Cyclooxygenase-2 Inhibition Attenuates Lipopolysaccharide-Induced Cardiovascular Failure

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Abstract—The present study aimed to determine the relevance of cyclooxygenase-2 (COX-2)–derived prostanoids for the adverse effects of lipopolysaccharides (LPSs) on cardiovascular function. For this goal, male Sprague-Dawley rats received a single intravenous dose of LPS (10 mg/kg) and were treated with different cyclooxygenase inhibitors. Injection of LPS caused a marked decrease of systolic arterial pressure, from 128 to 79 mm Hg, and a concomitant increase of heart rate, from 380 to 530 minutes⁻¹. Both the decrease of systemic arterial pressure and the increase of heart rate induced by LPS were almost absent if the animals also received the COX-2 blocker rofecoxib (20 mg/kg), regardless whether the drug was given 1 hour before or 1 hour after LPS. Although plasma and organ levels of prostanoids were lowered by rofecoxib, the characteristic LPS-induced increases of NO synthase II and COX-2 gene expression, as well as of plasma and tissue nitrate/nitrite concentrations, were not affected by rofecoxib. Although rofecoxib treatment did also not change LPS-induced tissue cytokine concentrations, it markedly improved LPS-induced liver damage, as indicated by the decrease of transaminases. Moreover, the overall well-being of the LPS-injected animals improved on concomitant treatment with the COX-2 inhibitor. Taken together, our data suggest that COX-2–derived prostanoids are major mediators for the detrimental effects of LPS on cardiovascular and organ function. 

Key Words: shock ■ cyclooxygenase ■ hemodynamics ■ prostaglandins ■ nitric oxide

It is well known that the release of lipopolysaccharides (LPS) from Gram-negative bacteria into the blood stream (eg, in the course of an infection) causes a number of serious adverse effects, such as an increase of body temperature, decrease of blood pressure, and multiorgan failure.1–3 There is consensus that the first mediators in the cascade of LPS-induced events are cytokines.1,2 These, in turn, induce the expression of a number of enzymes, generating a second class of mediators, which are meant to support the body’s defense against bacterial invaders but also mediate adverse effects. Characteristic cytokine-induced enzymes in this context are the inducible isoforms of NO synthase (NOS-II) and cyclooxygenase-2 (COX-2).2,4,5 Thus, it is clear that the increase of body temperature and local inflammatory reactions are triggered by COX-2–derived prostanoids.5,7

LPS septicemia leads to cardiovascular failure, as reflected by a strong decrease of arterial blood pressure that is resistant to vasoconstrictor hormones.8–12 The mechanisms leading to LPS-induced hypotension have not yet been clearly identified. In view of the ubiquitous and strong stimulation of NO formation through NOS-II, it has been assumed that this particular vasoconstrictor plays the major role for the decrease of arterial resistance,1,2,4 Moreover, it has been observed that inhibitors of NOS-II such as L-canavanine, S-methylisothiourea, or amino-

Received August 8, 2002; first decision September 12, 2002; revision accepted September 30, 2002.
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Hypertension is available at http://www.hypertensionaha.org
DOI: 10.1161/01.HYP.0000041221.13644.B9
COX-2 inhibition have been determined, we characterized the effects of selective inhibition of both COX isoforms on LPS-induced reactions such as generation of cytokines, induction of NOS-II and COX-2 with consecutive formation of NO and prostaglandins, and arterial hypotension and tachycardia.

Methods

Animal Experiments

All animal experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals (NIH publication No. 85-23, revised 1985) and German laws relating to the protection of animals, and were approved by the local ethics committee. Male Sprague-Dawley rats (200 to 250 g) received Ringer’s solution intravenously (control) or LPS (Escherichia coli, Sigma) 10 mg/kg IV and were killed by decapitation 0, 5, or 10 hours (n=8 per group) after injection. Animals (n=8 per group) also received LPS (10 mg/kg IV) 1 hour after oral administration of ketorolac (2 mg/kg, Cayman) or rofecoxib (2 mg/kg or 20 mg/kg MSD) and were killed 5 hours after injection. One group (n=8) received LPS (10 mg/kg) 1 hour before rofecoxib administration (20 mg/kg).

Systolic arterial blood pressure and heart rate were measured by the tail-cuff method 0, 5, and 10 hours after injection of LPS or vehicle. MAP was recorded for 5 hours after injection of LPS without or with administration of rofecoxib (20 mg/kg) or vehicle (n=5 per group) in anesthetized rats as described.

The dose of LPS was chosen from the literature to produce severe experimental endotoxemia leading to cardiovascular failure. Ketorolac (2 mg/kg) preferentially inhibits COX-1, whereas rofecoxib (20 mg/kg) selectively blocks COX-2.

Determination of NOS-II, COX-1, COX-2, and β-Actin mRNA

Total RNA (50 µg) from the heart, lung, and liver was extracted, and the abundance of NOS II mRNA, COX-1 mRNA, and COX-2 mRNA was semi-quantitated and related to β-actin mRNA (1 µg) by specific RNase protection assay as described.

Determination of Nitrate/Nitrite, PGE₂, and 6-Keto PGF₁α

Nitrate/nitrite concentrations and concentrations of PGE₂ and 6-keto prostaglandinF₁α in plasma and liver were determined using commercially available assay kits (Cayman Chemical).

Immunoblotting for NOS-II, COX-1, and COX-2 Protein

Immunoblotting was performed with modifications as described. Total protein (100 µg) from liver and lung was used by using a mouse monoclonal antibody against NOS II (BD Transduction Laboratories), rabbit polyclonal antibodies against COX-1 or COX-2 (Cayman), and a horseradish peroxidase–conjugated secondary antibody (BD Transduction Laboratories for NOS II and Santa Cruz for COX).

Cytokine Assays

Tissue concentrations of tumor necrosis factor-α, interleukin-1β, and interferon-γ were determined by using enzyme-linked immunosorbent assay kits (R&D Systems).

Determination of Glutamate-Oxaloacetate-Transaminase and Glutamate-Pyruvate-Transaminase

Plasma-concentrations of glutamate-oxaloacetate-transaminase and of glutamate-pyruvate-transaminase were determined by using commercially available colorimetric assay kits (Reflotron, Roche).

Results

Animals became lethargic and showed piloerection starting 2 hours after LPS injection. This condition persisted for the whole observation period and was not affected by ketorolac (2 mg/kg). Pretreatment of LPS-injected rats with rofecoxib (2 mg/kg) attenuated these symptoms. The behavior of animals treated with rofecoxib (20 mg/kg) did not differ from control rats. Whether rofecoxib (20 mg/kg) was administered 1 hour before or 1 hour after LPS injection did not affect any of the results obtained in the study (data not shown).

Characteristic Effects of LPS

Hemodynamic changes in the macrocirculation are shown in Figure 1. Blood pressure and heart rate remained stable in the control group. LPS challenge resulted in a decrease in blood pressure and increase in heart rate 5 and 10 hours after administration.

Gene expression of inducible isoforms of NOS and COX was strongly elevated 5 and 10 hours after LPS injection, whereas COX-1 gene expression was downregulated (Figure 2). Plasma and tissue nitrate/nitrite concentrations were increased after LPS administration, indicating an increased formation of NO owing to induced NOS-II protein. Concentrations of the prostanooids 6-keto PGF₁α and PGE₂ in plasma and liver tissue were also increased owing to LPS treatment (Figure 3).

Effect of COX-2 Inhibition on Hemodynamic Changes After LPS Injection

MAP in anesthetized rats was ~90 mm Hg and was not affected by treatment with rofecoxib (20 mg/kg) (Figure 4).
In control rats, MAP did not change during the investigation period of 5 hours. LPS injection caused an initial transient decrease in MAP to \( \text{50 mm Hg} \), which was not prevented by pretreatment with rofecoxib (20 mg/kg). After 20 minutes, MAP reached control level in both groups again. From 2 hours after LPS injection, there was a progressive decrease in MAP to \( \text{40 mm Hg} \), which was clearly attenuated by rofecoxib (20 mg/kg).

As shown in Figure 5, even a lower dose of rofecoxib (2 mg/kg) attenuated LPS-induced arterial hypotension and tachycardia, whereas ketorolac did not improve LPS-induced hemodynamic changes.

**Effect of COX Inhibition on LPS-Induced Formation of NO, Prostaglandins, and Cytokines**

Treatment of LPS-injected rats with rofecoxib (20 mg/kg) did not influence induction of NOS-II and COX-2 gene expression (Figure 6). Also downregulation of COX-1 gene expression was unchanged after rofecoxib (20 mg/kg).

The highly elevated plasma and tissue nitrate/nitrite levels after LPS injection were not significantly reduced by COX inhibition (Figure 7, left panel). The increased concentrations of 6-keto PGF\(_{1\alpha}\) and PGE\(_2\) in plasma and liver, respectively, tended to be lower after pretreatment with ketorolac but did not reach significance. However, rofecoxib dose-dependently diminished formation of both prostaglandin isoforms, which even reached control levels after 20 mg/kg in the plasma and in both doses in hepatic tissue after 5 hours (Figure 7, right panel).

Injection of LPS resulted in a time-dependent strong increase of plasma and tissue cytokine concentrations (Figure 8). Rofecoxib treatment (20 mg/kg) did not affect the LPS-induced cytokine formation in plasma and tissue after 5 hours.

**Effect of COX Inhibition on Liver Parameters**

LPS-treatment resulted in an elevation of transaminases, which was not affected by pretreatment with ketorolac or rofecoxib (2 mg/kg) (Figure 9). In contrast, rofecoxib (20 mg/kg) significantly diminished LPS-induced increase in transaminases.

![Figure 2](image-url)  
**Figure 2.** Effect of LPS (10 mg/kg) on NOS-II (top), COX-2 (middle), and COX-1 (bottom) mRNA in the rat heart, lung, and liver 5 and 10 hours after intravenous injection. Values are related to signals obtained for \( \beta \)-actin mRNA. Mean±SE of 8 animals per group. \( *P<0.05 \) vs 0 hours; \( †P<0.05 \) vs 5 hours.

![Figure 3](image-url)  
**Figure 3.** Effect of LPS (10 mg/kg) on nitrate/nitrite concentrations in the plasma and the liver (top) and on plasma concentration of 6-keto PGF\(_{1\alpha}\) and PGE\(_2\) in the liver (bottom) 5 and 10 hours after intravenous injection. Tissue concentrations are related to tissue wet weight. Mean±SE of 8 animals per group. \( *P<0.05 \) vs 0 hours; \( †P<0.05 \) vs 5 hours.

![Figure 4](image-url)  
**Figure 4.** Effect of vehicle, LPS (10 mg/kg), or LPS (10 mg/kg) 1 hour after oral administration of rofecoxib (20 mg/kg) on MAP in anesthetized rats during 5 hours after LPS injection. Mean±SE of 5 animals per group. \( *P<0.05 \) vs control; \( †P<0.05 \) vs LPS+rofecoxib (20 mg/kg).
Discussion

Sepsis and endotoxin shock in patients are still associated with a high mortality rate of 30% to 50%.\textsuperscript{34} LPS injection in our experiments caused cardiovascular failure with pronounced arterial hypotension and tachycardia, which was associated with an overall mortality rate of ≈44% (n = 75). LPS injection stimulated plasma and tissue cytokine formation, leading to a markedly enhanced expression of NOS-II with consecutive generation of great amounts of NO, as indicated by the elevated plasma and tissue levels of nitrate/nitrite concentrations. This constitution is well established and underlines the validity of our animal model.\textsuperscript{1–5}

We observed a differential regulation of COX-isoforms in the way that COX-2 expression was strongly induced by the LPS treatment, and COX-1 expression was downregulated. This is in accordance with previous studies,\textsuperscript{35,36} in which this differential regulation has been assumed to promote organ damage during endotoxemia owing to loss of microvascular control because of possibly diminished production of COX-1–derived prostanoids.\textsuperscript{36}

Plasma and tissue concentrations of vasodilatory prostanoids such as PGE\textsubscript{2} and 6-keto PGF\textsubscript{1α} are known to be elevated during endotoxemia, as was the case in the present experiments. Therefore, there has been some effort in the past decade to define the therapeutic effect of inhibitors of prostaglandin synthesis in septic individuals. In both human and animal studies, nonspecific inhibitors of COX, such as indomethacin or ibuprofen, showed improvement in either the morbidity or mortality associated with sepsis or endotoxin shock.\textsuperscript{37–41} However, a recent randomized, double-blind, placebo-controlled trial\textsuperscript{42} including 455 septic patients revealed no improved survival in the ibuprofen group. The fact that adverse effects of nonselective COX inhibitors (eg, on renal function) are reportedly pronounced in states of sepsis may be of relevance for the outcome in this context.\textsuperscript{43} In addition, opposite effects of COX-1 and COX-2 activity on the pressor response to angiotensin II, one of the most potent endogenous vasoconstrictors, have been reported.\textsuperscript{27} The pressor effect of angiotensin II was blunted by COX-1 inhibition, whereas it was enhanced by COX-2 inhibition. These results indicate a possible disadvantage of nonselective COX inhibition during septic shock.

One could imagine that selective inhibition of COX-2 might give some advantage in the treatment of sepsis. Our study demonstrates that treatment of rats with rofecoxib clearly attenuates LPS-induced arterial hypotension and tachycardia, whereby the time point of rofecoxib administration—1 hour before or 1 hour after LPS injection—had no influence. Ketorolac, at a dose preferentially inhibiting COX-1,\textsuperscript{31} did not improve LPS-caused cardiovascular failure. Rofecoxib treatment had no effect on cytokine formation, induction of NOS-II and COX-2 expression, downregulation of COX-1 expression, and increased NO production. These
parameters were also not affected by ketorolac. In contrast, LPS-induced enhanced production of PGE₂ and 6-keto PGF₁α, the stable metabolite of prostacyclin, was clearly and dose-dependently diminished by rofecoxib and even reached control values after administration of 20 mg/kg rofecoxib, whereas ketorolac had no significant effect on the formation of these prostanooids. This is in accordance with a recent study reporting control values of PGE₂ concentrations in plasma and lung after selective COX-2 inhibition in a murine model of endotoxemia. Our data provide convincing evidence that the hemodynamic improvement in the macrocirculation by rofecoxib treatment is mediated by the reduced generation of vasodilatory COX-2–derived prostanooids.

It is well established that PGE₂ in the central nervous system triggers a response, generally called ‘acute phase reaction,’ including fever and hyperalgesia. The diminished LPS-induced piloerection after treatment with rofecoxib (2 mg/kg), which was almost absent after administration of 20 mg/kg rofecoxib, and the improved overall well-being of the animals after treatment with rofecoxib may be owing to the reduced PGE₂ production. In addition, PGE₂-induced hyperpyrexia requires increased fluid uptake and could result in relative hypovolemia, which in turn provokes cardiovascular failure.

There is some evidence for an organ-protective effect of COX-2 inhibition during endotoxemia. LPS injection was associated with substantial rises in the plasma levels of glutamate-oxaloacetate-transaminase and glutamate-pyruvate-transaminase, indicating the development of acute liver injury, which is consistent with previous studies. Treatment of rats with rofecoxib dose-dependently attenuated the acute liver injury associated with endotoxin shock. This effect is possibly related to the dose-dependent diminished hepatic PGE₂ levels after rofecoxib administration.

Regarding the enormous and widespread induction of NOS-II with consecutive formation of high amounts of NO in nearly all organs, this vasodilatory molecule has been made responsible for the cardiovascular failure in septic and endotoxic shock states. This hypothesis was strengthened by findings that selective NOS-II inhibition and also nonselective NOS inhibition prevents cardiovascular collapse. However, nonselective NOS inhibition was associated with marked side effects—such as provoked renal dysfunction, increased number of bacteria in plasma and liver (bottom left) and liver (bottom right) 5 and 10 hours after intravenous injection. Effect of LPS (10 mg/kg) or LPS + rofecoxib (20 mg/kg) on plasma (top right) and tissue (bottom right) cytokine concentrations 5 hours after LPS injection. Tissue concentrations are related to total protein assayed. Mean±SE of 8 animals per group. P<0.05 vs 0 hours or control; †P<0.05 vs 5 hours.

Figure 7. Effect of LPS (10 mg/kg), LPS + ketorolac (2 mg/kg), LPS + rofecoxib (2 mg/kg), or LPS + rofecoxib (20 mg/kg) on nitrate/nitrite concentrations in the plasma (top left) and the liver (bottom left) and on plasma concentration of 6-keto PGF₁α (top right) and PGE₂ concentration in the liver (bottom right) 5 hours after LPS injection. Tissue concentrations are related to tissue wet weight. Mean±SE of 8 animals per group. *P<0.05 vs control; †P<0.05 vs LPS 5 hours; and ‡P<0.05 vs LPS + rofecoxib (2 mg/kg).

Figure 8. Effect of LPS (10 mg/kg) on concentrations of the cytokines tumor necrosis factor-α (TNF-α), interleukin-1β, and interferon-γ in plasma (top left) and liver (bottom left) 5 and 10 hours after intravenous injection. Effect of LPS (10 mg/kg) or LPS + rofecoxib (20 mg/kg) on plasma (top right) and tissue (bottom right) cytokine concentrations 5 hours after LPS injection. Tissue concentrations are related to total protein assayed. Mean±SE of 8 animals per group. *P<0.05 vs 0 hours or control; †P<0.05 vs 5 hours.

Figure 9. Effect of LPS (10 mg/kg) on concentrations of the cytokines tumor necrosis factor-α (TNF-α), interleukin-1β, and interferon-γ in plasma (top left) and liver (bottom left) 5 and 10 hours after intravenous injection. Effect of LPS (10 mg/kg) or LPS + rofecoxib (20 mg/kg) on plasma (top right) and tissue (bottom right) cytokine concentrations 5 hours after LPS injection. Tissue concentrations are related to total protein assayed. Mean±SE of 8 animals per group. *P<0.05 vs 0 hours or control; †P<0.05 vs 5 hours.
tissue with higher cytokine levels,54 and accelerated and enhanced fall in blood pressure52—and overall did not improve49,55 or even reduce54 survival in experimental sepsis, possibly owing to detrimental effects of the inhibition of NO generation in the microcirculation. In addition, a multicenter trial using a nonselective NOS inhibitor in septic patients was stopped by the safety committee because of adverse effects. Studies with selective NOS-II inhibitors revealed that arterial hypotension and vascular hyporeactivity were only slightly alleviated, indicating that there may be other or additional pathways involved in the pathogenesis of septic cardiovascular failure.29,56–58 Also our findings do not support a central role of NO mediating the LPS-induced arterial hypotension, especially supported by a grant from the Deutsche Forschungsgemeinschaft (BU 1360/1–1).

**Perspectives**

We found that COX-2 inhibitors prevented the fall of blood pressure and the rise of heart rate and in general improved the well-being of LPS-treated rats. We infer from our findings that COX-2–derived prostanoids are of particular relevance for the changes of cardiovascular function during endotoxemia. Further animal and human studies are necessary to clarify whether inhibition of the generation of COX-2–derived prostanoids is beneficial in the treatment of septic circulatory failure. Possible effects of COX-2 inhibitors on, eg, renal and pulmonary function and on hemostasis would be areas of interest in this context, as well as the question whether therapeutic strategies taken from experimental sepsis are useful under clinical conditions of sepsis.

**Acknowledgments**

The expert technical assistance provided by Gertraud Wilberg and Regina Menrath is gratefully acknowledged. The study was financially supported by a grant from the Deutsche Forschungsgemeinschaft (BU 1360/1–1).

**References**


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Hypertension. published online October 28, 2002:

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