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

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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 an 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).

Materials. Chemicals were purchased from Sigma (St. Louis, MO), and the 2.5 mg/kg dose of LPS (Escherichia coli 0111:B4) was purchased from LIST Biological Laboratories, Campbell, CA. The 2.5 mg/kg dose of LPS was injected intraperitoneally with a 1 or 2.5 mg/kg dose of LPS (Escherichia coli 0111:B4) from LIST Biological Laboratories, Campbell, CA. The 2.5 mg/kg dose of LPS was injected intraperitoneally with a 1 or 2.5 mg/kg dose of LPS (Escherichia coli 0111:B4) from LIST Biological Laboratories, Campbell, CA.
causes a dramatic decrease in glomerular filtration rate (GFR) of 
~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 m/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,
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 determina-
tion of renal blood flow (RBF) using a blood flowmeter and probe
(0.5 V) (Transonic Systems, Ithaca, NY), as described by Traynor and
Schnermann (20). 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 μl·g body
wt⁻¹·min⁻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 com-
bination of polymerase chain reaction (PCR) and standard hybridiza-
tion 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 λgt11 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

![Graph A](https://example.com/graph1.png)

![Graph B](https://example.com/graph2.png)

Fig. 1. A: effect of indomethacin (Indo) on urinary 6-keto-PGF₁α during endotoxemia in wild-type (WT) mice. LPS 1.0 mg/kg was injected intraperi-
toneally, and urine was collected 16 h after LPS administration. Urine 6-keto-
PGF₁α 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.

![Graph C](https://example.com/graph3.png)

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-PGF₁α and renal
cAMP in PGIs Tg mice compared with WT mice. Urine 6-keto-PGF₁α 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 Smal 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 PGIs. 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 antipain, 1 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, Cambridge, MA) diluted 1:250 in Tris-buffered saline 0.1% Tween 20 containing 5% dry milk. β-Actin protein expression was used as loading controls and was detected using a rabbit polyclonal antibody (Abcam, Cambridge, MA) diluted at 1:3,000. The secondary antibodies were conjugated to horseradish peroxidase. Antibody detection was made by enhanced chemiluminescence (Amersham, Arlington Heights, IL) with exposure to X-ray film.

**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 PGF2 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.
(196.5 ± 21.0 vs. 212.0 ± 10.0 μl/min) and MAP (91.1 ± 2.3 vs. 89.1 ± 4.1 mmHg). RBF (1.39 ± 0.12 vs. 1.08 ± 0.1 ml/min, P < 0.01) was increased in the PGI₃ Tg (Fig. 2A). There is a significant increase in urinary 6-keto-PGF₁α-to-creatinine ratio in the PGI₃ 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 PGI₃ Tg mice (11.9 ± 1.0 vs. 5.5 ± 0.28 pg/ml, P < 0.0001) (Fig. 2B). PGI₃ protein expression increased significantly in the kidney in the Tg mice (Fig. 3A). The PGI₃ 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 PGI₃ Tg mice compared with the wild-type mice. Despite comparable GFR and higher RBF and MAP at the baseline, PGI₃ 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 PGI₃ Tg mice. MAP also decreased significantly with 1.0 mg/kg LPS (71.8 ± 3.1 vs. 91.1 ± 2.3 mmHg, P < 0.05, 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 PGI₃ Tg mice and wild-type mice. To examine whether PGI₃-cyclic AMP-renin pathway accounted for the increased sensitivity to LPS in the PGI₃ 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,
29). PGIs expression also occurs in the endothelium, and PGI\textsubscript{2} has been shown to increase during renal ischemia and cause renal vasodilation (8). Since a decrease in PGI\textsubscript{2} 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 PGI\textsubscript{2} 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-PGF\textsubscript{1α}.

Studies were initially undertaken in wild-type mice. Low-dose LPS (1 mg/kg) was shown to significantly increase urinary 6-keto-PGF\textsubscript{1α}, and there was no effect on GFR. The possibility, however, existed that the increase in renal PGI\textsubscript{2} 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-PGF\textsubscript{1α}. 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 PGI\textsubscript{2} 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 PGI\textsubscript{2} 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 PGI\textsubscript{2} 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 PGI\textsubscript{2}. 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 endotoxemia-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 PGI\textsubscript{2}-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 PGI\textsubscript{2}.

REFERENCES


