Inhibition of COX-1 attenuates the formation of thromboxane A₂ and ameliorates the acute decrease in glomerular filtration rate in endotoxemic mice

Katharina Mederer, Manuel Meurer, Hayo Castrop, and Klaus Höcherl

Institute of Physiology, University of Regensburg, Regensburg, Germany; and Institute of Experimental and Clinical Pharmacology and Toxicology, Friedrich-Alexander-Universität Erlangen-Nürnberg (FAU), Erlangen, Germany

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Mederer K, Meurer M, Castrop H, Höcherl K. Inhibition of COX-1 attenuates the formation of thromboxane A₂ and ameliorates the acute decrease in glomerular filtration rate in endotoxemic mice. Am J Physiol Renal Physiol 309: F332–F340, 2015. First published May 27, 2015; doi:10.1152/ajprenal.00567.2014.—Thromboxane (TX) A₂ has been suggested to be involved in the development of sepsis-induced acute kidney injury (AKI). Therefore, we investigated the impact of cyclooxygenase (COX)-1 and COX-2 activity on lipopolysaccharide (LPS)-induced renal TXA₂ formation, and on endotoxemia-induced AKI in mice. Injection of LPS (3 mg/kg ip) decreased glomerular filtration rate (GFR) and the amount of thrombocytes to ~50% of basal values after 4 h. Plasma and renal cortical tissue levels of TxB₂ were increased ~10- and 1.7-fold in response to LPS, respectively. The COX-1 inhibitor SC-560 attenuated the LPS-induced fall in GFR and in platelet count to ~75% of basal levels. Furthermore, SC-560 abolished the increase in plasma and renal cortical tissue levels of TXB₂ in response to LPS. The COX-2 inhibitor SC-236 further enhanced the LPS-induced decrease in GFR to ~40% of basal values. SC-236 did not alter thrombocyte levels nor the LPS-induced increase in plasma and renal cortical tissue levels of TXB₂. Pretreatment with clopidogrel inhibited the LPS-induced drop in thrombocyte count, but did not attenuate the LPS-induced decrease in GFR and the increase in plasma TxB₂ levels. This study demonstrates that endotoxemia-induced TXA₂ formation depends on the activity of COX-1. Our study further indicates that the COX-1 inhibitor SC-560 has a protective effect on the decrease in renal function in response to endotoxin. Therefore, our data support a role for TXA₂ in the development of AKI in response to LPS.

Address for reprint requests and other correspondence: K. Höcherl, Institut für Experimentelle und Klinische Pharmakologie und Toxikologie, Friedrich-Alexander-Universität Erlangen-Nürnberg (FAU), Fahrstr. 17, D-91054 Erlangen, Germany (e-mail: klaus.hoecherl@fau.de).

SEPSIS STILL REMAINS a major cause of death in intensive care units (50). The development of acute kidney injury (AKI) is a common, serious complication of sepsis. AKI occurs in ~41% of septic patients, which results in higher morbidity and mortality. With regard to intensive care units, the mortality rate of septic patients with AKI is ~67%, compared with 43% in patients without sepsis (52). Thus, drugs for the treatment of sepsis-induced AKI are urgently required. An increase in serum creatinine with or without a concomitant decrease in urine output is used as criteria for the detection and classification of AKI (64). Since both parameters represent markers for a decrease in glomerular filtration rate (GFR), a hallmark of AKI in general and also of septic AKI is the loss of GFR. Although in experimental models of sepsis and in patients changes in renal vascular resistance (RVR) as well as in renal blood flow (RBF) vary widely, a decrease in GFR is commonly observed (8, 61). This decrease is largely independent of changes in blood pressure (14, 72). There is experimental evidence that early in sepsis-related AKI, the predominant pathogenetic factor is renal vasoconstriction (37). Micropuncture studies in rodents suggest that GFR and glomerular plasma flow are reduced in response to endotoxemia, likely due to an increase in afferent arteriolar resistance (46). Moreover, findings obtained from in vitro blood-perfused juxtamedullary nephron preparations suggest that endotoxin elicits vasoconstriction probably due to direct, endothelium-mediated effects of LPS on vascular smooth muscle cells (70). It has been assumed that an imbalance between vasodilatory and vasoconstrictive substances is responsible in the pathogenesis of septic AKI. In this regard, elevated renal and/or circulatory levels of vasoconstrictors, such as angiotensin II, catecholamines, endothelin, and thromboxane (TX), for example, may play an important role in septic AKI (5, 18, 40, 72).

TXA₂ is a proinflammatory, vasoconstrictive lipid mediator. The generation of TXA₂ involves three steps: 1) the release of arachidonic acid (AA) from membrane phospholipids by phospholipase A₂, 2) the formation of prostaglandin (PG) H₂ from AA by cyclooxygenase (COX), and 3) the conversion of PGH₂ into TXA₂ by TX synthase (TXS). TXA₂ mediates its effects through a G protein-coupled receptor, named TP receptor, which has been localized to the renal vasculature, glomerular mesangial cells, podocytes, and various nephron segments (49). TXA₂ is a likely candidate to play a role in sepsis-induced AKI. The TXA₂ agonist U-46619 mimics the fall in GFR and the increase in RVR commonly seen in response to injection of LPS (7). In addition, U-44069 preferentially constricts the afferent arteriole (28). Furthermore, genetic deletion of the TX TP receptor, TP receptor antagonism, and TXS inhibition have been shown to improve renal function in animal models of sepsis (5, 12, 68). During physiological conditions, TXA₂ mainly derives from platelets, where the formation depends on the activity of COX-1 (57). During inflammation, PGH₂ production is thought to depend mainly on the inducible form of COX, COX-2. Therefore, it has been suggested that COX-2 is responsible for the formation of TXA₂ during septic AKI (12). However, inhibition of COX-2 does not attenuate the fall in GFR in response to LPS (41). Furthermore, endotoxemia induces an immediate release of TXA₂, at a time point when COX-2 synthesis just begins (5, 35). Therefore, it is still unclear which isofrom of COX is responsible for the formation of TXA₂ during severe inflammation.
We investigated therefore the impact of COX-1 and COX-2 activity for the increased formation of TxA₂ in response to LPS, which is an experimental approach commonly used in examining the pathogenesis of septic AKI. We used a low, nonlethal dose, which has been found to induce AKI in the absence of severe hypotension (39). Because GFR decreases shortly after the injection of endotoxin (12), we examined the effect of LPS on COX-1 and COX-2 activity at an early time point, to gain insight into the development of septic AKI. Since we found that TxA₂ formation depends on the activity of COX-1 at this time point, we further investigated the effect of COX-1 inhibition on GFR. Because antiplatelet drugs such as the P2Y₁₂ receptor antagonist clopidogrel have been reported to attenuate the drop in platelet count and to improve end-organ damage in animal models of sepsis (26, 62), we further investigated the effect of clopidogrel on GFR.

MATERIALS AND METHODS

Animal experiments. All animal experiments were performed according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the local ethics committee. Male C57BL/6j mice (8 wk old) were obtained from Charles River (Sulzfeld, Germany). Mice were acclimated for at least 1 wk in the animal facility with free access to food and water. The animals were housed with a 12:12-h day-night cycle. Controls received intraperitoneal injections of 500 µl of 0.9% saline. To induce AKI, mice were injected intraperitoneally with 3 mg/kg LPS (intra-peritoneal injection of 500 µl of LPS from Escherichia coli serotype 0111:B4; Sigma Aldrich Chemical, dissolved in 0.9% of saline), a low, nonlethal dose, which has been found to worsen renal function in the absence of severe hypotension (39). Furthermore, mice received a single dose of SC-560 (20 mg/kg; Sigma Aldrich Chemical) was given orally 48, 24, and 2 h before the injection of LPS. Clopidogrel sulfate (20 mg/kg; Sigma Aldrich Chemical) was given as a single dose 30 min before the injection of LPS. SC-236 (COX-2 inhibitor) has been previously established (55, 65, 66) in studies demonstrating inhibition of constitutive prostaglandin synthesis with the primary antibody. Protein preparation and immunoblotting. Protein preparation and immunoblotting were performed as described previously (32). In brief, protein samples (20 µg) were electrophoretically separated on 10% polyacrylamide gels and transferred to nitrocellulose membranes, which were blocked overnight in 5% nonfat dry milk diluted in Tris-buffered saline with 0.1% Tween-20, and then incubated for 1 h at room temperature with antibodies against COX-1 and COX-2 (Cayman Chemical; 1:2,000). After being washed, the membrane was incubated for 2 h with the secondary antibody (Santa Cruz Biotechnology, Santa Cruz, CA); 1:2,000). After being washed, the membrane was incubated for 2 h with the secondary antibody (Santa Cruz Biotechnology, Santa Cruz, CA) and subjected to a chemiluminescence detection system. Quantitative assessment of band densities was performed densitometrically using ImageJ Software.

Immunohistochemistry for TxA₂, COX-1, and COX-2. Kidneys from vehicle- and LPS-treated mice were fixed in 4% paraformaldehyde solution by retrograde perfusion through the abdominal aorta. Immunolabeling was performed on 5-µm paraffin sections as described previously (32). In brief, after being boiled in a microwave oven followed by cooling, kidney sections were incubated with an anti-TxA₂ antibody (Proteintech Group; 1:2,000). After being washed, the membrane was incubated for 2 h with the secondary antibody (Santa Cruz Biotechnology; Santa Cruz, CA; 1:2,000) overnight at 4°C, followed by incubation with a secondary antibody. As a negative control, we used the secondary antibody without incubation with the primary antibody.

Statistical analyses. Data are expressed as means ± SE. Statistical significance was assessed with Student’s t-test or with one- or two-way ANOVA followed by Tukey’s post hoc test using GraphPad Prism version 6.05 (GraphPad Software, San Diego, CA). Significance was taken at P < 0.05.

RESULTS

Endotoxemia increases TxA₂ formation and renocorticoidal COX-2 expression. Already 1 h after the injection of LPS, endotoxemic mice were inactive and displayed piloerection. First, we investigated the time-dependent effect of a single
dose of LPS (3 mg/kg ip) on platelet count, the major physiological source of TxA2. We found that endotoxia decreased the amount of platelets to ~53, 41, and 35% of control values at 4, 8, and 16 h after the injection of LPS, respectively (n = 6, P < 0.05; Fig. 1A). Next, we determined plasma and renocortical tissue concentrations of TxB2, a stable metabolite of TxA2. Plasma levels of TxB2 were elevated ~7.4-, 2.7-, and 2.0-fold at 4, 8, and 16 h after the injection of LPS, respectively (n = 6, P < 0.05; Fig. 1B). We further found that endotoxia increased renocortical tissue levels of TxB2 ~2.3- and 1.7-fold at 4 and 8 h after the injection of LPS (n = 6, P < 0.05; Fig. 1B). Furthermore, we investigated the effect of LPS on enzymes involved in renocortical TxA2 biosynthesis. We found that endotoxia for 4 h did not alter renocortical COX-1 and TxS protein expression, but strongly increased renocortical COX-2 protein abundance about ~10-fold (n = 6, P < 0.05; Fig. 1C). COX-2 protein expression was still increased 5.6- and 4.0-fold at 8 and 16 h after the injection of LPS, respectively (n = 6, P < 0.05; Fig. 1B). In contrast, the expression of COX-1 and TxS was decreased to ~70% of control levels at 8 and 16 h after the injection of LPS (n = 6, P < 0.05; Fig. 1C). Endotoxia time-dependently decreased Tx TP receptor mRNA abundance to 60, 24, and 21% of control values at 4, 8, and 16 h after the injection of LPS, respectively (n = 6, P < 0.05). In addition, we determined renocortical tissue concentration of AA. Renocortical AA tissue concentration was increased 1.7-, 1.4-, and 1.5-fold at 4, 8, and 16 h after the injection of LPS (n = 6, P < 0.05; Fig. 1D).

**Immunolocalization of TxS, COX-1, and COX-2.** To determine the impact of endotoxia on the renocortical localization of these enzymes, TxS immunoreactivity was mainly detected in cortical arterioles of vehicle-treated mice. Only a mildly positive TxS-immunoreactive staining was observed in glomeruli. The distribution pattern was not altered in mice treated with LPS (3 mg/kg) at 4 h. Renocortical COX-1 immunoreactivity was detected in the glomerulus, extraglomerular mesangium, tubular segments (most likely distal convoluted tubule, connecting tubule, and the collecting duct), cortical interstitial fibroblasts, and in some endothelial cells of vehicle-treated mice. The distribution pattern was not altered in mice treated with LPS (3 mg/kg) at 4 h. Renocortical COX-2 immunoreactivity was detected in some macula densa cells of vehicle-treated mice (not shown). Endotoxia increased COX-2 immunoreactivity mainly in cortical interstitial cells. Vascular and arteriolar endothelial cells only showed a mildly positive COX-2-immunoreactive staining in response to LPS at 4 h (Fig. 2).

**COX-1 inhibition attenuates the LPS-induced increase in plasma and renocortical tissue TxB2 concentration and the decrease in platelet count.** Because Tx formation was highest at 4 h after LPS injection, subsequent studies were performed at this time point. To determine the impact of COX-1 and COX-2 on endotoxia-induced formation of TxA2, we used the COX-1 inhibitor SC-560 (20 mg/kg) and the COX-2 inhibitor SC-236 (10 mg/kg). Inhibition of COX-1 decreased

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**Fig. 1.** Time-dependent effects of endotoxia. Thrombocyte count (A) and plasma thromboxane (Tx) B2 levels (B) and renocortical TxB2 concentration at 4, 8, and 16 h after the injection of lipopolysaccharide (LPS; 3 mg/kg ip). C: expression of cyclooxygenase (COX)-1, COX-2, and Tx synthase (TxS) was determined 4, 8, and 16 h after the injection of LPS (insets show representative immunoblots for COX-1, COX-2, and TxS). The COX-2 blot is composed of 2 pieces: basal COX-2 protein expression was determined with 100 μg of total protein per sample and LPS-induced COX-2 protein expression was determined with 20 μg of total protein per sample. D: renocortical arachidonic acid (AA) concentration at 4, 8, and 16 h after the injection of LPS. Values are means ± SE for 6 animals. **P < 0.05 vs. LPS.
basal tissue and plasma levels of TxB2 and abolished the LPS-induced increase in renocortical tissue and plasma concentration of TxB2 at 4 h \((P < 0.05; \text{Fig. } 3, \text{A and B})\). Inhibition of COX-2 did not alter basal levels of TxB2 or the LPS-induced increase in renocortical tissue and plasma levels of TxB2 (Fig. 3, A and B). Neither SC-560 nor SC-236 altered the amount of thrombocytes in vehicle-treated mice. However, inhibition of COX-1, but not of COX-2, attenuated the LPS-induced fall in platelet count \((P < 0.05; \text{Fig. } 3C)\).

**COX-1 inhibition ameliorates endotoxemia-induced AKI.** Four hours after injection of LPS, GFR decreased to \(~52\)% of pretreatment values \((P < 0.05; \text{Fig. } 4A)\). Neither the COX-1 inhibitor SC-560 nor the COX-2 inhibitor SC-236 influenced basal GFR at 4 h after injection. However, inhibition of COX-1 attenuated the LPS-induced decrease of GFR to \(~77\)% of pretreatment values at 4 h after injection of endotoxin \((P < \text{Fig. } 4A)\).

![Fig. 2. Distribution of Txs, COX-1, and COX-2 in mouse renal cortex. A: Txs immunoreactivity in renal cortex of LPS-treated mice. Arrows indicate labeling of renal vasculature. B: COX-1 immunoreactivity in renal cortex of LPS-treated mice. #Labeling of renal tubule. *Labeling of cortical interstitial cells; arrows indicate labeling of renal arteries (RA) or renal veins (RV). C: COX-2 immunoreactivity in renal cortex of LPS-treated mice. Immunoreactivity from endotoxemic mice was determined 4 h after the injection of LPS (3 mg/kg ip). Arrows indicate labeling of RV; *labeling of cortical interstitial cells. Approximate magnification: \(\times 100 \ (A) \text{ or } \times 400 \ (B, C)\).

![Fig. 3. Effect of COX inhibition on Tx formation and on platelet count. Plasma TxB2 concentration (A), renocortical TxB2 levels (B), and platelet count (C) were determined 4 h after the injection of LPS (3 mg/kg ip) in mice treated with or without the COX-1 inhibitor SC-560 (20 mg/kg ip) or the COX-2 inhibitor SC-236 (10 mg/kg ip). Values are means ± SE for 6–12 animals per group. **P < 0.05 vs. pretreatment or vehicle-treated controls. #P < 0.05 vs. LPS.](http://ajprenal.physiology.org/)

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In contrast, inhibition of COX-2 enhanced the LPS-induced decrease of GFR to 41% of pretreatment values at 4 h after injection of endotoxin (P < 0.05; Fig. 4A). Furthermore, renal TNF-α mRNA abundance was increased ~7-fold 4 h after the injection of LPS (n = 6, P < 0.05). SC-560 did not alter basal or LPS-induced levels of TNF-α (Fig. 6B).

**DISCUSSION**

In the present study, we demonstrate that SC-560, an experimental COX-1 inhibitor, inhibited the formation of TxA2 and attenuated the early decrease in GFR in an animal model of endotoxemia-induced AKI without affecting the LPS-induced proinflammatory response.

In line with previous observations, we found that renocortical COX-2 expression was strongly increased and that renocortical COX-1 expression moderately decreased after the injection of LPS (30, 32, 34, 51). Furthermore, renocortical TxA2 expression was not changed at 4 h after injection of LPS.

**Effect of clopidogrel pretreatment on LPS-induced decrease in platelet count and GFR.** We further investigated the effect of clopidogrel pretreatment on platelet count, plasma TxA2 levels, and on GFR. Clopidogrel (10 mg·kg⁻¹·day⁻¹; orally) was administered 48, 24, and 2 h before injection of LPS (3 mg/kg ip). Thrombocyte count (A), plasma TxA2 levels (B), and GFR (C) 4 h after injection of LPS. Values are means ± SE for 6 animals. ★P < 0.05 vs. LPS.
confirming a previous report where no alteration in TxS mRNA abundance was observed in IL-1β/LPS-stimulated rat glomeruli (38). However, we now found that TxS expression decreased at later time points. Furthermore, we observed a time-dependent downregulation of the Tx TP receptor mRNA in response to LPS, which may be due to an activation of the NF-κB pathway (76).

Injection of LPS caused an increase in renocortical tissue TxB2 concentration as described before (5, 22). Therefore, one may conclude that LPS-induced renocortical TxA2 formation mainly depends on COX-2 activity. Such an implication was previously assumed by others with regard to renal and cardiac TxA2 formation in response to LPS (12, 24). To verify this assumption, we performed experiments with the selective COX-1 inhibitor SC-560 and with the selective COX-2 inhibitor SC-236 (55, 65). We now found that the COX-1 inhibitor SC-560, but not the COX-2 inhibitor SC-236, inhibited the LPS-induced increase in renocortical TxB2 concentration. Therefore, our data suggest that COX-1, but not COX-2, is responsible for the endotoxemia-induced increase in TxB2 formation. Since the expressions of COX-1 and of TxS were unaltered in the early response to LPS, one may conclude that an increased release of AA could be responsible for an increased formation of PGH2 and subsequently for Tx (33). In line with this, we found that renocortical AA concentration was increased in response to LPS. To outline the role of COX-1 for the formation of TxA2, we determined the amount of platelets and measured plasma levels of TxB2. Confirming previous findings we observed a strong increase in plasma levels of TxB2, which was accompanied by a drop in platelet count after injection of LPS (35, 63). In line with the concept of a COX-1-dependent formation of platelet-derived TxA2, we found that inhibition of COX-1, but not of COX-2, attenuated the drop in platelet count and inhibited the LPS-induced rise in plasma TxB2 levels. Since SC-560 did not completely inhibit LPS-induced thrombocytopenia, additional factors and/or a direct effect of LPS have to be involved in platelet activation in response to endotoxia (75).

There is growing evidence that LPS exerts a direct effect on platelets. It has been reported that platelets express the toll-like receptor (TLR) 4, the receptor for the bacterial endotoxin LPS, and that TLR4 expression is responsible for LPS-induced thrombocytopenia (1, 4). It has been found that TLR4 polymorphism decreases Tx biosynthesis (53), suggesting that a direct activation of platelets by LPS is responsible for the marked increase in systemic TxA2 formation. In addition to the direct effect of LPS on platelet activation, an inflammation-induced thrombin formation can activate platelets via protease-activated receptors. Furthermore, endothelial cell damage exposes subendothelial collagen and induces the expression of the von Willebrand factor and of tissue factor on endothelial cells, which in turn activates platelets and further stimulates thrombin formation (19). Activated platelets further stimulate platelet activation via the release of TxA2 and ADP (43), suggesting that platelets are the main source of Tx synthesis in response to endotoxia. However, because COX-1 and TxS are expressed not only in platelets but also in vascular cells, for example (21), also vascular-derived Tx may contribute in part to the increase in endotoxin-induced Tx formation (17). Since our study does not specifically address this issue, further studies are necessary to investigate the contribution of vascular-derived TxA2 for the overall formation of TxA2 in response to LPS.

Endothelial dysfunction is one of the hallmarks of sepsis and a role for platelets in endothelial dysfunction has been supposed (59). It is now well-accepted that platelets are major effector cells in both hemostasis and inflammation. Platelets are involved in the development of hyperinflammation, disseminated intravascular coagulation, and microthrombosis (19). In experimental endotoxia, inhibition of platelet function was found to reduce endothelial dysfunction and to ameliorate multiple organ failure including acute lung injury (26, 56, 62). Recently, a role for platelets in the development of renal failure has been proposed for septic patients (69). However, it is still unclear whether antiplatelet drugs such as clopidogrel improve renal function in response to endotoxia. Therefore, we used the P2Y12 receptor antagonist clopidogrel, which has been shown to inhibit LPS-induced thrombocytopenia and to improve acute lung injury in mice (74). Indeed, clopidogrel inhibited the drop in platelet count in response to endotoxia in our study. Although there is evidence that inhibition of platelet P2Y12 receptors decreases basal platelet TxA2 formation and inhibits platelet activation and aggregation mediated by TxA2 pathways, by reducing platelet formation of TxA2 and by inhibiting responses after TP receptor activation (2, 3, 10, 42, 54), clopidogrel did not affect the increase in plasma TxB2 levels after injection of LPS in our present study. A similar finding was previously observed for the P2Y12 receptor antagonist ticlopidine (35). We further found that clopidogrel did not improve the fall in GFR in response to LPS. Thus, our data indicate that inhibition of
P2Y₁₂ does not have a protective effect on endotoxemia-induced AKI. In line with this, it has been reported that LPS-induced systemic inflammation is more severe in P2Y₁₂-null mice (44).

Our data indicate that COX-1 is responsible for the increased formation of TxA₂ in response to LPS. Therefore, we investigated the effect of SC-560 on endotoxemia-induced AKI. Neither COX-1 nor COX-2 inhibition affected GFR in control mice as described before (15). We now found that inhibition of COX-1 attenuated the early decrease in GFR in response to LPS, without affecting the inflammatory response to LPS. It has been reported that inhibition of TxA₂, pharmacological blockade of TP receptors, or genetic disruption of TP receptors ameliorates the LPS-induced decrease in GFR (5, 12). Similarly to these reports, the endotoxemia-induced decrease in GFR in our present study was not completely prevented by SC-560 (5, 12). Since high concentrations of all five primary prostanooids are reported to be low-affinity agonists at the TP receptor (29), one may argue that also COX-2-derived prostanooids could contribute to the decrease in GFR in response to endotoxemia. Furthermore, an increased formation of TP receptor-activating isoprostanes could be involved in the decline in GFR (6, 73). However, this assumption seems to be unlikely, because genetic disruption of TP receptors is also not sufficient to fully reverse the decline in GFR (12). Interestingly, a very similar effect was recently reported after ischemic AKI in rats (11). In this study, SC-560 at a dose of 1 mg/kg, but not the COX-2 inhibitor SC-58125, attenuated the decrease of GFR 24 h after renal ischemia. Taken together, it seems likely that COX-1-derived prostanooids negatively influence renal function in animal models of AKI. However, the precise mechanism for the protective effect of COX-1 inhibition has to be determined in future studies. In addition, other vasoconstrictive factors like angiotensin II, endothelin-1, or the renal nervous system could also be involved in the LPS-induced decrease in GFR (46, 58, 72).

A limitation of our study is that neither blood pressure nor RBF was measured. In normal conditions, autoregulatory mechanisms attempt to maintain RBF constant during hypotension. With regard to endotoxemia, the altered renal function has been attributed predominantly to renal hypoperfusion, and it has been suggested that the reduction of GFR and perfusion pressure are secondary to the decline in RBF after induction of sepsis or endotoxemia (36). Thus, one may argue that a possible strong hypotensive effect of LPS in our study negatively influences renal function. Indeed, it has been found that an intravenous dose of 2 mg/kg LPS reduces blood pressure from 106 to ~75 mmHg 6 h after the injection of LPS in conscious freely moving mice (66). Recently, we determined blood pressure in endotoxicemic mice by radiotelemetry. In this study, we found that 1 h after intraperitoneal injection of LPS at a dose of 3 mg/kg mean arterial pressure (MAP) dropped significantly from 104 to 93 mmHg. However, during the following 2 to 3 h, MAP recovered in the wild-type mice (48). The different route of administration may explain the discrepancies obtained in conscious mice. Furthermore, one may argue that the two different COX inhibitors have different effects on blood pressure in response to LPS. However, it has recently been found that the early decrease in blood pressure was attenuated by pretreatment with either SC-560 or SC-236 in rats treated with LPS at a dose of 1 mg/kg iv (67). Although we did not measure RBF, one may speculate from our findings that COX-1 inhibition, like TxA₂ inhibition and TP receptor antagonism, may also improve RBF. In line with this assumption, it has been found that SC-560 improved RBF after renal ischemia and reperfusion (11). In contrast to COX-1 inhibition, inhibition of COX-2 enhanced the effect of LPS on GFR, suggesting that COX-2 produces mainly vasodilatatory prostanooids, which are of importance to maintain GFR by opposing vasoconstrictive influences. The role of COX-2-derived prostanooids for the LPS-induced decrease in GFR is somewhat confusing. We recently found that the COX-2 inhibitor parecoxib ameliorated the LPS-induced decrease in creatinine clearance in rats, probably because of the attenuation of LPS-induced hypotension (30, 32), whereas others did not observe an effect of COX-2 inhibition on plasma urea and plasma creatinine levels (41). The discrepancies may be due to the higher doses of LPS used in previous studies, differences in fluid infusion, the time point of the determination, and the determination in conscious or anesthetized animals, for example. Moreover, it has recently been demonstrated that a reduced production of creatinine limits its use as marker of kidney injury in sepsis (20). Since nonselective COX inhibition is expected to decrease both vasodilatory and vasoconstrictive derivatives of AA metabolism, our data may also explain why nonselective COX inhibition fails to improve renal function in human sepsis (9, 27). Together with previous studies, our data support therefore a role for TxA₂ in the development of AKI in response to LPS. However, it should be noted that this study was not designed to specifically address this issue.

In summary, we found that inhibition of COX-1 during basal conditions has a minor role on GFR. However, inhibition of COX-1 ameliorates the reduction in GFR in response to endotoxemia. This effect may result from a decrease in the formation of TxA₂ via COX-1. Thus, inhibition of COX-1, but not of P2Y₁₂ receptors, may be a potential preventive intervention for sepsis-induced AKI.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


REFERENCES


