Prostacyclin in endotoxemia-induced acute kidney injury: cyclooxygenase inhibition and renal prostacyclin synthase transgenic mice

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Wang W, Zolty E, Falk S, Summer S, Stearman R, Geraci M, Schrier R. Prostacyclin in endotoxemia-induced acute kidney injury: cyclooxygenase inhibition and renal prostacyclin synthase transgenic mice. Am J Physiol Renal Physiol 293: F1131–F1136, 2007. First published July 25, 2007; doi:10.1152/ajprenal.00212.2007.—Sepsis-related acute kidney injury (AKI) is the leading cause of AKI in intensive care units. Endotoxin is a primary initiator of inflammatory and hemodynamic consequences of sepsis and is associated with experimental AKI. The present study was undertaken to further examine the role of the endothelium, specifically prostacyclin (PGI2), in the pathogenesis of endotoxemia-related AKI. A low dose of endotoxin (LPS, 1 mg/kg) in wild-type (WT) mice was associated with stable glomerular filtration rate (GFR) (164.0 ± 16.7 vs. 173.3 ± 6.7 μl/min, P = not significant) as urinary excretion of 6-keto-PGF1α, the major metabolite of PGI2, increased. When cyclooxygenase inhibition with indomethacin abolished this rise in 6-keto-PGF1α, the same low dose of LPS significantly decreased GFR (110.7 ± 12.1 vs. 173.3 ± 6.7 μl/min, P < 0.05). The same dose of indomethacin did not alter GFR in WT mice. To further study the role of PGI2, in endotoxemia, renal-specific PGI synthase (PGIs) transgenic (Tg) mice were developed that had increased PGIs expression only in the kidney and decreased renal PGI2 synthase glomerular filtration rate (GFR) (164.0 ± 16.7 vs. 173.3 ± 6.7 μl/min, P = not significant). An elevation in renal cAMP, however, suggested an elevation in renal cAMP synthase transgenic mice (10, 23). When cyclooxygenase inhibition with indomethacin abolished this rise in 6-keto-PGF1α, the same low dose of LPS significantly decreased GFR (110.7 ± 12.1 vs. 173.3 ± 6.7 μl/min, P < 0.05). The same dose of indomethacin did not alter GFR in WT mice. To further study the role of PGI2 in endotoxemia, renal-specific PGI synthase (PGIs) expression was increased only in the kidney and increased urinary 6-keto-PGF1α. These Tg mice, however, demonstrated endotoxemia-related AKI with low-dose LPS (1 mg/kg) (GFR: 12.6 ± 3.9 vs. 196.5 ± 21.0 μl/min, P < 0.01), which did not alter GFR in WT mice (164.0 ± 16.7 vs. 173.3 ± 6.7 μl/min, P = not significant). An elevation in renal cAMP, however, suggested an activation of the PGI2-cAMP-renin system in these Tg mice. Moreover, angiotensin-converting enzyme inhibition afforded protection against endotoxin-related AKI in these Tg mice. Thus endothelial PGIs-mediated PGI2, as previously shown with endothelial nitric oxide synthase-mediated nitric oxide, contributes to renal protection against endotoxemia-related AKI. This effect may be overridden by excessive activation of the renin-angiotensin system in renal-specific PGIs Tg mice.

Endotoxin [lipopolysaccharide (LPS)] has been shown to induce nitric oxide synthase (20, 24). The resultant nitric oxide-mediated arterial vasodilation activates the sympathetic and renin-angiotensin systems, which maintain blood pressure at the expense of renal vasoconstriction (27). The renal vasoconstriction predisposes to AKI, and protection has been demonstrated with renal denervation (27).

There is experimental evidence that the endothelium is involved in the renal vasoconstriction-associated endotoxemia. Specifically, endothelial nitric oxide synthase (eNOS) knockout mice have been shown to have increased blood pressure and renal vascular resistance (9, 29). In this setting of renal vasoconstriction, a small dose of endotoxin that has no effect on wild-type mice causes AKI in the eNOS knockout mice. There is also evidence that endotoxin-related increase in tumor necrosis factor-α (2, 11, 30) and oxygen radicals (28) is involved in endotoxemia-related endothelial damage and AKI.

With the importance of the endothelium in endotoxemia-related AKI, a potential renal protective role of prostacyclin (PGI2) must be considered. In that regard, renal sympathetic stimulation and angiotensin, events that occur with endotoxemia (27), are known to increase PGI2 synthesis (8).

The present study was, therefore, undertaken to examine the effect of 1) decreased renal PGI2 with cyclooxygenase (COX) inhibition, and 2) increase in renal PGI2 with renal-specific PGIs transgenic (PGIs) Tg mice on endotoxin-induced AKI.

METHODS

Animals. The experimental protocol was approved by the Animal Ethics Review Committee at the University of Colorado Health Sciences Center, C57BL/6 mice were purchased from Jackson Laboratories (Bar Harbor, ME). In collaboration with Dr. Mark Geraci’s laboratory, a kidney-specific (KSP)-cadherin/PGLs transgenic construct was developed. This construct has been shown to express transgenes in the kidney (10, 23). University of Cincinnati Transgenic Mouse Core Facility produced Tg mice, which overexpress PGIs. The background strain of the PGIs Tg mice is C57BL/6. Male mice aged 8–10 wk were used throughout the study. Mice were maintained on a standard rodent chow and had free access to water.

Materials. Chemicals were purchased from Sigma (St. Louis, MO), unless otherwise specified.

Animal protocol. Wild-type mice were injected intraperitoneally with a 0.5 or 2.5 mg/kg dose of LPS (Escherichia coli 0111:B4 from LIST Biological Laboratories, Campbell, CA). The 2.5 mg/kg dose
causes a dramatic decrease in glomerular filtration rate (GFR) of \( \sim 75\% \) (27, 28), whereas no fall in GFR occurs with the 1 mg/kg LPS dose (29). In preliminary studies, the PGIs Tg mice showed increased sensitivity to endotoxin with the 2.5 mg/kg LPS dose that caused anuria in those mice. Thus 1.0 mg/kg dose of LPS was used, which did not affect GFR in wild-type mice, but caused a significant decrease in GFR in PGIs Tg mice. As a potential protective intervention, the angiotensin-converting enzyme inhibitor (ACEI), 1 mg/kg enalapril, or vehicle was injected subcutaneously 30 min before LPS. The COX inhibitor, 5 mg/kg indomethacin, was injected intraperitoneally 30 min before LPS. Functional studies were carried out 16 h after LPS administration. In this model, AKI has been shown to be established at 16 h (27–29).

**Measurement GFR, renal blood flow, and mean arterial pressure.** The animals were anesthetized with pentobarbital (60 mg/kg) and placed on a thermostatically controlled surgical table. A tracheotomy was performed in all mice. Catheters (custom pulled from PE-250) were placed in the jugular vein for maintenance infusion and in the carotid artery for blood pressure measurement. The kidney was exposed by a left subcostal incision and was dissected free from perirenal tissue, and the renal arteries were isolated for the determination of renal blood flow (RBF) using a flowmeter and probe (0.5 V) (Transonic Systems, Ithaca, NY), as described by Traynor and Schnermann (26). Mean arterial pressure (MAP) was measured via a carotid artery catheter connected to a Transpac IV transducer and monitored continuously using Windaq Waveform recording software (Dataq Instruments). An intravenous maintenance infusion of 2.25% bovine serum albumin in normal saline at a rate of 0.25 ml/g body wt \(^{-1}\) was started 1 h before experimentation. FITC (0.75%)-inulin was added to the infusion solution for the determination of GFR, as described by Lorenz and Gruenstein (13). A bladder catheter (PE-10) was used to collect urine. Two 30-min collections of urine were obtained under oil and weighed for volume determination. Blood for plasma inulin determination was drawn between urine collections. FITC in plasma and urine samples were measured using CytoFluor plate reader (PerSeptive Biosystems, Foster City, CA).

**Construction of the KSP-cadherin/PGIs transgene.** Using a combination of polymerase chain reaction (PCR) and standard hybridization library screening, the entire coding sequence for the rat PGIs cDNA has been determined and submitted to GenBank/EMBL with accession no. U53855 (6). The coding region of the rat PGIs cDNA shows 82% homology to the bovine sequence (7, 17) and 82.5% homology to the human (15) cDNA. A full-length cDNA for the rat PGIs was amplified by PCR from a rat lung 5'-stretch cDNA library constructed in a \( \lambda gt11 \) vector (CLONTECH Laboratories, Palo Alto, CA) using a high-fidelity \( pfu \) DNA polymerase (Stratagene, La Jolla, CA) with PCR primers that encompass the start (5'-CTG GAA TTC...)

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**Fig. 1.** A: effect of indomethacin (Indo) on urinary 6-keto-PGF\(_{1\alpha}\) during endotoxemia in wild-type (WT) mice. LPS 1.0 mg/kg was injected intraperitoneally, and urine was collected 16 h after LPS administration. Urine 6-keto-PGF\(_{1\alpha}\) was measured using enzyme immunoassay kit. B: effect of Indo on glomerular filtration rate (GFR) during endotoxemia in WT mice. LPS 1.0 mg/kg was injected intraperitoneally, and GFR was measured 16 h after LPS administration. GFR was measured by FITC-inulin clearance. Values are means ± SE. *P* value <0.05 is considered statistically significant.

**Fig. 2.** A: baseline and LPS response of GFR, renal blood flow (RBF), and mean arterial pressure (MAP) in PGI synthase (PGIs) transgenic (Tg) mice compared with WT mice. GFR was measured by FITC-inulin clearance, RBF by renal flow probe, and MAP through carotid artery. B: increased urine 6-keto-PGF\(_{1\alpha}\) and renal cAMP in PGIs Tg mice compared with WT mice. Urine 6-keto-PGF\(_{1\alpha}\) levels were measured using enzyme immunoassay kit. Renal cAMP levels were measured using Correlate-EIA Direct cAMP enzyme immunoassay kit. NS, not significant. Values are means ± SE. *P* value <0.05 is considered statistically significant.
CGG GAG CCA TG-3') and stop (5'-AGT GTC TGC TCC ACA GGT CA-3') codons of the PGIs cDNA. A complete open-reading frame is demonstrated by sequence analysis using Applied Biosystems 377 and 373 sequencers (Perkins-Elmer, Foster City, CA). The KSP-1 vector was a kind gift from Dr. Igarashi at Southwestern Medical Center at Dallas. It contains 3.55 kb of flanking sequence of the mouse KSP-cadherin promoter. This construct has been shown to express transgenes in the renal tubular epithelium (23). The rat PGIs cDNA was cloned into the SmaI site, creating the KSP-cadherin-PGIs-Bovine Growth Hormone vector. The proper cloning orientation of our construct was confirmed by direct sequence analysis.

Geraci et al. (6) have shown that Tg animals with epithelial overexpression of PGIs will have profound vascular effects by the paracrine action of PGI2. Animals with lung epithelial overexpression of PGIs demonstrated a vascular phenotype by inhibiting pulmonary vasoconstriction during hypoxia. The authors conclude that this eicosanoid acts through autocrine and paracrine mechanisms.

PGIs protein expression. Organs were homogenized in 250 mM sucrose, 25 mM imidazole, 1 mM ethylenediaminetetraacetic acid, and 1/10 volume of a protease solution consisting of 25 μg/ml aprotinin, 0.5 μg/ml leupeptin, 0.7 μg/ml pepstatin, 0.1 mg/ml soybean trypsin inhibitor, and 200 μM phenylmethylsulfonyl fluoride. SDS-PAGE immunoblotting was performed on 50 μg of protein extract. Samples were electrophoresed through a 10% acrylamide gel for detection of PGIs. PGIs protein was detected using a rabbit polyclonal antibody (Cayman, Ann Arbor, MI) diluted 1:250 in Tris-buffered saline (TBS) and a goat polyclonal antibody (Abcam, Cambridge, MA) diluted at 1:3,000. The secondary antibodies were conjugated to horseradish peroxidase. Antigenic detection was made by enhanced chemiluminescence (Amersham, Arlington Heights, IL) with exposure to X-ray film.

Fig. 3. PGIs protein expressions in the kidney (A), lung (B), heart (C), and liver (D). Western blots were used to examine PGIs and β-actin protein expressions in different organs in the WT and PGIs Tg mice. Rabbit polyclonal anti-PGIS and anti-β-actin antibodies were used as the primary antibodies, and protein bands were detected using enhanced chemiluminescence method.

**RESULTS**

**Effect of indomethacin on urine 6-keto-PGF1α and renal function during endotoxemia in the wild-type mice.** LPS administration (1.0 mg/kg) in wild-type mice was associated with an increase in urinary 6-keto-PGF1α (Fig. 1A), the major PGI2 metabolite, and no change in GFR (Fig. 1B). Next, studies were undertaken to examine whether the COX inhibitor, indomethacin, in a dose to decrease urinary 6-keto-PGF1α, would alter the renal response to endotoxemia. In these studies, 1 mg/kg LPS did not alter GFR in untreated mice, while indomethacin-treated mice demonstrated a significant decrease in GFR (Fig. 1B). The decrease in GFR was not associated with an increase in urinary albumin excretion.

**Characterization of PGIs Tg mice.** Baseline data for these PGIs Tg indicated no differences from wild-type mice for GFR

![Image](http://ajprenal.physiology.org/)
(196.5 ± 21.0 vs. 212.0 ± 10.0 µl/min) and MAP (91.1 ± 2.3 vs. 89.1 ± 4.1 mmHg). RBF (1.59 ± 0.12 vs. 1.08 ± 0.1 ml/min, P < 0.01) was increased in the PGI-Tg (Fig. 2A). There is a significant increase in urinary 6-keto-PGF1α-to-creatinine ratio in the PGI-Tg animals (3.8 ± 0.38 vs. 2.2 ± 0.4, P < 0.05) (Fig. 2B). Renal cyclic AMP concentrations were also significantly greater in the PGI-Tg mice (11.9 ± 1.0 vs. 5.5 ± 0.28 pg/ml, P < 0.0001) (Fig. 2B). PGI protein expression increased significantly in the kidney in the Tg mice (Fig. 3A). The PGI expression levels were similar in the wild-type mice and Tg mice in other organs, such as lung (Fig. 3B), heart (Fig. 3C), and liver (Fig. 3D).}

**Increased sensitivity to endotoxin in PGI-Tg mice compared with the wild-type mice.** Despite comparable GFR and higher RBF and MAP at the baseline, PGI-Tg animals were more sensitive to LPS. While GFR remained unchanged [189.7 ± 19.0 vs. 212.0 ± 10.0 µl/min, P = not significant (NS)] with 1.0 mg/kg dose of LPS in the wild-type control mice, it decreased significantly (12.6 ± 3.9 vs. 196.5 ± 21.0 µl/min, P < 0.01, Fig. 4A) in the PGI-Tg mice. MAP also decreased significantly with 1.0 mg/kg LPS (71.8 ± 3.1 vs. 91.1 ± 2.3 mmHg, P < 0.01, Fig. 4B), while RBF remained unchanged (1.36 ± 0.20 vs. 1.59 ± 0.12 ml/min, P = NS, Fig. 4C).

**Effect of enalapril on decreased GFR during endotoxemia in PGI-Tg mice and wild-type mice.** To examine whether PGI-cyclic AMP-renin pathway accounted for the increased sensitivity to LPS in the PGI-Tg mice, they were pretreated with the ACEI enalapril. Enalapril (1 mg/kg) was administrated subcutaneously 30 min before LPS injection (1.0 mg/kg ip). Renal function was examined 16 h after LPS administration. GFR improved significantly with enalapril (101.7 ± 17.2 vs. 12.6 ± 3.9 µl/min, P < 0.001, Fig. 5A), even though there were no significant differences in MAP (80.6 ± 5.5 vs. 71.8 ± 3.1 mmHg, P = NS, Fig. 5B) and RBF (1.56 ± 0.08 vs. 1.36 ± 0.2 ml/min, P = NS, Fig. 5C). There was no protective effect of enalapril on GFR or RBF in wild-type mice with the 2.5 mg/kg LPS dose, which causes AKI (Fig. 6). There was no difference in MAP.

**DISCUSSION**

The integrity of renal eNOS in the endothelium has been shown to be important in endotoxemia-related AKI (21, 22,
PGIs expression also occurs in the endothelium, and PGI2 has been shown to increase during renal ischemia and cause renal vasodilatation (8). Since a decrease in PGI2 by inhibition of COX can be deleterious to renal function in settings in which renal vasoconstriction already exists, such as congestive heart failure (25) and cirrhosis (1, 5, 16), the potential role of PGI2 in endotoxemia-related AKI was investigated.

Of interest, lung-specific PGIs Tg mice have been shown to be protected against hypoxia-related acute lung injury (6). The possibility, therefore, arose that renal-specific PGIs Tg mice would be protected against endotoxemia-related AKI. Such mice were, therefore, developed and investigated during LPS administration. These Tg mice demonstrated an increase in renal PGIs and urinary 6-keto-PGF1α.

Studies were initially undertaken in wild-type mice. Low-dose LPS (1 mg/kg) was shown to significantly increase urinary 6-keto-PGF1α, and there was no effect on GFR. The possibility, however, existed that the increase in renal PGI2 was affording renal protection in wild-type mice. Studies were, therefore, undertaken using a dose of the COX inhibitor, indomethacin, which significantly blocked the increase in urinary 6-keto-PGF1α. In these experiments, the administration of low-dose LPS (1 mg/kg) was shown to cause AKI as assessed by a significant fall in GFR. These results, therefore, indicated that, along with eNOS, endothelial synthesis of PGI2 affords renal protection during endotoxemia.

These results in wild-type mice suggested that an upregulation of renal-specific PG synthase might afford renal protection during endotoxemia, as has been reported in the lung with pulmonary-specific PGIs (6). The present results of the experiments in the renal-specific PGIs Tg mice were, however, quite surprising. These Tg mice were found to be more, not less, sensitive to endotoxin-related AKI.

These provocative results in the Tg mice were, therefore, in need of explanation. Of interest were previous studies in mice lacking the PGI2 receptor (4). Renal artery stenosis in these mice was found to be associated with decreased responses of plasma renin activity compared with wild-type mice or mice lacking any of the four PGE subtype receptors (4). Since PGI2 is known to increase renal cyclic AMP and renin (12), the possibility existed that the PGIs Tg mice were exhibiting increased cyclic AMP, and the resultant enhanced renin-angiotensin activity was obscuring any renal vasodilating effect of PGI2. This could explain the increased renal sensitivity of the PGIs Tg mice to endotoxin. This possibility was pursued in two ways. First, the renal cyclic AMP levels were measured and compared with wild-type mice. The Tg mice exhibited significantly higher renal concentrations of cyclic AMP than wild-type mice. Second, the effect of the ACEI enalapril to afford renal protection against endotoxemia-related AKI in the PGIs Tg mice was examined. The results demonstrated that enalapril afforded renal protection in these Tg mice. In the 2.5 mg/kg LPS model of AKI in wild-type mice, there was no observed effect of enalapril. The decrease in MAP in PGIs Tg mice with 1.0 mg/kg of LPS may also have contributed to the decrease in GFR observed in the Tg mice.

In summary, the present results provided further evidence for the importance of the endothelium in the pathogenesis of endotoxin-related AKI. As with the previously demonstrated protective effect of eNOS, an increase in renal PGIs attenuates renal injury during endotoxemia. An excess of renal PGIs in Tg mice, however, may obscure beneficial effects against endotoxemia-related AKI by activating the PGI2-cyclic AMP-renin pathway. While renal-specific PGIs Tg mice are not protected against LPS-related AKI, we cannot exclude a potential renal protective effect of upregulated systemic PGI2.

REFERENCES


