Experimental strategies in the treatment of acute renal failure in sepsis
Johannes, T.

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CHAPTER 5

ILOPROST PRESERVES RENAL OXYGENATION AND RESTORES KIDNEY FUNCTION IN ENDOTOXEMIA-INDUCED ACUTE RENAL FAILURE IN THE RAT

Tanja Johannes, Can Ince, Karin Klingel, Klaus E Unertl, and Egbert G Mik

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Abstract

Objective: To investigate that exogenous prostacyclin would counterbalance an endotoxemia-induced intrarenal vasoconstriction and would therefore have beneficial effects on kidney function.

Design: Prospective, randomized, controlled study.

Setting: University medical center research laboratory.

Subjects: Eighteen male Wistar rats.

Interventions: In anesthetized and ventilated animals, arterial blood pressure (mean arterial blood pressure [MAP]) and renal blood flow (RBF) were recorded. Renal microvascular PO$_2$ (µPO$_2$) and renal venous PO$_2$ were continuously measured by phosphorescence lifetime technique. All animals received a 30-minute infusion of lipopolysaccharide (LPS) (2.5 mg/kg) to induce endotoxemia. One group of rats was not resuscitated. A second group received fluid resuscitation 90 minutes after stop of LPS infusion. In a third group of rats, the prostacyclin analogue iloprost (100 ng/kg/min) was continuously infused in addition to fluid resuscitation. Furthermore, in all the animals, plasma NOx levels, renal inducible nitric-oxide synthase (iNOS) messenger RNA (mRNA) expression, and creatinine clearance were determined.

Measurements and Main Results: During LPS infusion, MAP and RBF progressively dropped to 50% of baseline at 120 minutes. After an initial increase in MAP and RBF, start of fluid resuscitation with iloprost resulted in the stabilization of both parameters. All animals became anuric during endotoxemia. Only in animals receiving iloprost was creatinine clearance totally restored at the end of the experiment. Iloprost had no significant effects on average µPO$_2$, but prevented the occurrence of cortical microcirculatory hypoxic areas. NOx levels and iNOS mRNA expression were significantly increased in all animals receiving LPS after 5 hours. There was no difference in NOx concentration between the different groups. In animals receiving iloprost, iNOS mRNA expression was significantly suppressed in the inner medulla.

Conclusions: Iloprost significantly restored kidney function of endotoxemic rats to baseline values. This beneficial effect of iloprost on renal function might be addressed to an improvement in intrarenal oxygenation.
Introduction

Acute renal failure (ARF) is a serious condition in patients with septic shock with 70% mortality (1, 2). Unfortunately, the pathogenesis is only partly understood, and there is need to further identify the mechanisms responsible for the development of ARF in sepsis and define strategies to prevent the development of ARF due to septic conditions. So far, it is known that sepsis-associated kidney dysfunction is characterized by a reduction in renal blood flow (RBF) and glomerular filtration rate (3). Morphology might range from endothelial damage in glomerular and interstitial vessels, disseminated fibrin thrombi, and aggregation of leukocytes and platelets to acute tubular necrosis (4). In sepsis, there is a release of a vast array of inflammatory cytokines, vasoactive mediators (nitric oxide [NO] adenosine, etc.), thrombogenic substances, and arachidonic acid metabolites (5). Overall, the predominant pathogenetic factor in the development of ARF seems to be renal hypoperfusion due to an imbalance between renal vasoconstriction and vasodilation (6). Whether this results in hypoxia as a key pathogenic factor remains controversial (7); but recently, we demonstrated that endotoxemia is associated with the occurrence of local hypoxia in the renal cortex in rats (8).

In the kidney, prostaglandins uphold the balance between vasodilator and vasoconstrictor to maintain homeostasis and physiologic kidney function (9). Because of its potent vasodilatory effects, prostacyclin (PGI\textsubscript{2}) must be considered to play a potential renal protective role in septic ARF. The synthesis of PGI\textsubscript{2} along the nephron is quite significant and highest in the glomeruli and the inner medulla. The documented role of PGI\textsubscript{2} in the kidney is the regulation of renal and glomerular hemodynamics, renin secretion, and tubular transport processes (6). In animal experimental studies, the stable PGI\textsubscript{2} analogue iloprost preserved kidney function against anoxia in rabbits (10), and had beneficial effects in ischemia/reperfusion-induced renal injury in a rat model (11). Furthermore, in a rat study of endotoxemia, iloprost inhibited an increase in plasma NO\textsubscript{2}/NO\textsubscript{3} and lung tissue inducible nitric-oxide synthase (iNOS) expression (12). In a clinical study, iloprost was successfully used to prevent contrast-mediated nephropathy (13). On the basis of the physiologic actions of PGI\textsubscript{2} and the described renal protective effects of iloprost, we hypothesized that this PGI\textsubscript{2} analogue might have a potential role in a renal protective strategy in sepsis.

This study in a rat model of endotoxemia was undertaken to examine whether 1) application of exogenous PGI\textsubscript{2} (iloprost) would counterbalance a sepsis-induced intrarenal vasoconstriction and would therefore have beneficial effects on kidney function; 2) this effect would be related to an intrarenal shift in microvascular oxygenation; and 3) iloprost-mediated effects were associated with changes in renal iNOS messenger RNA (mRNA) expression and total body NO production.

Material and methods

Experiments were conducted in accordance with the guidelines for Institutional and Animal Care and Use Committees’ care and handling of the animals and approved by the Animal Research Committee of the Academic Medical Center at the University of Amsterdam.

Animal Preparation and Monitoring.

Male Wistar rats (259 ± 42 g; Harlan, Horst, The Netherlands) were anesthetized with a mixture of ketamine (90 mg/kg), medetomidine (0.5 mg/kg), and atropine-sulfate (0.05 mg/kg) intraperitonally. A tracheostomy was performed, and the animals were mechanically ventilated (FIO\textsubscript{2} 0.4). The right
carotid artery was cannulated and used for monitoring of arterial blood pressure and heart rate. To allow continuous central venous pressure measurement, a catheter was inserted into the right jugular vein to a level of the right atrium. A catheter in the right femoral artery was used for withdrawal of blood via the right femoral vein for continuous infusion of Ringer’s lactate (15 mL/kg/h; Baxter, The Netherlands) and ketamine (50 mg/kg/h; Nimetek; Eurovet, The Netherlands). Rectal temperature was maintained at 37°C. Through flank incision, the left kidney was exposed, decapsulated, and immobilized. Left RBF was measured with a perivascular flow probe connected to a flow meter (T206; Transonic Systems, Ithaca, NY). The left ureter was isolated, ligated, and cannulated for urine collection. At the end of the experiment, the left kidney was removed and weighed. All preceding steps are described in detail in a previous study (14).

**Experimental Protocol.**

At the start of the experiment, rats were randomized between control (n = 4), nonresuscitation (n = 6), fluid resuscitation (n = 6), and iloprost (n = 6) groups. All animals receiving iloprost received standard fluid resuscitation with hydroxyethyl starch (130 kDa). At the end of surgery (60 minutes), two optical fibers for phosphorescence measurements were placed both 1 mm above the kidney surface and 1 mm above the renal vein and an intravenous infusion of Oxyphor G2 (10 mg/kg in 15 minutes; Oxygen Enterprises, USA) was started. Forty minutes later, µPO\(_2\) and rvPO\(_2\) were continuously recorded. Then, a baseline blood sample (0.4 mL) was taken. In 18 rats, a 30-minute infusion of lipopolysaccharide (LPS, 2.5 mg/kg; serotype 0127:B8, Sigma, The Netherlands) was given to induce septic shock. Ninety minutes after stop of LPS infusion, two groups of animals received fluid resuscitation (5 mL/kg followed by 5 mL/kg/hr; Voluven, 6% hydroxyethyl starch 130/0.4; Fresenius Kabi, The Netherlands). Additional to the fluid resuscitation, in one group of animals iloprost (100 ng/kg/min; Ilomedine, Schering, The Netherlands) was continuously infused. Continuous infusion was chosen because of the pharmacokinetic properties of the drug (a short plasma half-life of 20–30 minutes) and continued throughout the end of the protocol. Another group (nonresuscitation) did not receive fluid resuscitation after LPS infusion. All animals received the same fluid volume except the nonresuscitation group. The experiment was ended 10 minutes after stop of treatment or a corresponding time point for the control groups. One animal of the nonresuscitation group died during the experiment and was replaced.

**Measurement of Renal Microvascular Oxygenation and Renal Venous PO\(_2\).**

The renal microvascular PO\(_2\) (µPO\(_2\)) within the kidney cortex and outer medulla was measured by oxygen-dependent quenching of phosphorescence using a dual-wavelength-phosphorimeter (15). The renal venous PO\(_2\) (rvPO\(_2\)) was detected by the same method (16). Briefly described, Oxyphor G2 (Oxygen Enterprises, Philadelphia, PA), intravenously infused, binds to albumin (17). If excited by a flash of light (wavelength ~440 or 630 nm), the Oxyphor G2-albumin complex emits phosphorescence (wavelength ~800 nm). Dependent on the oxygen concentration, the phosphorescence intensity decreases and the relationship between the measured decay-time and the PO\(_2\) can be estimated using the Stern-Volmer relation (18–20). Heterogeneity in oxygen pressure was analyzed by fitting a sum of small rectangular distributions to the distributions of quencher concentration in the phosphorescence data (21).

**Blood Gas Measurements.**

An arterial blood sample (0.4 mL) was taken via the femoral artery at three different time points: first time point, 0 minutes = baseline (t\(_b\)); second time point, 120 minutes = endotoxemia (t\(_1\)); and third
time point, ~300 minutes = resuscitation ($t_2$). The same volume of hydroxyethyl starch replaced the sampling volume of blood. Samples were analyzed for the determination of blood gas values (ABL505 blood gas analyzer; Radiometer, Denmark), hematocrit, Hb concentration, and HbSO$_2$ (OSM3; Radiometer, Denmark). Furthermore, in each sample sodium and potassium concentration, plasma creatinine concentration, and NOx plasma levels were measured.

**Calculations of Renal Oxygenation.**
Renal oxygen delivery ($DO_{2ren}$) was calculated as RBF x arterial oxygen content: RBF x (1.31 x Hb x SaO$_2$) + (0.003 x PaO$_2$). Renal oxygen consumption ($VO_{2ren}$) was calculated as RBF x (arterial - renal venous oxygen content difference). Renal venous oxygen content is: (1.31 x Hb x SrvO$_2$) + (0.003 x rvPO$_2$) (16). An estimation of the renal vascular resistance (RVR) of the renal artery flow region was made: $RVR = MAP/\text{RBF}$ (22).

**Measurement of Renal Function.**
Creatinine clearance ($Cl_{crea}$) was assessed as an index of glomerular filtration rate. Calculations of the clearance were performed with standard formula: $Cl_{crea}$ (mL/min) = (U x V)/P, where U is the urine creatinine concentration, V is the urine volume per unit time, and P is the plasma creatinine concentration. The specific elimination capacity for creatinine of the left kidney was normalized to the organ weight. For analysis of urine volume and creatinine concentration, urine samples from the left ureter were collected at 10-minute intervals. At the midpoint of each 10-minute interval, plasma creatinine concentration was analyzed. Analysis of samples was performed using Jaffe method. In all, the urine samples’ sodium concentration was determined. To calculate sodium excretion (UNa$^+$ x V), sodium concentration in urine (UNa$^+$; mmol/L) was multiplied by the urine flow (V). The cost of sodium transport ($VO_2/TNa^+$) is the relation of the total amount of $VO_2$ over the total amount of sodium reabsorbed ($TNa^+$). $TNa^+$ (mmol x min$^{-1}$) was calculated according to: ($Cl_{crea} x PNa^+$)– UNa$^+$ x V, where PNa$^+$ is the plasma concentration of sodium.

**Plasma NOx Measurement.**
NOx (nitrate/nitrite/S-nitrosothiols) in plasma was determined using a NO analyzer (Sievers Instruments, Boulder, CO). For measurement of NOx plasma, samples (50 µL) were centrifuged at 20,200g for 10 minutes and immediately frozen at -80°C. A detailed description of this method is published elsewhere (23). Briefly described, NOx is determined using vanadium (III) chloride (Sigma-Aldrich, Zwijndrecht, The Netherlands). Vanadium (III) chloride reduces nitrate/nitrite to NO gas, which is released in a closed system apparatus of an ozone-based chemiluminescent assay at a temperature of 90°C. A calibration curve of sodium nitrate was constructed using the mean of ten measured values for each calibration point. As concentrations of plasma nitrate are 20–40 µM/L, of nitrite 0.2–1 µM/L and of S-nitrosothiols 0.002–0.005 µM/L, the value obtained by the vanadium assay is mostly nitrate. The raw data were analyzed using the integration method for estimating the area under the curve.

**iNOS mRNA Expression.**
After termination of the experiment, the kidney was immediately fixed in 4% paraformaldehyde overnight and embedded in paraffin. iNOS mRNA expression was determined by in situ hybridization. $^{35}$S-labeled antisense RNA probes for the detection of iNOS mRNA were synthesized by in vitro transcription from the dual promoter plasmid pSPT18 containing a 725-bp murine iNOS cDNA fragment. Control mRNA probes were obtained from the vector pSPT18. Pretreatment, hybridization, and washing conditions of dewaxed 5-µm paraffin renal tissue sections were...
performed as described previously (24). Slide preparations were subjected to autoradiography, exposed for 3 weeks at 4°C, and counterstained with hematoxylin and eosin. Image capturing was performed using phase-contrast microscopy (20x objective, DMIRB; Leica, Bensheim, Germany) and a digital camera (ProgRes C10; JenaOptik, Jena, Germany). Quantitative analysis of autoradiographic signals obtained by in situ hybridization representing iNOS mRNA expressing renal tissue was done by self-written image analysis software. Areas of hybridization-positive renal tissue were referred to the total area of tissue sections and expressed in percentage of iNOS mRNA positive (% iNOS+) pixels per 0.24 mm² renal tissue (n = three kidneys for each group).

Data Presentation and Statistics.
Values are presented as mean ± SD, unless otherwise indicated. Analysis of the mono-exponential fit procedures of the phosphorescence curves was performed using Labview 6.1 software (National Instruments, Austin, TX). For PO₂ histogram recovery and statistics, GraphPad Prism version 4.0 for Windows (GraphPad Software, San Diego, CA) was used. For data analysis within each group and intergroup differences, two-way analysis of variance for repeated measurements with Bonferroni posttest was performed. \( p < 0.05 \) were considered significant.

Results

Systemic and Regional Hemodynamics.
The baseline values in the experimental groups and the control group were not different (Table 1). In the nonresuscitation group, MAP decreased 43% at t₁ and dropped further to 60% at t₂ compared with baseline \( (p < 0.05 \) vs. control). The heart rate was significantly decreased compared with control 5 hours after start of LPS infusion. The central venous pressure was significantly increased at t₁ and t₂. After LPS infusion, RBF dropped 62% \( (t₁) \) and reached its lowest reading at t₂ with 80% compared with baseline \( (p < 0.05 \) vs. control). The RVR increased by 61% at t₂.

Fluid resuscitation could not restore MAP. There was a significant increase in central venous pressure at t₁ and t₂ compared with baseline. After fluid resuscitation, RBF was still 63% lower than at baseline. After a significant increase in RVR at t₁ \( (p < 0.05 \) vs. control), RVR was normalized by fluid resuscitation.

In the iloprost group, MAP decreased 39% after LPS infusion compared with baseline. A further drop in MAP was prevented in animals receiving iloprost as a supplement to standard fluid resuscitation. In this group, MAP was 38% lower than at baseline at t₂ \( (p < 0.05 \) vs. control). The heart rate remained unchanged during the experimental period. There was a significant increase in central venous pressure at t₁ and t₂ compared with baseline. In the iloprost group, RBF was restored after resuscitation, slightly but not significantly, and 48% lower than at baseline. At t₂, RVR was normalized to baseline values.
### TABLE 1. Systemic hemodynamic parameters

<table>
<thead>
<tr>
<th></th>
<th>Baseline ($t_0$)</th>
<th>Endotoxemia ($t_1$)</th>
<th>Resuscitation ($t_2$)</th>
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<tbody>
<tr>
<td><strong>MAP (mmHg)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>non-resuscitation</td>
<td>114 ± 5</td>
<td>65 ± 5 *†</td>
<td>46 ± 7 *†</td>
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<tr>
<td>fluid resuscitation</td>
<td>108 ± 7</td>
<td>64 ± 16 *†</td>
<td>58 ± 12 *†</td>
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<tr>
<td>iloprost</td>
<td>107 ± 3</td>
<td>65 ± 8 *†</td>
<td>67 ± 4 *†</td>
</tr>
<tr>
<td>time control</td>
<td>109 ± 4</td>
<td>107 ± 7</td>
<td>103 ± 7</td>
</tr>
<tr>
<td><strong>HR (bpm)</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>non-resuscitation</td>
<td>262 ± 21</td>
<td>279 ± 32</td>
<td>250 ± 62 †</td>
</tr>
<tr>
<td>fluid resuscitation</td>
<td>261 ± 20</td>
<td>263 ± 21</td>
<td>277 ± 50</td>
</tr>
<tr>
<td>iloprost</td>
<td>258 ± 30</td>
<td>271 ± 27</td>
<td>290 ± 24</td>
</tr>
<tr>
<td>time control</td>
<td>271 ± 28</td>
<td>274 ± 35</td>
<td>321 ± 28</td>
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<tr>
<td><strong>CVP (mmHg)</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>non-resuscitation</td>
<td>5.7 ± 2.4</td>
<td>7.2 ± 1.4 *</td>
<td>7.9 ± 1.9 *</td>
</tr>
<tr>
<td>fluid resuscitation</td>
<td>6.8 ± 0.9</td>
<td>8.1 ± 0.7 *</td>
<td>8.0 ± 2.0 *</td>
</tr>
<tr>
<td>iloprost</td>
<td>5.9 ± 2.0</td>
<td>7.0 ± 2.0 *</td>
<td>7.4 ± 2.2 *</td>
</tr>
<tr>
<td>time control</td>
<td>5.5 ± 1.1</td>
<td>6.5 ± 1.4</td>
<td>6.8 ± 1.4 *</td>
</tr>
<tr>
<td><strong>RBF (mL·min⁻¹)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>non-resuscitation</td>
<td>5.5 ± 0.3</td>
<td>2.1 ± 1.0 *†</td>
<td>1.1 ± 0.6 *†</td>
</tr>
<tr>
<td>fluid resuscitation</td>
<td>5.8 ± 0.9</td>
<td>1.6 ± 0.9 *†</td>
<td>2.2 ± 1.4 *†</td>
</tr>
<tr>
<td>iloprost</td>
<td>6.0 ± 0.6</td>
<td>2.2 ± 1.0 *†</td>
<td>3.1 ± 1.4 *†</td>
</tr>
<tr>
<td>time control</td>
<td>6.3 ± 0.8</td>
<td>5.6 ± 0.3</td>
<td>6.1 ± 0.9</td>
</tr>
<tr>
<td><strong>RVR (dyne·sec/cm⁵)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>non-resuscitation</td>
<td>21 ± 2</td>
<td>38 ± 19 *</td>
<td>54 ± 27 *†</td>
</tr>
<tr>
<td>fluid resuscitation</td>
<td>19 ± 3</td>
<td>51 ± 28 *†</td>
<td>36 ± 18</td>
</tr>
<tr>
<td>iloprost</td>
<td>18 ± 2</td>
<td>41 ± 32 *</td>
<td>26 ± 12</td>
</tr>
<tr>
<td>time control</td>
<td>18 ± 2</td>
<td>19 ± 2</td>
<td>17 ± 3</td>
</tr>
</tbody>
</table>

Values represent mean ± SD. * $P < 0.05$ vs baseline. † $P < 0.05$ vs control. MAP = mean arterial blood pressure; HR = heart rate; CVP = central venous pressure; RBF = renal blood flow; RVR = renal vascular resistance.

**Example Experiment.**

A typical example of an experiment is shown in Figure 1. During LPS infusion, MAP and RBF progressively dropped. At 120 minutes, MAP and RBF are about 50% decreased compared with baseline values. With the start of fluid resuscitation and continuous infusion of iloprost there was an initial increase in MAP and RBF followed by stabilization of both parameters. In four of six animals, there was an undulation in MAP and RBF during resuscitation with iloprost. After a period of anuria during septic shock the animal started to urinate under resuscitation. $C_{\text{crea}}$ was totally restored at the end of the experiment. After an initial increase in microvascular $\text{PO}_2$ ($\mu\text{PO}_2$) during LPS infusion, cortical ($c\mu\text{PO}_2$) and outer medullary ($m\mu\text{PO}_2$) microvascular $\text{PO}_2$ slightly dropped during resuscitation. Furthermore, the renal venous $\text{PO}_2$ ($r\text{PO}_2$) progressively decreased during the experimental period.
FIGURE 1. Example experiment. (A) During lipopolysaccharide (LPS) infusion mean arterial pressure (MAP) and renal blood flow (RBF) progressively drop. Compared with baseline values MAP and RBF are about 50% decreased at 120 minutes. After an initial increase in MAP and RBF with start of fluid resuscitation + iloprost both parameters are undulating and stabilizing. Following anuria during septic shock the animal starts to urinate under resuscitation. Creatinine clearance (Cl_{crea}) is totally restored at the end of the experiment. (B) After an initial increase in microvascular PO\textsubscript{2} (\(\mu\)PO\textsubscript{2}) during LPS infusion cortical (C) and outer medullary (m) \(\mu\)PO\textsubscript{2} slightly drop during resuscitation. Renal venous PO\textsubscript{2} (rvPO\textsubscript{2}) progressively decreases with start of LPS application.

Renal Oxygenation Parameters.

There was a significant decrease in \(\mu\)PO\textsubscript{2} and m\(\mu\)PO\textsubscript{2} at t\textsubscript{2} compared with baseline in the control group (Fig. 2). The renal oxygen delivery, consumption, and extraction (DO\textsubscript{2ren}, VO\textsubscript{2ren}, and renal oxygen extraction) remained unchanged over time.

In the nonresuscitation group, \(\mu\)PO\textsubscript{2} was stable around baseline values at t\textsubscript{1}. However, 5 hours post-LPS, \(\mu\)PO\textsubscript{2} and m\(\mu\)PO\textsubscript{2} were 22 ± 8 and 18 ± 8 mm Hg, respectively (\(p < 0.05\) vs. control). This significant reduction in the average \(\mu\)PO\textsubscript{2} was accompanied by a significant left shift in both the cortical and outer medullary oxygen histogram at t\textsubscript{1} and t\textsubscript{2} (Fig. 3). DO\textsubscript{2ren} decreased from 1.20 ± 0.23 at baseline to 0.42 ± 0.20 at t\textsubscript{1} and to 0.20 ± 0.12 mL/min at t\textsubscript{2} (\(p < 0.05\) vs. control) in the nonresuscitation group. In the same group, VO\textsubscript{2ren} was significantly decreased compared with baseline at t\textsubscript{2}, whereas renal oxygen extraction in- creased from 50 ± 15 at t\textsubscript{1} to 82% ± 9% at t\textsubscript{2} (\(p < 0.05\) vs. control).

After fluid resuscitation, \(\mu\)PO\textsubscript{2} and m\(\mu\)PO\textsubscript{2} were 41 ± 13 and 35 ±11 mmHg, respectively, which was only slightly but not significantly lower than in the control group. DO\textsubscript{2ren} was significantly decreased at t\textsubscript{1} and t\textsubscript{2} (\(p < 0.05\) vs. control). Furthermore, there was a significant drop in VO\textsubscript{2ren} with 0.13 ± 0.10 at t\textsubscript{1} and 0.16 ± 0.12 mL/min/g at t\textsubscript{2} compared with baseline in animals receiving fluid resuscitation. Renal oxygen extraction was 65% ± 15% at t\textsubscript{2} (\(p < 0.05\) vs. control).
In the iloprost group, cμPO$_2$ was slightly but not significantly reduced at $t_2$. The medullary PO$_2$ was at $t_2$ 42 ± 7 mm Hg and, therefore, as high in the control group. In this group, there was a significant prevention in the appearance of cortical microcirculatory hypoxic areas compared with the fluid resuscitation group. A drop in DO$_{2\text{ren}}$ could not be prevented by iloprost ($p < 0.05$ vs. control). However, iloprost restored VO$_{2\text{ren}}$ from 0.12 ± 0.07 mL/min/g at $t_1$ to baseline values (0.26 ± 0.10 mL/min/g). The renal oxygen extraction was with 87% ± 12% at $t_2$, the highest of all groups ($p < 0.05$ vs. control, fluid resuscitation).

**FIGURE 2.** Renal oxygenation during septic shock and resuscitation. Values represent mean ± SD. *$p < 0.05$ vs. baseline. **$p < 0.05$ vs. control. +$p < 0.05$ vs. fluid resuscitation. $T_0$, baseline; $t_1$, endotoxemia; $t_2$, resuscitation. cμPO$_2$, cortical microvascular PO$_2$; mμPO$_2$, outer medullary microvascular PO$_2$; DO$_{2\text{ren}}$, renal oxygen delivery; VO$_{2\text{ren}}$, renal oxygen consumption; O$_2$ER$_{2\text{ren}}$, renal oxygen extraction.
FIGURE 3. Renal microvascular oxygen histograms during septic shock and resuscitation. (A) Cortical microvascular oxygen histograms, (B) Outer medullary microvascular oxygen histograms. Values represent mean ± SD *p < 0.05 vs. baseline. †p < 0.05 vs. control. p < 0.05 vs. fluid resuscitation. t₀, baseline; t₁, endotoxemia; t₂, resuscitation.
FIGURE 3 B. (Continued).
Kidney Function, Tubular Sodium Resorption, and Metabolic Cost.

There was no change in Cl\text{crea}, TNa\text{a} and VO\text{2}/TNa\text{a} relation over time in the control group (Fig. 4). At t\text{1} all LPS-treated animals were anuric. This condition did not improve in the nonresuscitation group.

After fluid resuscitation, Cl\text{crea} was one third of baseline (p < 0.05 vs. control). The tubular sodium resorption was significantly reduced at t\text{2} (p < 0.05 vs. control) and related to a significant increase in metabolic cost (VO\text{2}/TNa\text{a}).

Resuscitation with iloprost restored Cl\text{crea} to baseline values (0.77 ± 0.24 mL/ min/g). Furthermore, the tubular sodium resorption was normalized with no increase in VO\text{2}/TNa\text{a} relation.

FIGURE 4. Kidney function, tubular sodium resorption, and metabolic cost during septic shock and resuscitation. Values represent mean ± SD. Changes in % between t\text{0} and t\text{2}. *p < 0.05 vs. baseline. †p < 0.05 vs. control. p < 0.05 vs. fluid resuscitation (1). Animal is anuric. Cl\text{crea}, creatinine clearance; TNa\text{a}, tubular sodium resorption; VO\text{2}/TNa\text{a}, oxygen consumption per sodium reabsorbed (metabolic cost).

Plasma NOx Concentration.

A calibration curve for sodium nitrate was constructed to determine the NOx concentration (Fig. 5). In n = 4 animals, plasma NOx was measured hourly for 5 hours following LPS infusion. After 4 hours, NOx was significantly higher than at baseline. At t\text{2} (5 hours) plasma NOx levels were significantly increased compared with time control. There was no change in plasma NOx concentration in the control group.

In the nonresuscitation group, NOx increased significantly from 17 ± 4 at baseline to 214 ± 67 µM/L at t\text{2} (p < 0.05 vs. control).

Fluid resuscitation had no influence on plasma NOx levels (223 ± 61 µM/L at t\text{2}). In animals receiving resuscitation with iloprost, NOx was with 255 ± 20 µM/L at t\text{2} highest of all experimental groups, but not significantly so.
FIGURE 5. Plasma NOx (nitrate/nitrite) levels during septic shock and resuscitation. Values represent mean ± SD *p < 0.05 vs. baseline. †p < 0.05 vs. control. T₀, baseline; T₁, endotoxemia; T₂, resuscitation. (A) Measurement of plasma NOx 1–5 hours postlipopolysaccharide (n = 4). Insert showing calibration curve of sodium nitrate. (B) Plasma NOx in control and experimental group for three different time points. AUC, area under curve.

iNOS mRNA Expression.

The expression of renal iNOS mRNA was increased in all animals receiving LPS (Fig. 6). In the nonresuscitation group, this increase was significant in all regions of the kidney. In the fluid resuscitation and iloprost group, iNOS mRNA expression did not reach significance in the inner medulla (p < 0.05 vs. control). For direct comparison of iNOS mRNA expression in the three experimental and the control groups, hematoxylin and eosin-stained kidney slices are shown for cortex, outer, and inner medulla (Figs. 6B–D).
Figure 6. Inducible nitric-oxide synthase (iNOS) messenger RNA (mRNA) expression of renal tissue determined by in situ hybridization. (A) iNOS mRNA expression in cortex, outer, and inner medulla. Results are expressed in percent of iNOS mRNA positive (% iNOS+) cells per 0.24 mm² renal tissue. Values represent mean ± SD *p < 0.05 vs. control. (B–D) iNOS mRNA expression as typical cluster formation of silver grains seen in the nonresuscitation, fluid resuscitation, and iloprost group in hematoxylin and eosin-stained renal tissue.
Discussion

In this study, we demonstrated that the prostaglandin analogue iloprost restored kidney function in a rat model of endotoxemia. The application of iloprost had beneficial effects on renal vascular resistance, normalized renal oxygen consumption, and preserved cortical and outer medullary microvascular oxygenation by preventing the occurrence of hypoxic regions.

Renal hypoperfusion because of intrarenal vasoconstriction is considered to be one of the most important pathogenetic factors in the development of ARF in sepsis (25). Therefore, the idea to use a vasodilator, like PGI$_2$, for the treatment of sepsis-related renal failure is understandable. In a recently published study of endotoxemia, the effects of decreased renal PGI$_2$ by cyclo-oxygenase inhibition and increased renal PGI$_2$ with transgenic mice were investigated (26). This study clearly demonstrated that endogenous PGI$_2$ contributed to renal protection in endotoxia-induced acute kidney injury because of the observation that glomerular filtration rate significantly decreased when a cyclooxygenase inhibitor was given. In transgenic animals, however, the protective vasodilating effect was probably overridden by excessive activation of the renin-angiotensin system because of an increase in renal cyclic AMP and renin. These negative effects are possibly related to an excess in PGI$_2$. Unfortunately, there are no data about plasma concentrations of PGI$_2$.

On the basis of the findings and the postulated underlying mechanisms of this study, we wanted to investigate if exogenously given prostacyclin in the form of iloprost can be used as a therapeutic strategy in the treatment of endotoxia-related ARF.

In our model, rats received after 2 hours of LPS standard fluid resuscitation supplemented by a continuous infusion of 100 ng/kg/min iloprost. After 5 hours, the clearance of creatinine restored to baseline values in the iloprost group, whereas in animals receiving fluid resuscitation alone the creatinine clearance was only 30% of baseline. Similar results were shown in a study of endotoxin-shocked rabbits. Here, glomerular filtration rate was substantially maintained in animals receiving the PGI$_2$ analogue taprostene (27). In our investigations, RBF only slightly increased when iloprost was infused, besides normalization in renal vascular resistance. We could recently demonstrate the appearance of cortical microcirculatory hypoxic areas in endotoxin-induced renal failure in the rat (8). The shift in the cortical oxygen histogram toward anoxia could also be shown in this study in nonresuscitated animals 5 hours after LPS infusion, and here also the outer medullary region was affected. This shift was attenuated in rats receiving iloprost resulting in no occurrence of hypoxic areas in both the cortical and outer medullary regions. This phenomenon might contribute to the improvement in kidney function. We could demonstrate that the tubular sodium resorption normalized in the iloprost group with no increase in renal oxygen consumption. Theses observations presuppose an adequate oxygenation in that part of the kidney where most of the oxygen is needed for the absorption of solute (28). The finding that the renal oxygen extraction increased in the iloprost group can be easily explained by the fact that the renal oxygen consumption significantly increased despite an only slight increase in RBF.

Septic ARF is characterized by severe intrarenal vasoconstriction in face of a profound vasodilatation in the systemic circulation (29). This vasodilation is mediated by the release of large amounts of NO after the induction of iNOS (30). As nitrate and nitrite are the primary oxidation products of NO, plasma NOx (nitrate/nitrite) levels can be used as an indicator of NO formation. Under physiologic conditions, constitutive NOS is involved in the regulation of the intrarenal blood flow and tubular resorption (31). During sepsis, renal expression of iNOS is increasing extensively
followed by dysregulation of intrarenal vascular tone (2). However, many approaches of simply blocking NOS failed and had detrimental effects on kidney function (32, 33), whereas selective inhibition of iNOS seems to be beneficial (34, 35). In our model, there was a significant increase in plasma NOx levels 5 hours after LPS administration that did not differ in the different groups. With a more specific look at the kidney, we analyzed iNOS mRNA expression by *in situ* hybridization for cortex, outer, and inner medulla. iNOS expression was up-regulated after 5 hours of endotoxemia in all investigated zones of the kidney (36). In the iloprost group, iNOS expression was suppressed in the outer medulla compared with the nonresuscitation group. Whether in this region eNOS could have exhibited its inhibitory effects on the vasoconstrictive system (32) and therefore have normalized renal sodium resorption remains speculative. Potential interaction between iNOS and eNOS is postulated and supported by the finding that increased iNOS activity inhibits eNOS activity (37).

The renin–angiotensin–aldosterone system plays an important role in maintaining blood pressure, in electrolyte and fluid homeostasis of organisms, and depends on the concentration of the protease renin. Hyperactivation of systemic renin–angiotensin–aldosterone system during sepsis is well documented. However, LPS was demonstrated to significantly down-regulate intrarenal renin–angiotensin–aldosterone system through inhibition of renin activity (38). This observation could be related to the development of ARF in the context of endotoxemia. Endothelial autacoids such as PGI$_2$ are known to stimulate renin secretion (26) and might therefore be beneficial in endotoxemia-related ARF.

A potential application of iloprost in septic patients is given as iloprost is already in clinical use, for example, in the treatment of primary pulmonary hypertension (39). However, there are contradictory results in clinical studies where iloprost was administered in sepsis (40–43). To profit from the potential renal protective vasodilatory effect of iloprost, it is necessary to make sure that the patient receives an adequate fluid resuscitation to avoid hypoperfusion of vital organs. Inadequate filling might be a possible explanation why different clinical studies did not show uniformly positive effects of iloprost administration in sepsis. It is known that high plasma concentrations of prostacyclin are associated with headache, nausea, and emesis (44). However, most of the patients in septic renal failure are sedated and mechanically ventilated, and, therefore, these side effects are negligible.

Although our study solely focused on the kidney, for potential clinical application, it is mandatory to consider the effects of iloprost to other organs. However, at least for the liver and lungs positive effects of PGI$_2$ analogues have been reported in the literature. In a porcine model of endotoxemia, iloprost ameliorated hepatic metabolic disturbances, and thereby, hepatic energy balance (45). Furthermore, low-dose PGI$_2$ had beneficial in LPS-induced inflammation in the rat, by reducing albumin leakage in the lung and improving blood oxygenation (46). Another limitation of our study is that it does not investigate long-term survival and outcome.

Our data demonstrate that the continuous infusion of the stable PGI$_2$ analogue iloprost substantially restored kidney function in a rat model of endotoxemia. These observations were associated with preserved cortical and outer medullary microvascular oxygenation and a significant protection against the occurrence in microcirculatory hypoxic areas. On the basis of these results, one could hypothesize that iloprost infusion as adjuvant to standard fluid resuscitation might be useful as a renal protective strategy to preserve the renal oxygenation and kidney function in the early stage of sepsis. Adequate fluid resuscitation should then avoid potential iloprost-induced hypotension and an early start of continuous iloprost infusion (because of the short plasma half-life
of 20 to 30 minutes) could restore kidney function and oxygenation before the onset of structural kidney damage like acute tubular necrosis. Of course, the true clinical value of iloprost in this setting and the prolonged beneficial effects on kidney function remain to be evaluated.

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References