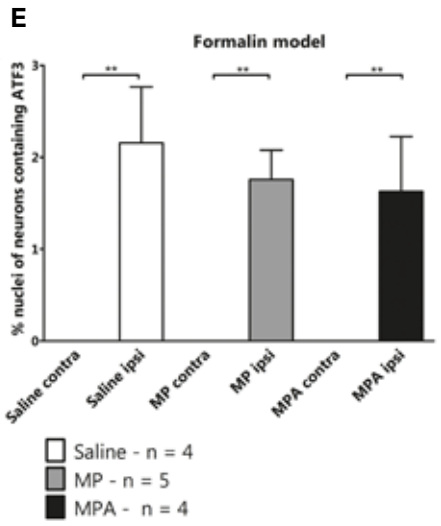
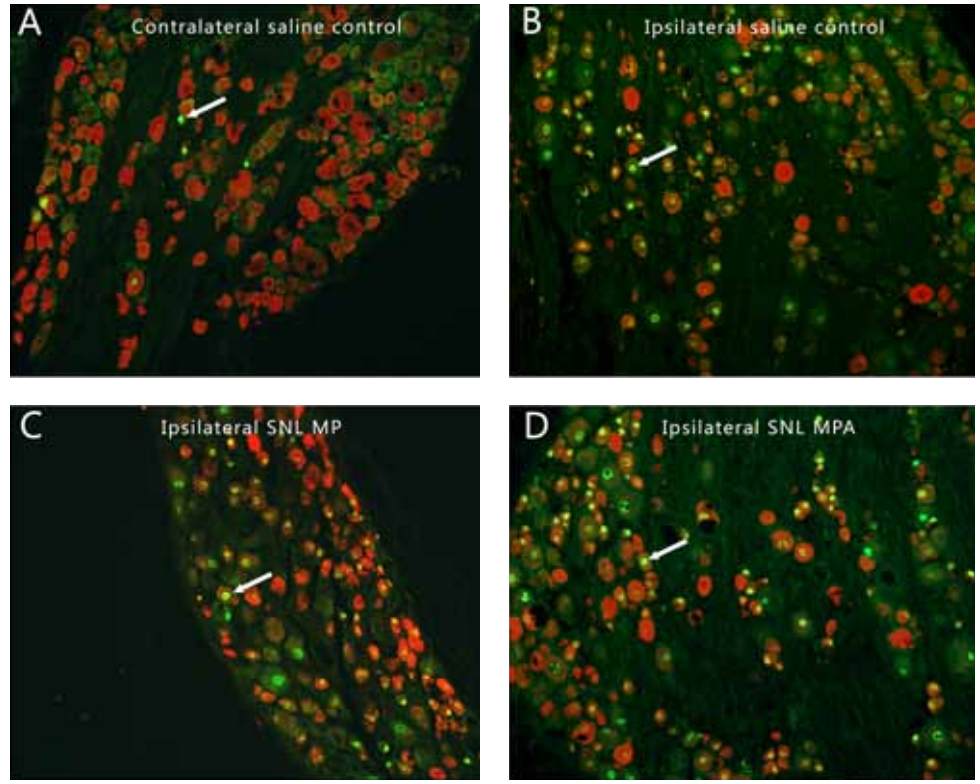


Figure 6 Effect of intrathecal methylprednisolone sodium succinate (MP) and methylprednisolone acetate (MPA) on neuronal injury (ATF3) in dorsal root ganglia (DRG) in the formalin model. A) Contralateral DRG of saline control rat. B) Ipsilateral DRG of saline control rat. C) Ipsilateral DRG of intrathecal MP treated rat. D) Ipsilateral DRG of intrathecal MPA treated rat. E) Percentage of neuronal nuclei containing ATF3 (y-axis) for saline (white), MP (grey) and MPA (black) treated animals in the formalin model, contra = contralateral DRG, ipsi = ipsilateral DRG. Data are plotted as mean, error bars are the standard deviation of the mean, ** 0.001 < p < 0.01.



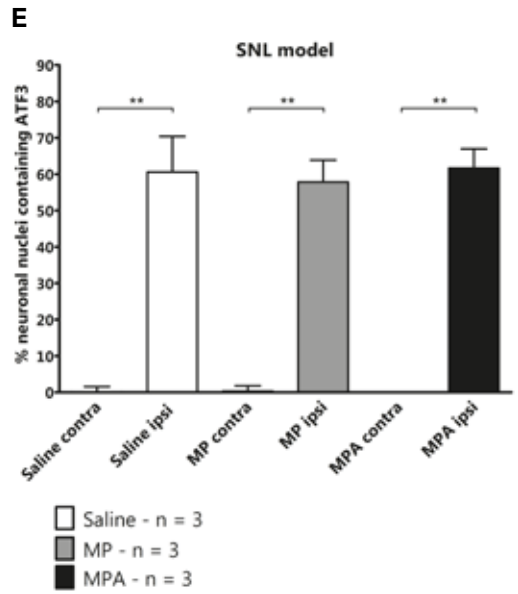


Figure 7 Effect of intrathecal methylprednisolone sodium succinate (MP) and methylprednisolone acetate (MPA) on neuronal injury (ATF3) in dorsal root ganglia (DRG) in the spinal nerve ligation (SNL) model. A) Contralateral DRG of saline control rat. B) Ipsilateral DRG of saline control rat. C) Ipsilateral DRG of intrathecal MP treated rat. D) Ipsilateral DRG of intrathecal MPA treated rat. E) Percentage of neuronal nuclei containing ATF3 (y-axis) for saline (white), MP (grey) and MPA (black) treated animals in the SNL model, contra = contralateral DRG, ipsi = ipsilateral DRG. Data are plotted as mean, error bars are the standard deviation of the mean, ** 0.001 < p < 0.01.

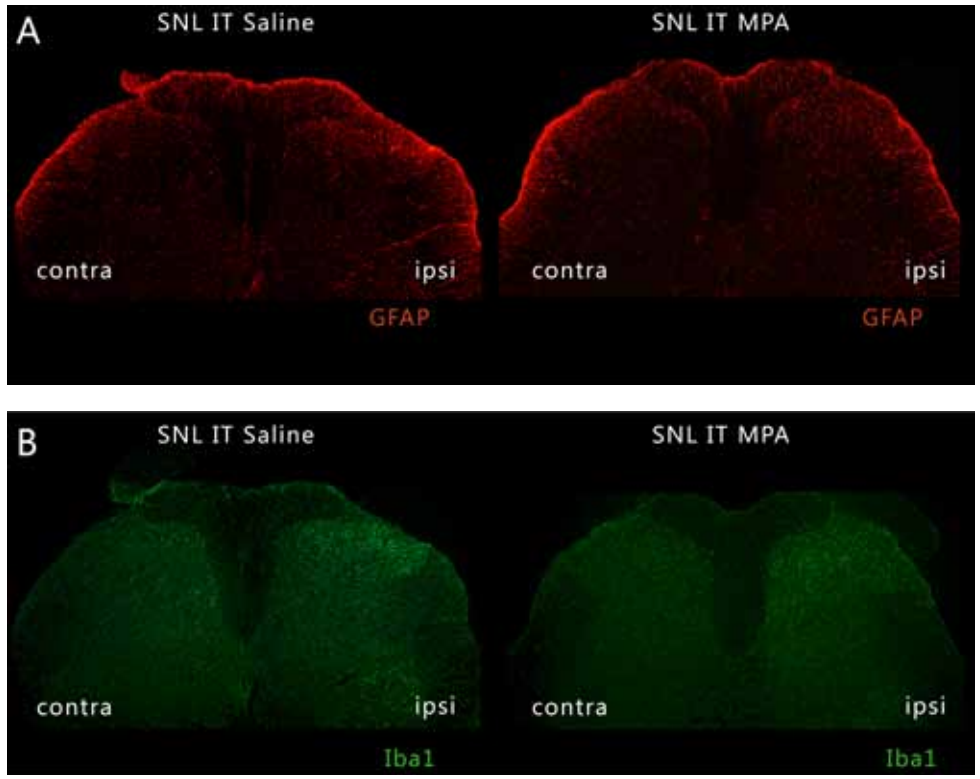
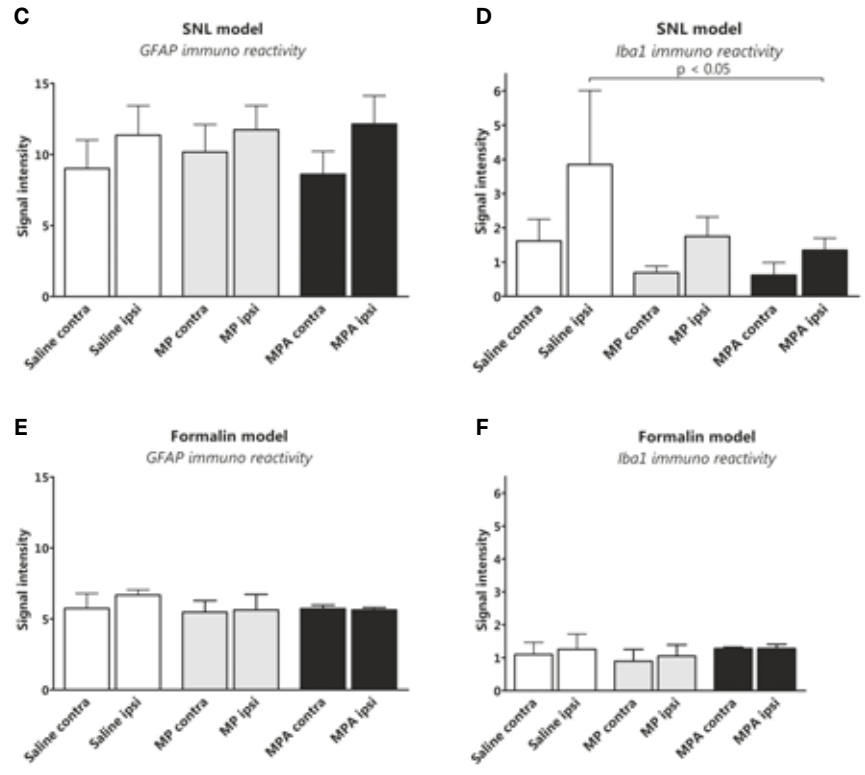


Figure 8 Effect of intrathecal methylprednisolone sodium succinate (MP) and methylprednisolone acetate (MPA) on glial cell activation (GFAP, Iba1) in the spinal nerve ligation (SNL) and formalin model. A) Represents the immuno reactivity of GFAP (red; astrocyte activation) in the spinal dorsal horn (SDH) of saline controls (left) and intrathecal MPA (right) treated animals in the SNL model, contra = contralateral side, ipsi = ipsilateral side. There is no difference between the contralateral and ipsilateral side and between the two treatment groups shown in C. C) Shows the quantitative signal intensity of GFAP immuno reactivity (y-axis) for saline (white), MP (grey) and MPA (black) treated animals. B) Represents the immuno reactivity of Iba1 (green; microglial activation) in the SDH of saline controls (left) and intrathecal MPA (right) treated animals in the SNL model. There is an increase in signal intensity on the ipsilateral side and a decrease in signal intensity comparing MPA treated animals with saline controls. D) Shows the quantitative signal intensity of Iba1 immuno reactivity (y-axis) for saline (white), MP (grey) and MPA (black) treated animals. E + F) Signal intensity of GFAP (E) and Iba1 (F) in the formalin model for saline (white), MP (grey) and MPA (black) treated animals. No difference between the contra and ipsilateral side and between the treatment groups are observed. Data are plotted as mean, error bars are the standard deviation of the mean.



DISCUSSION

OVERVIEW OF RESULTS

We observed severe generalized allodynia when high intrathecal doses of either MP or MPA were injected. At behaviorally tolerated doses, we did not observe analgesic or antihyperalgesic effects in any of the three models; carrageenan, formalin or SNL. There was a reduction in allodynia 7-days after formalin injection in intrathecal MP and MPA treated rats. No reduction was noted in indices of neuronal injury in DRG or astrocyte activation in the spinal dorsal horn with intrathecal MP or MPA. There was a decrease in microglial activation in spinal dorsal horn in intrathecal MPA treated rats in the SNL model. The importance of these observations will be considered below.

GENERALIZED ALLODYNIA

The severe generalized allodynia that was observed in the present study after intrathecal injections of glucocorticoids has been described only once before, after intrathecal bolus injections of doses of 400 µg MP in rats.[25] In addition, increased pain sensitivity in the paws has been reported after continuous intrathecal infusion of 0.02 mg/kg/day dexamethasone for 7 days in rats.[26] In the present study, rats tolerated a substantially higher dose of MPA (400 µg) as compared to MP (40 µg). MPA is hydrolyzed by cholinesterases into active methylprednisolone to become soluble and clinically active, giving the formulation its depot characteristics.[27] MP, methylprednisolone sodium succinate, is soluble and thereby biologically active when injected. The free fraction of methylprednisolone in the MP formulation is higher as compared to the MPA formulation. This explains why a lower dose of MP is tolerated intrathecally, as compared to the MPA dose. Why severe allodynia is observed at higher doses is unknown. As considered above, this hyperpathia is unlikely to be caused by pH, osmolarity, preservatives or particles in the suspension. It might be an effect of the glucocorticoid itself. Safety studies in dogs showed that intrathecal glucocorticoids cause neuro-inflammation when given at high doses[28-31] even with minimal concentrations of preservatives present.[24]

INFLAMMATORY MODEL AND GLUCOCORTICOID ACTION

In the carrageenan model there was no difference in the development of mechanical allodynia between intrathecal MPA treated rats and saline controls in the first eight hours after carrageenan injection. No studies are available examining the efficacy of intrathecal glucocorticoids in this model. The effect of systemic glucocorticoid administration has been described, showing a complete prevention of the development of hyperalgesia 3 hours after carrageenan injection.[32] Since the plasma levels of methylprednisolone were low after intrathecal MPA administration (18 ng/ml), this might have prevented a strong peripheral effect. However, while methylprednisolone plasma levels were low, MPA treated rats did have less development of paw edema as compared to saline controls confirming a drug action. Similar results were observed in a sciatic transection model where chronic high dose MP did not affect nociceptive thresholds but did prevent the development of neuropathic edema.[33] Reduced peripheral edema after a very low intrathecal dose of MPA in the carrageenan model could be explained by the fact that only low doses are necessary for edema reduction or that the reduction of peripheral edema can be mediated neuraxially. There are examples of neuraxial administered drugs reducing peripheral edema such as morphine and MAP kinase inhibitors.[34;35]

FORMALIN MODEL AND GLUCOCORTICOID ACTION

In agreement with previous work, there was no effect in flinching behavior during the first phase after formalin between intrathecal MPA and MP versus saline controls.[25;36;37] Also, phase 2 flinching behavior in intrathecal MPA and MP treated animals was not reduced as compared to saline controls. In other studies, intrathecal glucocorticoids; dexamethasone[36] and repeated triamcinolone 250 µg[25], but also systemic glucocorticoids; single dose dexamethasone 2.5 to 6.25 mg/kg[37] and 30 mg/kg[38] have been reported to reduce phase 2 flinching. There is also data showing modest or no reduction in flinching behavior after a single intrathecal dose of 400 µg MP[25] or twice intraperitoneal dosing of 250 µg/kg dexamethasone.[39] The single doses of intrathecal MP and MPA as used in our study, were the maximum tolerable intrathecal doses, but low as compared to the intraperitoneal or repeated intrathecal doses in studies showing analgesic effects. The late reduction in mechanical allodynia we observed in intrathecal MPA/MP treated rats compared to saline, has not been hitherto reported before. A possible explanation for the later analgesic onset of the treatment with MP is that a majority of the therapeutic effects of glucocorticoids are mediated through transactivation and repression of genes which takes 24 to 48 hours to occur.[10]

NERVE INJURY MODEL AND GLUCOCORTICOID ACTION

Of the six reports on intrathecal administration of glucocorticoids in neuropathic pain models (SNL, chronic constriction injury (CCI) or spared nerve injury (SNI)) in rats, three reports mention a reduction, two an increase of thermal hyperalgesia and/or mechanical allodynia, and one no effect.[26;40-44] Analgesic effects were observed with different glucocorticoid formulations (prednisolone, methylprednisolone, or dexamethasone) either bolus or continuous infusion, all started on the day of nerve injury or within three days after nerve injury.[40-42] After local (perineural)[43;45;46], epidural[47], or systemic[48-50] administration of glucocorticoids in neuropathic pain models (SNL, CCI, or SNI) in rats, most report a reduction of nociceptive behavior. However in three out of seven studies the analgesic effect was either very short lived (only at one time point) and/or less than 25% reduction of pain-like behavior.[43;47;50] Further, others have reported no effect[33;51] or that glucocorticoids have pain enhancing capabilities.[26;44;52] In conclusion, regarding the large body of data on glucocorticoid treatment for neuronal injury collected over more than 60 years and the contradicting results published, one may say that if there is any analgesic effect of glucocorticoids after nerve injury it is small and short lived. In our study with well documented preclinical models, no effect on pain behavior was observed after any route of administration.

EFFECT OF GLUCOCORTICOIDS ON SURROGATE MARKERS

The origin of afferent traffic associated with neuropathic pain after SNL reflects ectopic activity arising from the injury site and from the DRG of the injured axon. In the face of spinal nerve injury, local inflammatory products arising from the nerve injury site, activate DRG cell populations and induce activity of ATF3. ATF3, rapidly upregulated in all injured DRG neurons after peripheral nerve injury confirmed in our study, has a survival function driving neurite outgrowth.[18] Glucocorticoid treatment did not affect the ATF3 measured in DRG neurons as compared to saline control. This may be explained as the dose of MP reaching the DRG was too low. The peak plasma level of MP (18 ng/ml) and an unknown amount of local diffusion after intrathecal MPA administration are probably insufficient to prevent neuronal damage occurring in the DRG.

The inflammatory cascade activated after nerve injury may activate DRG cell populations such as satellite cells and pericellular glia, resulting in a further increase of local expression of pro-inflammatory substances such as cytokines, chemokines, and growth factors. Although injury is limited to the primary afferent and/or DRG, these interventions can lead to persistent changes in the spinal dorsal horn, including glial activation. Activation of spinal glia after peripheral nerve injury occurs in the first week after injury. Microglia are activated by day 3 with a maximum on day 7 and remain activated for at least three weeks.[26] Astrocytes follow a similar timeframe. It has been reported that glucocorticoids attenuate glial activation. The activation of astrocytes was reduced in the SNL and SNI model.[42;43;46] Microglia activation was attenuated after perineural treatment with 200 µg triamcinolone acetonide in the SNL model[46] but not after daily intraperitoneal injections of 2.5 mg/kg dexamethasone in the SNI model.[26] In the present study only microglial activation was attenuated after intrathecal MPA treatment in the SNL model.

STUDY STRENGTH AND WEAKNESSES

Combining all data, the number of rats without an analgesic effect after intrathecal glucocorticoid injection is substantial. We chose to pretreat rats one hour before carrageenan and formalin injection. This was based on the pharmacokinetic data of intrathecal MPA showing peak plasma levels from 1 to 3 hours after administration. The peak plasma levels would be present during the initial part of the pain stimulus/model. However, we are aware of the fact that glucocorticoids also have later onset therapeutic effects through transactivation and repression of genes, which can take over 24 hours.[12] This might explain why we did not observe any analgesic affect after glucocorticoid administration but only a later effect on mechanical allodynia 7 days after formalin injection. In the SNL model, we sacrificed rats receiving intraperitoneal and perineural glucocorticoids 11 days after nerve ligation. It is known that at this

time point glial cell activation is around its peak. However, since the glucocorticoid treatment was earlier we might have missed a treatment effect on glial cell activation.

CONCLUSION

In this study severe generalized allodynia was observed after high doses of MP and MPA in naive rats. No acute analgesic effects were observed with intrathecal MP and MPA in any of the three established rodent pain models; carrageenan, formalin or SNL model. Only late antiallodynic effects were observed in the formalin model 7 days after formalin injection. Our results do not support clinical use of intrathecal glucocorticoids in the treatment of pain.

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CHAPTER 7

Effect of glucocorticoids on the central glucocorticoid receptor in a rat nerve ligation model

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Submitted

ABSTRACT

Aims – Despite widespread use, the efficacy of neuraxial glucocorticoids for neuropathic pain is subject to debate. Since most glucocorticoid actions are mediated through its receptor, we explored the effects of intrathecal methylprednisolone acetate (MPA) on total glucocorticoid receptor (tGR) levels and activation of the glucocorticoid receptor (phosphorylated state = pGR) within the spinal dorsal horn (SDH) and dorsal root ganglion (DRG) in a spinal nerve ligation (SNL) model in rats.

Methods – Rats received unilateral ligation of the L5/L6 spinal nerves and were treated with two intrathecal doses of either 400 µg MPA or 0.9% saline with a 72-hour interval. Plantar tactile thresholds were measured over time. Seven days after drug treatment, DRG and SDH were harvested to assess tGR and pGR levels using immunohistochemistry and qPCR.

Results – Allodynia, defined by lowered tactile withdrawal thresholds after SNL, was unaltered by intrathecal MPA. In saline controls, mRNA levels

of tGR did not change after SNL in the DRGs or SDH. tGR and pGR protein levels in the SDH however, significantly increased on the ipsilateral side of SNL compared to the contralateral side and to naïve tissue. When treating rats with MPA, tGR mRNA levels were significantly reduced in the SDH compared to saline controls. tGR and pGR protein levels, however were not significantly lower compared to saline controls.

Conclusion – In intrathecal MPA treated rats, tGR mRNA levels decreased after SNL. However this did not result in lower tGR and pGR protein levels compared to saline controls, and did not decrease ligation-induced mechanical hypersensitivity.

INTRODUCTION

As glucocorticoids act upon a variety of crucial targets in pain pathways[1], they should be potent long acting analgesic agents. However, despite widespread use of neuraxial glucocorticoids in pain medicine, their efficacy is subject to debate. There is consensus only on a short lasting analgesic effect in low back pain patients[2], but not in sustained pain states or for use in neuropathic pain syndromes. Preclinical and clinical results, in fact show varying effects of glucocorticoids from analgesic to hyperalgesic effects.[3-10]

On a cellular level, glucocorticoids mediate their actions primarily by binding to the glucocorticoid receptor (GR). The GR also known as NR3C1, is a ligand-driven transcription factor. Upon binding with a glucocorticoid, GR phosphorylates into an active form (pGR) and translocates to the nucleus where it affects expression of specific sets of genes by transcriptionally activating or repressing them.[11] In addition, glucocorticoids may evoke fast non-genomic neuronal responses by binding to membrane-bound or cytosolic GR or by effects not mediated by a receptor.[11]

It is unclear exactly how glucocorticoids would act to regulate or modify pain signaling. After nerve injury, plasma cortisol levels and GR expression in the spinal cord are increased, indicating an elevated glucocorticoid activity.[3;10;12] Exogenous glucocorticoids may influence the endogenous increased plasma cortisol and GR expression in the spinal cord in several ways. They may increase GR binding and activity and stimulate its downstream actions, and down regulate endogenous cortisol levels and GR expression via a negative feedback mechanism. It is not known what the net effect of exogenous glucocorticoids on spinal GR levels in an acute pain state is. Therefore we examined if an intrathecal administered glucocorticoid, methylprednisolone acetate (MPA), alters a) pain-like behavior and b) total (tGR) and activated (pGR) glucocorticoid receptor levels within the spinal dorsal horn (SDH) and dorsal root ganglion (DRG) in a spinal nerve ligation (SNL) model in rats.

MATERIALS AND METHODS

The protocol of the present study has been approved by the AAALAC accredited (International, Association for Assessment and Accreditation of Laboratory Animal Care) Institutional Animal Care and Use Committee (IACUC) of the University of California, San Diego, USA.

ANIMALS

Male Harlan Sprague-Dawley rats, 80-100 gram (Indianapolis, IN, USA), were maintained 2 per cage in standard cages at room temperature on a 12:12 h light/dark cycle with free access to food and water. After arrival at the housing facility, they were allowed at least 2-3 days of acclimation before use. Experiments have been carried out during light cycle.

SPINAL NERVE LIGATION (SNL) MODEL

Spinal nerve injury was induced by the procedure described by Kim and Chung.[13] Briefly, the left L5 and L6 lumbar spinal nerves were exposed in isoflurane 2.4% / oxygen-anesthetized rats and tightly ligated with 6.0 silk suture at a point distal to their DRGs and proximal to their conjunction to form the sciatic nerve. Rats were given post-operative subcutaneous fluids including analgesics (lactated Ringers + 5 mg/kg Carprofen) and then housed 2 per cage for post-operative recovery. Withdrawal thresholds were obtained at 0, 1, 3, 7, 18, 21 and 25 days after SNL for all rats.

BEHAVIORAL MEASUREMENTS

All behavioral measurements were made by observer (MR) blinded to the treatment groups and were conducted at fixed times (9:00 a.m. to 5:00 p.m.). The thresholds for mechanical allodynia were measured with a series of calibrated von Frey filaments (Stoelting, Wood Dale, IL, USA), ranging from 3.16 to 5.18 (0.41-15.0 g). The animals were acclimated for 45 min in the test chamber with mesh floors and von Frey filaments were applied perpendicularly against the plantar surface of the paw. The 'up-down' method of Dixon as described by Chaplan[14] was used to determine the value at which paw withdrawal occurred 50% of the time, interpreted as the mechanical threshold.

INTRATHECAL CATHETER IMPLANTATION AND DRUG ADMINISTRATION

On postoperative day 13 after SNL, when all rats were weighing more than 200 grams, intrathecal catheters were implanted for drug injections. Rats were surgically implanted with intrathecal catheters under isoflurane 2.4% / oxygen inhalation anesthesia as described previously.[15] The intrathecal catheters were externalized for injection. Rats were given post-operative subcutaneous fluids including analgesics (lactated Ringers + 5 mg/kg Carprofen) and then housed individually for post-operative recovery. Following implantation, catheters were flushed with saline and rats were monitored daily for viability, allowing 5 days of recovery before testing. Animals showing any evidence of motor dysfunction or distress after catheter placement were immediately euthanized using a carbon dioxide chamber.

On postoperative day 18 after SNL, rats were randomized to either the methylprednisolone acetate (MPA) group or the saline control group. Before administration, the presence of preservatives in the MPA preparation (depo-medrol® from Pfizer) was minimized as described in more detail before.[16] The MPA group received 400 µg (10 µl) of the suspension followed by 10 microliter 0.9% saline flush through the intrathecal catheter. In the saline group a total of 20 microliters of 0.9% saline was injected. Intrathecal injections were given twice with a 3-day interval, on postoperative day 18 and 21.

TISSUE COLLECTION

On postoperative day 25 after SNL, seven days after the start of intrathecal drug treatment, spinal cord and dorsal root ganglia were collected from all rats and processed for either immunohistochemistry or quantitative real time PCR (qPCR). For immunohistochemistry, tissues were collected from rats subjected to a) SNL only, b) SNL + intrathecal saline, and c) SNL + intrathecal MPA (n=3 rats/ group). Three additional control groups were added; a) naive rats (n=4), b) naive rats + intrathecal saline (n=3), and c) naive rats + intrathecal MPA (n=3). Naive rats that received intrathecal drug treatment followed the same drug dosing protocol as the SNL rats. For qPCR analysis, SNL + intrathecal saline (n=5), SNL + intrathecal MPA (n=6) and naive rats (n=6) were included.

IMMUNOHISTOCHEMISTRY

Animals were anesthetized with isoflurane 4.0% in a room air/oxygen mixture and transcardially perfused with saline 1 ml/gram bodyweight followed by freshly prepared 4% paraformaldehyde in 0.1 M phosphate buffered saline 1 ml/gram bodyweight. Spinal cord and DRGs of L5 and L6 roots were isolated, post-fixed overnight in the same fixative and moved to 30% sucrose for at least 72 hours. Free floating transverse sections (30 µm) were taken from the spinal cord using a microtome. DRGs were embedded in Tissue-Tek® (O.C.T. Compound, Sakura® Finetek, PA, USA) frozen and cut (10 µm) on a Leica CM1800 Cryostat (IMEB, CA, USA) and directly mounted on glass slides. Both free floating spinal sections and mounted DRG sections were permeabilized with 0.3% Triton X-100 (Sigma, St. Louis, MO, USA), blocked with 5% goat serum in phosphate buffered saline and incubated with antibodies raised against the glucocorticoid receptor (3D5) in every form (tGR = total GR) (primary antibody made in mouse, 1:1500, cat. no. sc-56851, Santa Cruz Biotechnology INC, CA, USA) and its phosphorylated state pGR (Ser211) (made in rabbit, 1:1500, cat. no. #4161, Cell Signaling Technology INC, MA, USA) for 48 hours at 4 °C. Binding sites were visualized with secondary antibodies conjugated with fluoro-Alexa-594 (goat anti-mouse, 1:1000, cat. no. A11032, Invitrogen, NY, USA) and streptavidin conjugated fluoro-Alexa-488 (goat anti-rabbit, 1:1000, cat

no. S-32354, Life Technologies, CA, USA). A streptavidin/ biotin blocking kit (cat. No. SP-2002, Vector Labs, CA, USA) was utilized as appropriate before biotinylated pGR. tGR and pGR antibodies were incubated simultaneously and with markers for astrocytes (GFAP) (made in mouse, 1:4000, cat. no. #MAB360, Chemicon, USA), microglia (Iba1) (made in rabbit, 1:2000, cat. no. #019-19741, WAKO, VA, USA) satellite cells (Vimentin) (made in mouse, 1:100, cat. no. 180052, Invitrogen, NY, USA), and neurons (NeuN) (made in mouse, 1:1000, cat. no. MAB377, EMD Millipore Corporation, MA, USA) to examine cellular localization. The tGR and pGR location within the diffent celltypes was also noted since the activated pGR is mainly found within the nucleus of cells. Images were captured using a Nikon TE300 fluorescence microscope (Nikon Corp, Tokyo, Japan) and overlay performed with Adobe Photoshop Creative suite (CS6; Adobe Systems Incorporated) or confocal microscopy. The investigator (MR) was blinded for the experimental conditions during quantification of GR and pGR staining. Quantification of tGR in the spinal cord was performed by measuring the total integrated signal intensity of pixels in lamina I-II of the SDH after substraction of the background signal intensity in this area using ImageJ 1.47 software. The total signal intensity was measured in at least 6 sections of the L5 to L6 area of the ipsi- and contralateral SDH (total of 12 sections per animal). Per section, 3 background signal intensity measurements were performed in the lamina I-II area and pooled to a mean for substraction. An increase in the signal intensity for tGR was interpreted as receptor upregulation. In addition, the number of pGR positive cells in lamina I and II were manually counted in at least 6 sections of the L5 to L6 area in the ipsi- and contralateral SDH (total of 12 sections per animal) and the results expressed as the mean count per side per group.

QUANTITATIVE REAL-TIME PCR (QPCR)

For qPCR measurements, rats were anesthetized with isoflurane 4.0% in a room air/oxygen mixture and spinal cords harvested from the vertebral column by hydro-extrusion using a saline-filled syringe after decapitation. The lumbar spinal cord (L3-L6) was divided into four parts; the ipsilateral ventral and dorsal parts and the contralateral ventral and dorsal parts. The ipsi and contralateral L5 and L6 DRGs were also immediately harvested at necropsy. Both spinal and DRG tissues were rapidly frozen on dry ice after dissection and kept at –70 °C until analysis. For analysis, samples were homogenized using pestle and mortle techniques in TRIzol and RNA extracted according to the manufacturer's protocol (Invitrogen, Carlsbad, CA, USA). All samples exhibited 260/280 absorbance ratios of approximately 2.0. Complementary DNA was prepared using MultiScribe reverse transcriptase (Applied Biosystems, CA, USA) according to the manufacturer's protocol. To determine RNA levels of tGR a SYBR® Green-Based Gene Expression Analysis (Applied Biosystems, CA, USA) was used. The primer for tGR costume made (by Eurofins MWG Operon, Ebersberg, Germany) with a design based on a paper by Dubois et al.[17] had the following sequence:

Common forward primer: GCCCTGGGTTGGAGATCATAC
Common Reverse primer: CATGCAGGGTAGAGACATTCTC

Serially diluted cDNA samples synthesized from C6 cell line collected after 24 hours of TNFα stimulation, which expresses tGR, were used as standard curve material. The threshold cycle values was determined and the number of cell equivalents in each sample calculated with the standard curve method.[18] Data was normalized to Hprt1 values.

DATA ANALYSIS

Data are presented as means with 95% confidence intervals. Significance was ascribed for $p < 0.05$. Behavioral time-course data in the SNL model (tactile thresholds) was analyzed using two way ANOVA with repeated measures across time. Differences in signal intensity for immunohistochemistry tGR, pGR cell counts and qPCR mRNA levels on the ipsilateral side between treatment groups were calculated with a t-test. Differences within groups between the ipsilateral and contralateral side were analyzed with a paired t-test. Graphics and statistical analyses were carried out using Prism 6 for Windows.

RESULTS

INTRATHECAL MPA DOES NOT REDUCE PAIN-LIKE BEHAVIOR AFTER SNL

After SNL, reduced tactile thresholds were observed from post-operative day 1, remaining decreased throughout the study. Pain-like behavior was well established on postoperative day 18, when the drug treatment was initiated. Intrathecal administration of MPA did not increase tactile thresholds in the allodynic hindpaw of SNL rats as compared to saline controls in the following 6 days (Figure 1).

INTRATHECAL MPA DECREASED MRNA LEVELS OF THE GR IN THE SDH RATS AFTER SNL

In DRGs, tGR mRNA levels did not significantly change after SNL, comparing levels in naïve rats (mean tGR mRNA 0.014; 95% CI 0.003 to 0.024) to levels in the ipsilateral DRGs in saline controls (mean 0.019; 95% CI 0.011 to 0.027; $p = 0.35$; Figure 2A). When treating SNL rats with intrathecal MPA, tGR mRNA levels in the ipsilateral DRGs did not change (mean 0.023; 95% CI 0.009 to 0.036; $p = 0.58$) compared to saline controls (Figure 2A).

In the SDH, there was no significant increase in the ipsilateral tGR mRNA levels after SNL in intrathecal saline rats (mean 0.24; 95% CI 0.21 to 0.27) compared to naïve tissue (mean 0.25; 95% CI 0.17 to 0.33; p = 0.80; Figure 2B). However, the ipsilateral tGR mRNA levels following intrathecal MPA (mean 0.15; 95% CI 0.11 to 0.20) significantly decreased compared to saline controls (p = 0.003) and naïve tissue (p = 0.017; Figure 2B).

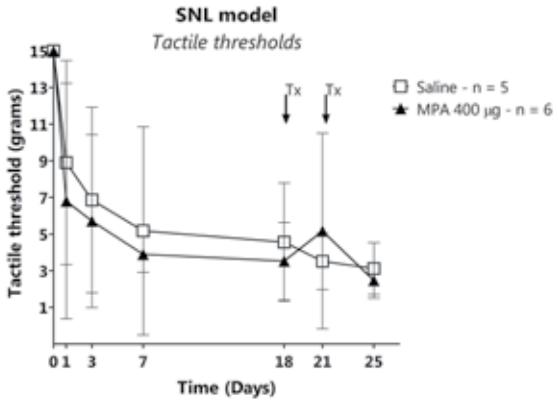


Figure 1 Effect of intrathecal methylprednisolone acetate (MPA) treatment on pain-like behavior in the spinal nerve ligation (SNL) model. Tactile thresholds in grams (y-axis) over time in days (x-axis). Tx with arrow indicates drug treatment on day 18 and on day 21 after SNL. White square = saline controls, black triangle = MPA treatment, n = number of animals, data are plotted as mean, error bars are the standard deviation of the mean.

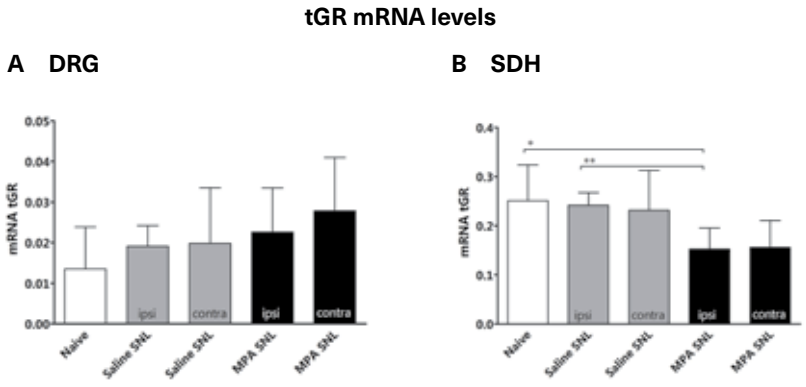


Figure 2 Effect of intrathecal methylprednisolone acetate (MPA) on glucocorticoid receptor mRNA levels (*tGR* mRNA) in the spinal nerve ligation (SNL) model in the A) dorsal root ganglion (DRG) and B) spinal cord dorsal horn (SDH). *tGR* mRNA levels (y-axis) are plotted for the different groups; white = naïve animals (number of animals (n) = 6), grey = intrathecal saline treated animals after SNL (n = 5), black = intrathecal MPA treated animals after SNL (n = 6). Ipsi = ipsilateral side of the nerve ligation, contra = contralateral side of the nerve ligation. Data are plotted as mean, error bars are the standard deviation of the mean. * p-value < 0.05, ** p < 0.01

TGR AND PGR IMMUNOREACTIVE SIGNAL INTENSITY INCREASED AFTER SNL IN BOTH INTRATHECAL SALINE AND MPA TREATED ANIMALS

In the SDH, tGR immunoreactive signal intensity did not significantly increase after SNL on the ipsilateral side in intrathecal saline treated rats (mean 12.8; 95% CI 8.6 to 16.9) compared to naïve tissue (mean 9.9; 95% CI 7.1 to 12.6; p = 0.076). However, using a paired comparison for assessing tGR immunoreactive signal intensity on the ipsilateral versus the contralateral side (mean 10.0; 95% CI 3.7 to 16.2) in intrathecal saline treated rats, a significant increase was observed (p = 0.030), indicating a nerve ligation effect (Figure 3A).

Intrathecal MPA treatment by itself, in naïve rats, did not change tGR signal intensities (mean 11.8; 95% CI 8.5 to 15.1; p = 0.17). After SNL, a significant increase in tGR signal intensities was observed comparing the ipsilateral (mean 11.7; 95% CI 10.6 to 12.8) versus the contralateral (mean 8.2; 95% CI 6.0 to 10.4) sides in intrathecal MPA treated rats (p = 0.040). There were no significant differences in

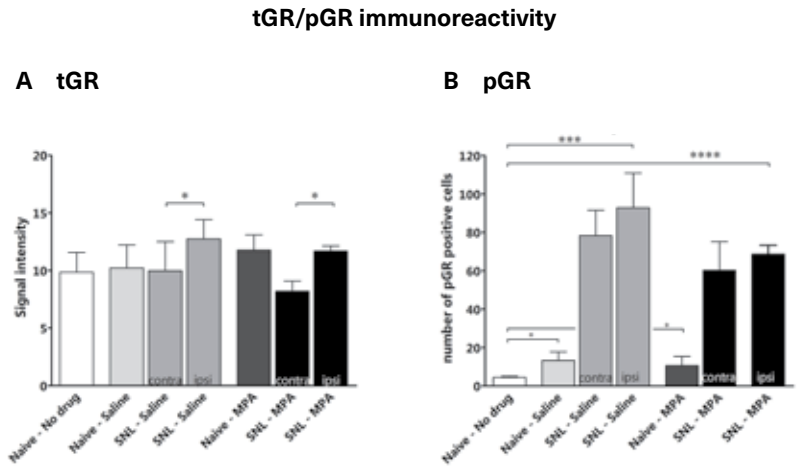


Figure 3 Effect of intrathecal methylprednisolone acetate (MPA) in the spinal nerve ligation (SNL) model on glucocorticoid receptor (GR)immunoreactive signal intensity of A) the total GR (tGR) and B) phosphorylated GR (pGR) in the spinal cord dorsal horn. A) Signal intensity of tGR immunohistochemistry staining (y-axis) and B) number (n) of pGR positive cells are plotted for the different groups; white = naïve animals (Naïve - no drug, n = 4), light grey = intrathecal saline treated animals without SNL (Naïve – Saline, n = 3), darker grey = intrathecal saline treated animals after SNL (SNL – Saline, n = 3), off black = intrathecal MPA treated animals without SNL (Naïve – MPA, n = 3), black = intrathecal MPA treated animals after SNL (SNL – MPA, n = 3). Ipsi = ipsilateral side of the nerve ligation, contra = contralateral side of the nerve ligation. Data are plotted as mean, error bars are the standard deviation of the mean. * p-value < 0.05, ** p < 0.01, *** p < 0.001, **** p < 0.0001.

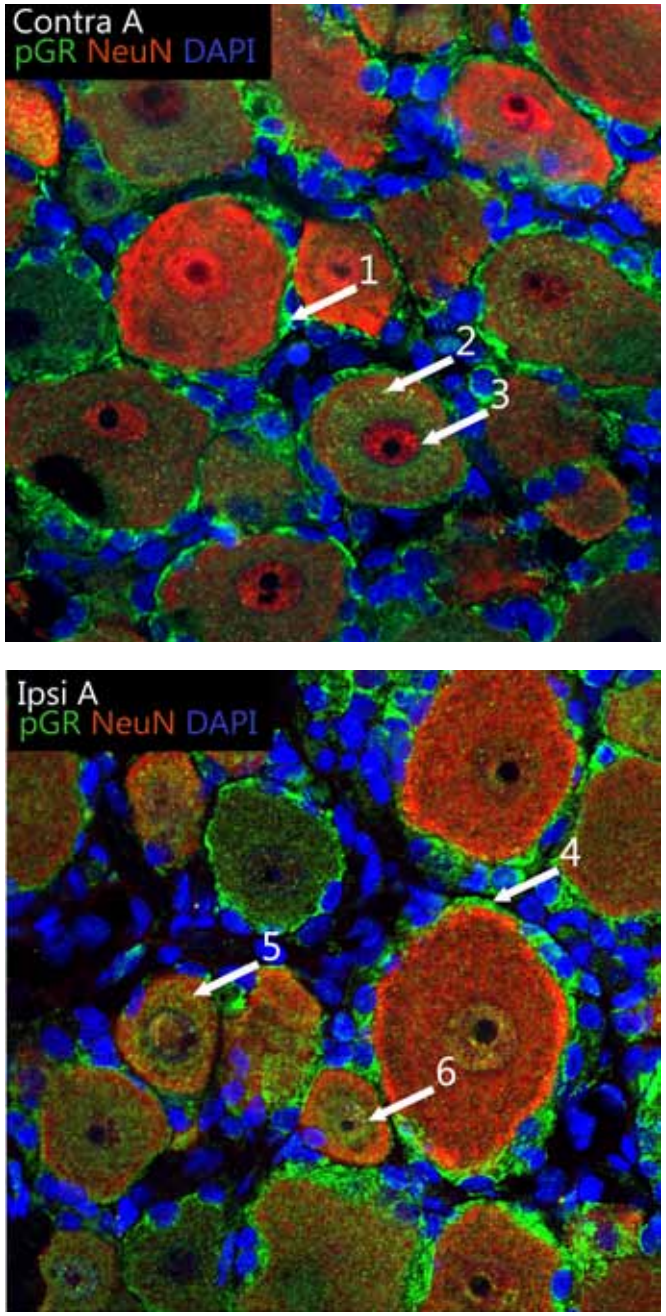
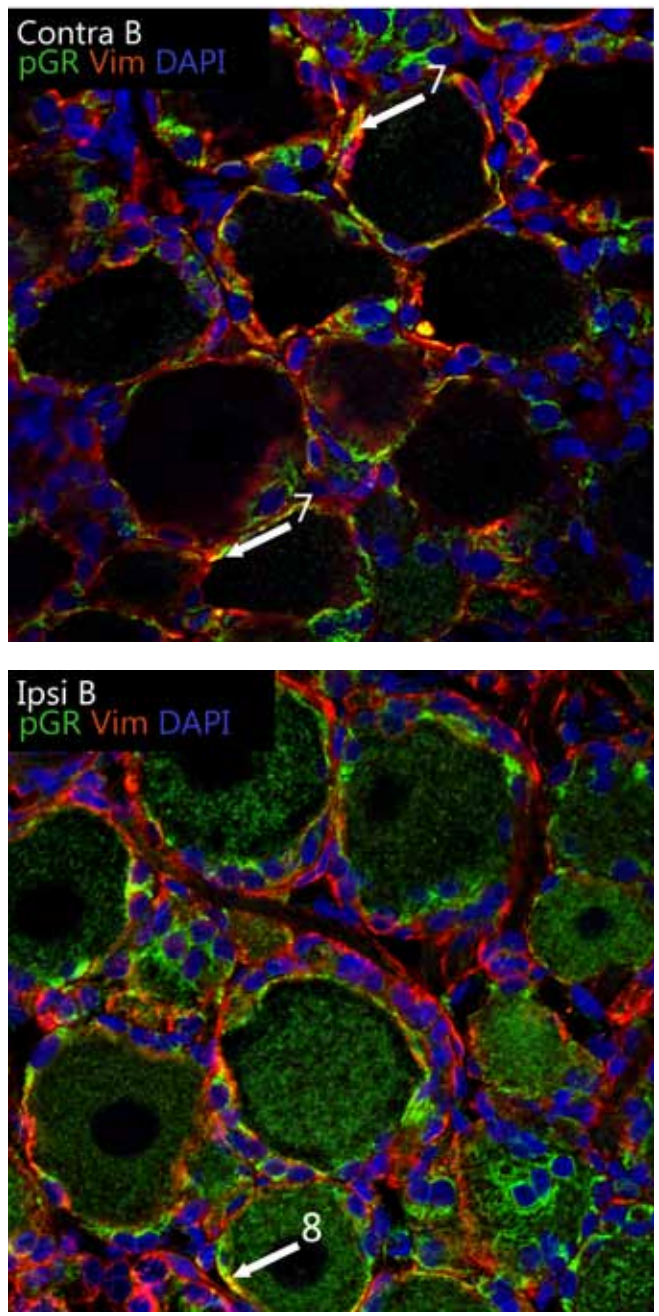


Figure 4 Co-localization of the activated (phosphorylated) glucocorticoid receptor (pGR) in the dorsal root ganglion (DRG) of a spinal nerve ligated saline control rat. pGR (green) co-localizes with A) neurons (NeuN = red) and B) satellite cells (Vimentin (Vim) = red). **Contra A)** Contralateral DRG to the nerve ligation site showing 1. pGR outside the neuron, 2. pGR in the neuronal cytosol and 3. nearly no pGR in neuronal nucleus. **Ipsi A)** Ipsilateral DRG to the nerve ligation site showing



4. pGR outside the neuron similar to the contralateral side, 5. increased pGR staining in the neuronal cytosol compared to contralateral side and 6. pGR located in the neuronal nucleus. **Contra B)** Contralateral DRG to the nerve ligation site showing 7. co-localization of pGR with satellite cells. **Ipsi B)** Ipsilateral DRG in the nerve ligation site showing 9. co-localization of pGR with satellite cells not visibly increased compared to the contralateral side. Nuclei staining with DAPI = blue.

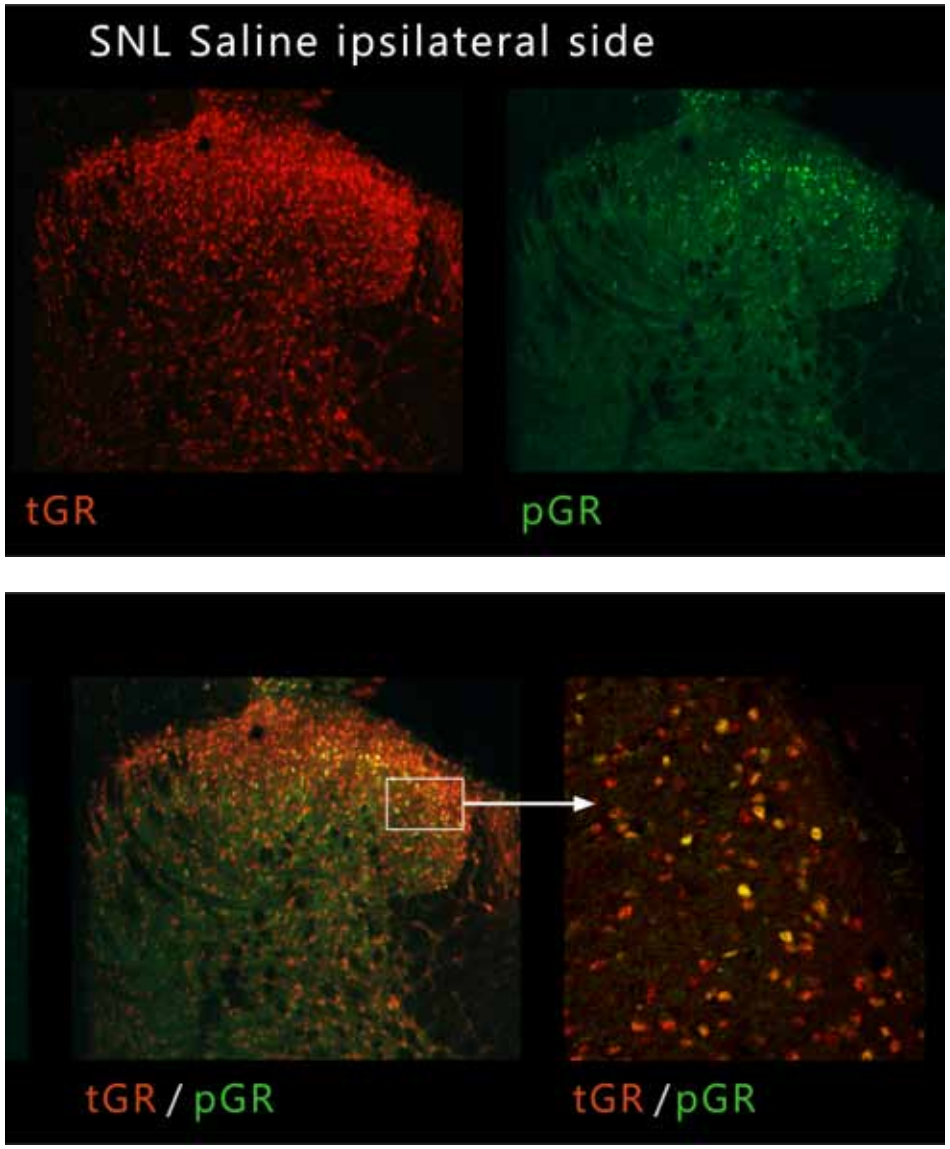


Figure 5 Co-localization of the total glucocorticoid receptor (tGR) with the activated (phosphorylated) glucocorticoid receptor (pGR) on the ipsilateral side of the spinal dorsal horn in a spinal nerve ligated (SNL) intrathecal saline treated rat. tGR = red, pGR = green, co-localization of tGR/pGR = yellow. The picture on the far right is an enlargement of the area surrounded by the white box on the picture on the mid right. All pGR staining is co-localized with tGR staining.

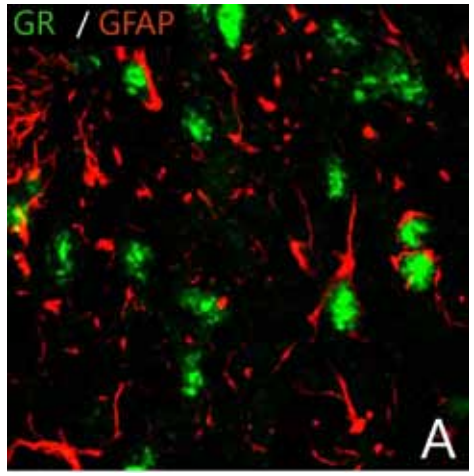
ipsilateral tGR signal intensities after SNL between intrathecal MPA treated rats and saline controls ($p = 0.35$; Figure 3A).

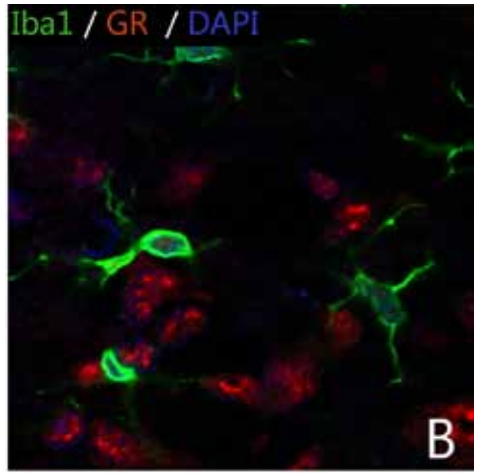
In the SDH, the number of pGR immunopositive cells significantly increased after intrathecal catheter implantation and saline (13.3; 95% CI 2.1 to 24.5; $p = 0.010$) or MPA (mean 10.7; 95% CI -1.1 to 22.4; $p = 0.044$) treatment compared to naïve tissue (mean 4.5; 95% CI 3.6 to 5.4). After SNL, the number of pGR immunopositive cells increased significantly on the ipsilateral side in both intrathecal saline (mean 92.8; 95% CI 47.7 to 138; $p = 0.0002$) and MPA treated animals (mean 68.8; 95% CI 57.4 to 80.1; $p < 0.0001$) compared to naïve tissue. Intrathecal MPA treatment did not change the number of SNL evoked pGR immunopositive cells compared to saline controls ($p = 0.090$; Figure 3B).

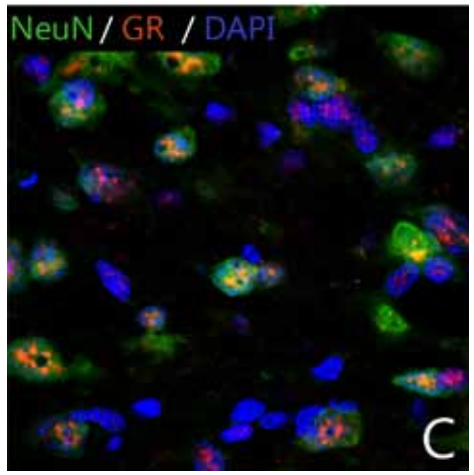
PGR AND GR CO-LOCALIZATION

In DRGs, tGR and pGR co-localized with neurons and satellite cells (Figure 4). In neurons on the contralateral side of the nerve ligation, pGR was observed in the cytosol and barely in the nucleus. On the ipsilateral side, pGR staining in the neuronal nucleus was observed.

In the SDH, tGR and pGR co-localized as expected (Figure 5). tGR predominantly co-localized with the neuronal marker NeuN, and had only minor co-localization with the microglial marker Iba1. Co-localization of tGR with GFAP, the astrocyte marker, was not observed (Figure 6). pGR expression was also localized in a similar fashion.







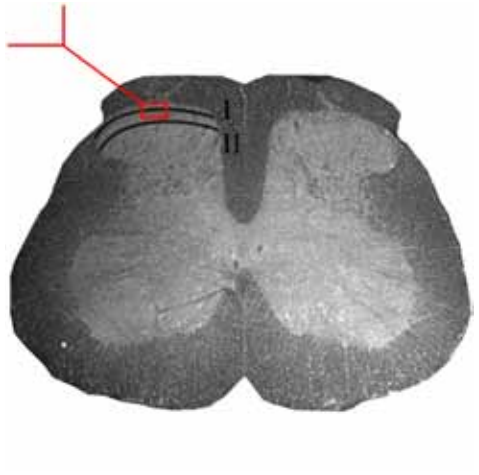


Figure 6 Glucocorticoid (GR = green or red) co-localization with A) astrocytes (GFAP = red), B) microglia (Iba 1 = green), and C) neurons (NeuN = green) in lamina I and II in the spinal dorsal horn indicated in the lower right. Nuclei staining with DAPI = blue. GR predominantly co-localized with the neuronal marker NeuN (C), and had only minor co-localization with the microglial marker Iba1 (B). No co-localization of GR with GFAP (A), the astrocyte marker, was observed.

DISCUSSION

OVERVIEW OF RESULTS

In the present study, no analgesic effect with two doses of intrathecal MPA was observed 18 to 25 days after SNL when ligation-induced mechanical hypersensitivity was well established in rats. Seven days after drug treatment, rats were euthanized and decreased tGR mRNA levels in intrathecal MPA treated animals compared to saline controls were observed. However this did not result in lower tGR and pGR immunoreactive signal intensities in intrathecal MPA treated animals compared to saline controls.

EFFICACY OF INTRATHECAL MPA IN NEUROPATHIC PAIN STATES

The lack of an analgesic effect with intrathecal glucocorticoid treatment in rats with pain-like behavior after SNL and spared nerve injury model has been described before.[7;10] In agreement with our findings in rats, there are also clinical studies showing no effect of intrathecal MPA treatment in postherpetic neuralgia patients and patients suffering from complex regional pain syndrome.[5;6]

GLUCOCORTICOID RECEPTOR EXPRESSION AFTER NERVE INJURY

After nerve injury in rats, plasma cortisol levels are significantly increased compared to baseline, lasting at least 21 days.[3;10] In our study, intrathecal MPA was administered on day 18 and day 21 after SNL, when plasma cortisol levels are still known to be high. Also an increased GR expression in the SDH has been reported after nerve injury with a time course parallel to the development of pain behavior.[10;12;19;20] In our study we show that tGR and pGR immunoreactivity is still upregulated 25 days after SNL in both saline controls and intrathecal MPA treated animals. Surprisingly, there was no significant difference between both groups in tGR and pGR immunoreactivity signal intensities seven days after intiation of intrathecal glucocorticoid treatment. We did however measure a decreased tGR mRNA level in intrathecal MPA treated animals. The reduced tGR mRNA expression after intrathecal MPA administration might be the result of a feedback mechanism after relative high doses of glucocorticoids at the target tissue. It however did not lead to significantly less pGR protein expression compared to saline controls.

We did not measure the effect of intrathecal MPA on downstream markers of GR activity in the present study. Several studies report effects of intrathecal glucocorticoids on surrogate markers such as microglial and actrocyte activation and changes in pro-inflammatroy cytokine (tumor necrosis factor α, interleukin-1β) levels after nerve injury with varying and sometimes contradicting results summarized in our review.[11]

The pGR we measured is one of several post-translational modifications of the GR. GR consists of a constant component, from exon 2 through 8, and a variable component exon 9. Alternative splicing of exon 9 gives rise to 5 different (human) protein subtypes that have been termed hGRα, hGRβ, hGRγ, hGR-A and hGR-P.[21] These protein subtypes have several different subisoforms (e.g. GRα-A, GRα-B, GRα-C1)[22] which are subject to post-translational modifications for example phosphorylation, which further modulates the transcriptional activity of the receptor. In the present study, we have chosen to focus on the total GR receptor (tGR), using an antibody directed to the constant component of the GR staining all the present glucocorticoid receptor subtypes. The activated GR (pGR), we studied is a GR phosphorylated at serine 211 in humans (corresponding with phosphorylation at serine 232 in the rat).[23;24] Phosphorylation at this site occurs to a greater extent in the presence of glucocorticoids and leads to translocation of the receptorcomplex to the nucleus.[24] Ser211 phosphorylation is therefore seen as a biomarker for activated GR in vivo.[23] An explanation for the observed reduction in tGR mRNA expression after intrathecal MPA administration, not leading to reduced pGR immunoreactive signal intensities, is that other pGR posttranslational modificated isoforms were downregulated after intrathecal MPA administration.

COLOCALISATION OF THE GLUCOCORTICOID RECEPTOR IN THE CENTRAL NERVOUS SYSTEM

The GR is located in every cell in the body but higher GR expression in the neuraxis is observed in the hippocampus, hypothalamus, and in the spinal cord in the substantia gelatinosa.[25] Pain is modulated in these areas and could indicate that pain pathways are under regulation of these receptors. Focusing on the spinal cord, GR was primarily increased in lamina I/II of the SDH in the development of pain and a majority of GR-expressing cell profiles expressed NeuN, corresponding with previous findings.[10;25] We find it surprising that GR is mainly expressed in neurons since most inflammatory processes have been studied in microglia and astrocytes. GR co-localization with astrocytes and microglial cells and oligodendrocytes has also been observed.[25]

STUDY STRENGTH AND WEAKNESSES

Although larger sample size per group for measurement of GR activity would have been useful, we did find significant differences in tGR and pGR immunoreactive signal intensities between the contra and ipsilateral side after SNL. Regarding the lack of difference in pGR immunoreactive signal intensities between MPA treated and saline control rats, it would be interesting to study different posttranslational modificated glucocorticoid receptor isoforms in nerve injury models to shed light on the varying and contradicting results of glucocorticoid therapy in neuropathic pain states.

CONCLUSION

In conclusion, our study shows that intrathecal MPA at the maximum usable dose employed, had no effect on the established tactile allodynia in a SNL model. tGR and pGR are upregulated in the spinal cord dorsal horn in the face of neuronal damage in both intrathecal MPA treated animals and saline controls. Intrathecal MPA treatment decreased tGR mRNA levels in the spinal cord dorsal horn after SNL, not lowering tGR and pGR immunoreactivity intensities, and not decreasing ligation-induced mechanical hypersensitivity in rats.

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4

GENERAL DISCUSSION

CHAPTER 8

THESIS SUMMARY

In this thesis we have studied the pharmacokinetics, safety and analgesic properties of intrathecal administration of methylprednisolone acetate (MPA) suspension in neuropathic pain states, both in patients and preclinical models.

Part I provides a literature overview of the development of neuropathic pain, the characteristics of glucocorticoids and their mechanism of action, the effect of glucocorticoids on neuropathic pain in patients and preclinical studies, with an emphasis on the glucocorticoid MPA.

In **Part II, Chapter 3**, we present the results of a pharmacokinetics study on intrathecal administration of MPA in patients. Peak plasma methylprednisolone (MP) levels were observed in the first six hours after intrathecal MPA injection, **Chapter 4 & 6**. While plasma levels of MP decreased rapidly within the following 24 hours, MP and MPA levels in the cerebrospinal fluid (CSF) remained elevated for over eight weeks. The dissolution of MPA and the conversion to MP seems to be the rate-limiting step for the clearance of MPA from the CSF. We hypothesize that the early MP plasma peak is caused by free MP already present in the MPA formulation before injecting the drug and by the early conversion of a part of the MPA formulation to MP. We conclude that after intrathecal administration of MPA, the conversion of MPA to MP is in general slow (which is an intended effect of the formulation as an acetate suspension), leading to prolonged exposure of neuraxial tissue to MPA and MP.

In **Chapter 4** we describe a preclinical safety study in dogs. Dogs were treated with four injections of intrathecal MPA with 7-day intervals according to the clinical protocol described in **Chapter 5**. We did not observe any clinical adverse events, but noted dose-dependent intrathecal inflammatory reactions – indicating neurotoxicity – at doses and injectate concentrations comparable to those employed in patients. These results suggest that repeated intrathecal administration of this particulate glucocorticoid formulation may be unsafe and continued use of this treatment modality in patients cannot be recommended.

Part III presents the results of clinical and preclinical efficacy studies on intrathecal MPA treatment. The clinical study was performed in patients suffering from postherpetic neuralgia (PHN), **Chapter 5**. The preclinical studies were performed using three well established rat models, **Chapter 6**. The clinical study, a randomized controlled double blinded trial (RCT) in PHN patients, was designed to replicate a trial by Kotani et al.[1] published in the New England Journal of Medicine in 2000. The trial showed profound reduction of pain in PHN patients after intrathecal MPA (92% of patients had 'good or excellent' pain relief at one year follow-up) without

any side effects or complications. In contrast to this trial published in 2000, the results of our replication trial indicated no beneficial effects of intrathecal MPA. It increased pain and interleukin-8 levels in all six treated PHN patients.

In the preclinical studies, we observed severe generalized allodynia during dose finding with high intrathecal doses of both MPA and MP in naive rats (rats not submitted to a pain model). In three separate pain models in rats, we also did not observe any acute analgesic effects of intrathecal MPA. We did observe biological activity of the MPA formulation after intrathecal administration in rats (a delayed 7-day reduction of tactile allodynia in the formalin model, and a decrease in microglial activation (ionized calcium-binding adapter molecule 1) in the spinal dorsal horn without effect on pain-like behavior after spinal nerve ligation), suggesting that the drug indeed reached the target tissue and generated down-stream effects.

In **Chapter 7** we present results showing that spinal nerve ligation in rats increased glucocorticoid receptor (GR) expression in the spinal cord dorsal horn. Intrathecal MPA reduced the GR mRNA levels without having an effect on the total or activated GR receptor levels, not decreasing ligation-induced mechanical hypersensitivity. We hypothesize that the GR mRNA levels are reduced after intrathecal MPA by a negative feedback mechanism. The clinical relevance of these findings remains to be studied.

In conclusion, intrathecal MPA does not decrease pain or pain-like behavior in severe neuropathic pain states in patients or rats, respectively, and is a potentially harmful treatment that should not be offered to patients.

GENERAL DISCUSSION

Pain physicians are frequently confronted with patients suffering from postherpetic neuralgia (PHN).[2] In a substantial number of PHN patients, even combinations of different drug treatments are not sufficient to achieve a satisfactory level of pain relief, leading to a reduced quality of life.[3;4] Therefore it is not surprising that desperate PHN patients sometimes ask their physician for new or experimental treatment options. After publication of the Kotani trial[1] in 2000 in the New England Journal of Medicine in which intrathecal administration of a glucocorticoid, MPA, was associated with profound pain reduction in PHN patients, several pain clinicians in the Netherlands started offering this new treatment to their patients. However, intrathecal MPA never became a standard of care in pain clinics in Europe and the United States in the years following publication of the trial. A call for a replication trial was repeatedly made.[4-7]

Because we were interested in the potential of the treatment, but not convinced by the unusually good results described in the Kotani trial,[1] we decided to verify their results in a replication trial, before routinely offering this treatment to more PHN patients in the pain clinic of our hospital. Since there had not been any adverse events in the 270 patients included in the Kotani trial, and we were able to remove the potentially neurotoxic preservatives from the MPA formulation (referred to as reformulated MPA formulation), the risks of our replicate study were considered acceptable. With approval of the medical ethics committee, we proceeded with the inclusion of patients in 2008.

During the first inclusions of our replicate trial, a discussion had risen among experts on the off-label use of drugs in the intrathecal space and preclinical safety studies were advised.[8;9] As early as the 1970s, intrathecal MPA was reported to cause adverse events.[10;11] For example, after repeated intrathecal injections at short intervals in multiple sclerosis patients, serious adverse events such as cerebral hemorrhage, meningitis, conus syndrome, progressive weakness, reversible bladder dysfunction, and paresthesia had been observed.[10;11] Although we had started our RCT, we were compelled to generate safety data and a parallel safety study was designed studying the reformulated MPA in a dog model. In dogs, a dose-dependent inflammatory meningeal reaction, accompanied by inflammatory masses suggesting abscess formation was observed after administration of both 10 and 40 mg of MPA with lidocaine. The safety study was completed in 2011. At the same time, the first planned interim analysis after ten included PHN patients, who had received either intrathecal MPA combined with lidocaine or lidocaine only, was performed. The results of the safety study, in combination with the clinical deterioration of PHN in all patients treated with MPA and evidence for futility led to the decision to end the trial.

In the next sections, the data of the pharmacokinetics, preclinical safety, and clinical and preclinical efficacy studies will be discussed in more detail.

PHARMACOKINETICS

While designing our clinical replication trial, we reviewed the literature for pharmacokinetic data on intrathecal MPA administration. Besides one study in 1963 by Sehgal et al.[12] no other pharmacokinetic studies had been published. That study showed that after intrathecal MPA administration, MP plasma concentrations were measurable up to 14 days after injection and beyond, and that there was considerable individual variation in the rate of absorption of the glucocorticoid from the CSF into the blood.[12] We were interested if the once weekly intrathecal dosing schedule, as suggested in the Kotani trial, provided optimum exposure of neural tissue to the drug. Therefore we collected CSF at different time points after MPA injection in our RCT to measure MP and MPA concentrations.

After administering MPA in a biological matrix, MPA slowly converts to MP, the biologically active form. In both the preclinical dog studies (Chapter 4) and rat studies (Chapter 6), we observed peak MP plasma levels early, around one and three hours after intrathecal administration of MPA, respectively. A steep decrease in MP plasma levels was noted after 24 hours in both rats and dogs. It is highly likely that this peak MP plasma level is generated by the free MP already present in the injectate that can freely diffuse into the systemic circulation and has a plasma half-life of 1.8 – 2.5 hours.[13-15] The slow decline of MP plasma levels in rats and dogs and in the CSF in humans shows us converted MP from the intrathecal MPA. Depots of MPA particles were observed up to six weeks after intrathecal injection in dogs (Chapter 4). We were able to show that most of the precipitated MPA was found 1 to 3 cm below the lumbar catheter tip on the spinal cord of the dog. After four intrathecal MPA injections with 7-day intervals, MP plasma levels were detectable up to two weeks after the last injection in dogs (Chapter 4) and MP CSF levels up to eight weeks in humans (Chapter 3). These values corresponded with earlier studies after intra-articular MPA administration in cows and horses.[16;17] We hypothesize that the slow conversion of MPA to MP in the intrathecal space is due to the relatively low concentration of butyrylcholinesterase in the CSF compared to plasma. In an in vitro study, butyrylcholinesterase was identified as one of the enzymes involved in hydrolysis of MPA to MP, increasing the speed of hydrolyzation.[18]

Summarizing these findings, we observed that after intrathecal administration of MPA, the MPA particles precipitate at or below the injection site in the intrathecal space, and that from that collection of MPA particles, a slow release of MP is facilitated. Long exposures of the surrounding tissue to the drug, up to eight weeks after intrathecal MPA administration, were observed. A dosing regimen of weekly intrathecal MPA injections, thus gives rise to drug accumulation and potential overdose and toxicity.

SAFETY

The long exposure time of neuraxial tissue to MPA and MP may provide one of the explanations for the toxicity observed in the preclinical safety study in dogs (Chapter 4). After four intrathecal reformulated MPA injections with minimal preservative concentrations with 7-day intervals in dogs, dose-related neurotoxicity was noted. All dogs treated with intrathecal MPA (regardless of the dose) had diffuse infiltrates of macrophages and other inflammatory cells along the inner surface of the dura and near the arachnoid, and elevated nuclear cell counts, protein levels and decreased glucose levels in the cisternal CSF samples suggesting an ongoing inflammatory process. In the highest MPA dose group (40 mg MPA), 2 out of 3 dogs developed large aseptic inflammatory masses on the inner surface of the dura and arachnoid. Abscess formation was predominantly observed in the caudal sac, the lowest point of the intrathecal space, where the MPA particles precipitated and formed a depot.

Neurotoxicity after intrathecal glucocorticoid administration has been reported previously in preclinical safety studies.[19-22] In all these studies, preservatives were not removed from the glucocorticoid formulation. Accordingly, one explanation for the observed neurotoxicity is the presence of preservatives. Our preclinical safety study, is the first to describe neurotoxicity after intrathecal MPA with minimal preservative concentrations, suggesting an additional covariate in the development of neurotoxicity such as a) the excitatory actions of MP on neuronal tissue and/or b) the presence of MPA particles.

Excitatory effects of MP have been observed in rats (Chapter 6). Generalized allodynia, diagnosed when animals spontaneously vocalized and adapted aggressive behavior when the experimenter stroked their fur, was noted with high intrathecal doses of reformulated MPA and the soluble methylprednisolone sodium succinate. Especially methylprednisolone sodium succinate, which only contains the free MP and no depot, caused general allodynia[23] at 10 fold lower doses compared to MPA. Since the free fraction of MP in the methylprednisolone sodium succinate formulation is higher as compared to the MPA formulation, this explains why a lower maximum tolerable intrathecal dose of methylprednisolone sodium succinate is tolerated, as compared to MPA. Also in humans an increase in pain was reported in the first days after intrathecal MPA administration[12] in agreement with our observed increase in PHN, and after epidural administration of the drug.[24] An in vitro study has shown that application of MP to spinal cord neurons can cause a concentration-dependent increase in firing rate and thus act as an excitatory agent.[25] In clinical studies there is evidence that MP, independent of the route of administration, can generate neuropsychiatric side effects such as mania and delirium[26-28] and proconvulsant and epileptogenic effects.[29;30] Most glucocorticoid receptors in the spinal cord dorsal horn were present in neurons, as we describe in Chapter 7, implicating a relation between

glucocorticoids and neuronal actions. We hypothesize that the reports of increased pain in humans and the increase in pain-like behavior in rats is caused by the glucocorticoid itself. It has been suggested that the excitatory effects of glucocorticoids can induce excitotoxicity, but if this effect is sufficient to explain the pathology observed in our preclinical safety study remains unclear.[25;31]

Besides the long exposure time to MP and MPA and the excitatory potential of MP in neuronal tissue, the particles in the MPA formulation might play a role in the development of neuroinflammation. Particulate materials such as carbon particles, particles from biomaterials, and other environmental toxins including nanoparticles can initiate an inflammatory reaction, as evidenced by cytokine release. That in turn leads to activation of a variety of cell adhesion factors, resulting in macrophage and neutrophil immigration in systems such as the lung and prosthetic interfaces of joints.[32] The extent of the inflammatory reaction is inversely proportional to particle size and directly proportional to surface area.[33] In the reformulated MPA suspension, 15% of particles are between 10 and 60 μm in diameter (Chapter 3). The majority of particles is between the molecule size of MPA (polar surface area of 101 angstrom) and an aggregated form of 10 μm in diameter. The critical size range for 'wear' particles from prosthetic joints to cause an inflammatory response has been estimated to be from 0.2 to 10 μm.[34] This suggests that most particles in the MPA suspension have the potential to cause a severe inflammatory response. Increased levels of IL-8 have been observed in the CSF after intrathecal administration of MPA in humans (Chapter 5). It is important to note that IL-8 has been hypothesized to be a pivotal mediator of particle-induced neutrophilic inflammation.[32;35]

In conclusion, intrathecal MPA has a dose-dependent neuro-inflammatory effect, possibly induced by the long exposure time of the neuraxial tissue to MP and MPA, the presence of low concentrations of preservatives in the MPA formulation, the excitatory actions of MP on neuronal tissue and/or the presence of particles in the intrathecal space.

EFFICACY

The neuroinflammatory reaction observed after repeated intrathecal MPA injections in the preclinical safety study was one of the reasons to end the RCT in patients immediately. It coincided with statistical evidence for futility at the planned interim analysis after the inclusion of only ten PHN patients. We observed an increase in the visual analogue scale (VAS) for global pain in all six PHN patients treated with intrathecal MPA. Our sequential analysis was based on the assumption that at least half of the effect as described by Kotani[1] would be observed in our trial (i.e. 50% of the patients in the treatment group experiencing good or excellent pain relief after one year, compared to 92% in the Kotani trial). Also, we assumed that the pain relief

in the lidocaine-only group would double to 10% compared to the effect described,[1] leading to a conservative estimate of the sample size. With six PHN patients experiencing an increase in global pain, the probability of finding a decrease in global pain in 50% of patients was negligible (i.e. < 0.00003). In conclusion, we could not replicate the results from the Kotani trial.
Our RCT with a final sample size of 10 patients, had no statistical power to detect pain relief in less than 50% of patients or a reduction of global pain less than 50% with intrathecal MPA treatment. However, it is unlikely that intrathecal MPA has a clinically relevant analgesic effect in intractable pain states, since also in our preclinical rat studies no effect with the maximum tolerable dose of MPA was observed in a total of 31 rats in two pain models (the inflammatory carrageenan model and the neuropathic spinal nerve ligation model). Combined with the potential risks of the treatment, intrathecal MPA should not be offered to patients with severe PHN.

In order to better understand the magnitude of the treatment effect observed in the Kotani trial, we compared their reported treatment effect with treatment effects observed in other trials in the PHN population. In patients suffering from PHN an average treatment effect size, expressed as number needed to treat (NNT), of 2.1 to 4.8 has been observed in large well-designed trials.[36] In the Kotani trial a NNT of 1.1 was reported.[1;4] With the aforementioned pre-study probabilities (NNT 2.1 to 4.8) of a relationship between treatment and effect being true,[37] the effect in the Kotani trial (NNT 1.1) could be interpreted as 'too good to be true'.

Contributing to the low NNT in the Kotani trial, is the small placebo effect of 5% (only 5 out of 91 patients in the control group, receiving an intrathecal injection with lidocaine only, reported >50% pain relief at 1-year follow-up). The average placebo effect (at least 50% pain relief) reported in PHN trials is approximately 15%.[36;38] In the group receiving no treatment at all in the Kotani trial, there was >50% pain relief in 3% of patients. It is important to note that the natural history of PHN shows that more than 50% of patients with PHN one year following the herpes zoster skin rash recover from PHN in the following years.[39-42] We tried to contact the authors in the hope of receiving more detailed information, but were unable to reach any of the lead authors.

There are several potential pathophysiologic explanations for the lack of an analgesic effect observed with intrathecal MPA treatment in PHN patients. First, it is unlikely that glucocorticoids are able to directly decrease central sensitization and/or ongoing ectopic impulses from the damaged peripheral nerve or DRG. To the best of our knowledge there is no evidence that glucocorticoids can generate conduction blocks comparable to local anesthetics. The most plausible mechanism of action for pain relief with glucocorticoids is that they act upon inflammatory targets in pain pathways.

If MPA would act upon a variety of crucial inflammatory targets in pain pathways, a long acting analgesic effect is to be expected.[43] Another possible explanation for the lack of an analgesic effect of intrathecal MPA in both our preclinical and clinical studies is that the anti-inflammatory actions of the drug could be outweighed by its neurotoxic effects. Finally, development of glucocorticoid resistance has been proposed in literature. From research in asthmatic patients we know that with increasing severity of the disease the beneficial response to glucocorticoids decreases.[44] Also, in other inflammatory diseases such as acute respiratory distress syndrome, cystic fibrosis and severe rheumatoid arthritis, no clinical benefit of glucocorticoid treatment is observed.[44] These disease entities have a severe state of inflammation in common. Studies have shown that this clinical phenomenon is caused by glucocorticoid resistance.[44;45] This glucocorticoid resistance has several distinct molecular mechanisms that contribute to the decreased clinical effect. Two important molecular mechanisms are:

a) Defective glucocorticoid receptor (GR) binding and translocation to the nucleus
b) Increased expression of glucocorticoid receptor β (GRβ). The GR has two main isoforms; GRα and GRβ. The glucocorticoid anti-inflammatory response is mediated through GRα. Binding of a glucocorticoid to GRα causes translocation of the glucocorticoid-receptor complex from the cytoplasm to the nucleus of a cell and binding to the DNA. This leads to transcription of anti-inflammatory products providing its clinical beneficial effect. GRβ is unable to bind glucocorticoids, fails to activate transcription, thus lacking a clinical beneficial effect. The relative levels of GRα and GRβ in a cell influence the cell's sensitivity to glucocorticoids, with higher levels of GRβ leading to glucocorticoid resistance. Studies have reported increased expression of GRβ in glucocorticoid-resistant patients of several diseases.[45]

During the research for current thesis we have attempted to find evidence for this hypothesis. Using the spinal nerve ligation model in rats, we tried to compare GR binding and translocation to the nucleus, and increased expression of GRβ in the ipsilateral versus the contralateral side of the DRG and spinal dorsal horn in spinal nerve ligated rats versus naïve rats treated with and without intrathecal MPA. Unfortunately, we were unable to obtain reliable GR subtype quantification with the custom made GRα and GRβ primers in our experiments. We only could reliably determine total GR and activated GR protein levels (Chapter 7). These data showed us that spinal nerve ligation in rats increases both total and activated GR expression in the spinal cord dorsal horn. Intrathecal MPA reduced the GR mRNA levels without having an effect on the total or activated GR receptor levels, not decreasing ligation-induced mechanical hypersensitivity. From these findings we hypothesize that the GR mRNA levels are reduced after intrathecal MPA by a negative feedback mechanism. The clinical meaning of these findings is unclear en further research is necessary to understand the effects of exogenous glucocorticoids on the DRG and spinal dorsal horn in severe pain states and to determine to what extent glucocorticoid resistance plays a role in the lack of an analgesic effect in severe pain states.

EPIDURAL GLUCOCORTICOID TREATMENT

In this thesis we focused on the efficacy of intrathecal MPA, an experimental treatment reserved for patients with intractable pain. Epidural MPA treatment, in contrast, is widely used in pain practice, frequently for the treatment of radiculitis.[46;47] In a recent systematic review on epidural glucocorticoid treatment, half of the more than 45 RCTs showed benefit from the injections and half did not.[47] In the trials that did show a positive effect of epidural MPA, the effect was often short lived (shorter than three months).[46-48] Nevertheless, the popularity of the treatment is high among clinicians and paramedical personnel and it is used as standard practice in the treatment work-up of low back pain in most clinics.

Although case reports on serious adverse events have been published, the treatment is regarded as safe.[24;49;50] From 1965 to 2014 a total of 131 serious adverse events have been reported through the 'FDA Adverse Event Reporting System' (FAERS); 41 cases of arachnoiditis and 90 cases of serious nervous system disorders. Recently the Food and Drug Administration published their conclusions on a risk assessment on epidural glucocorticoid treatment. They state that; 'Given the large number of epidural procedures performed, these serious adverse events appear to be rare.' They have decided not to modify their warning about serious neurologic events.[51] Because of safety issues, MPA is mainly used for lumbar interlaminar epidural injections. For cervical epidural injections and epidural injections with a transforaminal approach, MPA was recently replaced by dexamethasone after reports of cerebrovascular events after inadvertent intravascular injection of MPA.[52;53] Animal studies examining the effects of intracarotid injections demonstrate that MPA as well as its non-particulate carrier, and methylprednisolone succinate can produce significant injury to the blood-brain barrier.[54]

In conclusion, there is a substantially lower risk on adverse events after epidural compared to intrathecal administration of MPA.

CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE RESEARCH

In conclusion, intrathecal MPA treatment should not be performed in patients because of the combined safety concerns and lack of efficacy in severe neuropathic pain states. When a single RCT presents extraordinary large beneficial treatment effects, a replicate trial is needed to verify these results before the treatment can be implemented in clinical practice.[55;56] In addition, before embarking on clinical evaluation of novel neuraxial treatments, high-quality preclinical safety data are mandatory.[8;9]

The present thesis provides an example of an irreproducible trial studying a neuraxial treatment that in a later preclinical safety study was proven to be unsafe. This emphasizes the need for both replication studies and preclinical safety data.

The results of our preclinical safety study were unexpected and raised the question how an anti-inflammatory drug, MPA, could cause an inflammatory response observed with neurotoxicity. We considered several hypotheses, such as the presence of low concentrations of neurotoxic preservatives in the glucocorticoid formulation, excitatory actions of the drug on neuronal tissue combined with prolonged exposure of neuronal tissue to the drug and/or microscopic mechanical injury to neuronal tissue by the presence of particles in the intrathecal space. It remains unclear which of the proposed mechanisms contributes most to the neurotoxic effects of MPA.

For future drug development it would be interesting to know if and under which conditions a suspension can safely be administered in the intrathecal space. Secondly, the question if the anti-inflammatory drug, MPA, is capable of inducing inflammation when administered in the intrathecal space, remains unanswered. An additional relevant study question is whether glucocorticoid resistance plays a role in the lack of anti-inflammatory actions in the central nervous system in a severe pain state.

We now know that intrathecal MPA does not provide sufficient pain relief for PHN patients. This underscores the need for an alternative treatment regimen to alleviate the pain experienced by some PHN patients. Treatment strategies that can desensitize both the peripheral and central nervous system are required. These should generally involve a multimodal approach, so that therapies may target the peripheral drivers of central sensitization and the related central pathological changes.[5]

NEDERLANDSE SAMENVATTING

Pijn is een onaangename sensorische en emotionele ervaring, geassocieerd met weefselschade of dreigende weefselschade.[57] Het is een fenomeen dat iedereen kent en in het leven zal ervaren. Pijn kan acuut optreden of chronisch zijn. Acute pijn blijft een relatief korte periode aanwezig totdat de onderliggende oorzaak verdwijnt. Van chronische pijn spreekt men als de pijn een lange tijd blijft bestaan (meestal gedefinieerd als meer dan drie maanden) en/of bestaat zonder dat er een aantoonbare oorzaak aan te wijzen is die de aanwezigheid en de mate van pijn kan verklaren. Chronische pijn komt vaak voor. In Europa varieert de gerapporteerde prevalentie van chronische pijn in de volwassen bevolking van 12% tot 30%.[58;59] In Nederland lijden ongeveer 3 miljoen volwassenen (18% van de bevolking) aan chronische pijn.[59]

Er zijn grofweg drie soorten pijn; nociceptieve/inflammatoire pijn, neuropathische pijn en pijn van gemengde origine. Dit proefschrift richt zich op chronische neuropathische pijn[60] dat bij 6,9 tot 10% van de volwassenen voorkomt.[58;61]

Neuropathische pijn is pijn die veroorzaakt wordt door een beschadiging of ziekte van het somatosensore zenuwstelsel.[57] Een van de vier meest voorkomende neuropathische pijnsyndromen is postherpetische neuralgie.[2;61] Postherpetische neuralgie wordt gedefinieerd als pijn in een nauw omschreven huidgebied (dermatoom) die gedurende meer dan drie maanden blijft bestaan, nadat de rode blaasjes van een herpes zoster (gordelroos) infectie zijn verdwenen.[62] Herpes zoster is het gevolg van een reactivatie van het varicella zoster virus, het virus dat we kennen als de veroorzaker van waterpokken. Nadat iemand waterpokken heeft gehad, houdt het zoster virus zich schuil in de dorsale wortelganglia (zenuwknopen) in het lichaam. Als de specifieke immuniteit van het lichaam voor het varicella zoster virus afneemt, bijvoorbeeld op oudere leeftijd, dan kan het virus reactiveren. Wanneer het virus zich hierbij gaat vermenigvuldigen, verspreidt het zich vanuit het dorsale wortelganglion naar de perifere zenuwen van het betreffende huidgebied, het dermatoom.
Dit resulteert in inflammatoire schade aan het zenuwweefsel en kan leiden tot neuropathische pijn.

Bij het merendeel van de patiënten verdwijnt de pijn veroorzaakt door het herpes zoster virus spontaan met het verstrijken van de tijd. Bij een aantal patiënten echter niet. Het risico op het ontwikkelen op postherpetische neuralgie varieert van 10 tot 25% afhankelijk van de toegepaste definitie.[62-64] Patiënten die lijden aan postherpetische neuralgie beschrijven sensaties zoals continue, brandende en kloppende pijn met af en toe scherpe, elektrische pijnscheuten en pijn bij aanraking van de huid (allodynie).[62;65] De pijn is vaak hevig en daardoor invaliderend.[3]

Behandeling van postherpetische neuralgie is lastig en vaak zijn er verschillende medicamenten nodig om de pijn onder controle te krijgen. Behandelingen van eerste keuze zijn middelen die ook bij depressies worden toegepast, de tricyclische antidepressiva, geneesmiddelen die worden gebruikt om epilepsie te behandelen, anti-epileptica (zoals gabapentine en pregabaline) en een plaatselijk verdovend middel, lidocaïne 5% pleisters.[36;65] Opiaten (morfine-achtige), tramadol en capsaïcine pleisters worden als tweede- of derdelijns therapie aangeraden.[4;66;67] Het effect van deze medicamenteuze behandelingen is vaak teleurstellend en er treden frequent bijwerkingen op.[4;65;68] Er is geen bewijs voor de gunstige effecten van invasieve pijnbehandelingen zoals epidurale en paravertebrale zenuwblokkades met lokaal anesthetica en/of glucocorticoïden, of sympathicus blokkades.[7;69]

In 2000 gloorde er opeens hoop voor patiënten die leden aan onbehandelbare postherpetische neuralgie. Er werd een artikel gepubliceerd in het vooraanstaande tijdschrift 'the New England Journal of Medicine' door een Japanse onderzoeksgroep, waarin werd beschreven dat de intrathecale toediening (toediening middels een ruggenprik) van methylprednisolon acetaat in combinatie met lidocaïne pijnvermindering gaf bij onbehandelbare postherpetische neuralgie patiënten.[1] De gedachte achter deze behandeling was dat methylprednisolon acetaat, een ontstekingsremmer, pijn verminderde door de aanhoudende ontstekingsreactie (inflammatie) te onderdrukken. Het bewijs voor een aanhoudende inflammatoire staat van het centrale zenuwstelsel als oorzaak van postherpetische neuralgie, was volgens de auteurs a) het feit dat bij postmortem (kadaver) studies in postherpetische neuralgie patiënten inflammatie was geobserveerd ter plaatse van het ruggenmerg[70] en b) de daling van de interleukine-8 concentratie in de cerebrospinale vloeistof na behandeling met methylprednisolon acetaat.[71]

Na vier wekelijkse intrathecale injecties met 60 mg methylprednisolon acetaat en 90 mg lidocaïne 2% rapporteerden 82 van de 89 postherpetische neuralgie patiënten dat hun pijn sterk tot zeer sterk was afgenomen, wat aanhield tot minstens een jaar na de behandeling. Dit in vergelijking met 5 van de 91 en 3 van de 90 patiënten na behandeling met respectievelijk alleen lidocaïne of zonder behandeling. Bij de 270 geïncludeerde patiënten traden geen bijwerkingen of complicaties op. Ook op MRI scans, gemaakt 4 weken, 1 jaar en 2 jaar na de behandeling, werden geen schadelijke effecten aangetoond.

Deze fantastische resultaten bereikten zowel de Nederlandse pijnbehandelaars als wanhopige patiënten die leden aan onbehandelbare postherpetische neuralgie. De behandeling werd patiënten aangeboden, echter de resultaten in de kliniek vielen tegen. De behandeling werd in de jaren die erop volgden dan ook niet geïmplementeerd als standaard behandeling voor uitbehandelde postherpetische

neuralgie patiënten. In de literatuur werd er meerdere keren verzocht om een onafhankelijke herhaling van het Japanse onderzoek.[4-7]

Wij waren geïnteresseerd in de potentie van de behandeling, maar twijfelden aan de onwaarschijnlijk goede resultaten van de Japanse studie. Derhalve besloten we de studie te herhalen met enkele aanpassingen in het studie protocol om de veiligheid van de behandeling te vergroten. We ontwikkelden met de apotheek een methode om de concentratie van het potentieel neurotoxische conserveringsmiddel myristyl-gamma-picolinium chloride aanzienlijk te verlagen, **hoofdstuk 5**. Daarnaast werd er een farmacokinetische studie, **hoofdstuk 3**, opgezet met als doel om meer inzicht te krijgen of het geadviseerde wekelijkse doseringsschema optimale weefselblootstelling zou geven aan het biologisch actieve methylprednisolon na intrathecale toediening van methylprednisolon acetaat. Met deze aanpassingen en gezien de afwezigheid van bijwerkingen of complicaties in de Japanse studie, ging de medisch ethische commissie akkoord met het studie protocol van de replicatie studie, **hoofdstuk 5**.

Tijdens de eerste patiënten inclusies, was er in de literatuur een discussie ontstaan over de veiligheid van 'off-label' toedienen van medicatie in de intrathecale ruimte.[8;9] Dat betekent: het toedienen van een geneesmiddel via een toedieningsweg of voor een indicatie waarvoor het geneesmiddel nog niet is goedgekeurd. Voorafgaand aan de Japanse studie[1] was er geen preklinische veiligheidsstudie verricht naar de eventuele neurotoxische effecten van methylprednisolon acetaat op zenuwweefsel. Er werd ons geadviseerd om deze veiligheidsstudie alsnog te verrichten, **hoofdstuk 4**. De studies beschreven in **hoofdstuk 3, 4** en **5** vonden simultaan plaats.

De resultaten van de farmacokinetische studie, **hoofdstuk 3**, toonden aan dat er gedurende meer dan acht weken na de laatste intrathecale toediening nog methylprednisolon in de cerebrospinale vloeistof van patiënten gemeten kon worden. Onze hypothese is dat methylprednisolon acetaat als suspensie slechts heel langzaam wordt omgezet tot het biologisch actieve, oplosbare methylprednisolon. De enzymen die deze omzetting bevorderen zijn slechts in geringe mate aanwezig in de cerebrospinale vloeistof. Het wekelijkse doseringsschema resulteert in accumulatie van methylprednisolon acetaat in de intrathecale ruimte en heeft potentiële overdosering en toxiciteit tot gevolg.

In **hoofdstuk 4** worden de resultaten beschreven van de preklinische veiligheidsstudie in honden naar de effecten van intrathecale methylprednisolon acetaat toediening op zenuwweefsel. De honden werden behandeld volgens hetzelfde protocol als de patiënten; vier, wekelijkse intrathecale injecties met methylprednisolon acetaat gecombineerd met lidocaïne. Er werden geen klinische bijwerkingen geobserveerd. Bij onderzoek van het zenuw- en omliggende weefsel ter plaatse van de toediening, zagen we zes weken na de laatste injectie een depot van methylprednisolon deeltjes

liggen. Dit bevestigde de zeer langzame omzetting van methylprednisolon acetaat naar methylprednisolon en de daardoor lange blootstelling van zenuwweefsel aan het middel. Daarnaast werden er dosis-afhankelijke inflammatoire reacties geobserveerd die op neurotoxiciteit wezen. Deze resultaten suggereerden dat herhaalde intrathecale toediening van methylprednisolon acetaat niet veilig is en dat het gebruik ervan in patiënten niet aanbevolen kan worden.

Bij het bekend worden van de resultaten van de veiligheidsstudie, **hoofdstuk 4**, is de inclusie van de patiënten voor de replicatie studie, **hoofdstuk 5**, direct gestaakt. **Hoofdstuk 5** beschrijft een gerandomiseerde gecontroleerde dubbel geblindeerde studie waarin uitbehandelde patiënten met postherpetische neuralgie werden behandeld met ofwel vier wekelijkse injecties met 60 mg methylprednisolon acetaat gecombineerd met 60 mg lidocaïne ofwel met vier wekelijkse injecties met 60 mg lidocaïne alleen. Het primaire eindpunt, pijnreductie acht weken na de laatste injectie, verschilde niet tussen beiden groepen. De pijn was in alle patiënten die behandeld waren met methylprednisolon acetaat toegenomen. Daarnaast was er een stijging in de interleukine-8 waarden in de cerebrospinale vloeistof in de met methylprednisolon acetaat behandelde patiënten, wat duidt op inflammatie.

Het optreden van neurotoxiciteit was zeer onverwacht, gezien het uitblijven van bijwerkingen en complicaties in de Japanse studie.[1] Er zijn een aantal oorzaken voor aan te wijzen; de lange blootstelling van zenuwweefsel aan methylprednisolon, de aanwezigheid van partikels (methylprednisolon acetaat is een suspensie) en de aanwezigheid van lage concentraties van het neurotoxische conserveringsmiddel myristyl-gamma-picolinium chloride. In de Japanse studie is echter een methylprednisolon acetaat preparaat gebruikt met een meer dan tien keer hogere concentratie van het conserveringsmiddel.

Omdat we het verschil in resultaten tussen onze studies en de Japanse studie niet konden verklaren en we geïnteresseerd waren of er een pijnstillend effect van intrathecaal methylprednisolon acetaat bestond, hebben we een dierexperimentele studie opgezet, **hoofdstuk 6**, waarin we in drie verschillende pijnmodellen ratten behandelden met intrathecaal methylprednisolon acetaat. Bij het zoeken naar de maximaal tolereerbare dosis in de rat werd er hevige gegeneraliseerde allodynie (pijngedrag na het aaien van hun vacht) geobserveerd bij hogere doseringen van zowel methylprednisolon acetaat als het oplosbare methylprednisolon natrium succinaat. Ook met de maximaal tolereerbare dosis werd er geen pijnstillend effect geobserveerd in twee van de drie verschillende pijnmodellen en in het derde model was de pijn afname gering en trad deze pas na zeven dagen op.

Om te beoordelen of de glucocorticoïd receptor een rol speelde in het uitblijven van een pijnstillend effect onderzochten we in **hoofdstuk 7** of intrathecale toediening van methylprednisolon acetaat invloed had op de glucocorticoïd receptor expressie

na spinale zenuwligatie. Het is bekend dat het aantal glucocorticoïd receptoren in de dorsale hoorn na spinale zenuwligatie (het onderbinden van een zenuw waardoor schade ontstaat) toeneemt. Behandeling met intrathecaal methylprednisolon acetaat deed de mRNA spiegels voor de glucocorticoïd receptor dalen, echter dit had geen effect op de daadwerkelijke hoeveelheden glucocorticoïd receptoren in de dorsale hoorn. Die bleven onveranderd toegenomen. Een mogelijke verklaring voor de daling in mRNA voor de glucocorticoïd receptor is een negatief terugkoppelingsmechanisme. De klinische relevantie van deze bevindingen dient nog verder onderzocht te worden.

Concluderend blijkt uit de studies in dit proefschrift dat intrathecale toediening van methylprednisolon acetaat pijn in patiënten met postherpetische neuralgie en ook pijngedrag van dieren in een experimenteel pijnmodel niet vermindert. We kunnen de resultaten uit de Japanse studie niet repliceren. Tot slot is de intrathecale toediening van methylprednisolon acetaat een potentieel schadelijke behandeling die niet aan patiënten moet worden aangeboden.

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ABOUT THE AUTHOR

Mienke Rijsdijk was born in 1983 on August the 21st in the Hague, the Netherlands. After graduating from secondary school (Coornhert Gymnasium, Gouda), she started her medical training in Utrecht in 2001. During her study she performed a research project at the Radiology Department of the University Medical Center Utrecht under supervision of dr. I.C. van der Schaaf and prof. dr. G.J. Rinkel. She focused on the detection of delayed cerebral ischemia after aneurysmal subarachnoid hemorrhage using perfusion CT. Her second research internship under supervision of dr. A.J.C. Slooter at the Intensive Care Department of the University Medical Center Utrecht was on continuous EEG monitoring during carotid endarterectomy for the detection of ischemic EEG changes using synchronization likelihood analysis. She graduated from medical school in March 2008.

In June 2008 she started her clinical residency in Anesthesiology under supervision of prof. dr. J.T.A. Knape and dr. R.G. Hoff. She combined her residency with research on intrathecal glucocorticoid treatment for neuropathic pain under supervision of prof. dr. C.J. Kalkman and dr. A.J.M. van Wijck. During her thesis project she performed two preclinical research projects, spending time in San Diego at the Department of Anesthesiology of the University of California, USA under supervision of prof. dr. T.L. Yaksh and in Stockholm at the Department of Physiology and Pharmacology of the Karolinska Institutet, Sweden under supervision of dr. C.I. Svensson. In 2016 she completed her residency program and postgraduate master in Clinical Epidemiology at the University of Utrecht.

Mienke is married to Jorrit Huisman. In their weekends and holidays they enjoy spending time on the water, sailing and windsurfing.

LIST OF PUBLICATIONS

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M. Rijsdijk, A.J.M. van Wijck, C.J. Kalkman, P.C.W. Meulenhoff, M.R. Grafe, J. Steinauer, T.L. Yaksh. Safety assessment and pharmacokinetics of intrathecal methylprednisolone acetate in dogs. Anesthesiology. 2012 Jan;116(1):170-181.

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GRANTS AND SCIENTIFIC AWARDS

2008 Philips CT NetForum Publication of the Year. Honorable mention 'Global and focal cerebral perfusion after aneurysmal subarachnoid hemorrhage in relation with delayed cerebral ischemia; Neuroradiology 50(9), 813-820. (2008). M. Rijsdijk, I.C. van der Schaaf, B.K. Velthuis, M.J. Wermer, G.J.E. Rinkel.'

2010 Western Anesthesiology Residents Congress. 2nd prize: Best poster presentation 'Safety assessment and pharmacokinetics of intrathecal of preservative-free methylprednisolone acetate in dogs.' M. Rijsdijk, A.J.M. van Wijck, C.J. Kalkman, P.C.W. Meulenhoff, M.R. Grafe, J. Steinauer, N. Tozier, T.L. Yaksh.

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