Effect of Cyclooxygenase-2 Inhibition on Renal Function After Renal Ablation

Pedro Lopez Sanchez, Luis Miguel Salgado, Nicholas R. Ferreri, Bruno Escalante

Abstract—Kidney failure is the common final stage of several diseases, and it is not a steady state but a progressive entity that ends in total absence of renal function. The factors responsible for this progression are not well known. However, single-nephron glomerular filtration rates and glomerular capillary plasma flow rates are increased after renal ablation. Although these alterations may provide protection against the fall in glomerular filtration rate, there is evidence suggesting that heightened single-nephron filtration and plasma flow rates and hydraulic pressures exert damage on the glomerulus, leading to proteinuria and glomerulosclerosis. It has been suggested that vasodilatory prostaglandins may mediate alterations in glomerular hemodynamics after renal ablation. Renal ablation produced an increase in urinary volume, protein, and prostaglandin E2, whereas urinary sodium and potassium were not affected and urinary osmolarity decreased; treatment with indomethacin or NS-398 partially prevented the renal functional changes elicited by renal ablation. Immunoblots for COX showed an increase in the expression of COX-2 protein 2 days after renal ablation. Furthermore, COX-2 mRNA expression was increased 1 day after renal ablation. These data suggest that COX-2–dependent prostaglandins participate in the renal mechanisms associated with the development of renal functional changes after renal ablation.

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Key Words: prostaglandins • cyclooxygenase • renal ablation • kidney failure • renal physiology

Kidney failure is the common final stage of several diseases, and it is not a steady state but a progressive entity that ends in total absence of renal function. The factors responsible for this progression are not well known. However, single-nephron glomerular filtration rates and glomerular capillary plasma flow rates are increased after renal ablation. Although these alterations may provide protection against the fall in glomerular filtration rate, there is evidence suggesting that heightened single-nephron filtration and plasma flow rates and hydraulic pressures exert damage on the glomerulus, leading to proteinuria and glomerulosclerosis. It has been suggested that vasodilatory prostaglandins may mediate alterations in glomerular hemodynamics after renal ablation. Renal synthesis of vasodilatory prostaglandins is increased in renal damage. Renal synthesis of prostaglandins in uremic rabbits and glycerol-induced acute kidney failure has been shown to compromise renal blood flow. These data further support the idea that prostaglandins participate in the development of kidney failure. However, it has been reported that renal problems for patients with a variety of diseases can become especially severe if prostaglandin synthesis is inhibited by anti-inflammatory nonsteroidal drugs.

The prostaglandins are a diverse group of autocrine and paracrine hormones that mediate many cellular and physiological processes. Cyclooxygenase (COX), an enzyme with 2 isoforms, catalyzes formation of endoperoxides from arachidonic acid. The 2 isoforms are known as COX-1 and COX-2. They are similar in amino-acid sequence and enzymatic function, although their physiological functions are thought to be quite different. COX-1 is constitutively expressed in most tissues but at different levels in various cell types. COX-2 normally is undetectable in most tissues, but it can be expressed at high levels after induction with a variety of substances. Selective inhibition has also shown differences between the 2 isoforms. COX-2 inhibitors have anti-inflammatory properties with little gastrointestinal ulceration, whereas COX-1 inhibitors have anti-inflammatory properties but lead to gastrointestinal distress.

In the kidney, there are several reports that suggest a possible role of COX-2 in the physiology or pathology of renal tissue. It has been shown that during the development of kidney failure by renal ablation, there is an increase in COX-2 mRNA associated with increased levels of protein and enzyme activity. In animals lacking the COX-2 gene, the
The kidney shows abnormalities that cause a progressive renal deterioration as the animal ages. These data suggest the relevance of COX for kidney function and the possibility that during development of kidney damage, there is induction of COX-2 mRNA expression, which would increase COX-2 synthesis, thereby increasing the level of vasodilatory prostaglandins that would participate in the renal hemodynamic changes associated with kidney pathology. Therefore, in the present study, we evaluated whether inhibition of COX-1 and COX-2 by indomethacin or selective inhibition of COX-2 by NS-398 ameliorates the renal functional changes associated with renal ablation and whether increased prostaglandin E$_2$ (PGE$_2$) synthesis is related to early changes in the expression of COX mRNA and COX protein.

**Methods**

Male Wistar rats weighing 250 to 300 g were used. For ablation of renal mass, animals were anesthetized with ethyl ether and placed on a warming table, and their kidneys were exposed under aseptic conditions via 2 dorsal incisions. The right kidney was completely removed after vessels and urethra were ligated. The left kidney underwent dissection of the renal artery branches. Two or 3 of these branches were ligated as described previously, and thus, two thirds of the kidney became ischemic (renal ablation). After this, incisions were closed. Sham-operated animals were used as controls. After surgery, animals were placed in metabolic cages, and samples of urine were taken daily for a period of 7 days.

When we tested the effect of indomethacin or NS-398, we used the following experimental design. Each treatment had 4 groups of rats: control, control plus treatment, nephrectomized, and nephrectomized following experimental design. Each treatment had 4 groups of rats: control, control plus treatment, nephrectomized, and nephrectomized plus treatment. When inhibitors were used, treatment was started on the day of surgery, and 3 mg/kg of either indomethacin or NS-398 was administered daily for 7 days. Urinary volume, Na$^+$, K$^+$, protein, urinary PGE$_2$ excretion, and urinary osmolality were measured daily during this period. However, only results for days 2, 5, and 7 are presented, to avoid repetition of the information. Urinary excretion of Na$^+$ and K$^+$ was measured in samples diluted 1:5, by use of an IL943 Flame Photometer (Instrumentation Laboratory). Urinary protein excretion was measured by the Bradford method. Samples were diluted 1:100 and read to 595 nm in a Beckman DU 650 spectrophotometer. A standard curve was prepared with bovine serum albumin. Urinary osmolality was measured in samples diluted 1:4 in a Vapro Vapor Pressure Osmometer (model 5520, Wescor Inc.). Urinary excretion of PGE$_2$ was measured with a monoclonal antibody enzyme immunoassay kit from Cayman Chemical. All data were normalized to 24-hour excretion.

**Blood Pressure Measurements**

Ten days before 5/6 nephrectomy, rats were subjected to implantation of an intraperitoneal device (TA11PA-C40 transducer) for telemetric measurements of blood pressure according to the manufacturer’s instructions (DataSciences International). Blood pressure was measured daily during a 10-minute period for 1 week. Systolic and diastolic blood pressure and mean blood pressure were recorded in a personal computer for later analysis.

**Immunoblotting for COX-1 and COX-2**

We obtained kidneys from sham-operated rats or nephrectomized rats (renal ablation) 2 days after nephrectomy was performed. To explore whether changes in COX expression were present early in the development of the renal functional changes induced by renal ablation, the kidneys were dissected in 2 areas: the ischemic or nonperfused area and the nonischemic or perfused area. Renal tissue was homogenized in 100 mmol/L Tris-HCl, pH 7.4, 100 μmol/L PMSF. Microsomes were prepared. The microsomes were resuspended in SDS-Tris-EDTA buffer, and proteins were measured by the Bradford method. Protein (100 μg) was mixed with loading buffer (glycerol 50% vol/vol; Tris-HCl/pH 6.5; SDS 1% wt/vol; bromophenol blue 0.1% wt/vol; 2-mercaptoethanol) heated to 100°C for 2 to 3 minutes, and the proteins were separated on 2 10% SDS/PAGE gels under reducing conditions and transferred to Hybond-P (Amersham) transfer membranes. The blots were blocked for 40 minutes with TBS containing 5% nonfat dry milk and 0.5% Tween 20. A polyclonal antibody raised against COX-2 (Cayman Chemical) and monoclonal antibody raised against COX-1 (Cayman Chemical) were applied, 1 to each gel, at a dilution of 1:1000 for 1 hour. After they were washed, visualization was achieved by use of peroxidase-labeled goat anti-rabbit antibody and an enhanced chemiluminescence technique (ECL; Amersham). The autoradiography was scanned with a densitometer system (KODAK EDAS 120 system). Values of each band are expressed in arbitrary units (AU). All samples were run simultaneously to eliminate intra-assay variation.

**RNA Isolation and Reverse Transcription–Polymerase Chain Reaction**

To establish whether mRNA expression was present and induced early after nephrectomy, we studied the expression of COX mRNA as early as 4 hours after surgery, as well as for the next several days. Rats were subjected to kidney extraction under pentobarbital anesthesia. Kidneys were extracted and frozen under liquid nitrogen. Tissue was homogenized with TRIzol reagent (Gibco Inc) in an UltraTurrax 25 homogenizer. Total RNA (2 μg) was converted to cDNA by use of the SuperScript II kit from Gibco. Polymerase chain reaction (PCR) conditions were optimized such that only the desired product was produced. PCR was performed by use of a Gene Cycler (Bio-Rad) thermocycler. Initial denaturation was done at 94°C for 5 minutes followed by 35 cycles of amplification. Each cycle consisted of 1 minute of denaturation at 94°C, 1 minute of annealing at 53°C, and 2 minutes for enzymatic primer extension at 72°C; after the final cycle, the temperature was held at 72°C for 7 minutes to allow reannealing of the amplified products. PCR products were then size fractionated through 1% agarose gel, and the bands were visualized with ethidium bromide. Gels were analyzed with a densitometer system (KODAK EDAS 120 system). Values of each band are in arbitrary units.

**Results**

We induced renal functional changes by the method of renal ablation. In our studies, mean arterial blood pressure increased in a time-dependent manner after renal ablation from values of 97 ± 4, 94 ± 4, and 98 ± 4 mm Hg in sham-operated rats to 105 ± 5, 119 ± 5, and 129 ± 4 mm Hg (P < 0.05; n = 5) in rats with renal ablation at 2, 5, and 7 days, respectively. Kidney function parameters also changed after renal ablation. Urinary volume and urinary protein increased from 13 ± 5 to 39 ± 7 mL/24 hours (P < 0.05; n = 5) and from 25 ± 7 to 48 ± 9 mg/24 hours (P < 0.05; n = 5), respectively, 2 days after renal ablation, whereas urinary sodium and urinary potassium were not affected (from 2.13 ± 0.25 to 2.55 ± 0.33 mmol/24 hours and from 4.3 ± 0.45 to 4.85 ± 0.67 mmol/24 hours, respectively; P < 0.05; n = 5) and urine osmolality decreased (from 1746 ± 69.7 to 578 ± 51 mmol/kg; P < 0.05; n = 5). Similar
results were also seen at 5 and 7 days after renal ablation. Concomitantly with these changes in blood pressure and renal function, we observed a time-dependent increment in urinary PGE$_2$ excretion from 15.8±6.7, 13.6±2.5, and 15.9±5.7 ng/24 hours to 31.7±7, 44.4±8.3, and 25.2±6 ng/24 hours (P<0.05; n=5) in rats with renal ablation at 2, 5, and 7 days, respectively.

**Effect of COX Inhibition on Renal Ablation–Induced Renal Function Changes**

To explore the role of COX in the development of kidney function after renal ablation, we used indomethacin (3 mg · kg$^{-1}$ · d$^{-1}$ PO) to inhibit COX-1 and COX-2 and NS-398 (3 mg · kg$^{-1}$ · d$^{-1}$ IP) to selectively inhibit COX-2$^{19,20}$ and evaluated the effects of these treatments on renal ablation–induced renal function changes. Treatment with vehicle, indomethacin, or NS-398 did not affect renal function parameters in sham-operated rats at any time during the 7 days of treatment. However, 2 or 5 days of indomethacin or NS-398 treatment partially prevented all renal ablation–induced renal function changes (Table). Urinary volume or urinary protein increments produced by nephrectomy were partially prevented by treatment with indomethacin or NS-398. Urinary osmolality reduction caused by nephrectomy was also partially prevented by treatment with indomethacin or NS-398 (Table).

**Effect of COX Inhibition on Urinary PGE$_2$ Excretion**

Both COX inhibitors used, indomethacin and NS-398, inhibited the renal ablation–induced increase of urinary PGE$_2$ excretion after 2 or 5 days of treatment. Inhibition of PGE$_2$ synthesis by indomethacin was higher than NS-398 inhibition (indomethacin inhibited by 46±4% and 40±3%, whereas NS-398 inhibited by 30±4% and 41±1% at 2 and 5 days, respectively) (Figure 1). Neither indomethacin nor NS-398 produced a total inhibition of PGE$_2$ synthesis. However, when higher doses of indomethacin (5 and 10 mg/kg) were used to totally abolish PGE$_2$ synthesis, sham-operated and renal ablation animals died 2 to 3 days after indomethacin treatment started.

Immunoblotting with COX-1–specific antiserum demonstrated no changes in the expression of COX-1 immunoreactive protein in microsomes prepared from renal tissue from the perfused and ischemic areas 2 days after renal ablation. COX-1 immunoreactivity was 9061 AU in sham-operated and 10 242 and 10 798 AU in renal-ablation rats from the perfused and ischemic areas, respectively (Figure 2). In contrast, immunoblotting with COX-2–specific antiserum demonstrated an increase in the expression of COX-2 immunoreactive protein in microsomes of renal tissue from the ischemic and perfused areas of rats with renal ablation. COX-2 immunoblotting was 5162 AU in sham-operated rats and 4367 and 20 118 AU in renal-ablation rats in perfused and ischemic tissue, respectively (Figure 2). When we evaluated the expression of renal COX mRNA, there were no changes in renal COX-1 mRNA expression after renal ablation (Figure 2). However, COX-2 mRNA expression in perfused and ischemic tissue was significantly increased after renal ablation (Figure 2).

### Table: Effect of COX Inhibition on Renal Function After Renal Ablation

<table>
<thead>
<tr>
<th></th>
<th>UV (ml/24 h)</th>
<th>UP (mg/24 h)</th>
<th>UNa (mmol/24 h)</th>
<th>UK (mmol/24 h)</th>
<th>UOsm (mmol/Kg)</th>
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<tbody>
<tr>
<td><strong>Indomethacin (3 mg · Kg$^{-1}$ · d$^{-1}$ PO)</strong></td>
<td></td>
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<tr>
<td>2 days</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Sham</td>
<td>14.4±3.4</td>
<td>27.7±4.2</td>
<td>2.13±0.25</td>
<td>4.3±0.45</td>
<td>1746±69.7</td>
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<tr>
<td>Npx</td>
<td>48±5.2$^*$</td>
<td>66.7±6.8$^*$</td>
<td>2.55±0.33</td>
<td>4.85±0.67</td>
<td>578±51$^*$</td>
</tr>
<tr>
<td>NpxTx</td>
<td>28.5±4.5†</td>
<td>49.3±5.3†</td>
<td>2.46±0.3</td>
<td>4.45±0.4</td>
<td>855±155†</td>
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<tr>
<td>5 days</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Sham</td>
<td>12.8±5.8</td>
<td>35.2±6.06</td>
<td>1.59±0.4</td>
<td>4.7±0.32</td>
<td>1673±115.2</td>
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<td>Npx</td>
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<td>56.28±6.6$^*$</td>
<td>2.45±0.15</td>
<td>5.18±0.46</td>
<td>560±29.5$^*$</td>
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<td>1.52±0.08</td>
<td>3.08±0.24</td>
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<td><strong>NS-398 (3 mg · Kg$^{-1}$ · d$^{-1}$ IP)</strong></td>
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<td>2 days</td>
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<tr>
<td>Sham</td>
<td>16±4.7</td>
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<td>4.37±0.44</td>
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<td>39.7±4.9$^*$</td>
<td>53.8±6.2$^*$</td>
<td>0.96±0.19</td>
<td>3.43±0.3</td>
<td>816±61.4$^*$</td>
</tr>
<tr>
<td>NpxTx</td>
<td>27.3±5†</td>
<td>39.4±9.2†</td>
<td>1.59±0.29</td>
<td>4.06±0.3</td>
<td>922.6±60.7†</td>
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<td>5 days</td>
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<tr>
<td>Sham</td>
<td>19±5.4</td>
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<td>2.67±0.3</td>
<td>6.5±0.3</td>
<td>1076±131†</td>
</tr>
</tbody>
</table>

UV indicates urinary volume; UP, urinary protein; UNa, urinary sodium; UK, urinary potassium; UOsm, urinary osmolarity; Sham, control rats; Npx, nephrectomized rats; and NpxTx, nephrectomized rats with treatment.

*P<0.05 vs control (Sham); † P<0.05 vs Npx.
Discussion

We have found that inhibition of COX-2 ameliorates the development of functional changes in the kidney after renal ablation. This study quantifies the magnitude and directional responses of kidney function after renal ablation and examines COX-2–dependent mechanisms by which renal prostaglandins in the remnant kidney may be regulated.

Support for the possibility that prostaglandins with known vasoactive properties (in particular, PGE\(_2\)) are directly relevant to the acute hyperfunctional state in remnant nephrons comes from data analysis of the urinary excretory rate of PGE\(_2\) and the effect of inhibition of COX-1 and COX-2. Additionally, several authors have reported similar observations of increased urinary PGE\(_2\) excretion in other experimental studies performed in chronic or acute renal ablation. Furthermore, our data suggest that increased prostaglandin renal excretion after renal ablation may be the result of increased expression of COX-2 mRNA. We based our suggestion on the observation that inhibition of inducible COX-2 with the specific inhibitor NS-398 prevented the increase in urinary PGE\(_2\) excretion and ameliorated renal ablation–induced changes in kidney function. Moreover, we found increased expression of COX-2 mRNA associated with increased content of COX-2 protein early in the development of functional changes in the kidney after renal ablation.

Increased prostaglandin production in renal tissue in kidney diseases has been suggested to be mediated by increased phospholipase A\(_2\) activity, associated with increased arachidonic acid release. However, our data suggest that increased prostaglandin renal excretion after renal ablation may be the result of increased expression of COX-2 mRNA. We based our suggestion on the observation that inhibition of inducible COX-2 with the specific inhibitor NS-398 prevented the increase in urinary PGE\(_2\) excretion and ameliorated renal ablation–induced changes in kidney function. Moreover, we found increased expression of COX-2 mRNA associated with increased content of COX-2 protein early in the development of functional changes in the kidney after renal ablation.

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COX-2 is one of the isoforms that metabolizes arachidonic acid to the prostaglandins and is thought to be undetectable in most tissues, but it can be expressed at high levels during inflammatory process. Accordingly, COX-2 expression would not be predicted in normal kidney. Our data (Figure 2) and that of other authors, however, have shown low but measurable levels of both COX-2 mRNA and COX-2 protein in normal adult rat kidney. Furthermore, recent reports have shown that depletion of dietary sodium increased the number of thick ascending limb cells expressing COX-2 in the kidney and that COX-2 mRNA and COX-2 protein are present in neonates and decline to adult levels by the third month of age, which suggests that COX-2 can be a constitutive enzyme and that it can be regulated by noninflammatory factors and participate in the process of differentiation.
The relevance of COX-2 in renal function was demonstrated in studies in animals lacking the COX-2 gene, in which severe renal abnormalities were present. The precise role of renal prostaglandins in the renal response to partial nephrectomy was not elucidated in these studies. However, recent publications suggest several possible mechanisms by which prostaglandins participate in the development of kidney failure after renal ablation. Several authors have suggested that renal blood flow may play a key role in the initiation of compensatory glomerular hyperfiltration. The known ability of the prostaglandins to affect vascular tone (vasodilation or vasoconstriction), the demonstration of the presence of COX-2 enzyme in the vascular bed and in cells of the vasa recta participating in the regulation of renal perfusion and glomerular hemodynamics, and reports showing that COX-2–dependent vasodilatory prostaglandins dilate afferent arterioles, which are the effectors of tubular glomerular feedback, suggest that COX-2–dependent vasodilatory prostaglandins are critical for the increases in renal blood flow after renal ablation. Alternatively, indomethacin or NS-398 may inhibit synthesis of prostaglandins, which are essential to the regulation of cell hypertrophy and hyperplasia, because several studies have demonstrated the importance of prostaglandins in cell growth. Additionally, it has recently been shown that low-sodium-diet induction of COX-2 mediates increased renal renin content. Thus, inhibition of the COX-2–dependent prostaglandins eliminates the angiotensin II increment after renal ablation, thereby protecting the kidney from the effects of angiotensin II. Additional experiments must be performed, however, to establish which prostaglandin (E₂, TXA₂, or the endoperoxides) and which exact mechanism affects regulation of the changes in kidney function elicited by renal ablation.

We have provided evidence that suggests that synthesis of COX-2 accounts for the increased release of PGE₂ in the 5/6 nephrectomy, whereas the physiological production of PGE₂ in the normal kidney derives from COX-1. Indeed, increased production of PGE₂ was blocked by the specific COX-2 inhibitor and was associated with amelioration of the renal effects of the renal ablation. Moreover, we visualized increased expression of COX-2 protein and mRNA in the early phases of renal damage after 5/6 nephrectomy. Therefore, we postulate that physiological prostaglandin production is the result of renal COX-1 activity, whereas proinflammatory prostaglandins are formed as a consequence of COX-2 induction at the site of renal injury, and that the proinflammatory prostaglandins contribute to the pathophysiology of the development of functional changes in the kidney after renal ablation.

Acknowledgments

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References


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