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Effects of inhibitors of the activity of cyclo-oxygenase-2 on the hypotension and multiple organ dysfunction caused by endotoxin: A comparison with dexamethasone

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1 Endotoxaemia is associated with the expression of the inducible isoform of cyclo-oxygenase, cyclo-oxygenase-2 (COX-2), and an overproduction of arachidonic acid (AA) metabolites. The role of the AA metabolites generated by COX-2 in the circulatory failure and multiple organ dysfunction caused by endotoxin is unclear. Dexamethasone prevents the expression of COX-2 and exerts beneficial effects in animal models of shock.

2 Here we compare the effects of two inhibitors of COX-2 activity, namely NS-398 (5 mg kg^{-1}, i.p., $n=7$) and SC-58635 (3 mg kg^{-1}, i.p., $n=9$) with those of dexamethasone (3 mg kg^{-1}, i.p., $n=9$) on the circulatory failure and organ dysfunction caused by lipopolysaccharide (LPS, *E. coli*, 6 mg kg^{-1}, i.v., $n=11$) in the rat.

3 Endotoxaemia for 6 h caused hypotension, acute renal dysfunction, hepatocellular injury, pancreatic injury and an increase in the plasma levels of 6-keto-$PGF_{1\alpha}$ (indicator of the induction of COX-2) and nitrite/nitrate (indicator of the induction of iNOS).

4 Pretreatment of rats with dexamethasone attenuated the hypotension, the renal dysfunction, the hepatocellular and pancreatic injury and the induction of COX-2 and iNOS caused by LPS. In contrast, inhibition of COX-2 activity with SC-58635 or NS-398 neither attenuated the circulatory failure nor the multiple organ failure caused by endotoxin.

5 Thus, the prevention of the circulatory failure and the multiple organ injury/dysfunction caused by dexamethasone in the rat is not due to inhibition of the activity of COX-2. Our results suggest that an enhanced formation of eicosanoids by COX-2 does not contribute to the development of organ injury and/or dysfunction in rats with endotoxaemia.

Keywords: Endotoxin shock; glucocorticosteroids; multiple organ failure; NS-398; prostaglandins; SC-58635

Introduction

There is substantial evidence that endotoxaemia is associated with an increase in arachidonic acid (AA) metabolites in blood and tissues (Feuerstein & Hallenbeck, 1987). In 1990, Needleman and colleagues discovered that endotoxin or lipopolysaccharide (LPS) substantially increases the formation of AA metabolites in human monocytes *in vitro* (Fu *et al*., 1990) and in mouse peritoneal macrophages *in vivo* (Masferrer *et al*., 1990). This increase was associated with the *de novo* synthesis of a new cyclo-oxygenase (COX) protein, termed COX-2, which is encoded by a different gene (located on chromosome 1) from that which encodes for the constitutive enzyme (COX-1), which is located on chromosome 9 (Yokoyama & Tanabe, 1989; Kosaka *et al*., 1994; Mitchell *et al*., 1995). The expression of COX-2 afforded by LPS in many different cell types is secondary to the activation of protein tyrosine kinases (Akarasereenont *et al*., 1995) and of the transcription factor NF-κB (Xie *et al*., 1992; see Mitchell *et al*., 1995 for reviews). The role of the overproduction of AA metabolites in the pathophysiology of endotoxic shock is not clear, as (i) many of the non-steroidal anti-inflammatory drugs (NSAIDs; e.g. indomethacin, ibuprofen) which have been tested in animal models of shock are at best non-selective inhibitors of COX-1 and COX-2 (Mitchell *et al*., 1993) and (ii) there are no studies evaluating the effects of selective inhibitors of COX-2 activity in animal models of shock.

We have recently demonstrated that the expression of COX-2 caused by LPS in the rat *in vivo* is (i) mediated by the nuclear transcription factor NF-κB and (ii) prevented by inhibitors of the activation of this transcription factor (e.g. calpain inhibitor I) as well as by dexamethasone (Ruetten & Thiemermann, 1997). Interestingly, prevention by dexamethasone and calpain inhibitor I of the expression of COX-2 was associated with beneficial effects on the circulatory and multiple organ failure caused by endotoxin. However, it is unclear whether the prevention by dexamethasone of the expression of COX-2 contributes to the beneficial effects of this glucocorticosteroid in rats with endotoxaemia.

Here we investigated whether an enhanced formation of AA metabolites contributes to the circulatory failure and the multiple organ injury and dysfunction caused by LPS in the rat. In addition, we have evaluated whether the beneficial effects of dexamethasone in endotoxaemia are secondary to the prevention of COX-2 expression. To achieve these two goals, we have compared the effects of two novel and selective inhibitors of COX-2 activity with those of dexamethasone on haemodynamics and organ (e.g. kidney, liver, pancreatic) injury and/or dysfunction in rats with endotoxaemia. We have also investigated the effects of these drugs on the alterations in the plasma levels of 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$; an indicator of the overproduction of AA metabolites by COX-2) and nitrite and nitrate (an indicator of the formation of nitric oxide (NO) by iNOS) in rats with endotoxaemia. The inhibitors of COX-2 activity used in this study were SC-58635,

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a recently discovered, highly selective inhibitor of COX-2 activity (Penning *et al*., 1997), and NS-398, a well-documented, selective inhibitor of COX-2 activity *in vitro* and *in vivo* (Futaki *et al*., 1994).

Methods

Measurement of haemodynamic changes

This study was carried out on 68 male rats (Tuck, Rayleigh, Essex, U.K.) weighing 240–320 g receiving a standard diet and water *ad libitum*. All animals were anaesthetized with thiopentone sodium (Intraval Sodium; 120 mg kg^{-1}, i.p.) and anaesthesia was maintained by supplementary injections of thiopentone sodium as required. The trachea was cannulated to facilitate respiration and rectal temperature was maintained at 37°C with a homeothermic blanket (BioScience, Sheerness, Kent, U.K.). The left carotid artery was cannulated and connected to a pressure transducer (Senso-Nor 840, Senso-Nor, Horten, Norway) for the measurement of phasic and mean arterial blood pressure (MAP) and heart rate (HR) which were displayed on a data acquisition system (MacLab 8e, ADI Instruments, Hastings, U.K.) installed on an Apple Macintosh computer. The femoral vein was cannulated for the administration of drugs. The bladder was also cannulated to facilitate urine flow and to prevent the possibility of the development of post-renal failure. Upon completion of the surgical procedure, cardiovascular parameters were allowed to stabilize for 15 min.

Experimental design

After the recording of baseline haemodynamic parameters and 30 min before the injection of LPS, animals were pretreated with either dexamethasone (3 mg kg^{-1}, i.p., $n=9$) or vehicle (saline, $n=11$). This dose of dexamethasone prevents the expression of COX-2 in rats challenged with LPS (10 mg kg^{-1}, i.v.) (Ruetten & Thiemermann, 1997). To elucidate the effects of the inhibition of COX-2 activity in endotoxic shock, two further groups of animals received at 60 min before LPS a bolus injection of the COX-2 inhibitors SC-58635 (4-[5-(4-methylphenyl) -3-(trifluoromethyl) - 1H-pyrazol -1 -yl] benzene-sulphonamide; Penning *et al*., 1997; Riendeau *et al*., 1997) (3 mg kg^{-1}, i.p., $n=9$) or NS-398 (N-[2-(cyclohexyloxy)-4-nitrophenyl]methanesulphonamide; Futaki *et al*., 1994; Panara *et al*., 1995) (5 mg kg^{-1}, i.p., $n=7$). The dose regimens of SC-58635 and NS-398 used here have been previously shown to abolish the rise in the plasma levels of 6-keto-$PGF_{1\alpha}$ caused by infusion of LPS (0.2 mg kg^{-1} h^{-1} for 6 h) in the rat (Hamilton *et al*., 1997; 1998). As both of these COX-2 inhibitors were dissolved in dimethyl sulphoxide (DMSO), a further group of animals was pretreated with DMSO (1 μg ml^{-1}, 10% v/v, $n=5$). Pretreatment of rats with DMSO did not alter the effects of LPS on haemodynamics or organ dysfunction (data not shown). At time 0 (e.g. 1 h after administration of the COX-2 inhibitors or 30 min after administration of dexamethasone), all animals received *E. coli* lipopolysaccharide (LPS, 6 mg kg^{-1}, i.v.) as a slow injection over 15–20 min. It should be noted that all animals received a total fluid replacement of 4 ml kg^{-1} h^{-1} (as an i.v. infusion into the femoral vein) throughout the experiment. To elucidate the effects of the above interventions on any of the parameters measured in rats injected with vehicle rather than LPS, these animals received bolus injections of either saline ($n=8$), dexamethasone (3 mg kg^{-1}, $n=5$), SC-58635 (3 mg kg^{-1}, $n=4$), NS-398 (5 mg kg^{-1}, $n=5$ or DMSO (1 μg ml^{-1}, 10% v/v, $n=5$).

Quantification of organ function and injury

Six hours after the injection of LPS, 1.5 ml of blood was collected into a serum gel S/1.3 tube (Sarstedt, Germany) from a catheter placed in the carotid artery. The blood sample was centrifuged ($1610 \times g$ for 3 min at room temperature) to separate serum. All serum samples were analysed within 24 h by a contract laboratory for veterinary clinical chemistry (Vetlab Services, Sussex, U.K.). The following marker enzymes were measured in the serum as biochemical indicators of multiple organ injury/dysfunction: (1) Liver injury was assessed by measuring the rise in serum levels of alanine aminotransferase (ALT, a specific marker for hepatic parenchymal injury) and aspartate amino-transferase (AST, a non-specific marker for hepatic injury) (Baue, 1993; Hewett *et al*., 1993). (2) Renal dysfunction was assessed by measuring the rises in serum levels of creatinine (an indicator of reduced glomerular filtration rate, and hence, renal failure) and urea (an indicator of impaired excretory function of the kidney and/or increased catabolism). (3) Pancreatic injury was assessed by measuring the rise in serum lipase (see Thiemermann *et al*., 1995; Ruetten & Thiemermann, 1997).

Measurement of the plasma levels of 6-keto-$PGF_{1\alpha}$

At 6 h after the injection of LPS, 0.5 ml of blood was collected from the catheter placed in the carotid artery. The blood sample was centrifuged ($1610 \times g$ for 3 min at room temperature) to separate serum. The concentration of 6-keto $PGF_{1\alpha}$ present in the serum was measured by radioimmunoassay (Salmon, 1978). Briefly, eicosanoid standards (prepared in control plasma) or test samples (100 μl) were mixed with 100 μl aliquots of the antiserum for 6 keto-$PGF_{1\alpha}$ which was prepared in Tris-buffer (50 mM) containing 0.05% gelatin and 0.1% sodium azide. Aliquots of [^{3}H] 6 keto-$PGF_{1\alpha}$ (100 μl) which was also prepared in Tris-buffer containing gelatin and sodium azide (as above), were then added to the samples or standards in addition to 100 μl of Tris-buffer, giving a final reaction volume of 400 μl. Following vortex mixing, the tubes were left overnight at 4°C. After this incubation period, unbound radioactivity was removed by the addition of 200 μl of Tris-buffer (50 mM, pH 7.4) containing 0.02% charcoal and 0.004% dextran. The charcoal was incubated with the standards and samples at 4°C for 10 min. Samples were then centrifuged at $800 \times g$ for 10 min in a refrigerated (4°C) centrifuge (Beckman; Type TJ-6, High Wycombe, Bucks, U.K.). The supernatant containing the antigen-antibody complex was then decanted and mixed with 4 ml of scintillation fluid (Picofluor, Canberra Packard, Pangbourne, Berkshire, U.K.). Bound radioactivity within the samples was determined using a liquid scintillation counter (Beckman; Type 3801, High Wycombe, Bucks, U.K.).

The % cross-reactivity (at 50% displacement) of the antibody against 6-keto $PGF_{1\alpha}$ used in this study against other eicosanoids was the following: PGE_2, 11%; $PGF_{2\alpha}$, 10%; thromboxane B_2 (TXB_2), 0.05%. The detection limit of this assay for 6-keto $PGF_{1\alpha}$ is approximately 10 pg per tube.

Measurement of serum nitrate and nitrite

Nitrite and nitrate are the primary oxidation products formed when NO reacts with oxygen and, therefore, the nitrite/nitrate concentration in plasma can be used as an indicator of NO synthesis. Blood was collected into heparin-treated capillary tubes and centrifuged ($1610 \times g$ for 5 min at room temperature) to separate cells and plasma. The nitrate in the sample was enzymatically converted to nitrite according to the method of Schmidt *et al*. (1992). Briefly, nitrate was

stoichiometrically reduced to nitrite by incubation of sample aliquots (50 μl) for 15 min at 37°C, in the presence of nitrate reductase (1 u ml^{-1}, E.C. 1.6.6.2), NADPH (500 μM) and flavine adenine dinucleotide (FAD, 50 μM) in a final volume of 80 μl. When nitrate reduction was complete, the unused NADPH which interferes with the subsequent nitrite determination, was oxidized by lactate dehydrogenase (100 iu ml^{-1}) and sodium pyruvate (100 mM), in a final reaction volume of 100 μl and incubated for 5 min at 37°C. Subsequently, total nitrite in plasma was assayed by adding 100 μl of Griess reagent (4% sulphanilamide and 0.2% naphtylenediamide in 10% phosphoric acid) to 100 μl samples of serum (Green *et al*., 1981). The increase in optical density was measured at 550 nm (reference filter: 650 nm) with a Molecular Devices microplate reader (Richmond, CA, U.S.A.). Total nitrite/nitrate concentrations (μM) were calculated by comparison with the optical density of standard solutions (0–200 μM) of sodium nitrate (also stoichiometrically converted to nitrite) prepared in plasma.

Materials

Bacterial lipopolysaccharide (*E. coli* serotype 0.127:B8), trifluoroacetic acid, hydrochloric acid, sulphuric acid, sulphanilamide, naphthylethylenediamide, phosphoric acid, charcoal, antibody against 6-keto-PGF$_{1\alpha}$ and dexamethasone were obtained from Sigma Chemical Co. (Poole, Dorset, U.K.). Sodium thiopentone (Intraval) was obtained from Rhone Mérieux Ltd. (Harlow, Essex, U.K.). [^{3}H]-6-keto-PGF$_{1\alpha}$ (specific activity: 6.55 TBq mmol^{-1}) was obtained from Amersham International (Little Chalfont, Bucks, U.K.).

Statistical evaluation

All values in figures and text are expressed as mean ± s.e.mean of n observations, where n represents the number of animals or blood samples studied. The data were analysed by use of ANOVA followed by Dunnett's test for multiple comparisons. A P value of less than 0.05 was considered to be statistically significant.

Results

Effects of dexamethasone and two selective inhibitors of COX-2 activity on the circulatory failure caused by endotoxaemia

Baseline values of MAP in all groups of animals ranged from 118 ± 5 to 130 ± 4 mmHg, and were not significantly different between groups (Figure 1). In animals which received an injection of vehicle for LPS rather than LPS, administration of saline, DMSO, dexamethasone or of the COX-2 inhibitors SC-58635 or NS-398 did not result in any significant alterations in MAP (Table 1).

Infusion of LPS (6 mg kg^{-1}, i.v.) produced a progressive fall in MAP from 130 ± 4 mmHg (baseline, before infusion of LPS) to 83 ± 4 mmHg at 6 h ($P<0.05$, $n=11$, Figure 1). Treatment of rats which had received LPS with dexamethasone attenuated the hypotension caused by LPS ($P<0.05$ at 240 to 360 min, Figure 1). The fall in MAP in LPS-rats which had received NS-398 was more rapid in onset and significantly greater than the one observed in the LPS-control group between 120 min and 300 min after injection of LPS ($P<0.05$ at 120 to 300 min, Figure 1). Similarly, the fall in MAP observed in LPS-rats treated with SC-58635 was significantly greater than in the LPS-control group at 180 min and 240 min ($P<0.05$, Figure 1). Although the MAP in LPS-rats treated with either of these two COX inhibitors was lower at the end of the experiment than in the LPS-control group, this effect did not reach statistical significance (Figure 1).

Baseline values of heart rate in all groups of animals ranged form 359 ± 7 to 398 ± 10 beats min^{-1}, and were not significantly different between groups (Table 2). In animals which received an injection of vehicle for LPS rather than LPS, administration of saline, DMSO, dexamethasone or of the COX-2 inhibitors SC-58635 or NS-398 did not result in any significant alterations in heart rate (Table 1).

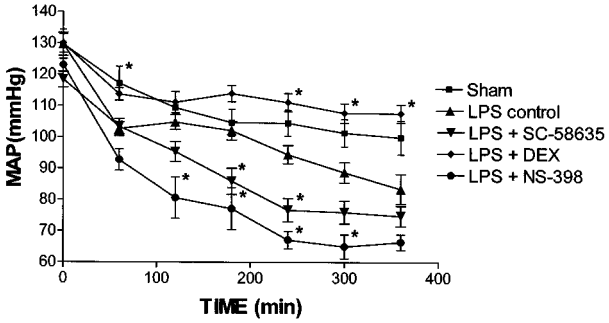

Figure 1 Comparison of the effects of dexamethasone with those of two inhibitors of COX-2 activity on the fall in mean arterial blood pressure (MAP) caused by LPS (6 mg kg^{-1}, i.v.) in the anaesthetized rat. Rats which had received LPS were treated with (i) vehicle (saline) rather than LPS (sham, $n=8$), (ii) vehicle (saline, 1 ml kg^{-1}, i.p.) plus LPS (LPS, $n=11$), (iii) LPS plus dexamethasone (DEX, $n=9$), (iv) LPS plus SC-58635 (SC, $n=9$) and (v) LPS plus NS-398 (NS, $n=7$). *$P<0.05$ represents significant difference when compared to LPS-control.

Table 1 Effects of different treatments on the serum levels of urea, creatinine, AST, ALT and lipase, the mean arterial blood pressure (T6 MPA) and heart rate (T6 HR) at 6 h after injection of vehicle (for LPS), and the plasma levels of nitrite/nitrate or 6-keto-PGF$_{1\alpha}$ in rats which had received vehicle for LPS (saline) rather than LPS

Treatment	*Urea* (mmol l^{-1})	*Creatinine* (μmol l^{-1})	*AST* (iu l^{-1})	*ALT* (iu l^{-1})	*Lipase* (iu l^{-1})	*T6 MAP* (mmHg)	*T6 HR* (beats min^{-1})	*[NO$_2$]/[NO$_3$]* (μmol l^{-1})	*6-keto-PGF$_{1\alpha}$* (ng ml^{-1})
Saline ($n=8$)	8 ± 1	35 ± 2	241 ± 19	83 ± 5	5 ± 3	100 ± 5	377 ± 10	16 ± 2	0.04 ± 0
DMSO ($n=5$)	6 ± 1	33 ± 5	221 ± 18	98 ± 17	47 ± 18*	114 ± 3	372 ± 19	21 ± 4	0.06 ± 0
Dexamethasone ($n=5$)	6 ± 0	32 ± 2	210 ± 234	70 ± 4	2 ± 2	105 ± 4	379 ± 5	15 ± 3	0.04 ± 0
SC-58635 ($n=4$)	6 ± 1	35 ± 2	311 ± 52	112 ± 19	20 ± 9	117 ± 3	407 ± 9	18 ± 3	0.04 ± 0
NS-398 ($n=5$)	6 ± 1	23 ± 3*	157 ± 16	77 ± 5	18 ± 8	102 ± 3	375 ± 5	24 ± 5	0.04 ± 0

*$P<0.05$ when compared to saline-control.

Table 2 Alterations in heart rate (HR) in rats treated with vehicle rather than LPS (saline), and LPS-rats pretreated with saline (LPS), dexamethasone (DEX), SC-58635 or NS-398

	Time (min)		
Group	*0*	*180*	*360*
Saline ($n=8$) HR (beats min^{-1})	368 ± 14	378 ± 9	377 ± 10
LPS ($n=11$) HR (beats min^{-1})	387 ± 8	409 ± 9	411 ± 14
DEX ($n=9$) HR (beats min^{-1})	344 ± 12	383 ± 8	395 ± 9
SC-58635 ($n=9$) HR (beats min^{-1})	345 ± 9	371 ± 14	368 ± 18
NS-398 ($n=7$) HR (beats min^{-1})	405 ± 14	410 ± 11	427 ± 18

When compared to rats which had received vehicle rather than LPS, endotoxaemia for 6 h did not result in a significant change in heart rate (Table 2). In rats treated with LPS, neither dexamethasone nor any of the two inhibitors of COX-2 activity had any significant effect on heart rate (Table 2).

Effects of dexamethasone and two selective inhibitors of COX-2 activity on the multiple organ dysfunction syndrome caused by endotoxaemia

In animals which received an injection of vehicle for LPS rather than LPS, administration of saline, DMSO, dexamethasone or of the COX-2 inhibitors SC-58635 or NS-398 did not result in any significant alterations in the serum levels of urea or creatinine (Table 1). When compared to rats which had received vehicle (saline) rather than LPS, endotoxaemia for 6 h resulted in significant rises in the serum levels of urea and creatinine (Figure 2a and b). Treatment of rats which had received LPS with dexamethasone attenuated the renal dysfunction caused by LPS ($P<0.05$, Figure 2a and b). In contrast, the COX-2 inhibitors SC-58635 or NS-398 did not affect the rise in the serum levels of urea and creatinine caused by LPS ($P>0.05$, Figure 2a and b).

In animals which received an injection of vehicle for LPS rather than LPS, administration of saline, DMSO, dexamethasone or of the COX-2 inhibitors SC-58635 or NS-398 did not result in any significant alterations in the serum levels of ALT and AST (Table 1). When compared to rats which had received vehicle (saline) rather than LPS, endotoxaemia for 6 h resulted in significant rises in the serum levels of ALT and AST (Figure 2c and d). Treatment of rats which had received LPS with dexamethasone attenuated the liver injury caused by LPS ($P<0.05$, Figure 2c and d). In contrast, the COX-2 inhibitors SC-58635 or NS-398 did not affect the rise in the serum levels of ALT and AST caused by LPS ($P>0.05$, Figure 2c and d).

In animals which received an injection of vehicle for LPS rather than LPS, administration of saline, dexamethasone or of the COX-2 inhibitors SC-58635 or NS-398 did not result in any significant alterations in the serum levels of lipase (Table 1). Surprisingly, DMSO caused a significant increase in the serum levels of lipase (indicating the development of pancreatic injury). When compared to rats which had received vehicle (saline) rather than LPS, endotoxaemia for 6 h resulted in a significant rise in the serum levels of lipase (Figure 2e). Treatment of rats which had received LPS with dexamethasone attenuated the pancreatic injury caused by LPS ($P<0.05$, Figure 2e). In contrast, the COX-2 inhibitors SC-58635 or NS-398 did not affect the rise in the serum levels of lipase caused by LPS ($P>0.05$, Figure 2e).

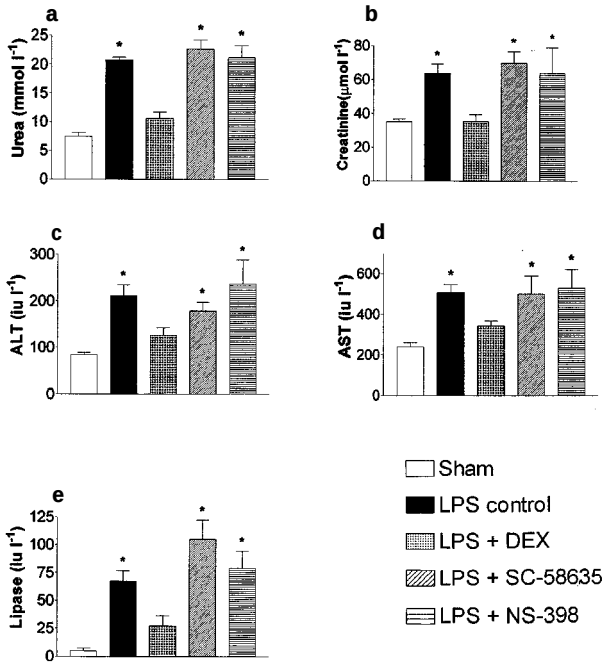

Figure 2 Comparison of the effects of dexamethasone with those of two inhibitors of COX-2 activity on the increase in the serum levels of (a) urea, (b) creatinine (indicators of renal dysfunction), (c) ALT, (d) AST (indicates the liver injury/dysfunction) and (e) lipase (indicates the pancreatic dysfunction) caused by LPS (6 mg kg^{-1}, i.v.) in the anaesthetized rat. Rats which had received LPS were treated with (i) vehicle (saline) rather than LPS (sham, $n=8$), (ii) vehicle (saline, 1 ml kg^{-1}, i.p.) plus LPS (LPS, $n=11$), (iii) LPS plus dexamethasone (DEX, $n=9$), (iv) LPS plus SC-58635 (SC, $n=9$) and (v) LPS plus NS-398 (NS, $n=7$). $*P<0.05$ represents significant difference when compared to sham.

Effects of dexamethasone and two selective inhibitors of COX-2 activity on the rise in the plasma levels of 6-keto-$PGF_{1\alpha}$ and nitrite/nitrate caused by endotoxaemia

In animals which received an injection of vehicle for LPS rather than LPS, administration of saline, DMSO, dexamethasone or of the COX-2 inhibitors SC-58635 or NS-398 did not result in any significant alterations in the plasma levels of 6-keto-$PGF_{1\alpha}$ (Table 1). When compared to rats which had received vehicle (saline) rather than LPS, endotoxaemia for 6 h resulted in a substantial rise in the plasma levels of 6-keto-$PGF_{1\alpha}$ (Figure 3). Treatment of rats which had received LPS with either dexamethasone or with the COX-2 inhibitors SC-58635 or NS-398 abolished the rise in the plasma levels of 6-keto-$PGF_{1\alpha}$ caused by LPS ($P<0.01$, Figure 3).

In animals which received an injection of vehicle for LPS rather than LPS, administration of saline, DMSO, dexamethasone or of the COX-2 inhibitors SC-58635 or NS-398 did not result in any significant alterations in the plasma levels of nitrite/nitrate (Table 1). When compared to rats which had received vehicle (saline) rather than LPS, endotoxaemia for 6 h resulted in significant rises in the plasma levels of nitrite and nitrate (Figure 4). Treatment of rats which had received LPS with dexamethasone attenuated the rise in the plasma levels of nitrite and nitrate caused by LPS ($P<0.05$, Figure 4). In contrast, the COX-2 inhibitors SC-58635 and NS-398 did not affect the rise in the serum levels of nitrite/nitrate caused by LPS ($P>0.05$, Figure 4).

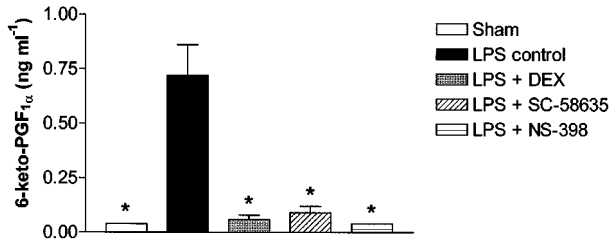

Figure 3 Comparison of the effects of dexamethasone with those of two inhibitors of COX-2 activity on the rise of the plasma levels of 6-keto-$PGF_{1\alpha}$ (an indicator of COX-2 activity) caused by LPS (6 mg kg^{-1}, i.v.) in the anaesthetized rat. Rats which had received LPS were treated with (i) vehicle (saline) rather than LPS (sham, $n=8$), (ii) vehicle (saline, 1 ml kg^{-1}, i.p.) plus LPS (LPS, $n=11$), (iii) LPS plus dexamethasone (DEX, $n=9$), (iv) LPS plus SC-58635 (SC, $n=9$) and (v) LPS plus NS-398 (NS, $n=7$). $^*P<0.05$ represents significant difference when compared to LPS-control.

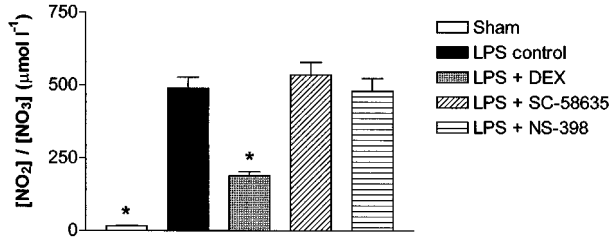

Figure 4 Comparison of the effects of dexamethasone with those of two inhibitors of COX-2 activity on the increase in the plasma levels of nitrite/nitrate caused by LPS (6 mg kg^{-1}, i.v.) in the anaesthetized rat. Rats which had received LPS were treated with (i) vehicle (saline) rather than LPS (sham, $n=8$), (ii) vehicle (saline, 1 ml kg^{-1}, i.p.) plus LPS (LPS, $n=11$), (iii) LPS plus dexamethasone (DEX, $n=9$), (iv) LPS plus SC-58635 (SC, $n=9$) and (v) LPS plus NS-398 (NS, $n=7$). $^*P<0.05$ represents significant difference when compared to LPS-control.

Discussion

The role of arachidonic acid (AA) metabolites in endotoxic shock have been extensively studied and reviewed (Fletcher & Ramwell, 1980; Bult & Herman, 1982), but results have often proved conflicting and inconclusive. Although it is well documented that circulating levels of prostanoids are elevated in experimental shock models (Cook *et al*., 1980; Feuerstein *et al*., 1981), their role in the pathogenesis of shock remains equivocal.

It has been suggested that the rise in the plasma levels of AA metabolites in rodents with endotoxaemia is due to the expression of COX-2 (in various organs and in the vasculature), as dexamethasone prevents both the expression of COX-2 protein as well as the increase in the plasma levels of 6-keto-$PGF_{1\alpha}$ caused by endotoxin in the rat (Masferrer *et al*., 1990; Ruetten & Thiemermann, 1997). We report here for the first time that two selective inhibitors of COX-2 activity (Futaki *et al*., 1994; Panara *et al*., 1995; Riendeau *et al*., 1997; Penning *et al*., 1997) abolish the rise in the plasma levels of 6-keto-$PGF_{1\alpha}$ caused by endotoxin in the rat. Thus, the increase in 6-keto-$PGF_{1\alpha}$ associated with endotoxaemia (in this model) requires the upregulation of COX-2.

This study provides the first evidence that selective inhibition of COX-2 activity in endotoxic shock does not attenuate the fall in blood pressure caused by endotoxin in the rat. On the contrary, the fall in blood pressure in LPS-rats which had been treated with either NS-398 or SC-58635 was more pronounced between 3 and 4 h after injection of LPS (between 2 and 5 h for NS-398). The mechanism(s) of this surprising finding is unclear. It is possible that the generation of vasoconstrictor metabolites of COX-2 (e.g. thromboxane A_2) counteracts the hypotension caused by endotoxin in the rat. However, this is unlikely, as there is substantial evidence that inhibitors of the biosynthesis or effects of thromboxane A_2 exert beneficial effects in endotoxin shock (Feuerstein & Hallenbeck, 1987). There is also evidence that several non-selective inhibitors of COX activity enhance the formation of TNFα in cultured cells (Kunkel *et al*., 1988) as well as in mice (Sironi *et al*., 1992; Pettipher & Wimberly, 1994) and humans challenged with endotoxin (Spinas *et al*., 1991; Martich *et al*., 1991). These findings strongly suggest that AA metabolites reduce the formation of TNFα. Thus, it is conceivable that the inhibition of COX-2 activity by NS-398 or SC-58635 may augment the fall in blood pressure by enhancing the formation of TNFα, as (injection of) TNFα causes hypotension by enhancing the formation of NO (see below; Kilbourn *et al*., 1990). This may well contribute to the augmentation by NS-398 or SC-58635 of the hypotension caused by endotoxin in the rat. Clearly, further studies are warranted to elucidate whether inhibitors of COX-2 activity augment the generation of pro-inflammatory cytokines *in vitro* and *in vivo*.

It should be noted that the hypotension caused by endotoxin in rodents is largely due to the enhanced formation of NO by the inducible NO synthase (iNOS), as selective inhibitors of iNOS activity (e.g. 1400W or L-NIL) abolished the delayed fall in blood pressure caused by endotoxin in the rat (Wray *et al*., 1998). Similarly, the hypotension afforded by endotoxin is substantially reduced in mice in which the gene for iNOS has been deleted by gene-targeting (MacMicking *et al*., 1995).

We also showed that selective inhibition of COX-2 activity does not affect the renal dysfunction, liver injury or pancreatic injury caused by severe endotoxaemia, suggesting that enhanced formation of AA metabolites by COX-2 does not contribute to the development of multiple organ failure in these animals. In patients with septic shock, there is a good correlation between the number of organs failing and mortality (see Baue, 1993). The inability of the COX-2 inhibitors used here to attenuate the organ dysfunction/failure associated with shock indicates that these agents may not be useful in the therapy of circulatory shock. Interestingly, the generation of AA metabolites by COX-2 plays (at least in the stomach) an important role in the protection of tissues against noxious stimuli (Gretzer *et al*., 1998) as well as in angiogenesis and wound healing (Schmassmann *et al*., 1998). Thus, it is possible that the generation of AA metabolites by COX-2 is of beneift in conditions of systemic inflammation, such as endotoxic shock.

While our study was conducted, Bernard and colleagues reported the outcome of a randomized, double-blind, placebo-controlled trial of intravenous ibuprofen in 455 patients with sepsis. In these patients, ibuprofen attenuated the over-production of urinary AA metabolites, reduced fever, tachycardia, oxygen consumption and lactic acidosis. However, ibuprofen did not reduce the circulatory failure, the organ dysfunction (measured as the occurrence of acute respiratory distress syndrome) or the mortality associated with severe sepsis (Bernard *et al*., 1997). Like many other NSAIDs which have been used in animal models of endotoxaemia, ibuprofen is a non-selective inhibitor of COX-1 and COX-2

activity (Meade *et al*., 1993; Mitchell *et al*., 1993). The dose of ibuprofen used in the above clinical trial was certainly sufficient to inhibit COX-2 activity, as ibuprofen attenuated the overproduction of urinary AA metabolites.

Our study also confirms that dexamethasone prevents the hypotension as well as the multiple organ dysfunction caused by endotoxin in the rat (Ruetten & Thiemermann, 1997). The reduction of the organ injury/dysfunction afforded by dexamethasone in the rat is not due to prevention by this glucocorticosteroid of the expression of COX-2, as selective inhibition of COX-2 activity with NS-398 or SC-58635 was unable to reduce the degree of organ injury/dysfunction caused by endotoxin in the rat (this study). Prevention of the expression of iNOS activity may account for the beneficial haemodynamic effects, but does not explain the attenuatiaon of organ injury/dysfunction, caused by dexamethasone, as selective inhibitors of iNOS activity (1400W or L-NIL) also abolish the hypotension, but do not affect the organ injury/dysfunction caused by endotoxin in the rat (Wray *et al*., 1998). The mechanism(s) by which dexamethasone attenuates the liver injury/dysfunction or the pancreatic injury (Ruetten & Thiemermann, 1997, this study) is unclear. There is evidence that dexamethasone induces the transcription of the IκB α gene resulting in an increase in the synthesis of IκB α protein. Stimulation by TNFα causes the release of NFκB from IκBα (i.e. the activation of NFκB). In cells pretreated with dexamethasone, the NFκB released by TNFα rapidly re-associates with (the newly synthesized) IκB α (Scheinman *et al*., 1995). Thus, dexamethasone significantly reduces the amounts of NFκB which are able to translocate to the nucleus to initiate transcription of early response genes, many of which play an important role in the pathophysiology of SIRS and septic shock; these include the genes for tumour necrosis factor α (TNFα), interleukin-1 (IL-1), IL-2, IL-6, IL-8, granulocyte-colony stimulating factor, macrophage-colony stimulating factor, COX-2, iNOS as well as certain chemokines and adhesion molecules, such as ICAM-1 and VCAM-1 (see Barnes & Karin, 1997 for review). It is possible that the prevention of the expression of the formation of pro-inflammatory cytokines or of chemokines and/or adhesion molecules contributes to the reduction in organ injury afforded by dexamethasone in this study.

In conclusion, this study demonstrates that (i) the increased levels of prostanoids which occur as a result of COX-2 induction in response to LPS exposure, do not play a significant role in the pathogenesis of the circulatory failure or the MODS associated with endotoxic shock, and (ii) the ability of dexamethasone to attenuate the circulatory failure and organ dysfunction associated with endotoxaemia cannot be attributed to its ability to prevent the expression of COX-2.

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Abbreviations:
AA, arachidonic acid; ALT, alanine aminotransferase; AST, aspartate aminotransferase; COX-1, cyclo-oxygenase-1; COX-2, cyclo-oxygenase-2; FAD, flavine adenine dinucleotide; HR, heart rate; ICAM-1, intercellular adhesion molecule-1; IL, interleukin; iNOS, inducible nitric oxide synthase; LPS, lipopolysaccharide; MAP, mean arterial blood pressure; MODS, multiple organ dysfunction syndrome; NSAIDS, non-steroidal anti-inflammatory drugs; SIRS, systemic inflammatory response syndrome; TNFα, tumour necrosis factor α; VCAM-1, vascular cell adhesion molecule-1

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