- CHAPTER VI -

EFFECTS OF KETAMINE ON LPS-INDUCED COX-2 EXPRESSION AND PGE_2 PRODUCTION IN AN EQUINE MACROPHAGE CELL LINE

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SUMMARY

Ketamine has been reported to reduce lipopolysaccharide (LPS)-induced tumour necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) production in an equine macrophage cell line (e-CAS cells) by directly inhibiting nuclear factor-kappa B (NF-κB) expression.[1] NF-κB is a key regulatory transcription factor associated with the expression of various pro-inflammatory mediators, including cytokines and inducible enzymes like cyclooxygenase-2 (COX-2). Prostaglandin E_2 (PGE_2), the main product of COX-2, significantly contributes to the clinical symptoms of inflammation. Hence, COX-2 inhibitors such as the non-steroidal anti-inflammatory drugs (NSAIDs) are commonly used to reduce PGE_2 production. Considering the effect of ketamine on LPS-induced NF-κB expression, it was hypothesised that ketamine might have also an effect on LPS-induced COX-2 expression.

To this end, we studied the effect of ketamine (0-1000 μM) on LPS-induced (1 μg/ml) COX-2 expression and PGE_2 production in e-CAS cells. Furthermore, the intracellular signalling molecules, c-Jun N-terminal kinase (JNK), mitogen-activated protein kinase p38 (p38), extracellular signal-regulated kinase (ERK), and NF-κB were selectively inhibited, to elucidate the molecular mechanism underlying LPS-induced COX-2 expression and subsequent PGE_2 production in e-CAS cells. For comparison RAW 264.7 cells were used.

In LPS-treated e-CAS cells, ketamine did not affect COX-2 expression at any of the concentrations tested. Ketamine significantly reduced LPS-induced PGE_2 production only at the highest ketamine concentration (1000 μM) investigated. The inhibition experiments suggest that COX-2 expression and the subsequent PGE_2 production are mainly regulated at the level of JNK, p38 and ERK in the e-CAS cell model and that activated NF-κB does not significantly contribute to COX-2 induction in e-CAS cells. In previous experiments with e-CAS cells, ketamine was found to inhibit the LPS-induced signalling pathways only at the level of NF-κB,[1] which is in line with the finding that ketamine does not inhibit COX-2 expression in LPS-treated e-CAS cells. Hence, despite its potency to reduce LPS-induced TNF-α and IL-6 production, the role of ketamine in COX-2 and PGE_2 associated equine inflammatory disorders might be limited.

1. INTRODUCTION

Ketamine is commonly used in a variety of equine anaesthesia procedures. Besides the induction of anaesthesia,[2] ketamine can be used as an adjunct to inhalant anaesthesia,[3-5] in total intravenous anaesthesia (TIVA) protocols,[6-8] for epidural analgesia[9] and peripheral nerve blocks.[10] More recently, studies focus on the antinociceptive effects of a subanaesthetic continuous rate infusion (CRI) of ketamine in conscious horses.[11] Besides its anaesthetic and analgesic effects, ketamine has been found to possess cytokine-modulating effects in rodents[12-14] and humans.[15,16] Previously, we demonstrated that in an equine macrophage cell line (e-CAS cells), ketamine inhibits the lipopolysaccharide (LPS)-induced release of both tumour necrosis factor alpha (TNF-α) and interleukin-6 (IL-6) production by directly inhibiting nuclear factor kappa B (NF-κB) expression in a concentration-dependent manner, even at subanaesthetic concentrations.[1,17] NF-κB is a key regulatory transcription factor, which is primarily sequestered in the cytoplasm in an inactive form by association with its inhibitory protein, inhibitor of kappa B (IκB). Exposure to LPS results in phosphorylation and degradation of IκB, allowing released NF-κB to translocate to the nucleus, to bind to its cognate DNA element and to initiate the transcription of multiple pro-inflammatory mediators such as cytokines, adhesion molecules and enzymes like inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2).[18-20] Alternatively, the transcription of many these pro-inflammatory mediators can be regulated by mitogen-activated protein kinases (MAPKs), including extracellular signal-regulated kinase (ERK), mitogen-activated protein kinase p38 (p38) and c-Jun N-terminal kinase (JNK).[21]

COX enzymes catalyse the biosynthesis of prostanoids such as prostaglandin E_2 (PGE_2), prostacyclin (PGI_2), prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and thromboxane A_2 (TXA_2), by sequential oxygenation and reduction of membrane-derived arachidonic acids. COX enzymes (72 kDa) consist of two isoforms: COX-1 and COX-2, sharing a 61% homology at the amino acid sequence.[22,23] COX-1 is ubiquitously expressed in mammalian cells and mainly involved in 'housekeeping' functions like maintaining gastric mucosal integrity, initiating platelet aggregation, regulating renal blood flow and glomerular filtration.[22] COX-2 is an inducible enzyme primarily expressed by cells that mediate inflammation such as macrophages, monocytes and neutrophils.[24] In response to inflammation, COX-2 expression results in an increased synthesis of prostaglandin E_2 (PGE_2), which contributes to the clinical syndrome of inflammation including vasodilation, oedema, fever and hyperalgesia.[25-27] Hence, to reduce PGE_2 production in clinical human and animal

patients, COX-2 inhibitors as non-steroidal anti-inflammatory drugs (NSAIDs) are used in the treatment of inflammatory disorders.[28]
Considering the effect of ketamine on TNF-α and IL-6 release by directly inhibiting NF-κB, it was hypothesised that ketamine might also reduce LPS-induced COX-2 expression and the subsequent PGE_2 production. Hence, these effects as well as the involvement of JNK, p38, ERK, and NF-κB on LPS-induced COX-2 expression and PGE_2 production were investigated in an equine macrophage cell line (e-CAS cells). To support the validity of the e-CAS model, RAW 264.7 cells were used for comparison.

2. MATERIALS AND METHODS

2.1. CHEMICALS

Ketamine, lipopolysaccharide (LPS; Escherichia coli, O111:B4), bovine serum albumin (BSA), Tris, ethylenediaminotetraacetic acid (EDTA), Igepal (CA-630), sodium deoxycholate, sodium dodecyl sulphate (SDS), glycerol, bromphenolblue, nitroblue tetrazolium (NBT), 5-bromo-4-chloro-3-indolyl phosphate (BCIP), dithiothreitol (DTT) and anti-PGE_2 antiserum were purchased from Sigma chemicals (St. Louis, MO, USA). Roswell Park Memorial Institute (RPMI) 1640 medium, Dulbecco's modified Eagle's medium (DMEM), penicillin, streptomycin, glutamine and phosphate buffered saline (PBS) were obtained from Biocambrex (Verviers, Belgium). Horse serum (HS), fetal bovine serum (FBS), non-essential amino acids and sodium pyruvate were from Gibco Invitrogen (Breda, The Netherlands). Alamar blue was purchased from BioSource International (Etten-Leur, The Netherlands). Helenalin, SP600125, SB203580 and U0126 were from Biomol (Plymouth, USA). Polyvinylidene fluoride (PVDF) membranes were from Millipore (Billerica, Massachusetts, USA). Dimethyl Sulfoxide (DMSO) was from Fisher Emergo B.V. (Landsmeer, The Netherlands). Tween-20 was from Merck KGaA (Darmstadt, Germany). Bradford Protein assay kit was from Bio-Rad Laboratories (Hercules, CA, USA). MgCl2.6H20 was from VWR International (Amsterdam, The Netherlands). Rabbit polyclonal anti-COX-2 was from Abcam (Cambridge, UK). Alkaline phosphatase conjugated with polyclonal goat anti-rabbit immunoglobulin was purchased from Dako Cytomation (Glostrup, Denmark). [^{3}H]PGE_2 was obtained from Amersham-Pharmacia Biotech (Veenendaal, The Netherlands). Ultima Gold was from Perkin Elmer (Wellesley, MA, USA).

2.2. CELL LINES AND CULTURE CONDITIONS

Two cell lines were used: the equine bone-marrow-derived macrophage cell line (e-CAS cells) described by Werners et al.,[29] and the murine macrophage-like cell line (RAW 264.7; ATCC No TIB-71).

The e-CAS cells were grown in RPMI 1640 medium supplemented with 10% HS, 100 U/mL penicillin, 100 µg/mL streptomycin, 2 mM glutamine, 1 mM sodium pyruvate and 1% non-essential amino acids. Cells were maintained at 37 ^{o}C, in a humidified atmosphere of 5% CO_2/ 95 % air.

The RAW 264.7 cells were cultured in DMEM, supplemented with 10% FBS, 100 U/mL penicillin, 100 µg/mL streptomycin, 2 mM glutamine and 1 mM sodium pyruvate. Cells were maintained at 37 ^{o}C, in a humidified atmosphere of 5% CO_2/ 95 % air as well.

To determine the relative expression of COX-2 protein, cells were cultured in 6-wells plates at a density of $1.0x10^6$/well (2 mL). For measuring PGE_2 production, cells were seeded in 24-well plates at a density of $0.2x10^6$/well (1 mL). Following 24 h of culturing, different experiments were performed as described below.

2.3. TIME RESPONSE CURVE

To determine the time-dependent PGE_2 production in e-CAS cells, cells were incubated with serum-free RPMI 1640 medium containing 1 µg/mL LPS. Cells exposed to medium only served as control. Production of PGE_2 and cell viability was determined every 4 hrs following 4 - 48 h of incubation.

2.4. EXPOSURE TO LPS AND KETAMINE

To study the influence of ketamine on LPS-induced COX-2 expression and PGE_2 production, e-CAS cells were exposed to serum-free medium containing LPS (1 µg/mL) and ketamine concentrations at a range of 0-36 µM or 0-1000 µM, comprising clinically relevant as well as high experimental concentrations, respectively. Based on the results of the time-response curves, COX-2 expression, PGE_2 production and cell viability were determined following 24 h of exposure.

For control experiments, RAW cells were exposed to 1 µg/mL LPS and the high concentration range of ketamine (0-1000 µM).

2.5. INHIBITION EXPERIMENTS

To investigate the potential involvement of MAP kinases and NF-κB on LPS-induced COX-2 expression and PGE_2 production, cells were exposed to serum-free medium containing LPS (1 µg/mL) and specific inhibitors of JNK (SP600125; 0-10

μM), p38 (SB203580; 0-10 μM), pERK (U0126; 0-10 μM) or NF-κB (helenalin; 0-1 μM). To control samples, equal amounts of inhibitor solvent (0.1% DMSO) and/or LPS solvent (PBS) were added. COX-2 expression, PGE_2 production and cell viability were determined following 24 h of incubation.

2.6. WESTERN BLOT ANALYSIS

Expression of COX-2 protein was determined by Western blot analysis. In brief, after exposure, cellular extracts were prepared in RIPA buffer [150 mM NaCl, 1% Igepal, 0.5% sodium deoxycholate, 0.1% SDS and 50 mM Tris-HCl (pH 8)]. Protein concentrations were measured using the Bradford protein assay (Bio-Rad Laboratories, Hercules, CA, USA). Cell pellets were resuspended in sample buffer [12.5% glycerol, 1.25 % SDS, 0.004% bromophenol blue, 95 mM DTT and 62.5 mM Tris-HCl (pH 8.8)]. Aliquots containing 40 μg of protein were separated by SDS-PAGE (10%) and transferred to polyvinylidene fluoride membranes. Membranes were blocked in blocking solution [150 mM NaCl, 0.1 M Tris-HCl (pH 8.0), 0.03% Tween-20 and 1% BSA] overnight at 4 °C and then incubated with primary antibodies (rabbit polyclonal anti-COX-2 diluted 1:500) for 1 h at room temperature (RT). After washing, blots were incubated with secondary antibodies (horse radish peroxidase conjugated polyclonal goat anti-rabbit IgG diluted 1:1000) for 1 h at RT. Immunoreactive protein bands were developed by using nitroblue tetrazolium (NBT) and 5-bromo-4-chloro-3-indolyl phosphate (BCIP) as substrates and quantified through densitometric analysis.

2.7. PGE_2 RADIO-IMMUNOASSAY

Production of PGE_2 was measured by radio-immunoassay (RIA). In brief, anti-PGE_2 antiserum and [^{3}H]PGE_2 were added to each standard or sample. The tubes were incubated for 18-24 h at 4^{o}C. After incubation, dextran-coated charcoal suspension (20 mg/ml charcoal, 4 mg/ml dextran) was added and tubes were centrifuged (3000 rpm, 4^{o}C, 15 min) to separate bound and unbound fractions. Supernatants, containing the bound fraction, were transferred into scintillation-vials and scintillation fluid (Ultima Gold) was added. After mixing, samples were analysed using a liquid scintillation counter (Tri-carb 2900TR, Packard). Concentrations of PGE_2 were determined by comparing the values of the samples with a standard curve.

2.8. CELL VIABILITY

The Alamar Blue ™ (AB) reduction cytotoxicity assay was used as an indicator of cell viability.[30] The assay is based on the reduction of resazurin to fluorescent resorufin. In brief, Alamar Blue solution was added to each well (1:10 dilution) and the plate was incubated at 37 ^{o}C for 4 h. Thereafter, resorufin formation was measured fluorometrically at 530-560 nm excitation wavelength and 590 nm emission wavelength.

2.9. STATISTICAL ANALYSIS

Statistical analysis was performed using the SPSS® 12.01 statistical package (SPSS© Inc., Chicago, IL, USA). Data are presented as mean ± standard deviation (S.D.) obtained from three independent experiments. One-way analysis of variance (ANOVA) was used to test for significant differences between group means. When appropriate, post hoc multiple comparisons were performed to test for differences between experimental groups (Dunnett's). Data were considered to be statistically significant when $p<0.05$.

3. RESULTS

3.1. TIME-DEPENDENT PGE_2 PRODUCTION IN LPS-STIMULATED E-CAS CELLS

To determine the time response for PGE_2 production in LPS-treated e-CAS cells, PGE_2 production and cell viability were determined every 4 hrs following 4 - 48 h of LPS (1 μg/mL) exposure. Over time, a significant increase in LPS-induced PGE_2 production was demonstrated (Fig. 1). Cell viability remained above 90% until 28 h of LPS exposure. Thereafter, viability significantly decreased towards the end of the experiment. Based on these results, COX-2 expression, PGE_2 production and cell viability were determined following 24 h of LPS exposure in the further experiments with e-CAS cells.

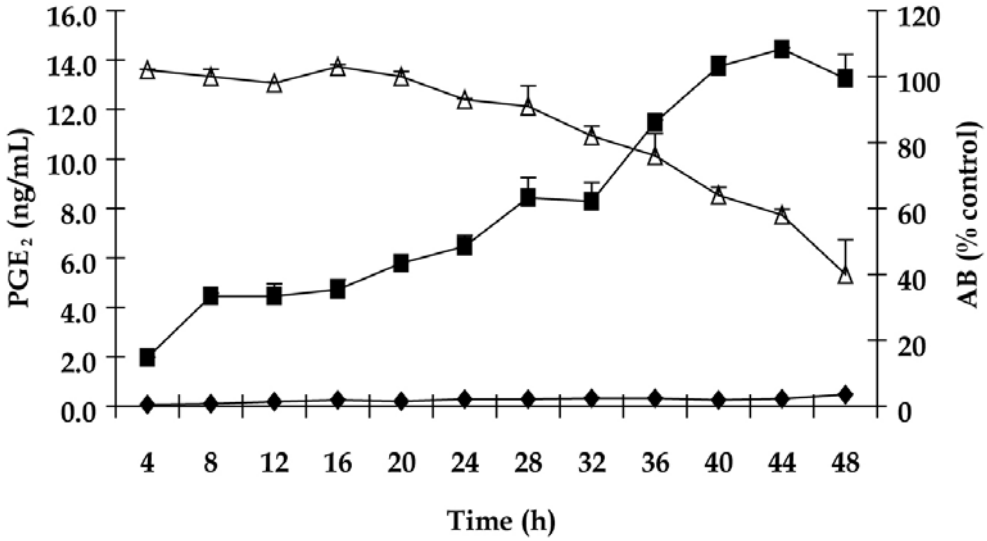

Figure 1. Time response curve of PGE_2 production in e-CAS cells. Cells were incubated with serum-free RPMI 1640 medium containing no (♦) or 1 μg/mL (■) LPS. PGE_2 production and cell viability (△) were determined every 4 h following 4 - 48 h of incubation. Data are expressed as mean ± S.D. of two independent experiments carried out in duplicate.

3.2. CELL VIABILITY

In all forthcoming experiments, cell viability was also tested by using the Alamar Blue cytotoxicity assay, but no significant differences were found (data not shown).

3.3. INFLUENCE OF KETAMINE ON LPS-INDUCED COX-2 EXPRESSION IN E-CAS AND RAW 264.7 CELLS

To study the influence of ketamine on LPS-induced COX-2 expression in e-CAS cells, cells were initially exposed to LPS (1 μg/mL) in the presence of a clinically relevant ketamine concentration range (0-36 μM). Western blot analysis showed that COX-2 expression was undetectable in non-stimulated e-CAS cells, but significantly increased in the presence of LPS (1 μg/mL). At clinical concentrations, ketamine did not significantly affect LPS-induced COX-2 expression in e-CAS cells. Subsequently, LPS-treated e-CAS and RAW 264.7 cells were exposed to high experimental ketamine concentrations (0-1000 μM). Again, no significant influence on COX-2 expression was found (Figs 2A and 3A).

Control experiments revealed that in absence of LPS, ketamine did not induce COX-2 expression in the tested concentration range (data not shown).

3.4. EFFECT OF NF-κB AND MAPK INHIBITORS ON LPS-INDUCED COX-2 EXPRESSION IN E-CAS AND RAW 264.7 CELLS

In both e-CAS and RAW 264.7 cells, helenalin (NF-κB inhibitor) did not significantly influence LPS-induced COX-2 expression (Figs 4A and 6A). In contrast, SP600125 (JNK inhibitor), SB203580 (p38 inhibitor) and U0126 (ERK inhibitor) significantly reduced COX-2 expression in LPS-treated e-CAS cells and RAW 264.7 cells (Figs 4 and 6, BCD, respectively). In LPS-treated e-CAS cells, SP600125 significantly inhibited COX-2 expression at 10 μM, while SB203580 and U0126 significantly blocked LPS-induced COX-2 expression at 1 and 10 μM (Fig. 4 BCD, respectively). Similar results were found in LPS-treated RAW 264.7 cells, although U0126 significantly blocked COX-2 expression only at 10 μM (Fig. 6 BCD).

Control experiments confirmed that in absence of LPS, none of the inhibitors induced COX-2 expression in e-CAS and RAW 264.7 cells (data not shown).

3.5. INFLUENCE OF KETAMINE ON LPS-INDUCED PGE_2 PRODUCTION

Exposure to LPS significantly increased PGE_2 production in e-CAS cells (from 0.17 ± 0.05 to 3.21 ± 0.24 ng/mL) and RAW 264.7 cells (from 0.04 ± 0.04 to 2.9 ± 0.7 ng/mL) when compared to non-treated cells.

Similarly to the effect of ketamine on LPS-induced COX-2 expression, ketamine at clinically relevant concentrations did not significantly inhibit LPS-induced PGE_2 production.

Following exposure to high experimental concentrations, PGE_2 production was only significantly reduced in e-CAS cells at a concentration of 1000 μM (Fig. 2B). In RAW 264.7 cells, no significant influence of experimental ketamine concentrations on LPS-induced PGE_2 production was found (Fig. 3B).

3.6. INFLUENCE OF MAPK AND NF-κB INHIBITORS ON LPS-INDUCED PGE_2 PRODUCTION

Corresponding to the influence of the selective inhibitors on LPS-induced COX-2 expression, PGE_2 production was only significantly reduced following inhibition of JNK, p38 and ERK in LPS-treated e-CAS and RAW 264.7 cells (Figs 5 and 7, BCD, respectively). Again, inhibition of NF-κB did not significantly reduce LPS-induced PGE_2 production in e-CAS and RAW 264.7 cells (Figs 5A and 7A).

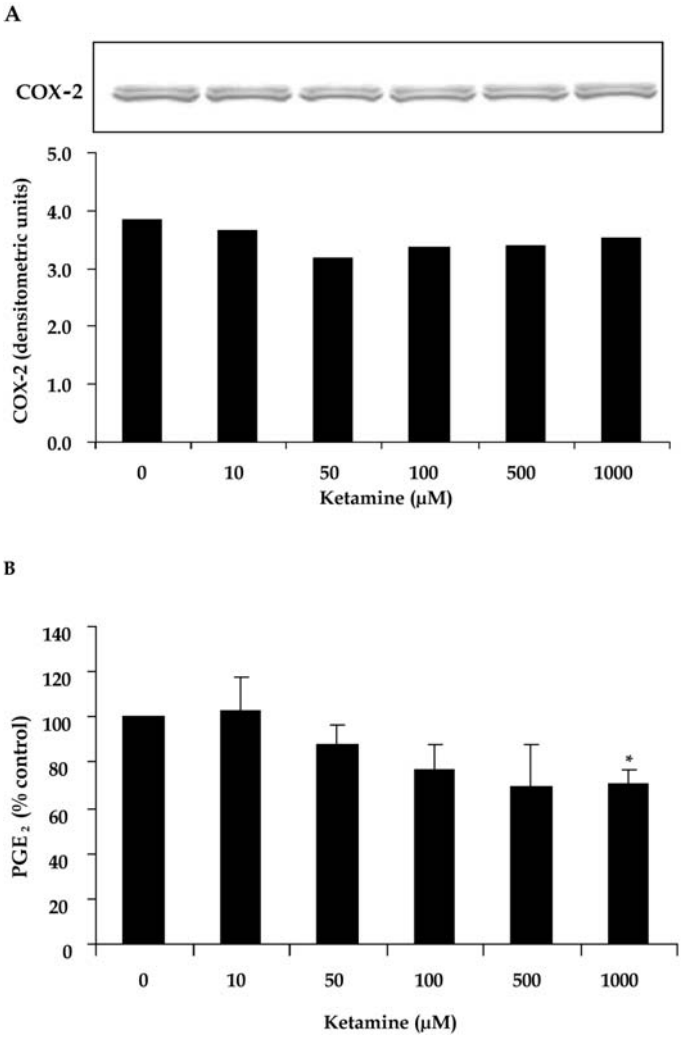

Figure 2. Effect of high experimental ketamine concentrations on LPS-induced (A) COX-2 expression and (B) PGE_2 production in e-CAS cells. (A) Cells were stimulated with LPS (1 μg/mL) and ketamine (0-1000 μM) for 24 h. Following incubation, cells were lysed and lysates were assayed for COX-2 expression by Western blot analysis. A representative Western blot of three independent experiments with densitometric quantification of the COX-2 bands is shown. (B) PGE_2 production in e-CAS cells was measured by radio-immunoassay (RIA) after 24 h of exposure to LPS (1 μg/mL) and ketamine (0-1000 μM). LPS alone was regarded as a positive control and set at 100%. Data are expressed as mean ± S.D. of three independent experiments carried out in triplicate. * statistically different from LPS alone ($p<0.05$).

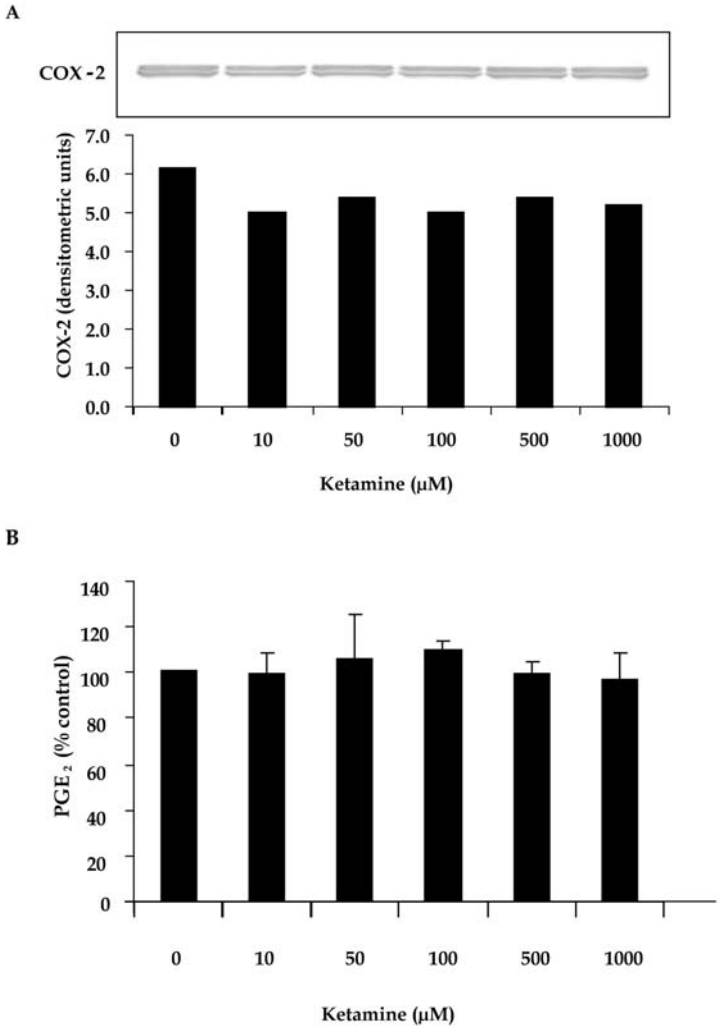

Figure 3. Effect of high experimental ketamine concentrations on LPS-induced (A) COX-2 expression and (B) PGE_2 production in RAW 264.7 cells. (A) Cells were stimulated with LPS (1 μg/mL) and ketamine (0-1000 μM) for 24 h. Following incubation, cells were lysed and lysates were assayed for COX-2 expression by Western blot analysis. A representative Western blot of three independent experiments with densitometric quantification of the COX-2 bands is shown. (B) PGE_2 production in RAW 264.7 cells was measured by radio-immunoassay (RIA) after 24 h of exposure to LPS (1 μg/mL) and ketamine (0-1000 μM). LPS alone was regarded as a positive control and set at 100%. Data are expressed as mean ± S.D. of three independent experiments in triplicate.

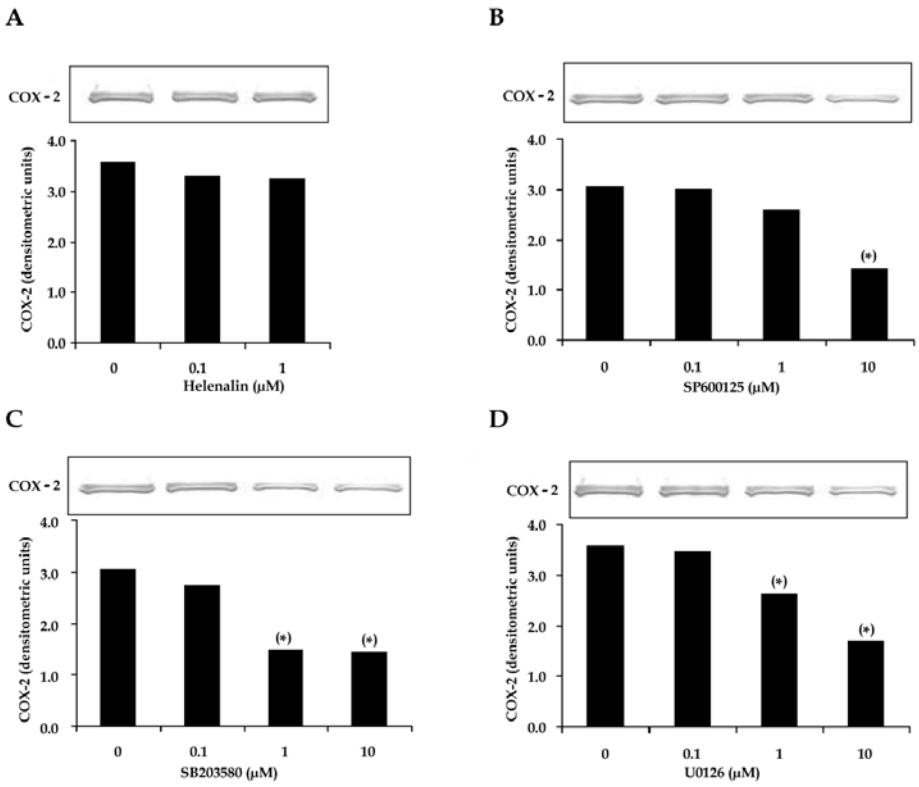

Figure 4. Effect of (A) helenalin, (B) SP600125, (C) SB203580 and (D) U0126 on LPS-induced COX-2 expression in e-CAS cells. Cells were incubated with the specific inhibitors of NF-κB (helenalin; 0-1 μM), JNK (SP600125; 0-10 μM), p38 (SB203580; 0-10 μM) and ERK (U0126; 0-10 μM) and LPS (1 μg/mL) for 24 h. Following incubation, cells were lysed and lysates were assayed for COX-2 expression by Western blot analysis. Representative Western blots of three independent experiments with densitometric quantification of the COX-2 bands are shown. (*) statistically different to LPS alone in the corresponding experiments ($p<0.05$).

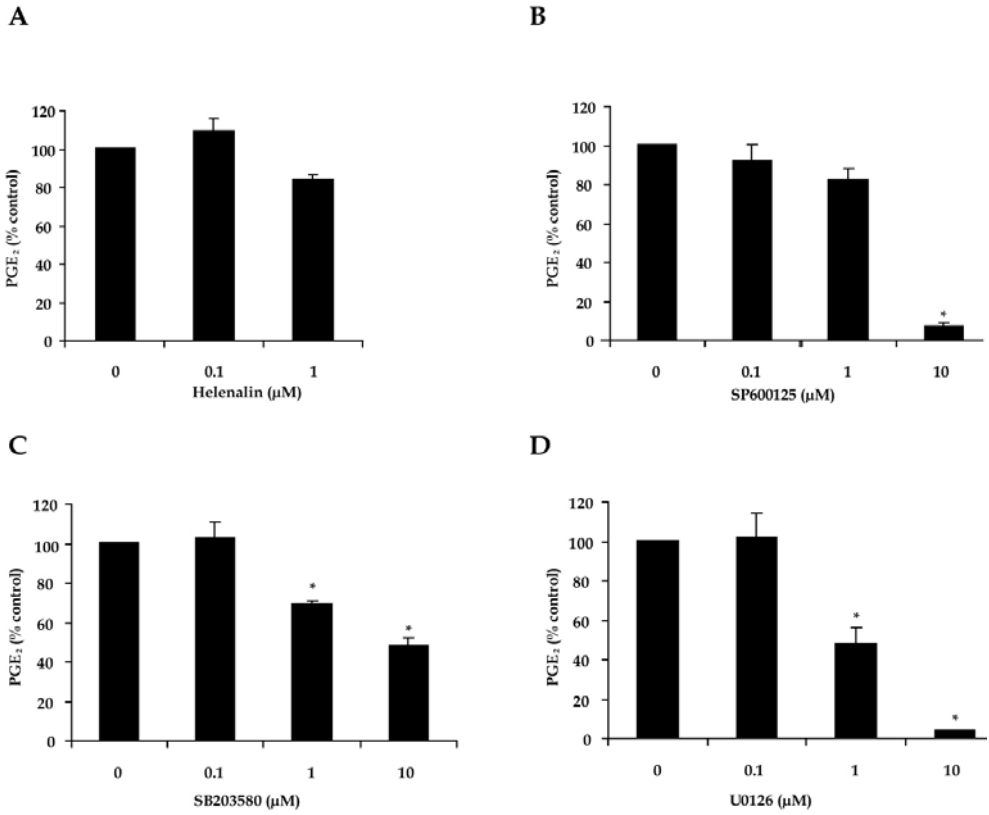

Figure 5. Effect of (A) helenalin, (B) SP600125, (C) SB203580 and (D) U0126 on LPS-induced PGE_2 production in e-CAS cells. Cells were incubated with the specific inhibitors of NF-κB (helenalin; 0-1 μM), JNK (SP600125; 0-10 μM), p38 (SB203580; 0-10 μM) and ERK (U0126; 0-10 μM) and LPS (1 μg/mL) for 24 h. Following incubation, PGE_2 production in RAW 264.7 cells was measured by radio-immunoassay (RIA). LPS alone was regarded as a positive control and set at 100%. Data are expressed as mean ± S.D. of three independent experiments in triplicate. *statistically different from LPS alone ($p<0.05$).

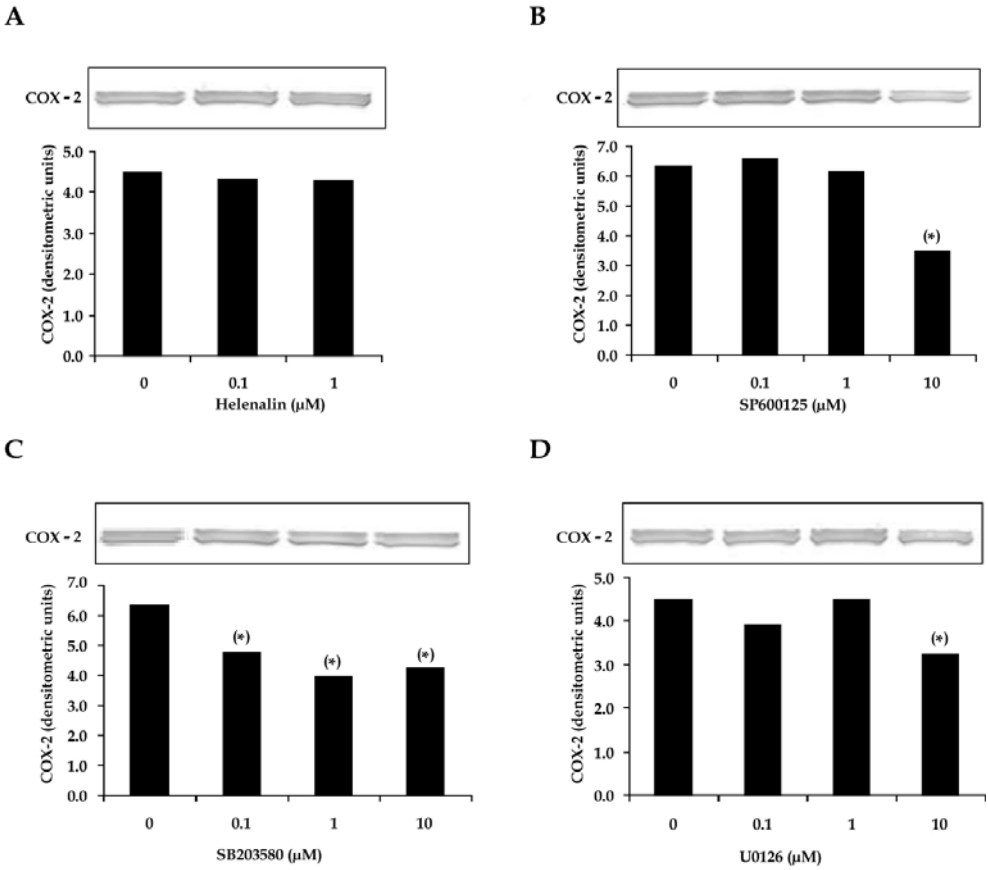

Figure 6. Effect of (A) helenalin, (B) SP600125, (C) SB203580 and (D) U0126 on LPS-induced COX-2 epxression in RAW 264.7 cells. Cells were incubated with the specific inhibitors of NF-κB (helenalin; 0-1 μM), JNK (SP600125; 0-10 μM), p38 (SB203580; 0-10 μM) and ERK (U0126; 0-10 μM) and LPS (1 μg/mL) for 24 h. Following incubation, cells were lysed and lysates were assayed for COX-2 expression by Western blot analysis. Representative Western blots of three independent experiments with densitometric quantification of the COX-2 bands are shown. (*) statistically different to LPS alone in the corresponding experiments ($p<0.05$).

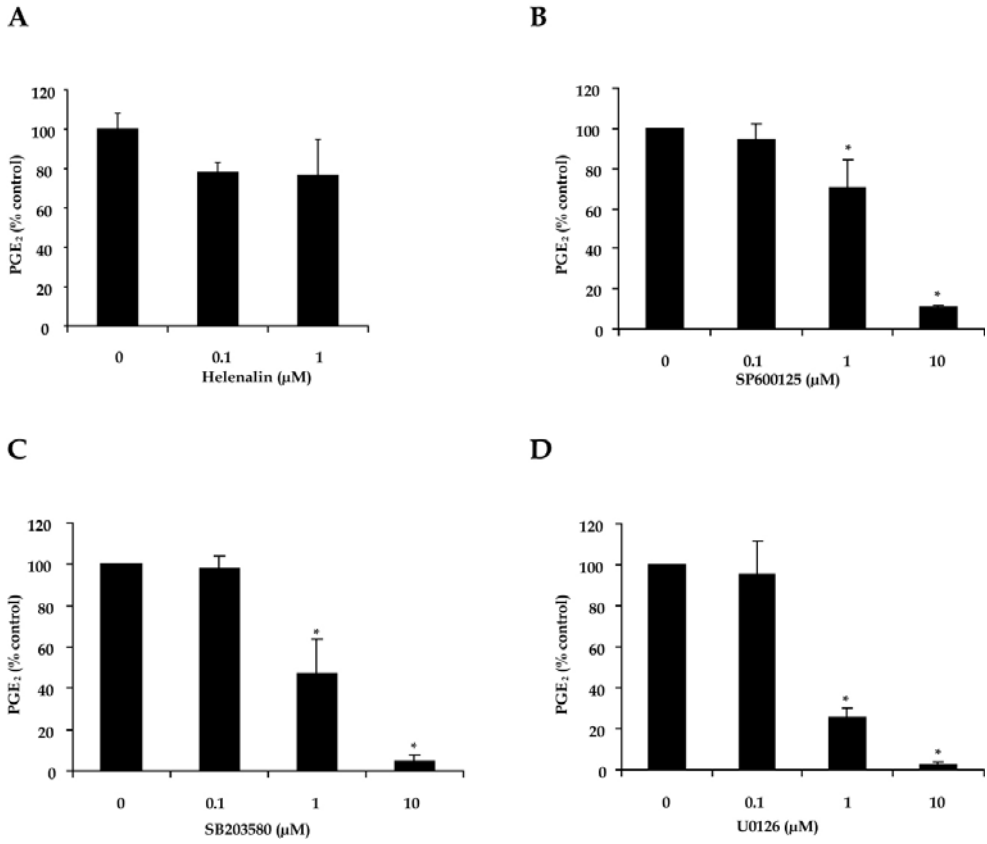

Figure 7. Effect of (A) helenalin, (B) SP600125, (C) SB203580 and (D) U0126 on LPS-induced PGE_2 production in RAW 264.7 cells. Cells were incubated with the specific inhibitors of NF-κB (helenalin; 0-1 μM), JNK (SP600125; 0-10 μM), p38 (SB203580; 0-10 μM) and ERK (U0126; 0-10 μM) and LPS (1 μg/mL) for 24 h. Following incubation, PGE_2 production in RAW 264.7 cells was measured by radio-immunoassay (RIA). LPS alone was regarded as a positive control and set at 100%. Data are expressed as mean ± S.D. of three independent experiments in triplicate. *statistically different from LPS alone ($p<0.05$).

4. DISCUSSION

Expression of COX-2 and subsequent PGE_2 synthesis have been associated with different inflammatory conditions such as colitis,[31] ischemia-reperfusion injury,[32] synovitis,[33] osteoarthritis,[34] osteochondrosis dissecans[35] and equine endotoxaemia.[36]

Inhibitors of COX-2, like the non-steroidal anti-inflammatory drugs (NSAIDs), are commonly used to reduce COX-2 expression and subsequent PGE_2 production, resulting in reduction of the clinical signs of inflammation.[37] In the present study, it was hypothesised that ketamine might reduce COX-2 expression as well, since COX-expression has been described to be regulated by NF-κB and ketamine has been found to possess cytokine-modulating effects by directly inhibiting NF-κB expression.[1,17,38,39] However, in both e-CAS and RAW 264.7 cells, ketamine did not reduce COX-2 expression at any of the tested concentrations.

It was previously assumed that LPS-induced COX-2 expression is controlled by an array of signalling molecules, including NF-κB and the three MAPKs (i.e., JNK, p38 and ERK).[21,24,40] However, the results of the present study demonstrate that LPS-induced COX-2 expression is not regulated via the NF-κB pathway, which is in line with the results found by Wadleigh et al.[41] The inhibition experiments in e-CAS and in RAW 264.7 cells revealed that LPS-induced COX-2 expression and PGE_2 production are predominantly regulated by the MAPKs ERK, p38 and JNK. In a previous experimental study regarding LPS-treated e-CAS cells, ketamine was found to significantly affect the LPS-induced signal transduction pathway only at the level of NF-κB.[1] This finding and the fact that COX-2 expression is not regulated by NF-κB, explains the failure of ketamine to significantly reduce LPS-induced COX-2 expression in the present study. However, since the highest concentration of ketamine (1000 μM) did significantly reduce PGE_2 production in LPS-treated e-CAS cells, an effect of this ketamine concentration on LPS-induced MAPK expression can not be fully excluded.

The clinically relevant ketamine concentrations used in this study were selected to mimic the plasma level of 5 μg/ml (18 μM) following the IV induction dose of 2.2 mg/kg body weight commonly used in horses.[42] Moreover, at these concentrations ketamine has been shown to reduce NF-κB expression in e-CAS cells.[1] Since NF-κB regulates COX-2 expression, initially clinically relevant ketamine concentrations were tested. As these ketamine concentrations did not affect LPS-induced COX-2 expression and PGE_2 production in e-CAS cells, high experimental ketamine

concentrations up to 1000 μM were tested to study if ketamine could affect LPS-induced COX-2 expression and PGE_2 production at all.

Ketamine is primarily used for its anaesthetic and analgesic properties. The analgesic potency of ketamine is achieved by its binding to N-methyl-D-aspartate (NMDA) receptors at which ketamine acts as an antagonist, resulting in the reduction of pathological pain states such as hyperalgesia, allodynia and the 'wind-up' phenomenon.[43-45] Increased PGE_2 concentrations are known to mediate hyperalgesia. Nishihara et al.[25] found that ketamine inhibits PGE_2-induced hyperalgesia via a NMDA-dependent mechanism. In addition, the results of the present study strongly suggest that the reduced PGE_2-induced hyperalgesia following ketamine exposure is not mediated by inhibiting COX-2 expression.

In conclusion, in LPS-treated e-CAS cells COX-2 expression is not regulated via the transcription factor NF-κB, but via the three classical MAPK pathways. Moreover, ketamine does not affect LPS-induced COX-2 expression in e-CAS cells. These findings are in line with previous data showing that ketamine affects the LPS-induced signal transduction pathway only at the level of NF-κB.

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