- CHAPTER V -

EFFECTS OF KETAMINE ON INDUCIBLE NITRIC OXIDE SYNTHASE EXPRESSION AND NITRIC OXIDE SYNTHESIS IN AN EQUINE MACROPHAGE CELL LINE FOLLOWING LPS EXPOSURE

D.P.K. Lankveld[1] – M.A.M. van der Doelen[2] - P. van Dijk[1]
L.J. Hellebrekers[1,3] – J. Fink-Gremmels[2]

[1]Division of Anaesthesiology and Intensive Care and [2]Division of Veterinary Pharmacology Pharmacy and Toxicology, Department of Equine Sciences, Faculty of Veterinary Medicine, Utrecht University, The Netherlands
[3]Division of Anaesthesiology, Department of Clinical Sciences of Companion Animals, Faculty of Veterinary Medicine, Utrecht University, The Netherlands

SUMMARY

Ketamine has been found to reduce lipopolysaccharide (LPS)-induced tumour necrosis factor alpha (TNF-α) and interleukin-6 (IL-6) concentrations in an equine macrophage cell line (e-CAS cells). This effect could be attributed to a direct inhibition of nuclear factor-kappa B (NF-κB) expression. NF-κB is one of the regulatory transcription factors associated with the expression of pro-inflammatory mediators, including cytokines, and inducible enzymes like inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2). Inducible NOS and its product nitric oxide (NO) are involved in various inflammatory disorders. Inhibiting NO production by blocking iNOS expression reduces the clinical signs of inflammation.

In the present study, the effect of ketamine (0-1000 µM) on LPS-induced iNOS expression and NO production was investigated in e-CAS cells. Furthermore, the intracellular signalling pathways, c-Jun N-terminal kinase (JNK), mitogen-activated protein kinase p38 (p38), extracellular signal-regulated kinase (ERK) and NF-κB were selectively inhibited, with the aim to elucidate the mechanisms underlying LPS-induced iNOS expression and NO production. For comparison, RAW 274.6 cells were used.

The results show that ketamine does not affect the LPS-induced iNOS expression and NO production in e-CAS cells at any of the concentrations tested. Inhibition experiments revealed that following LPS activation, different mitogen-activated protein kinases (MAPKs), including JNK, p38, ERK, as well as NF-κB, contribute to the increased iNOS expression and NO production in e-CAS cells. In previous experiments with e-CAS cells, ketamine has been found to inhibit the LPS-induced signalling pathways only at the level of NF-κB,[1] which alone seems not to be sufficient to suppress iNOS induction. Hence, despite its potency to reduce LPS-induced TNF-α and IL-6 production, the role of ketamine in iNOS and NO associated equine inflammatory disorders might be limited.

1. INTRODUCTION

Ketamine is a dissociative anaesthetic agent which is frequently used in equine practice for both the induction and maintenance of general anaesthesia in horses.[2,3] As a non-competitive N-methyl-D-aspartate (NMDA) receptor antagonist it produces superficial sleep combined with profound analgesia.[2-4] Ketamine preserves respiratory and cardiovascular functions and is therefore advocated for use in cardiovascular depressed patients.[5] Furthermore, ketamine has been found to possess cytokine-modulating effects in rodents[6-8] and humans.[9,10] Previously, we demonstrated that in an equine macrophage cell line (e-CAS cells), ketamine inhibits the LPS-induced release of both tumour necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) by directly inhibiting nuclear factor-kappa B (NF-κB) expression in a concentration-dependent manner, even at subanaesthetic concentrations[1,11] NF-κB is a transcription factor, which is normally sequestered in the cytoplasm as a dimer, coupled to its inhibitory protein, inhibitor of kappa B (IκB). Exposure of responsive cells 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).[12-15] Alternatively, the transcription of many of these pro-inflammatory mediators can be induced 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).[16,17]

Inducible NOS (iNOS) catalyses the production of nitric oxide (NO) by conversion of L-arginine to L-citrulline.[12,18] It is absent in resting cells, but in response to LPS, expression of iNOS increases, thereby generating large amounts of NO within several hours and for as long as 5 days.[12,19-21] Excessive NO production by LPS-induced iNOS may lead to an array of detrimental effects such as vasodilation, increased vascular permeability and oedema.[22-24] In addition, excessive NO production might result in the formation of peroxynitrite ($ONOO^-$), that acts as a radical and thus contributes to tissue damage.[25,26] Therefore, inhibiting NO production by blocking iNOS expression reduces the pathophysiological consequences of (local) inflammatory processes.

Considering the effect of ketamine on LPS-induced TNF-α and IL-6 production by directly inhibiting NF-κB, it was hypothesised that ketamine might also reduce LPS-induced iNOS expression and NO production. In previous experimental

studies, ketamine has been found to exert conflicting effects on LPS-induced iNOS expression and NO production. For example, in vitro, ketamine inhibited LPS-induced iNOS expression and NO production in a murine macrophage-like cell line[27] and in rat alveolar macrophages.[28] Moreover, ketamine blunted iNOS expression in several abdominal organs of endotoxin-challenged rats.[29,30] In contrast, in an ex vivo study performed by Shibakawa et al.,[31] no significant inhibition of nitrite release in LPS-treated primary glial cell cultures following ketamine exposure was found.

In the present study, the influence of ketamine on LPS-induced iNOS expression and NO production in an equine macrophage cell line was investigated. Furthermore, the intracellular signalling pathways, JNK, p38, ERK and NF-κB were selectively inhibited, with the aim to elucidate the mechanisms underlying LPS-induced iNOS expression and NO production. To support the validity of the e-CAS model, a murine macrophage-like cell line (RAW 264.7) was used for comparison.

2. MATERIALS AND METHOD

2.1. CHEMICALS

Ketamine, lipopolysaccharide (LPS; Escherichia coli, O111:B4), sodium pyrophosphate, ethylenediaminotetraacetic acid (EDTA), Igepal (CA-630), sodium dodecyl sulphate (SDS), Tris, glycerol, bromphenolblue, bovine serum albumin (BSA), nitroblue tetrazolium (NBT), 5-bromo-4-chloro-3-indolyl phosphate (BCIP), dithiothreitol (DTT), sulphanilamide, N-(1-Naphtyl)ethylenediamine.2HCL (NED) 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). Phosphoric acid was obtained from Boom B.V. (Meppel, The Netherlands). Alamar Blue (AB) 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 and sodium nitrite were 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-iNOS was from Cayman Chemical Company (Tallinn, Estonia). Alkaline phosphatase conjugated with polyclonal goat anti-rabbit immunoglobulin was purchased from Dako Cytomation (Glostrup, Denmark).

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.,[32] 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 IU/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 IU/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 NO production and relative expression of iNOS protein, cells were seeded in 24-well plates at a density of 0.2 x 10^6 cells per well (1 mL) and in 6-wells plates at a density of 1.0 x 10^6 cells per well (2 mL), respectively. Following 24 h of culturing, different experiments were performed as described below.

2.3. TIME RESPONSE CURVE

To determine the time-dependent NO 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. Nitric oxide production and cell viability were determined every 4 h following 4 - 48 h of incubation.

2.4. EXPOSURE TO LPS AND KETAMINE

To study the influence of ketamine on LPS-induced iNOS expression and NO 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, iNOS expression, NO 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 experimental concentration range of ketamine (0-1000 µM).

2.5. INHIBITION EXPERIMENTS

To investigate the potential involvement of MAP kinases and NF-κB in LPS-induced iNOS expression and NO 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), ERK (U0126; 0-10 μM) or NF-κB (helenalin; 0-1 μM). To the control samples, equal amounts of inhibitor solvent (0.1% DMSO) and/or the LPS solvent (PBS) were added. iNOS expression, NO production and cell viability were determined following 24 h of incubation.

In RAW 264.7 cells, parallel inhibition experiments were only performed for LPS-induced iNOS expression in the presence and absence of the above mentioned inhibitors

2.6. DETERMINATION OF NITRIC OXIDE

Nitric oxide production was determined by measuring nitrite (NO^{2-}) accumulation in the culture medium using the spectrophotometric Griess assay.[33] Briefly, 200 μl of supernatant was transferred to a 96-well plate and incubated with 20 μl sulphanilamide (1 % w/v) in phosphoric acid (5%) for 10 minutes at room temperature (RT). Subsequently, 20 μl N-(naphtyl)-ethylenediamine dihydrochloride (1.4 % w/v) was added and 2 minutes later the optical density of the reaction mixture was read at 540 nm using a microplate reader (Bio Rad Model 3550 Microplate Reader). Nitrite concentrations were calculated by using a standard curve of sodium nitrite ($NaNO_2$) dissolved in culture medium.

2.7. WESTERN BLOT ANALYSIS

Expression of iNOS protein was determined by Western blot analysis. In brief, after exposure to the indicated test compounds (LPS and inhibitors), 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 according to the manufacturer's instruction (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 (8%) 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-iNOS diluted

1:1000) for 1 h at RT. After washing, blots were incubated with secondary antibodies (alkaline phosphatase conjugated polyclonal goat anti-rabbit IgG diluted 1:1000) for 1 h at RT. Immunoreactive protein bands were developed by using nitroblue tetrazolium and bromochloroindolyl phosphate as substrates and quantified through densitometric analysis.

2.8. CELL VIABILITY

The Alamar Blue ™ (AB) reduction cytotoxicity assay was used as an indicator of cell viability.[34] 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°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 of NO production are presented as mean ± standard deviation (S.D.) of three independent experiments carried out in triplicate. 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 significant differences between experimental groups (Dunnett's). Data were considered to be statistically significant when $p<0.05$.

3. RESULTS

3.1. TIME-DEPENDENT NO PRODUCTION IN LPS-STIMULATED E-CAS CELLS

To determine the time response for NO production in LPS-treated e-CAS cells, NO production and cell viability were determined every 4 h following 4 - 48 h of LPS (1 μg/mL) exposure. Over time, a significant increase in LPS-induced NO production was demonstrated (Fig. 1). An apparent steady state level was observed between 16 and 24 h of exposure, after which a second increase in NO production was documented. Cell viability remained above 90% until 28 h of LPS exposure. Thereafter, viability significantly decreased towards the end of the experiment ($p<0.05$). Based on these results, iNOS expression, NO production and cell viability were determined at 24 h of LPS exposure in the further experiments with e-CAS cells.

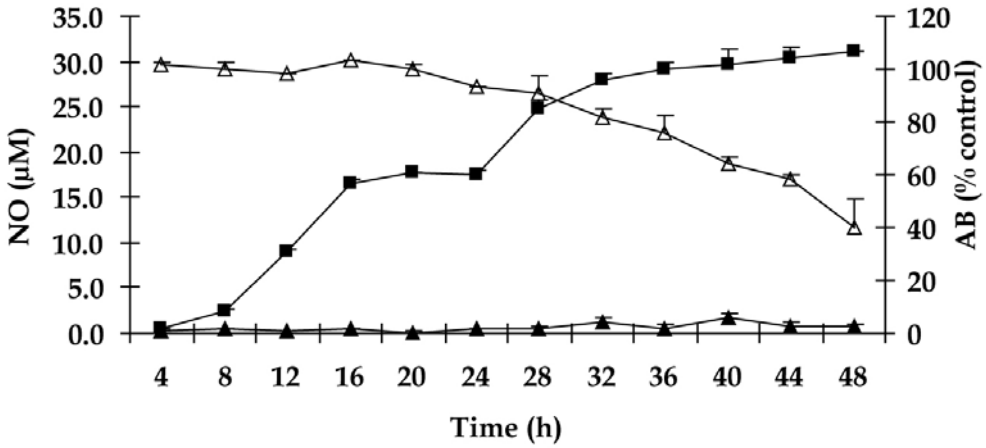

Figure 1. Time-response curve of NO production in e-CAS cells. Cells were incubated with serum-free RPMI containing no (▲) or 1 μg/mL (■) LPS. NO production and cell viability (△) were determined every 4 hrs following 4 - 48 h of incubation. Data are expressed as mean ± S.D. of two independent experiments 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 iNOS EXPRESSION AND NO PRODUCTION IN E-CAS CELLS

To study the influence of ketamine on LPS-induced iNOS expression and NO production 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). Exposure to LPS significantly increased iNOS expression and NO production (from 2.2 ± 0.5 to 25.2 ± 0.7 μM) in e-CAS cells. At the concentrations tested, ketamine did not affect the LPS-induced iNOS expression and NO production.

Subsequently, cells were exposed to high experimental ketamine concentrations (0-1000 μM), but again no effect on LPS-induced iNOS expression and NO production could be observed (Fig. 2). Control experiments revealed that ketamine alone, in the absence of LPS, did not alter iNOS expression or NO production (data not shown).

3.4. EFFECT OF KETAMINE ON LPS-TREATED RAW 264.7 CELLS

Western blot analysis showed that the iNOS protein is undetectable in resting RAW 264.7 cells, but significantly increased in the presence of LPS. Like in e-CAS cells, none of the ketamine concentrations tested significantly inhibited LPS-induced iNOS expression in RAW 264.7 cells (Fig. 3A). In non-stimulated RAW 264.7 cells, NO production was low, varying between 0.1 and 0.3 µM. After stimulation with LPS (1 µg/mL), NO production significantly increased to 10.0 ± 0.6 µM. When RAW 264.7 cells were incubated with LPS and high experimental concentrations of ketamine, significant inhibition of NO production was only observed at 1000 µM (Fig. 3B).

3.5. EFFECT OF INHIBITORS OF NF-κB AND MAPK PROTEINS ON LPS-INDUCED iNOS EXPRESSION AND NO PRODUCTION IN E-CAS AND RAW 264.7 CELLS

In e-CAS cells, a concentration-dependent inhibition of LPS-induced iNOS expression by helenalin (NF-κB inhibitor), SP600125 (JNK inhibitor), SB203580 (p38 inhibitor) and U0126 (ERK inhibitor) could be determined (Fig. 4). Co-incubation of inhibitors with LPS reduced the LPS-stimulated NO production in e-CAS cells in a similar concentration-dependent manner as observed for the iNOS expression (Fig. 5). Helenalin significantly inhibited NO production in LPS-treated e-CAS cells at 0.1 and 1 µM by 22% and 81%, respectively. The MAPK inhibitors, SP600125, SB203580 and U0126, significantly blocked LPS-induced NO production at only 10 µM by 50%, 30% and 36%, respectively. Control experiments confirmed that in absence of LPS, none of the inhibitors induced iNOS expression or NO production (data not shown).

Similarly, in RAW 264.7 cells, inhibition of NF-κB and the three MAPK isoforms significantly suppressed the stimulatory effect of LPS on iNOS expression (Fig. 6).

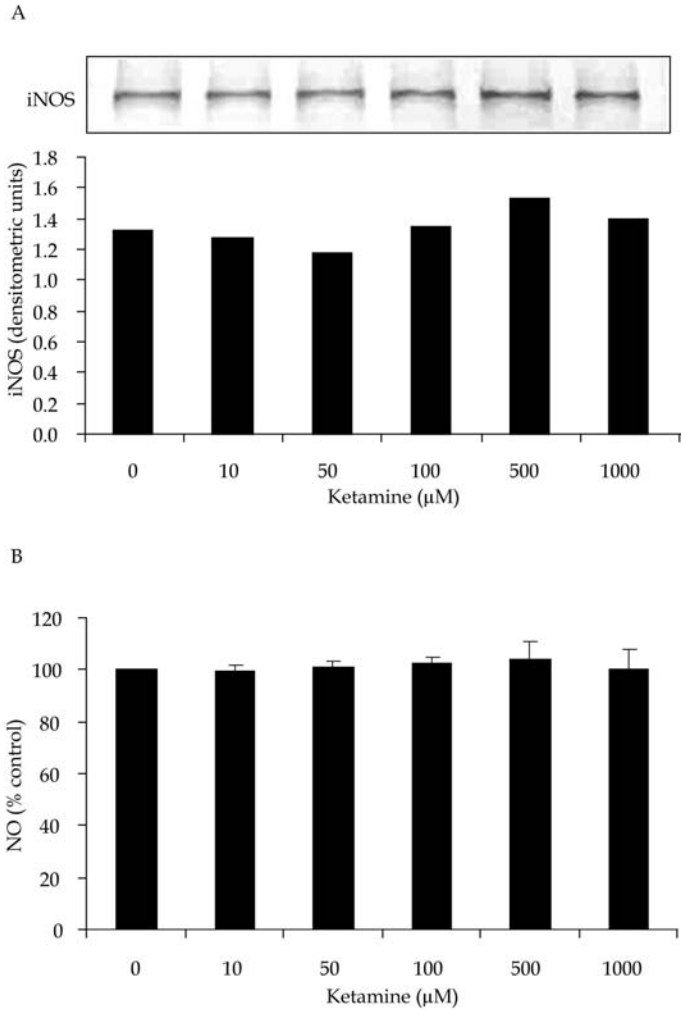

Figure 2. Ketamine at high experimental concentrations does not affect LPS-induced (A) iNOS expression and (B) NO production in e-CAS cells. (A) Cells were incubated with LPS (1 μg/mL) and ketamine (0-1000 μM) for 24 h. Following exposure, cells were lysed and lysates were analysed for iNOS expression by Western blotting. A representative Western blot of three independent experiments with densitometric quantification of the iNOS bands is shown. (B) Cells were incubated with LPS (1 μg/mL) and ketamine (0-1000 μM) for 24 h. Following exposure, supernatants were analysed for NO production by Griess reaction. 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.

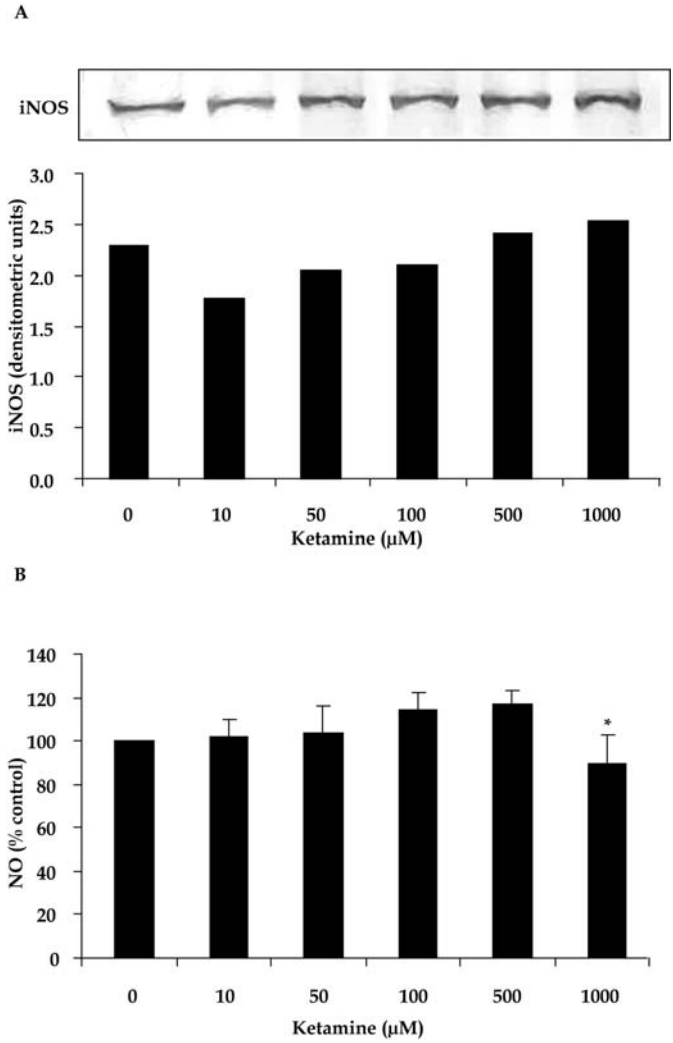

Figure 3. Ketamine at high experimental concentrations does not affect LPS-induced (A) iNOS expression and (B) NO production in RAW 264.7 cells. (A) Cells were incubated with LPS (1 μg/mL) and ketamine (0-1000 μM) for 24 h. Following exposure, cells were lysed and lysates were analysed for iNOS expression by Western blotting. A representative Western blot of three independent experiments with densitometric quantification of the iNOS bands is shown. (B) Cells were incubated with LPS (1 μg/mL) and ketamine (0-1000 μM) for 24 h. Following exposure, supernatants were analysed for NO production by Griess reaction. 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 to LPS alone ($p<0.05$).

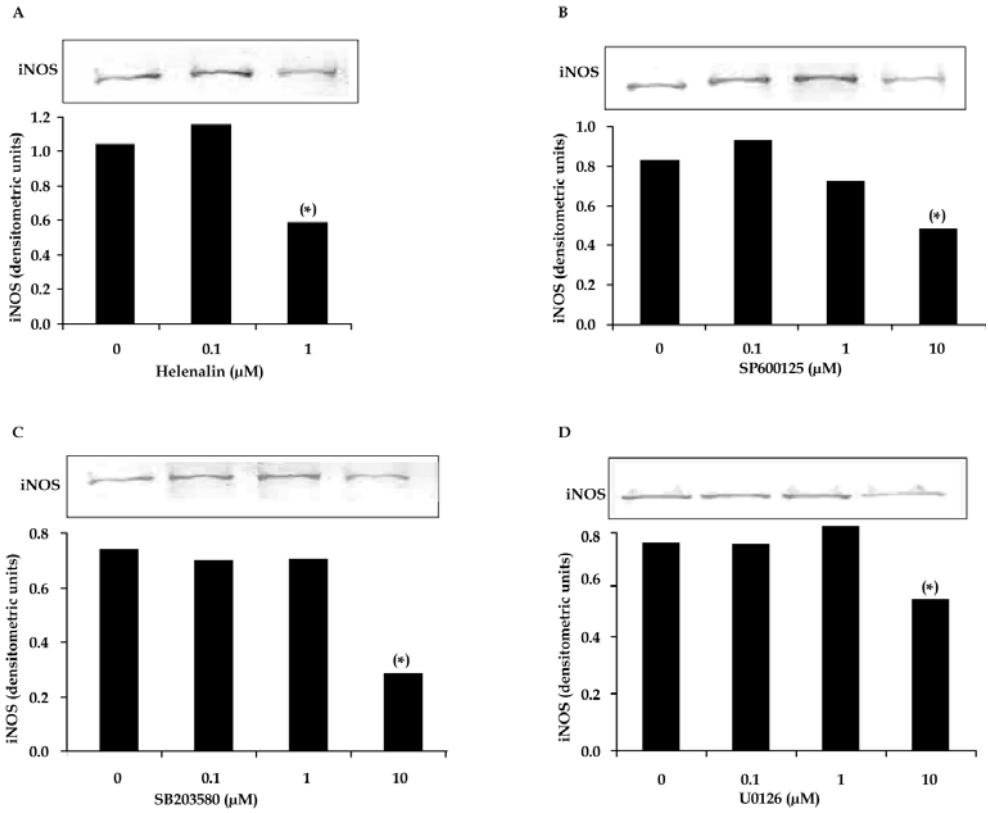

Figure 4. Inhibition of LPS-induced iNOS expression in e-CAS cells by NF-κB and MAPK inhibitors. Cells were incubated with LPS (1 μg/mL) and signal transduction inhibitors against (A) NF-κB (helenalin; 0-1 μM), (B) JNK (SP600125; 0-10 μM), (C) p38 (SB203580; 0-10 μM) or (D) ERK (U0126; 0-10 μM). Following 24 h of exposure, cells were lysed and lysates were analysed for iNOS expression by Western blotting. Representative Western blots of three independent experiments with densitometric quantification of the iNOS bands are shown. (*) statistically different to LPS alone in the corresponding experiments ($p<0.05$).

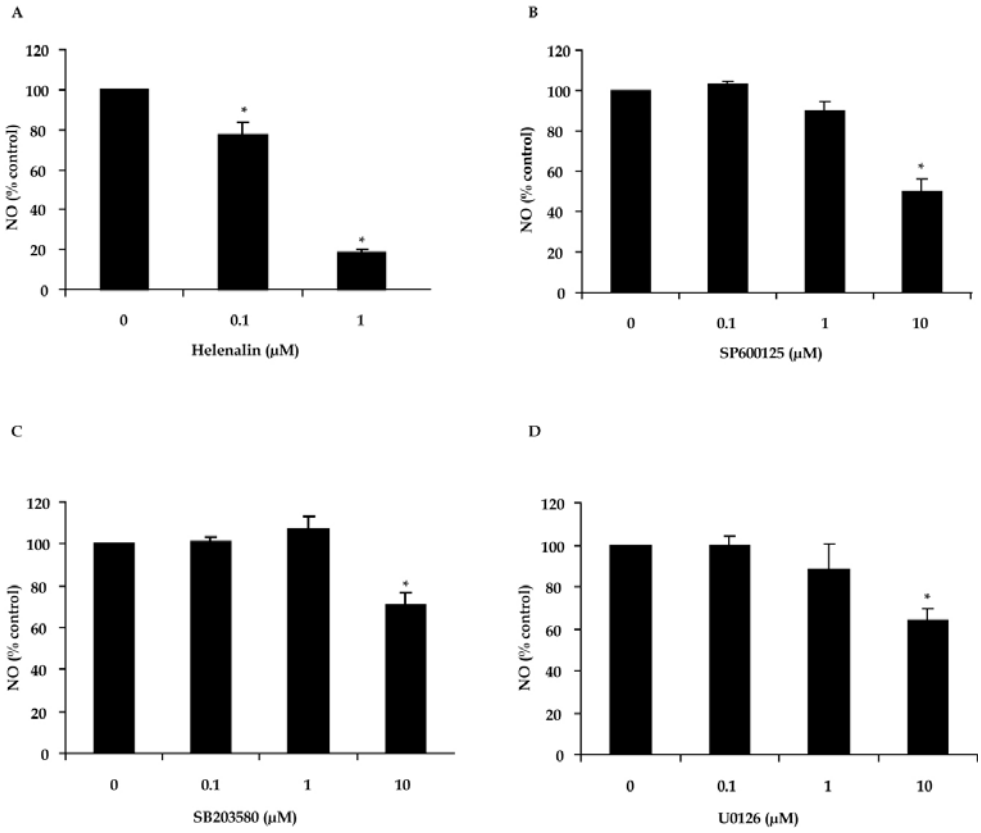

Figure 5. Inhibition of LPS-induced NO production in e-CAS cells by NF-κB and MAPK inhibitors. Cells were incubated with LPS (1 μg/mL) and signal transduction inhibitors against (A) NF-κB (helenalin; 0-1 μM), (B) JNK (SP600125; 0-10 μM), (C) p38 (SB203580; 0-10 μM) or (D) ERK (U0126; 0-10 μM). Following 24 h of exposure, supernatants were analysed for NO production by Griess reaction. Data are expressed as mean ± S.D. of three independent experiments carried out in triplicate. LPS alone was regarded as a positive control and set at 100%. * statistically different to LPS alone ($p<0.05$).

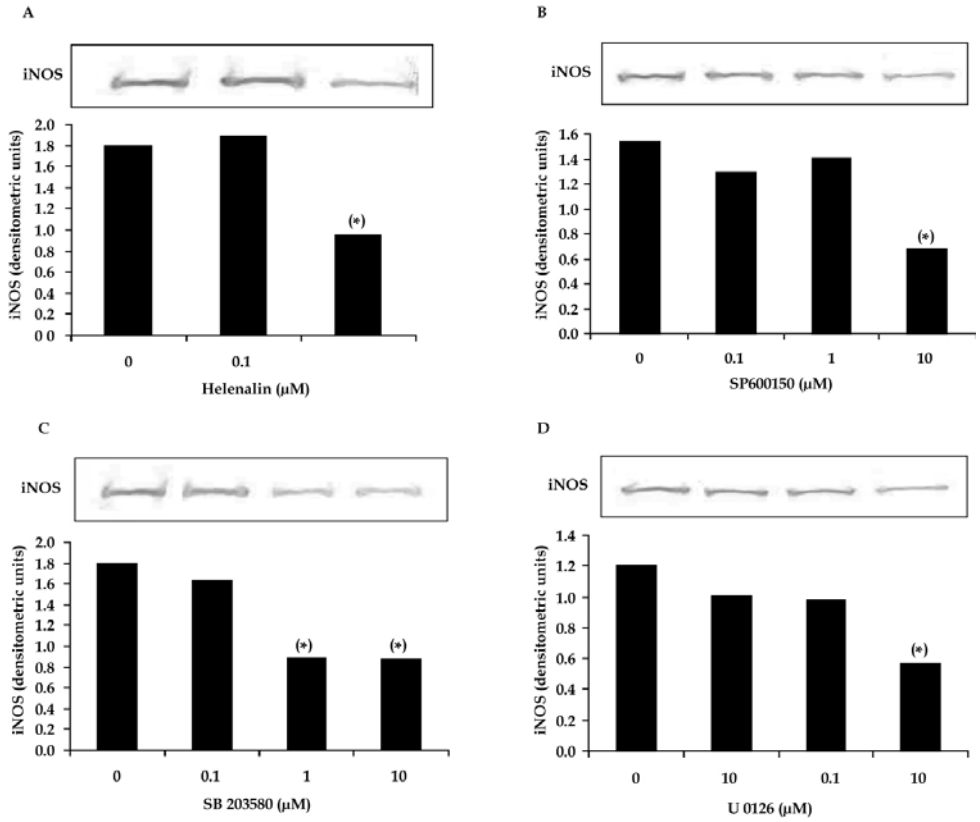

Figure 6. Inhibition of LPS-induced iNOS expression in RAW 264.7 cells by NF-κB and MAPK inhibitors. Cells were incubated with LPS (1 μg/mL) and signal transduction inhibitors against (A) NF-κB (helenalin; 0-1 μM), (B) JNK (SP600125; 0-10 μM), (C) p38 (SB203580; 0-10 μM) or (D) ERK (U0126; 0-10 μM). Following 24 h of exposure, cells were lysed and lysates were analysed for iNOS expression by Western blotting. Representative Western blots of three independent experiments with densitometric quantification of the iNOS bands are shown. (*) statistically different to LPS alone in the corresponding experiments ($p<0.05$).

4. DISCUSSION

In rodents and humans, iNOS and its product NO have been shown to be involved in many inflammatory disorders, such as arthritis, colitis, neurodegenerative diseases and septic shock.[16,19] In analogy to rodents and humans, it has been suggested that both iNOS and NO play a significant role in equine inflammatory diseases as well. Indeed, in vitro experiments showed that iNOS expression and NO production are significantly increased in LPS-treated equine alveolar macrophages,[35] and in articular chondrocytes.[36] In vivo, Mirza et al.[37] showed a significant increase in iNOS staining in mucosal leukocytes and mucosal vasculature in horses suffering from a strangulating large colon volvulus. Moreover, NO concentrations determined in articular cartilage, subchondral bone and the underlying trabecular bone were shown to be higher in horses suffering from osteoarthritis than in healthy horses.[38] In addition, both iNOS expression and NO production were shown to be higher in uterine biopsies and secretions of mares suffering from breeding-induced endometritis than in mares resistant to it.[39] The findings of these experiments suggest that inhibiting NO production by blocking iNOS expression might be beneficial in limiting the inflammatory response in horses.

Previous characterisation of e-CAS cells had already indicated that these cells are capable of expressing iNOS and producing NO in response to LPS.[32] In the present study, a time-dependent increase in NO production could be demonstrated. The delayed increase in NO production at 8 h following LPS treatment is consistent with the preceding onset of LPS-induced iNOS expression observed in the experimental studies carried out in murine macrophages.[40] The second increase following an apparent steady-state level between 16 and 24 h after LPS stimulation is thought to be related to an intracellular stress-response resulting from serum deprivation over a longer period. The latter is also reflected by simultaneous reduction in cell viability. Based on these data, the 24 h time point was selected to measure NO production in e-CAS cells in the further experiments.

The clinically relevant ketamine concentrations used in this study were selected in consideration of 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.[41] Moreover, at these concentrations ketamine has been shown to reduce NF-κB expression in e-CAS cells.[1] Since NF-κB regulates iNOS expression, initially the clinically relevant ketamine concentrations were tested. As these ketamine concentrations did not affect LPS-induced iNOS expression and NO production in e-CAS cells, high

experimental ketamine concentrations up to 1000 μM were tested to study if ketamine could affect LPS-induced iNOS expression and NO production at all.

The fact that ketamine did not inhibit LPS-induced iNOS expression and NO production at any of the concentrations tested in e-CAS cells is in contrast with results obtained from other experimental studies. In vitro, ketamine (10-100 μM) reduced iNOS expression and NO production in a concentration-dependent manner in LPS-treated rat alveolar macrophages.[28] Moreover, in J774 macrophages, ketamine concentrations of 30-600 significantly decreased NO production in response to LPS and interferon-γ (IFN-γ).[27] In vivo, ketamine blunted iNOS expression in the stomach, ileum and liver of LPS-treated rats.[29,30] The disparities between the literature reports and our findings might be due to interspecies differences and specific sensitivity of individual cell types and organs, but are more likely reflecting differences in the experimental protocols used. The prototypical inducer of iNOS expression is LPS, but IFN-γ is frequently added to increase iNOS expression and NO production in LPS-primed cells.[40,42] However, the molecular pathway by which LPS induces iNOS expression and NO production differs from that of IFN-γ. Lipopolysaccharide binds to CD14 and activates the transcription factor NF-κB via the IKK-IκB pathway, whereas IFN-γ binds to IFN-γ receptors at the cell membrane and stimulates the transcription factor IFN regulatory factor 1 via the JAK-STAT signalling pathway.[15,16,42] Hence, differences in outcome might be due to the fact that ketamine differently affects the distinct pathways involved in regulating iNOS expression.

The inhibition experiments presented in this paper indicate that both NF-κB and the MAPKs, JNK, p38, and ERK, are involved in the LPS-induced regulation of iNOS expression and NO production in e-CAS cells. Similar results were found in RAW 264.7 cells used in this study. These data correlate with previous inhibition experiments performed in other cell culture models.[43-47] However, in previous experiments with e-CAS cells, ketamine was found to significantly inhibit the LPS-induced signalling pathways only at the level of NF-κB.[1] This finding and the fact that NF-κB alone is not sufficient to suppress iNOS expression, explains the failure of ketamine to significantly reduce iNOS expression and the subsequent NO production.

In conclusion, ketamine does not affect the MAPK and NF-κB-mediated iNOS expression and subsequent NO production in LPS-treated e-CAS cells, which is in line with previous data showing that ketamine affects the LPS-induced signal transduction pathway only at the level of NF-κB.[1]

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