Cu/Zn-Superoxide Dismutase Gene Attenuates Ischemia-Reperfusion Injury in the Rat Kidney

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Abstract. Evidence has accumulated for a role of toxic oxygen radicals in the pathogenesis of ischemia-reperfusion injury in the kidney. The aim of this study was to evaluate the hypothesis that reducing posts ischemic renal injury is possible by delivery of the gene for the antioxidant enzyme superoxide dismutase (SOD). Female Sprague-Dawley rats received intravenous injections of recombinant adenovirus (1 × 10⁹ pfu) containing the transgenes for Escherichia coli β-galactosidase (Ad-LacZ, as control) or human Cu/Zn-SOD (Ad-SOD). Three days later, renal ischemia was produced by cross-clamping the left renal vessels for 60 min. The right kidney was removed before reperfusion and processed for the transgene. Renal SOD protein and activity in rats given Ad-SOD was 2.5-fold higher than from the animals receiving Ad-LacZ. Urinary lactate dehydrogenase concentrations were elevated by ischemia-reperfusion in the Ad-LacZ group (1403 ± 112 U/L), yet values were 50% lower in Ad-SOD–treated rats. Free radical production was elevated by ischemia-reperfusion but was significantly lower in SOD-treated animals. Importantly, on post-ischemic day 1, glomerular filtration rates were reduced to 0.21 ml/min per 100 g in the Ad-LacZ group, whereas values remained significantly higher (0.39) in the Ad-SOD group. Two weeks after ischemia-reperfusion, inflammation, interstitial fibrosis, tubular atrophy and tissue levels of necrosis factor alpha and interleukin-1 were significantly higher in the Ad-LacZ–treated than in Ad-SOD–treated rats. In conclusion, these results indicate that SOD expression can be increased by delivery of the sod gene to the kidney by intravenous injection and that sod gene transduction minimized ischemia-reperfusion–induced acute renal failure.

Acute renal failure affects as many as 5% of all hospitalized patients (1) and has an unacceptably high rate of mortality (2). Ischemia-reperfusion–induced renal injury is the most common cause of acute renal failure (3) and is characterized by pronounced depression of the glomerular filtration rates (GFR). After transplantation, decreased GFR due to ischemia-reperfusion may lead to delayed renal graft function, a reversible dysfunction, or even irreversible nonfunction. This problem is almost always observed in transplantation of cadaveric renal allografts that have been subjected to warm or cold ischemia, then reperfusion. Indeed, delayed graft function has adverse effects on long-term survival of allografts (4), hampering efforts to increase the donor pool by using marginal donor organs (5). Therefore, efforts to reduce damage to kidneys exposed to ischemia-reperfusion–induced injury are essential.

Oxygen-derived free radical species have been implicated in the pathogenesis of ischemia-reperfusion–induced renal injury (6,7). During ischemia, increased hypoxanthine and xanthine are produced as a result of ATP degradation (8), and xanthine dehydrogenase is converted into xanthine oxidase (9,10). With the reintroduction of molecular oxygen upon reperfusion, purines are metabolized by xanthine oxidase, leading to the production of superoxide radicals (6,10). Superoxide radicals can be converted into highly reactive hydroxyl radicals, which react with proteins, lipids, or nucleic acids, leading to renal damage. Moreover, the endogenous scavenger superoxide dismutase (SOD) is rapidly depleted during ischemia and reperfusion, especially the cytoplasmic copper-zinc form (11,12). Previously, exogenous SOD has been administered, but the protective effects on tissue injury have been minimal (13), probably because the half-time for plasma elimination of SOD is minutes (14). Therefore, clinical use of SOD as a scavenger for superoxide radicals has been limited, and therapeutic approaches designed to deliver SOD to intracellular sites continuously would be extremely valuable.

One such promising therapeutic approach involves gene transfer (15). It is therefore hypothesized that delivery of the sod gene could provide high and long-lasting levels of SOD at the site of free radical generation (i.e., inside the cell). It has been demonstrated that adenovirus (Ad)-mediated transfer of Cu/Zn-SOD cDNAs protects human endothelial cells against...
oxidant stress in vivo (16). The purpose of this study therefore was to investigate whether levels of SOD in rat kidney can be increased after injection with Ad-SOD gene and to evaluate whether gene delivery could minimize acute renal failure caused by ischemia-reperfusion. Indeed, use of an adenoviral vector encoding Cu/Zn-SOD (Ad-SOD) protected rats against ischemia-reperfusion–induced renal injury. Preliminary accounts of this work have appeared elsewhere (17).

Materials and Methods

Adenoviral Vectors

Recombinant adenovirus containing the transgene for either human Cu/Zn-SOD (Ad-SOD) or Escherichia coli β-galactosidase (Ad-LacZ) was prepared as described elsewhere (18,19). In short, the plasmid shuttle vectors pAd5.CMV.LacZ and pAd5.CMV.SOD1 were constructed by standard cloning protocols as described by Sambrook et al. (20). The adenoviral shuttle plasmids were transfected into the permissive HEK 293 host cell line to generate recombinant Ad-LacZ. Ad-SOD was obtained as a viral seed stock as a kind gift from Dr. John Engelhardt (University of Iowa). Virus isolates were plaque purified and propagated in HEK 293 cells, isolated, concentrated, and titered by plaque assay. Viral vectors of different titers were diluted in 0.4 ml of saline and injected into the iliac vein via a local incision. The skin incision was closed with two stitches.

Animals and Surgical Procedures

Adult female Sprague-Dawley rats (200 to 250 g) had free access to standard rat chow and tap water and received humane care according to the criteria of the US National Institutes of Health. Three days after viral injections (1 × 10⁹ pfu), animals were anesthetized with ether and placed under a warm lamp to maintain body temperature. A midline laparotomy was performed, and the left kidney was dissected free from the surrounding tissue (21). A nontraumatic vascular clamp was placed across the renal pedicle to induce ischemia for 60 min, and the abdomen was temporarily closed during the ischemic period. Five minutes before the end of the ischemic period, a contralateral nephrectomy was performed. The excised kidney was cut into two pieces; one piece was frozen and one was fixed in formalin for transgene detection and histology. Reperfusion of the left kidney was achieved by releasing the vascular clamp. The abdominal wall was closed with double-layer sutures and the animals were allowed to recover. Rats were killed 14 d after reperfusion, and the kidneys were removed for histology.

Transgene Detection: Western Blot of SOD

Whole kidney tissue was homogenized and 30 μg of protein was resolved by electrophoresis via 16% sodium dodecyl sulfate–polyacrylamide gel electrophoresis. Proteins were blotted with anti–Cu/Zn-SOD antibody (Oxis, Portland, OR), followed by horseradish peroxidase–conjugated secondary antibody. Protein detection was visualized by chemiluminescent detection by ECL Western Detection Reagent (Amersham Life Science, Paisley, England).

Detection of β-Galactosidase Activity

Whole kidney tissue was homogenized in buffer A containing 40 mmol/L Tris, 140 mmol/L NaCl, and a protease inhibitor cocktail including 1 μg/mL aprotinin, 1 μg/mL leupeptin, 10 mg/ml phenylmethylsulfonyl fluoride (PMSF), and 1 mmol/L dithiothreitol. The homogenate was centrifuged at 10,000 × g for 10 min at 4°C, and the supernatant was collected and diluted with buffer A to a final volume of 150 μl. A 150-μl aliquot of assay buffer B containing 120 mmol/L Na₂HPO₄, 80 mmol/L NaH₂PO₄, 2 mmol/L MgCl₂, 100 mmol/L 2-mercaptoethanol, and 1.33 mg/ml of O-nitrophenyl-β-D-galactopyranoside was added to the supernatant and incubated for 30 min at room temperature. The reaction was stopped by addition of 500 μl of a 2.8% sodium carbonate solution. Activity of β-galactosidase was quantified by nitrophenol formation, which was measured spectrophotometrically at 420 nm. Values were normalized to total protein determined by the Bio-Rad Protein Assay (Bio-Rad, Hercules, CA).

Detection of SOD Activity

Kidney tissue was homogenized in a buffer containing 40 mM Tris, 140 mM NaCl, and the protease inhibitors aprotinin, leupeptin, PMSF, and dithiothreitol, then centrifuged at 10,000 × g for 10 min at 4°C. SOD activity was measured in the supernatant by the reduction of ferricytochrome c as described by McCord and Fridovich (9). Supernatant (10 μl) was added to a solution containing 50 mM K₂HPO₄, 0.1 mM Na₂-ethylenediaminetetraacetate, and 0.5 mg/ml xanthine oxidase (0.4 U/ml). The reduction of cytochrome c was measured spectrophotometrically at 550 nm. SOD activity was calculated on the basis of the millimolar extinction coefficient of 18.5 for reduced cytochrome c.

Immunohistochemical Staining

Because of the endogenous expression of β-galactosidase and SOD in normal kidney tissue (22,23), localization of recombinant transgenes was difficult via immunohistochemistry. Thus, an adenoviral vector carrying exogenous transgene linked to hemagglutinin (HA) epitope (Ad-HA–tagged IxBeα) was used as a reporter gene. Rats were injected with Ad-HA–tagged IxBeα (1 × 10⁹ pfu) intravenously and killed 3 d later. Sections (5 μm) of paraffin-embedded kidneys were stained by use of monoclonal antibody against HA-tagged recombinant protein conjugated with peroxidase (anti–HA-peroxidase, Roche Diagnostics GmbH, Mannheim, Germany) and the Dako Envision System (Dako Corporation, Carpinteria, CA).

GFR

Rats were placed in metabolic cages with free access to water and chow, and 24-h urine samples were collected. Blood samples were taken from the tail vein on days 1, 3, 7, and 14 after reperfusion. Creatinine levels in urine and serum were determined with a commercially available kit (Sigma Diagnostic, Inc., St. Louis, MO), and GFR were calculated from the ratio of creatinine in the urine/blood, the volume of urine produced in 24 h, and the body weight (24).

Histopathology

Renal tissue fixed in formalin was processed by dehydration and then embedded in paraffin. Sections were cut at 5 μm and stained with hematoxylin and eosin for histologic assessment in a blinded manner. In the evaluation of kidneys harvested 2 h after reperfusion, the severity of renal damage in terms of tubular epithelial injury and debris accumulation (cast formation) was graded with an arbitrary score of 0 to 3, where 0 was normal, 1 mild, 2 moderate and 3 severe (25). A similar scoring system was also used in the evaluation of tubular atrophy and interstitial inflammation and fibrosis in kidneys 2 wk after reperfusion. The total number of infiltrating leukocytes (e.g., neutrophils and mononuclear cells) in cortical interstitial spaces was counted. The mean values in five high-power fields were used for statistical analyses.
Detection of Free Radicals Adducts and Lactate Dehydrogenase in Urine

In a separate study to detect urinary free radicals and lactate dehydrogenase (LDH), rats were killed after collection of urine samples for 2 h, and kidneys were removed for histology. The spin-trapping reagent 4-POBN (1 g/kg body weight) was dissolved in 2 ml saline and injected slowly into the tail vein 15 min before ischemia. After reperfusion, urine was collected through a catheter placed in the urinary bladder for 2 h and samples were kept at −80°C until electron spin resonance (ESR) analysis. Samples were placed in an aqueous flat cell and bubbled with oxygen for 5 min to eliminate interfering ascorbyl free radical and with nitrogen for 5 min to eliminate line broadening by molecular oxygen. Free radical adducts were detected with a Bruker ESP 300 ESR spectrometer (Bruker Instrument, Billerica, MA). Instrument conditions were as follows: 20-mW microwave power; 1.01-G modulation amplitude, and 80-G scan range (26). LDH assays were performed via standard enzymatic techniques (27).

RNase Protection Assay

Total RNA was isolated from renal tissue by RNA STAT 60 (Tel-Test, Friendswood, TX). RNase protection assays were performed by the RiboQuant multiprobe assay system (Pharmingen, San Diego, CA). Briefly, [32P] RNA probes were transcribed with T7 polymerase with the multiprobe template set at rCK-1, and RNA (10 μg) was hybridized with 4 × 10^5 cpm of probe overnight at 56°C. Samples were then digested with RNase followed by proteinase K treatment, phenol:chloroform extraction, and ethanol precipitation, and were resolved on a 5% acrylamide-bisacrylamide (19:1) urea gels. After drying, gels were visualized by autoradiography.

Statistical Analyses

ANOVA that used Tukey’s post hoc test, one-way repeated-measures ANOVA, and Wilcoxon signed rank test were used for the determination of statistical significance as appropriate. Data are presented as means ± SEM. P < 0.05 was selected before the study as the level of significance.

Results

Expression of SOD after Gene Transfection

To find an optimal dose of Ad-SOD for this study, serum alanine transaminase and SOD protein expression in kidneys were evaluated in rats receiving 1 × 10^8, 1 × 10^9, or 1 × 10^10 pfu of Ad-SOD and 1 × 10^9 of Ad-LacZ (n = 4 per group). During the first 2 d after viral injection, animals treated with 1 × 10^8 or 1 × 10^9 pfu of adenoviral vectors looked healthy, but most of the rats given 1 × 10^10 pfu of Ad-SOD were sedentary and feverish. The administration of 1 × 10^8 or 1 × 10^9 of Ad-LacZ or Ad-SOD did not affect serum alanine transaminase levels (<40 U/L). However, alanine transaminase levels from rats receiving 1 × 10^10 pfu of Ad-SOD were about twofold higher than levels in animals receiving lower titers of virus. This is most likely due to viral-induced toxicity; therefore, a dose of 1 × 10^9 pfu was chosen for this study because of its lack of side effects.

In kidneys harvested 3 d after Ad-SOD injections, increased levels of human SOD protein were observed in rats receiving 1 × 10^8, 1 × 10^9, or 1 × 10^10 pfu of Ad-SOD and 1 × 10^9 pfu of Ad-LacZ (Figure 1A). Enzyme activity in renal tissue was also measured 3 d after injection of 1 × 10^9 pfu of either Ad-LacZ or Ad-SOD.
over, SOD activity in kidneys from animals receiving Ad-SOD was elevated about 2.5-fold (Figure 1C). Thus, Ad-LacZ and Ad-SOD gene delivery led to protein expression and increased enzyme activity in the kidney.

Localization of Gene Transfection

Three days after injection of an Ad-HA–tagged reporter gene, the tagged gene products could be detected almost everywhere in the kidney (Figure 2). More than 80% of proximal tubules in outer cortical areas showed strong positive staining (Figure 2B), and nearly all of the proximal tubules in the inner cortex were positive (Figure 2D). In glomeruli, capillary endothelial cells were positive for gene transfection, whereas mesangial cells were mostly negative. Nearly all of the epithelial cells of the tubules and loop of Henle in the outer medulla were transfected (Figure 2F). Moreover, the majority of cells in the inner medulla were also positive (Figure 2H), and nearly all of the peritubular endothelial cells were stained positive, although to varying extents. These results indicate that most tubular epithelial

Figure 2. Representative photomicrographs of kidneys from rats receiving saline or hemagglutinin (HA)-tagged adenoviral reporter gene. Animals were injected with saline (0.4 ml) or HA-tagged adenovirus (Ad)-reporter gene (1 × 10⁹ pfu) intravenously and killed 3 d later. Immunohistochemical staining was performed to locate the sites of gene delivery in kidneys as described under Materials and Methods. The left column is the kidney from rat given saline; the right column is the one receiving adenovirus. (A and B) Outer cortex. (C and D) Inner cortex. (E and F) Inner medulla. Magnification, ×200.
Figure 3. Representative photomicrographs of kidneys from rats receiving Ad-LacZ or Ad-SOD before and after ischemia and reperfusion. Animals were treated as described under Materials and Methods. (A) Kidney from rat 3 d after receiving Ad-LacZ. (B) Kidney 3 d after receiving Ad-SOD. (C) Kidney of Ad-LacZ–treated rat 2 h after ischemia and reperfusion. (D) Ad-SOD–treated kidney 2 h after ischemia and reperfusion. (E) Ad-LacZ–treated kidney 2 wk after ischemia and reperfusion. (F) Ad-SOD–treated kidney 2 wk after ischemia and reperfusion. (G) Ad-LacZ–treated kidney 2 wk after ischemia and reperfusion. Blue is due to fibrosis. (H) Ad-SOD–treated kidney 2 wk after ischemia and reperfusion. Stain, hematoxylin and eosin (A to F) and trichome (G and H). Magnification, ×200.
cells and vascular endothelial cells are transfected by intravenous administration of adenoviral vectors.

**Effects of Ad-SOD on Ischemia-Reperfusion–Induced Renal Injury**

Morphology of kidneys that were excised 3 d after injection of 1 × 10^9 pfu of Ad-LacZ or Ad-SOD is depicted in Figure 3, A and B. These photomicrographs demonstrate normal renal histology with intact integrity of tubules and interstitial space, indicating that viral vectors at the dose used here did not cause morphologic damage. Representative photomicrographs of kidneys 2 h after ischemia-reperfusion are shown in Figure 3, C and D. Kidneys from rats receiving Ad-LacZ exhibited dramatic renal injury (Figure 3C), with tubular cell swelling, loss of brush border, and cast formation. On the other hand, only mild tubular injury was observed in kidneys from rats treated with Ad-SOD (Figure 3D). Pathology scores of these kidneys 2 h after ischemia-reperfusion are shown in Figure 4. Nonischemic kidneys scored zero, whereas the extent of tubular epithelial injury in the kidneys of rats receiving Ad-LacZ was increased dramatically. Ad-SOD–treated kidneys, however, exhibited much less injury than kidneys receiving Ad-LacZ (P < 0.05). Furthermore, cast formation in kidneys from rats given Ad-LacZ was more than fourfold higher than values from Ad-SOD–treated kidneys (P < 0.05). As a result, the sum of pathology scores from Ad-LacZ–treated kidneys was 3.1 ± 0.4, a value about twofold higher than that of Ad-SOD–treated kidneys. This indicates that early ischemia-reperfusion–induced renal injury was largely reduced by the successful delivery of the Ad-SOD gene and expression of SOD protein.

Representative photomicrographs of kidneys treated with either Ad-LacZ or Ad-SOD 2 wk after ischemia and reperfusion are shown in Figure 3E and Figure 3F, respectively. Increased inflammation, interstitial fibrosis, and tubular atrophy were observed in kidneys from rats receiving the Ad-LacZ gene (Figure 3E); however, Ad-SOD–treated kidneys exhibited almost normal renal architecture (Figure 3F). Pathology scores...
quantitating the extent of tubular atrophy, interstitial infiltration, and fibrosis were significantly two- to threefold higher in the Ad-LacZ–treated kidneys than in kidneys from Ad-SOD–treated rats (Figure 5A). Quantitation of infiltrating leukocytes showed that there were only a minimal number of leukocytes in the nonischemic kidneys (Figure 5B); however, a large number of infiltrating leukocytes were observed in the renal cortex of rats receiving the Ad-LacZ gene (Figure 2E). sod gene transduction significantly reduced inflammatory cell infiltration about two- to threefold (Figure 5B). Figure 3G shows dramatic interstitial fibrosis around atrophic tubules in kidneys from Ad-LacZ–treated rats, whereas changes were minimal after Ad-SOD transfection (Figure 3H).

**Effects of Ad-SOD on GFR**

GFR during the first 2 wk after ischemia-reperfusion are shown in Figure 6. The values at day 0 (i.e., normal GFR measured before ischemia) were about 5.5 ml/min per 100 g. In rats receiving the Ad-LacZ gene, GFR was reduced to about 40% of normal values 1 d after ischemia-reperfusion and did not recover fully until 2 wk later. This was comparable to values of rats given saline (data not shown); however, GFR was reduced significantly less and increased much faster in rats receiving Ad-SOD.

**Effects of Ad-SOD on Urinary Release of LDH and Free Radical Adduct Formation after Ischemia-Reperfusion**

LDH release in urine from normal rats was minimal (Figure 7); however, 2 h after reperfusion, LDH levels were increased dramatically in Ad-LacZ–treated animals. Importantly, values were increased significantly less in rats receiving Ad-SOD (P < 0.05). Reperfusion subsequent to renal ischemia could lead to free radical formation. Accordingly, free radicals were trapped with the spin-trapping reagent 4-POBN and detected with ESR. Figure 8 shows representative ESR spectra due to free radical adducts in urine collected during the first 2 h of reperfusion. A six-line ESR spectrum due to radical adducts was detected in urine samples from both groups. However, free radical signals from urine of a rat receiving Ad-LacZ were almost threefold larger than Ad-SOD–treated rats, indicating that transfer of sod gene reduced free radicals.

**Effects of Ad-SOD on Tissue Levels of Inflammatory Cytokines**

Tissue levels of the inflammatory cytokines interleukin-1 (IL-1) and tumor necrosis factor alpha (TNF-α) could be detected in ischernically injured kidneys from rats receiving Ad-LacZ 2 wk after ischemia-reperfusion (Figure 9). However, in tissue from rats treated with Ad-SOD, IL-1 was reduced dramatically and TNF-α could not be detected.
**Discussion**

**Gene Delivery of SOD Reduces Ischemia-Reperfusion Injury in the Kidney**

Although ischemia will ultimately destroy living tissues, oxidant stress caused by reperfusion increases injury (6,7). The pathogenesis of ischemia-reperfusion–related changes involves reactive oxygen species (6,8,10), and the results of the study reported here support the harmful role of oxidants. It was observed that increased levels of intracellular SOD in kidney minimized ischemia-reperfusion–induced tubular injury and improved postischemic renal function (Figures 3 to 7). Importantly, SOD levels in kidney were elevated by delivering the Cu/Zn sod gene in vivo with an adenoviral vector as evidenced by increased tissue SOD protein expression and elevated enzyme activity (Figure 1). Moreover, attenuated postischemic renal failure in animals with overexpression of SOD was accompanied by reduced free radical adducts in urine (Figure 8). To our knowledge, this report is the first to demonstrate protection against kidney ischemia-reperfusion injury by antioxidant gene products by successful gene delivery.

Ischemia-reperfusion injury of the kidney involves components of a typical inflammatory reaction (28,29). Oxygen free radicals can directly trigger the activation of leukocytes and expression of adhesion molecules (30). Enhancement of inflammatory cytokine production (IL-1 and TNF-α) increases expression of cell adhesion molecules (ICAM-1), allowing neutrophils to adhere and accumulate in peritubular vessels causing capillary plugging and obstruction (31–33), leading to tissue destruction and reduced GFR. Indeed, the inflammatory reaction in ischemically injured kidney could still be detected 2 wk after the initial insult in kidneys from Ad-LacZ–treated rats (Figures 3E and 5B) and was accompanied by expression of the proinflammatory cytokines, TNF-α and IL-1 (Figure 9). Importantly, these phenomena were attenuated by SOD overexpression, which may be attributed both to extra enzyme in the kidney upon reperfusion and its continuous production during the recovery period.

**Advantages of Antioxidant Gene Delivery in Protection of Renal Ischemia-Reperfusion Injury**

SOD is a naturally occurring intracellular enzyme that catalyzes the breakdown of superoxide radicals (34). Ischemia and reperfusion leads not only to an increase in superoxide production, but also to a rapid depletion of SOD (11,12).

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**Figure 8.** Effects of delivery of Ad-SOD on electron spin resonance (ESR) spectrum of free radical adducts in urine. The spin trapping reagent 4-POBN (1 g/kg) was injected intravenously 10 min before ischemia. Urine samples were collected through a catheter placed in the urinary bladder during the first 2 h of reperfusion. Free radical adducts in urine were detected by ESR as described under Materials and Methods. Typical spectra are shown. (A) Rat received saline. (B) Rat received Ad-LacZ (1 × 10⁹ pfu). (C) Computer simulation of the radical adduct spectrum from Ad-LacZ–treated rat. (D) Rat received Ad-SOD (1 × 10⁹ pfu). n ≥ 3 per group.

**Figure 9.** Effects of delivery of Ad-SOD on inflammatory cytokines in postischemic kidneys. Tumor necrosis factor alpha (TNF-α) and interleukin-1 (IL-1) mRNA were determined in renal tissue after 2 wk of ischemia-reperfusion by RNase protection assay as detailed under Materials and Methods. Representative data are shown.
Numerous studies have assessed the potential benefits of exogenous SOD in preventing reperfusion injury, but data are conflicting (8,35). As a result, an efficient way to inhibit oxidative injury has not been established. One important factor responsible for unfavorable effects of exogenous SOD administration is most likely its short half-life in plasma (14). Gene transfer technology has the theoretical advantage of introducing SOD genes into kidney tissue, leading to continuous production of the gene product. Indeed, effective gene delivery was established by intravenous injection of the gene vectors (1 × 10^9 pfu) 3 d before the ischemic insult without toxic side effects (Figure 1). Moreover, nearly all components of renal tissue were transfected 3 d after intravenous administration of the adenoviral vector (Figure 2), most likely making vulnerable renal regions more resistant to the ischemia-reperfusion insults.

The use of adenovirus as a vector for kidney-directed gene therapy has made significant progress in the area of kidney biology, in particular in hereditary kidney disease and inflammatory and fibrotic disease (36). Adenoviral vectors have distinct advantages of high titer and high expression of the transgene. Because the adenovirus can infect both dividing and nondividing cells (37), the vector has a significant advantage in delivering genes into quiescent or terminally differentiated cells and is thus suitable for gene transfer into complex organs such as the kidney. Adenoviral vectors also have disadvantages. First, the expression of the transfected gene is limited to weeks or months because the adenovirus does not integrate into the host genome. Second, the adenovirus can elicit immunological responses; therefore, the vector cannot be administered repeatedly. Nonetheless, ischemia-reperfusion-induced renal injury usually occurs in emergency situations, with harmful effects maximal within a week (e.g., posttransplant acute renal failure). This makes the adenoviral vector especially suitable for antioxidant gene delivery in prevention of acute renal failure.

Previously, Heikkila and colleagues (38) tried to use ex vivo kidney perfusion system in adenoviral gene delivery. They found that about 75% of glomerular cells were transfected and no tubular cells expressed transgene. Apparently, this is a good model for gene therapy in kidney transplantation and allows renal allografts to be treated before implantation. Kidneys in a perfusion system may behave differently as a result of surgical procedures, temperature, and use of perfusion media. Moreover, Moullier and colleagues (39) achieved adenoviral gene transfer to tubular cells by infusion through renal artery. Although this technique enables the vector to go through kidney, then into bloodstream, it appears to be associated with trauma. In this study, virus circulates in the blood until it enters cells and has minimal side effects.

Clinical Implications

The study reported here demonstrates the feasibility of delivering exogenous SOD with an adenoviral gene transfer approach, with successful expression of the gene product. Moreover, overexpression of SOD in kidney by gene delivery provides protection against ischemia-reperfusion–induced renal injury. This study raises the intriguing possibility of the potential therapeutic use of SOD, not only in hypoxia-reoxygenation injury, but also in other inflammatory renal disease states. It has been reported that initial postsischemic injury and free radicals are involved in progression of acute and chronic rejection (35,40,41). The protection of free radical–mediated early injury by SOD gene transfection shown in this study may have beneficial effects on the long-term outcome of kidney allografts.

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