Effects of a Stable Prostacyclin Analog on Experimental Ischemic Acute Renal Failure

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The effect of OP-41483, a stable prostacyclin (PGI_2) analog, on ischemic acute renal failure (ARF) was investigated in dogs. Administration of OP-41483 for three days after ischemia significantly increased renal cortical blood flow (RCBF) when compared with dogs treated with the saline vehicle. In the OP-41483–treated group, serum creatinine levels remained relatively low during postoperative days 1–3 and mean survival time was prolonged. Injection of a silicone rubber vascular casting compound (Microfil) revealed increased numbers of visible renal cortical glomeruli and microvessels compared to the saline vehicle group. Histologic sections showed only very limited tubular necrosis, whereas sections of kidneys treated with saline showed extensive tubular necrosis. In conclusion, this stable prostacyclin analog provided a significant degree of protection for the kidneys from ischemic injury and may be useful in a clinical setting.

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THE PROSTACYCLIN/THROMBOXANE A_2 (PGI_2/TXA_2) system plays an important role in the control of platelet aggregation, vascular tone (vasoconstriction and dilation), distribution of tissue blood flow,[1,2] and glomerular filtration rate (GFR).[3] It has been reported that renal ischemia stimulates TXA_2 synthesis but not PGI_2 synthesis, leading to a decrease in the intrarenal PGI_2:TXA_2 ratio.[4] This imbalance in the PGI_2/TXA_2 system probably contributes to the renal cortical vasoconstriction, decrease in renal cortical blood flow (RCBF), and decrease in GFR seen in ischemic acute renal failure (ARF).

Treatment with a selective TXA_2 synthesis inhibitor during ischemic ARF not only inhibits TXA_2 synthesis but also stimulates PGI_2 synthesis, and this inhibitor has been shown to prevent acute tubular necrosis (ATN) after ischemia.[4] In contrast, cyclooxygenase inhibitors that block endoperoxide conversion to both TXA_2 and PGI_2 do not prevent ATN. These findings suggest that the PGI_2 level and the PGI_2:TXA_2 ratio play important roles in renal ischemic injury.

Several studies have demonstrated that PGI_2 infusion has a protective effect in experimental ischemic ARF,[5,6] but the potential clinical utility of PGI_2 is limited because it is very unstable at physiologic pH and temperature. 15-Cyclopentyl-ω-pentanor-5(E)-6,9 metano PGI_2 (OP-41483), a recently developed PGI_2 analog, is chemically stable for more than 48 hours at pH 7.4 and 37 C.[7] This agent is 3–10 times less potent than PGI_2 in terms of inhibiting platelet aggregation and 4 times less potent in terms of its vasodilatory effect.[8]

Another analog of PGI_2 (Iloprost) has been reported to have some protective effect for ARF,[9] and it has been suggested that this effect is mediated by preservation of renal perfusion and tissue oxygenation. Therefore, the effects of administration of OP-41483 on RCBF were examined using hydrogen washout and on the renal cortical microvasculature using Microfil injection, in addition to renal function and histology during the early stage of ARF.

Materials and Methods

Adult mongrel dogs of either sex weighing 10–15 kg were anesthetized with 30 mg/kg of sodium pentobarbital. The left kidney was exposed through a midline abdominal incision, and the renal artery was isolated. Preischemic RCBF (control flow) was measured as described below; then the left renal artery was clamped for 2 hours. Immediately after unclamping, postischemic RCBF (pretreatment flow) was measured, and the animals were divided into experimental and control groups. Group 1 comprised ten dogs that received intravenous (I.V.) stable PGI_2 analog, OP-41483 (Ono Phar-

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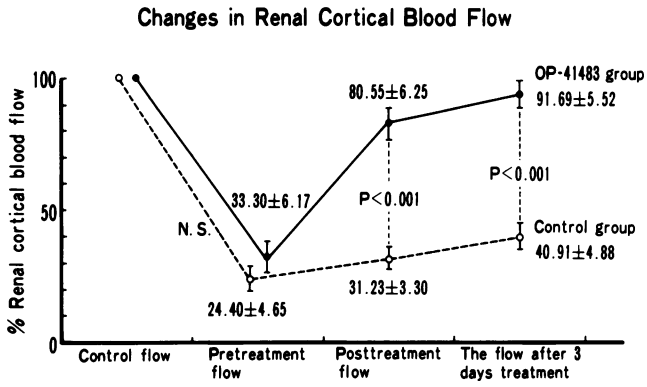


FIG. 1. Per cent change in renal cortical blood flow compared to the preischemic control flow levels. Post-treatment flow on days 0 and 3 is significantly higher in the OP-41483 group compared to the control group.

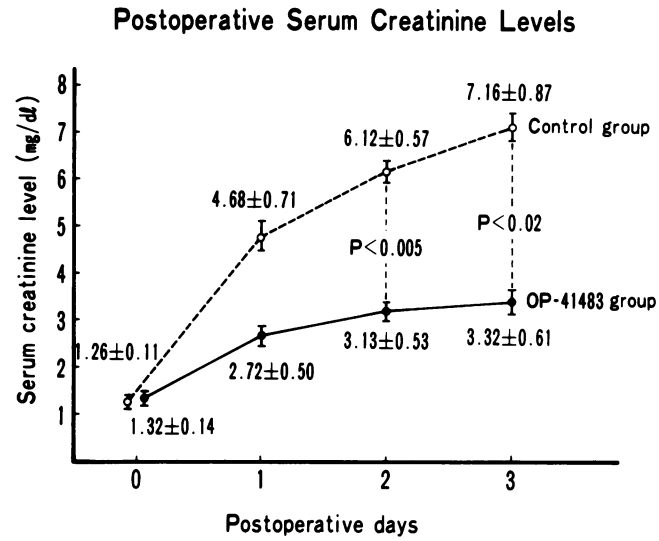


FIG. 2. Postoperative serum creatinine levels in Groups 1 and 2. Serum creatinine levels in the OP-41483 group on postoperative day 2 and 3 are significantly lower compared to the control group.

maceutical Co., Osaka, Japan) in 20 mL of saline solution at 30 ng/kg/min for 1 hour. After OP-41483 infusion, RCBF was measured again (post-treatment flow) and a contralateral nephrectomy was performed. During each of the next 3 days, these dogs received single infusions of OP-41483 (40 μg/kg) in 250 mL of saline solution over 3–4 hours. Serum creatinine levels were determined daily on postoperative days 0 to 3. The RCBF was determined again after the final treatment on postoperative day 3. RCBF was measured using a hydrogen washout technique[10] and calculated by the method of Kety.[11] Group 2 was the control group. Ten dogs were treated identically except that saline solution without OP-41483 was infused. The parameters were RCBF, serum creatinine levels, animal survival, renal histology, and the renal microvascular architecture.

After measurement of the final RCBF, the left kidney was removed from five dogs in each group and immediately perfused with a heparinized saline solution through the renal artery until the perfusate draining from the renal vein was essentially free of blood. Microfil MV-112 (Canton Bio-Medical Products, Boulder, CO) was then gently infused through the renal artery with a hydrostatic pressure of 100 cm. Microfil infusion was continued until the injected Microfil flowed freely from the renal vein. Injected kidneys were cured overnight in a freezer (−20 C), cut into 1-cm slices, and then the tissue was cleared for 6 days with ethyl alcohol-methyl salicylate. Photographs were taken through a stereoscopic dissecting microscope.

Kidney biopsies were taken on postoperative day 3 from the other five dogs in each group, and the animals were allowed to awaken for determination of mean survival time. Mean survival times were compared for these two groups of five dogs.

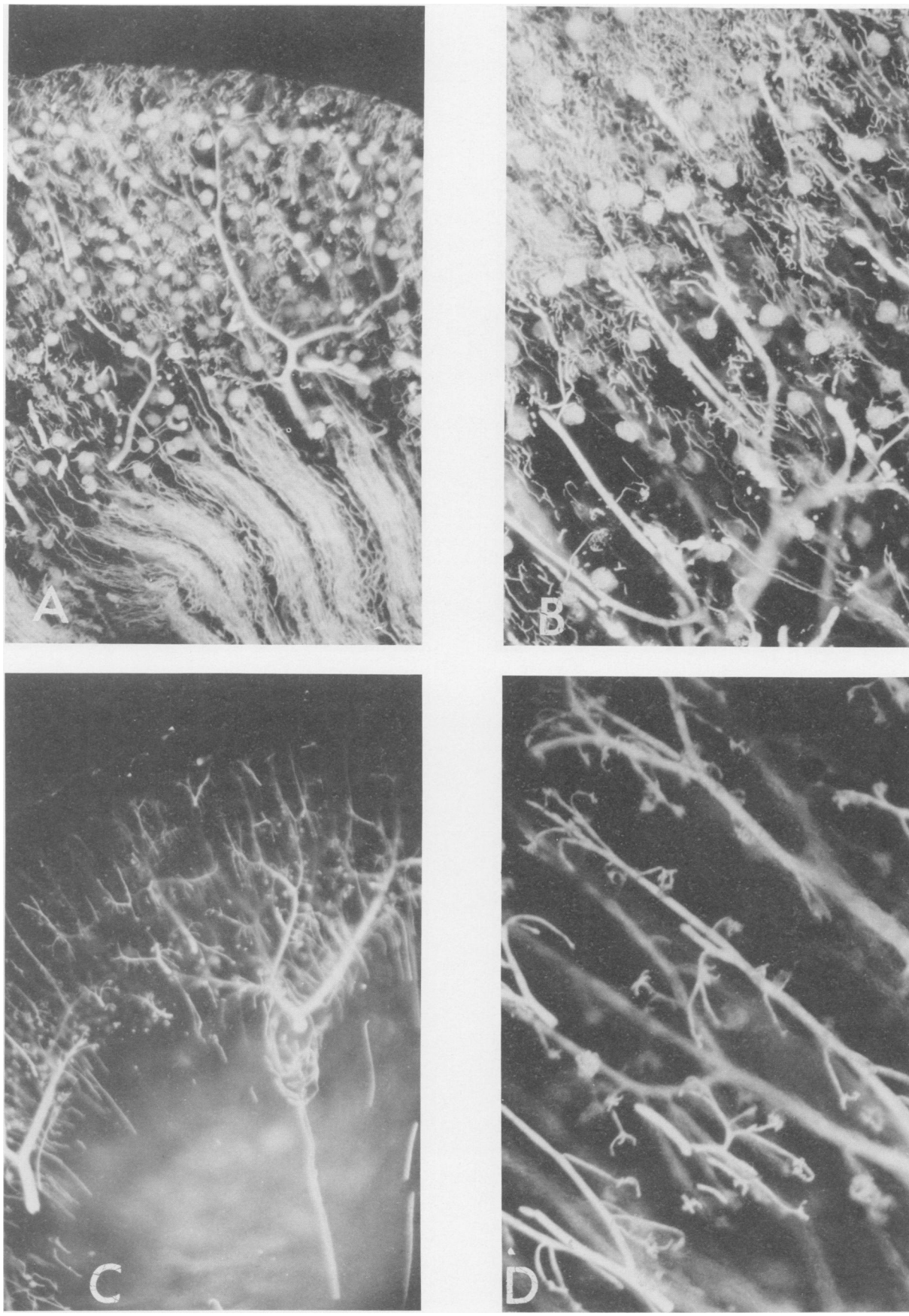
Kidney specimens were fixed in formalin and processed for light microscopy. Sections stained with hematoxylin and eosin or periodic acid-Schiff were examined.

Statistical analysis for all parameters were made by using the Student's t-test. A value of less than 0.05 was considered significant. All values are given as mean ± SEM.

Results

Figure 1 compares the mean RCBF values (percentage of control flow values) in the dogs receiving either the stable PGI_2 analog (OP-41483) or saline. RCBFs measured after 2 hours of ischemia were reduced to about 30% of the control flow levels in both groups ($p < 0.001$). Post-treatment flow in the OP-41483 group (Group 1) increased from 33.3% of the control level to 80.6% of the control flow level by day 0 ($p < 0.001$), whereas no significant change was seen in the control group (Group 2) ($p < 0.001$). RCBFs after 3 days of treatment reached 91.7% of the control level in the OP-41483 group, whereas the values in the control group remained at 40.9% of the control flow level ($p < 0.001$).

FIGS. 3A–D. Renal microvascular architecture visualized by Microfil (MV-112) injection 3 days after ischemia. *A.* In the OP-41483 group Microfil reached the outer cortex and many vasa recta were visualized. *B.* Higher magnification permitted visualization of many peritubular microvessels in the OP-41483 group. *C.* In the control group Microfil did not reach the outer cortex, the medullary region was obscure, and the vasa recta were not visualized. *D.* Higher magnification revealed very few glomeruli and peritubular microvessels in the control group.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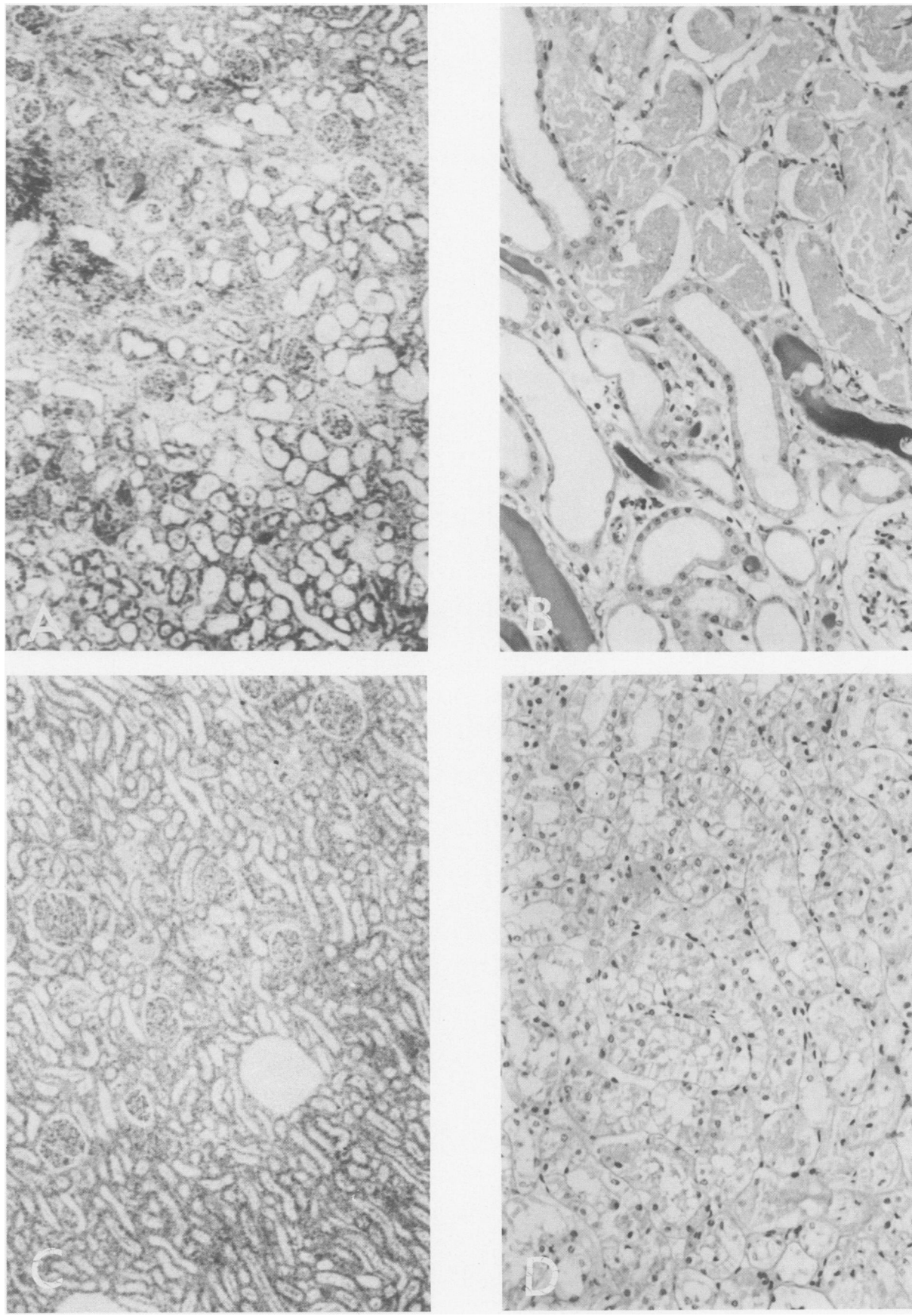
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Figure 2 shows the daily mean serum creatinine levels for both groups. Serum creatinine levels in Group 2 progressively increased from 1.3 mg/dL to 7.2 mg/dL during the 3 postoperative days. Serum creatinine values in Group 1 remained below 3.3 mg/dL during the 3 postoperative days. The differences in serum creatinine levels were statistically significant on postoperative days 2 and 3 ($p < 0.005$ and $p < 0.02$, respectively).

Figure 3 shows the microvascular architecture of the kidneys as visualized by Microfil injection on postoperative day 3. In Group 1 Microfil reached the outer cortex and the vasa recta was visualized (Fig. 3A); greater magnification permitted visualization of many glomeruli and peritubular microvessels (Fig. 3B). In Group 2 kidneys, Microfil remained predominantly in the relatively large intralobular vessels; the outer cortex, the medullary region, and the vasa recta were not visualized. Thus, the cortical vessels had a typical "dead tree" appearance as shown in Figure 3C. The number of visible glomeruli and cortical microvessels was greatly decreased (Fig. 3D).

Figure 4 shows the histologic appearance of kidney specimens obtained by wedge biopsy on postoperative day 3. Sections from Group 2 kidneys revealed extensive necrosis or flattening of cortical tubular epithelium (Fig. 4A) and acellular granular debris filling the lumens of some of the tubules (Fig. 4B). Sections from Group 1 kidneys showed only very limited tubular necrosis (Fig. 4C). The tubular structure was well preserved and tubular dilatation was minimal; flattening of epithelium or acellular debris was not found (Fig. 4D).

Mean survival time for dogs in Group 1 was 47.7 ± 22.5 days, whereas that in Group 2 was 4.1 ± 0.4 days. The difference in mean survival times was statistically significant ($p < 0.01$). Four of five dogs in Group 1 survived more than 30 days, and they were transferred from the postoperative surgical animal housing facility to a less "clean" facility where they later died of pulmonary infections. Kidneys removed at necropsy showed no histologic sign of ARF.

Discussion

The pathophysiology of ischemic ARF is not well understood. However, renal cortical vasoconstriction, preferential renal cortical ischemia (*i.e.*, decreased RCBF), and reduced GFR are characteristic of ischemic ARF.[12,13] Recent studies suggest that eicosanoids, especially an imbalance of the PGI_2/TXA_2 system, may play an important role in the pathogenesis of these phenomena.[4] It has been reported that renal ischemia stimulates TXA_2 synthesis but not PGI_2 synthesis, which leads to an intrarenal imbalance of PGI_2/TXA_2 system and a low PGI_2:TXA_2 ratio. Administration of selective TXA_2 synthesis inhibitor during experimental ARF inhibits the increase of intrarenal TXA_2 synthesis, stimulates intrarenal PGI_2 synthesis, and protects the kidney from ischemic injury.[4] In contrast, administration of cyclooxygenase inhibitors, which inhibit the synthesis of both TXA_2 and PGI_2, shows no protective effect during experimental ischemic ARF.[4] Furthermore, vascular permeability increases after ischemia,[14] and it has been reported that TXA_2 increases vascular permeability and PGI_2 decreases vascular permeability.[14] These data suggest that low PGI_2 levels and a low PGI_2:TXA_2 ratio are critical factors during renal ischemic injury.

Because PGI_2, the vasodilating eicosanoid, is produced by the renal cortex in response to agents that decrease cortical blood flow,[15] PGI_2 probably participates in the homeostatic compensatory response to the reduced renal perfusion and GFR seen during the early phase of ARF.[3] Administration of exogenous PGI_2 could help correct the imbalance of the PGI_2/TXA_2 system and protect the kidney from ischemic injury, but PGI_2 is not stable at physiologic pH and temperature; its half-life in the circulation is only 2–3 minutes.[1,2]

Because the clinical applicability of PGI_2 is limited, a stable PGI_2 analog OP-41483 was developed. OP-41483 is stable for more than 48 hours at physiologic pH and temperature.[8] Although somewhat less active than PGI_2, this analog has strong vasodilating and platelet aggregation inhibitory effects. A previous study using another PGI_2 analog, Iloprost, has been reported to prevent the decrease in GFR and the increase in renal vascular resistance associated with ARF.[9] These authors suggested that these effects might be mediated by preservation of renal blood flow and tissue oxygenation. The present study provides morphologic evidence confirming this hypothesis. In the current study, administration of OP-41483 for 3 days after ischemia reduced renal cortical vasoconstriction, improved RCBF and renal function, prolonged survival time, and limited the oc-

FIGS. 4A–D. Light photomicrographs of kidneys on days 3. *A.* Sections of kidneys from control dogs (Group 2) show widespread necrosis or flattening of tubular epithelium (hematoxylin and eosin, original magnification ×40). *B.* Higher magnification of sections from control dogs show flattened or absent tubular epithelium and acellular granular debris filling several tubules (hematoxylin and eosin, original magnification ×200). *C.* Sections of kidneys from dogs treated with OP-41483 (Group 1) show essentially normal tubular architecture (hematoxylin and eosin, original magnification ×40). *D.* Higher magnification of sections from dogs treated with OP-41483 show well-preserved tubular structure (hematoxylin and eosin, original magnification ×200).

currence of tubular necrosis in dogs with experimental renal ischemia. Microvascular architectural studies demonstrated that administration of OP-41483 increased the visability of glomeruli, peritubular microvessels, and vasa recta. Decreased vasoconstriction and increased glomerular perfusion might be the primary contributing factors improving GFR and renal function as well as subsequently protecting the kidney from ischemic injury.

OP-41483 was administered postischemically during the reperfusion period and showed a protective effect. It has been reported that reperfusion after ischemia produces more damage than that caused by the period of ischemia alone.[16] Recent studies suggest that it may actually be the reentry of oxygenated blood into the ischemic organ during reperfusion that initiates tissue damage.[16] Therefore, oxygen free radicals may play a critical role in the structural and functional damage to kidneys during reperfusion.[17] Oxygen free radicals can disrupt the integrity of the vascular endothelium.[18] Loss of endothelial integrity and the consequent extravasation of intravascular fluid and blood cells into a previously ischemia area could further contribute to reperfusion injury.[17] Because the PGI_2 that is normally synthesized by endothelium is known to protect the vascular wall and endothelium,[19] it is assumed that this is, in part, a mechanism by which administration of this exogenous PGI_2 analog protected the dog kidneys from damage by oxygen free radicals during reperfusion in the present study.

These results suggest that the PGI_2/TXA_2 system is an important mediator of the pathophysiologic phenomena seen in ischemic ARF. Administration of OP-41483 provided a degree of protection for the kidney from ischemic injury. These effects are probably related to the correction of an imbalance of the PGI_2/TXA_2 system (*i.e.*, elevation of PGI_2:TXA_2 ratio), which decreases vasoconstriction, platelet aggregation, and intrarenal thrombosis within the ischemic kidneys. Modulation of the PGI_2/TXA_2 system with OP-41483 might be one approach for protecting the kidney from ischemia and reperfusion injury in a clinical setting.

Acknowledgments

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