1. Current status glucocorticoids in the treatment of inflammatory disorders

It was more than half a century ago when glucocorticoids were discovered as miraculously active compounds against inflammatory disorders, such as rheumatoid arthritis, asthma, allergic reactions, systemic lupus erythematosus and vasculitis. Their dramatic anti-inflammatory activity and broad applicability have made the introduction of glucocorticoids into the clinic one of the major breakthroughs of drug discovery research in the last century (1).

The enthusiasm about this new group of drugs gradually tempered when the toxicity profile became more apparent (2). A range of endocrinal, cardiovascular, musculoskeletal, dermatological and gastrointestinal effects was discovered strongly limiting the use of glucocorticoids in the clinic (3). Among the most pronounced side effects are osteoporosis (leading to fractures), skin atrophy, Cushing’s syndrome, susceptibility for infections, hyperglycemia, ulceration of the stomach, psychiatric disorders, and hypertension (4-11). Due to the poor safety profile, systemic (i.e. oral, intravenous and intramuscular) treatment with glucocorticoids became only reserved for severe, refractive forms of inflammatory diseases in which other drugs were not sufficiently active.

The finding that the detrimental musculoskeletal and dermatological side effects of glucocorticoids were more related to long-term use of glucocorticoids offered some perspective (12). The disease course of inflammatory disorders is often characterized by exacerbations (relapses) of inflammation besides the long-term progression of tissue damage (13,14). Short-term treatment with repeated injections of high doses of glucocorticoids is one of the few strategies that can rapidly suppress exacerbations. This so-called ‘pulse therapy’ involves three to five (alternate) daily i.v. injections of up to 1 gram of (methyl)prednisolone or 0.1-0.2 g dexamethasone. Although cardiovascular, endocrinal and gastrointestinal side effects still do occur, pulse therapy is generally fairly well tolerated (15).

Topical administration proves to be the primary strategy to avoid (systemic) adverse effects. Application on the skin proved to be successful in treatment of inflammatory skin disorders while inhalation of glucocorticoids became the first choice treatment of asthma and allergic rhinitis (16). However, local treatment is only attractive when the target organ is easily accessible, which is often not the case. In rheumatoid arthritis local treatment can be realized by means of intra-articular injection (5-10 mg methylprednisolone acetate or 5 mg triamcinolone acetonide are most commonly used). However, intra-articular treatment requires a special technique and to prevent local reactions such as osteonecrosis, a particular joint should not be given more than 4-5 injections a year. Furthermore, this treatment approach is only attractive when single large joints are affected. In most cases rheumatoid arthritis involves a range of (smaller) affected joints and systemic therapy remains the only option (17).

Besides the poor safety profile, also the poor pharmacokinetic behavior limits the usefulness of glucocorticoids in systemic therapy (18). Glucocorticoids are drugs with a relatively high clearance rate and a large volume of distribution. This implies that to reach pharmacologically active drug levels at the site of inflammation, high and frequent doses
must be administered. The majority of these systemically administered doses localizes in healthy non-target tissues if not rapidly excreted from the body. To increase the amount of drug at the target site after systemic administration and to decrease localization at non-target tissues the so-called ‘drug targeting’ approach may offer perspective. Drug targeting makes use of colloidal carrier systems in which the drug is incorporated or to which the drug is attached. Distribution of the carrier-associated drug to organs/tissues is reduced, as the carrier cannot diffuse into extravascular tissues.

2. Drug targeting to inflamed sites

Drug targeting can be defined as a treatment approach leading to increased localization of a therapeutic agent at the target site in the body. Drug targeting can be realized by employing a drug carrier, which shows preferential affinity for the (pathological) target tissues. The increased target concentration of the carrier-associated drug as compared to the free drug may lead to a higher activity of the drug at the target site and/or reduced side effects at healthy non-target tissues.

Drug targeting may be considered as a means of local administration that makes use of the systemic route, since ideally drug targeting leads to 100% delivery of the systemically administered drug at the target site. Approaching this ideal situation depends on the type of drug carrier and target site. For instance, drug targeting to organs in which the mononuclear phagocyte system (MPS) is present (such as liver, spleen and bone marrow) can be highly efficient, as phagocytes in these organs are exposed to the blood circulation and rapidly take up the majority of the injected drug carrier (19). Meanwhile this phenomenon has compromised the use of drug carriers for targeting to non-MPS sites and additional modifications were necessary to enable significant drug delivery to these sites.

Over the last decades, a range of carriers has been investigated for the purpose of drug targeting. These include: plasma proteins, soluble antibodies, lipoproteins, nanoparticles, viruses, erythrocytes and erythrocyte-based structures, blood platelets, polymers, polymeric micelles and liposomes (20-31). To impose target selectivity to these carriers targeting ligands can be attached, such as antibodies or natural ligands for target site-specific receptors. This approach is often referred to as ‘active drug targeting’. Some of these carriers may show target selectivity without the use of targeting ligands. The above-mentioned affinity of several unmodified drug carriers for the mononuclear phagocyte system is just an example. This phenomenon is usually referred to ‘passive drug targeting’.

Interestingly, the approach of passive drug targeting can effectively be employed in inflammatory diseases. Inflammation generally results in locally increased vascular permeability, enabling cellular, (macro)molecular and colloidal blood components to extravasate and enter the inflamed site. I.v. injected colloidal drug carriers that stay long enough in the circulation spontaneously extravasate into sites of inflammation as well. This phenomenon of preferential localization at inflamed areas has been shown to occur with several colloidal drug carriers (32-34). Of these colloidal drug carriers, so-called ‘long-circulating liposomes’ may be among the most attractive ones.
3. Long-circulating liposomes

Liposomes are small lipid bilayer vesicles enclosing an aqueous core in which water-soluble drugs can be entrapped. The attractiveness of liposomes as drug carrier relates to their relatively high drug loading capacity, good biocompatibility, low toxicity, versatility and ease of preparation. Creating a lipid film by evaporation of a solution of membrane-forming lipids and subsequently hydrating this film with the aqueous drug solution spontaneously results in the formation of drug-encapsulating liposomes. Repeated extrusion, sonication or high-shear homogenization can be employed to produce liposomes of the desired size. The unencapsulated drug can easily be removed by dialysis, gel-filtration or centrifugation (35). Size and lipid composition are crucial parameters determining the in vivo behavior of liposomes after systemic administration. First, to prevent leakage of the drug from liposomes both upon storage and after injection in the circulation, stability of the drug-containing liposome formulation must be investigated and optimized. Second, the liposomes must be sufficiently small and have a sufficiently long circulation half-life so that they are allowed to extravasate at sites of pathology to a significant extent. Liposome formulations that meet these requirements/specifications are generally referred to as ‘long-circulating liposomes’ (31).

The most extensively studied long-circulating liposome systems are PEG-liposomes. This liposome type is created by grafting low-molecular poly(ethylene glycol) (PEG) to the lipid bilayer surface (Figure 1). It is generally thought that surface-grafted water-soluble polymers can oppose adhesion of plasma proteins to the liposomes that would otherwise ‘tag’ liposome for recognition and subsequent premature uptake by phagocytes of the mononuclear phagocyte system. PEG-liposomes can circulate with a half-life as long as 24 hrs in rats and up to 50 hours in humans. PEG-liposomes have been developed for the targeted delivery of cytotoxic agents to tumors, as the tumor vasculature is often loosely organized, providing increased access for blood components (36). Several liposomally encapsulated cytotoxic agents are on the market or in late phase clinical trials (37,38).

Preclinical and clinical studies have revealed that i.v. administered PEG-liposomes can indeed preferentially localize in inflamed tissues. For instance, Brouwers et al. showed that PEG-liposomes can be employed for the scintigraphic detection of inflamed sites in patients with Crohn’s disease (39). Despite these results, PEG-liposomes have hardly been used for the purpose of drug targeting in inflammatory disorders. Corvo et al. reported increased therapeutic activity of superoxide dismutase (SOD) upon encapsulation in PEG-liposomes and i.v. administration in an experimental rat model of arthritis (40). Disappointing results were obtained with the disease-modifying anti-arthritic drug methotrexate in PEG-liposomes (41). Although encapsulation of glucocorticoids in long-circulating liposomes for targeted delivery in inflammatory disorders seems logic and straightforward, the approach has never been proposed and investigated. Only a few studies on liposomal formulations of glucocorticoids for local, intra-articular treatment of inflamed joints in experimental arthritis exist (42,43).
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**Figure 1.** Schematic representation of a drug-containing long-circulating PEG-liposome
4. Aims and outline of this thesis

The primary aim of this thesis was to investigate application of PEG-liposomes as passive targeting system for the delivery of glucocorticoids to sites of inflammation. Chapter 2 provides a background to the field of advanced drug delivery with liposomes. The present status of both passive and active drug targeting with liposomes is reviewed.

Chapter 3 describes the pharmacokinetics, tissue distribution, degree of inflamed joint targeting and therapeutic activity of prednisolone phosphate-PEG-liposomes in rat adjuvant arthritis, a widely accepted animal model for rheumatoid arthritis. Chapter 4 also addresses the anti-arthritic effect of prednisolone phosphate-PEG-liposomes, but in a different model: murine collagen induced arthritis. This model allows a mechanistic study of the localization and anti-arthritic effect of liposomal glucocorticoid at a microscopic level. Besides application for arthritis treatment, we also studied the therapeutic activity of liposomal glucocorticoid in experimental models of multiple sclerosis. Chapter 5 describes pharmacokinetics, the degree of targeting to the inflamed nervous system and the beneficial effects of prednisolone phosphate-PEG-liposomes, both at a microscopic as well as a macroscopic level, in comparison to standard treatment with (free) methylprednisolone. To our surprise, liposomal prednisolone phosphate not only proved to be highly effective in models of inflammatory disorders, but also in murine tumor models, as reported in Chapter 6. The effect on tumor growth is evaluated both macroscopically as well as under the microscope. Some possible mechanisms are discussed that could explain this unexpected antitumor effect.

Chapter 7 describes the evaluation of glucocorticoids other than prednisolone in PEG-liposomes in experimental arthritis. Dexamethasone and budesonide are studied regarding both therapeutic activity and systemic adverse effects. An approach is proposed and evaluated to optimize the therapeutic index of liposomal glucocorticoid by encapsulating inhaled high-clearance glucocorticoids. Chapter 8 addresses the phenomenon of complement-related hypersensitivity reactions, which has been observed with PEG-liposomes in the clinic. The study in this chapter aims at finding the responsible key factor(s) and proposes a long-circulating liposome type that does not induce complement activation. Since the biological fate of liposomes-attached PEG after cellular uptake is not known, it was the objective of the work presented in Chapter 9 to design a biodegradable polymer-lipid conjugate for the preparation of long-circulating liposomes. Targeted delivery of glucocorticoids and therapeutic activity in experimental arthritis of this new type of liposome is also evaluated. Finally, Chapter 10 provides a summary and a general discussion of the results presented in this thesis.
REFERENCES
