Tempol, a membrane-permeable radical scavenger, reduces oxidant stress-mediated renal dysfunction and injury in the rat

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Renal ischemia/reperfusion injury is one of the most common causes of acute renal failure (ARF), initiating a complex and interrelated sequence of events, resulting in injury to and the eventual death of renal cells [1, 2]. Although reperfusion is essential for the survival of ischemic tissue, reperfusion itself causes additional cell injury (reperfusion-injury) [3], which has been attributed to the generation of reactive oxygen species (ROS) inter alia [3-5]. Furthermore, it appears that the proximal tubule (PT) appears to be particularly susceptible to the reperfusion-injury mediated by ROS [2, 3].

The hypothesis that the generation of ROS contributes to renal ischemia/reperfusion injury is supported by many studies demonstrating the beneficial effects of various interventions, which either reduce the generation or reduce the effects of ROS [3]. These therapeutic strategies include the administration of antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) [6, 7], ROS scavengers such as mannitol [8], and agents that prevent the generation of ROS such as desferrioxamine (DEF) [8, 9] and allopurinol [10]. However, the potential benefits of the systemic administration of these
agents, for example, SOD, are limited for the following reasons [11]: (1) SOD does not permeate biological membranes and is therefore not able to reduce the detrimental effects of superoxide anions, which are produced intracellularly. (2) Superoxide anions produced within cells (for example, via uncoupling of the mitochondrial respiratory chain) do not leave the cell in which they have been generated, but remain potentially injurious to intracellular cell structures. (3) SOD dismutates superoxide anions into hydrogen peroxide (H$_2$O$_2$), which, in turn, can generate the highly toxic hydroxyl radical via the Fenton or Haber-Weiss reactions and which also contribute to renal reperfusion injury [5]. To overcome these limitations, SOD mimetics, which scavenge radicals and permeate biological membranes, are of small molecular weight and have the potential to be useful in conditions associated with ischemia/reperfusion injury of the kidney and associated acute renal failure (ARF).

Tempol (4-hydroxy-2,2,6,6-tetramethylpiperidine-N-oxyl or 4-hydroxy-tempo) is a stable piperidine nitroxide and is a water-soluble analogue of the spin label tempo, which is widely employed in electron spin resonance spectroscopy [12]. It has a relatively low molecular weight (172) and permeates biological membranes [13]. Tempol scavenges superoxide anions in vitro [13] and may act as a genuine “SOD-mimetic” [14]. Tempol also reduces the formation of hydroxyl radicals either by scavenging superoxide anions or by reducing the intracellular concentrations of Fe$^{2+}$ and, hence, the formation of hydroxyl radicals via the Fenton or Haber-Weiss reactions [15-17].

To date, there has been relatively little research into any protective actions afforded by tempol within the kidney or in specific renal structures such as the PT, under normal or pathophysiologic conditions. One report has described how a two-week administration of tempol reduced both hypertension and renal excretion of 8-iso-prostaglandin F$_{2\alpha}$ (used as a marker of oxidative stress) in spontaneously hypertensive rats (SHRs) [18]. We have recently discovered that tempol attenuates renal dysfunction and injury caused by endotoxin in the rat [19] and during hemorrhagic shock [20]; the pathogenesis of which involves an enhanced formation of ROS. Furthermore, we have also shown that tempol reduces infarct size in rodent models of regional myocardial ischemia/reperfusion [20]. The aim of this study was therefore to investigate whether tempol (1) can reduce the renal dysfunction and injury associated with ischemia/reperfusion of the rat kidney in vivo, and (2) can protect cultured rat PT cells against oxidant stress in vitro. To elucidate putative mechanisms by which tempol may exert beneficial actions against renal ischemia/reperfusion injury, its effects were compared with those of DEF, an iron chelator that binds and subsequently reduces the availability of Fe$^{2+}$ and that has previously been shown to protect rabbit kidneys against ischemic injury [8, 9].

METHODS

Animal preparation

In vivo studies were carried out using 83 male Wistar rats (Tuck, Rayleigh, Essex, UK) weighing 230 to 320 g and receiving a standard diet and water ad libitum and cared for in accordance with the Home Office Guidance in the Operation of the Animals (Scientific Procedures) Act 1986, published by HMSO (London, UK). All animals were anesthetized with sodium thiopentone (Intraval® Sodium, 120 mg/kg intraperitoneally; Rhone Merieux Ltd., Essex, UK), and anesthesia was maintained by supplementary infusions of sodium thiopentone. The animals were placed onto a thermostatically controlled heating mat (Harvard Apparatus Ltd., Kent, UK), and body temperature was maintained at 38 ± 1°C by means of a rectal probe attached to a homeothermic blanket. A tracheotomy was performed to maintain airway patency and to facilitate spontaneous respiration. The right carotid artery was cannulated (PP50, I.D. 0.58 mm; Portex, Kent, UK) and connected to a pressure transducer (Senso-Nor 840; Senso-Nor, Horten, Norway) for the measurement of mean arterial blood pressure (MAP) and derivation of the heart rate (HR) from the pulse waveform, which were displayed on a data acquisition system (MacLab 8e; AD Instruments, Hastings, UK) installed on an Apple Macintosh computer. MAP and HR were monitored for the duration of each experiment. The jugular vein was cannulated (PP25, I.D. 0.40 mm; Portex) for the administration of drugs. A midline laparotomy was performed, and the bladder was cannulated (PP90, I.D. 0.76 mm; Portex). Both kidneys were located, and the renal pedicles, containing the artery, vein, and nerve supplying each kidney, were carefully isolated.

Renal ischemia/reperfusion

Rats were allowed to stabilize for 30 minutes before they were subjected to bilateral renal occlusion for 45 minutes using artery clips to clamp the renal pedicles. Reperfusion commenced once the artery clips were removed (control animals). Occlusion was verified visually by change in the color of the kidneys to a paler shade and reperfusion by a blush. Other rats, which underwent identical surgical procedures similar to control animals but did not undergo bilateral renal clamping, were subjected to sham operation (sham operated) and were maintained under anesthesia for the duration of the experiment. At the end of all experiments, animals were killed by an overdose of sodium thiopentone.

Experimental protocol

Upon completion of surgical procedures, the animals were randomly allocated to the eight groups described in Table 1. At one minute before commencement of reperfusion, animals received a bolus injection of either...
Table 1. Renal ischemia/reperfusion model and intervention: Experimental design

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Ischemia time min</th>
<th>Reperfusion time hours</th>
<th>Treatment</th>
<th>Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>11</td>
<td>a</td>
<td>a</td>
<td>Sham-operated</td>
<td>Saline only (4 mL/kg/h)</td>
</tr>
<tr>
<td>2</td>
<td>14</td>
<td>45</td>
<td>6</td>
<td>Control (45 min ischemia + 6 h reperfusion)</td>
<td>Saline only (4 mL/kg/h)</td>
</tr>
<tr>
<td>3</td>
<td>12</td>
<td>45</td>
<td>6</td>
<td>Control + tempol</td>
<td>30 mg/kg bolus</td>
</tr>
<tr>
<td>4</td>
<td>6</td>
<td>a</td>
<td>a</td>
<td>Tempol sham-operated</td>
<td>30 mg/kg bolus</td>
</tr>
<tr>
<td>5</td>
<td>12</td>
<td>45</td>
<td>6</td>
<td>Control + DEF</td>
<td>40 mg/kg bolus</td>
</tr>
<tr>
<td>6</td>
<td>6</td>
<td>a</td>
<td>a</td>
<td>DEF sham-operated</td>
<td>40 mg/kg bolus</td>
</tr>
<tr>
<td>7</td>
<td>12</td>
<td>45</td>
<td>6</td>
<td>DEF + tempol sham-operated</td>
<td>40 mg/kg bolus</td>
</tr>
<tr>
<td>8</td>
<td>6</td>
<td>a</td>
<td>a</td>
<td>DEF + tempol sham-operated</td>
<td>40 mg/kg bolus</td>
</tr>
</tbody>
</table>

Sham-operated or control animals were administered a bolus injection of either vehicle (saline), tempol, desferrioxamine (DEF), or a co-administration of tempol and DEF one minute before commencement of reperfusion. The corresponding groups then received a continuous infusion of either vehicle (saline), tempol, DEF, or a combination of tempol and DEF throughout the reperfusion period.

*These animals did not undergo renal clamping (sham-operated).*

vehicle (saline, 4 mL/kg, IV), tempol (30 mg/kg in saline, IV), DEF (40 mg/kg in saline, IV), or DEF (40 mg/kg in saline, IV) in combination with tempol (30 mg/kg in saline, IV). The corresponding groups then received a continuous infusion of one of the following throughout the reperfusion period: vehicle (saline, 4 mL/kg/h, IV), tempol (30 mg/kg/h in saline, IV), DEF (40 mg/kg/h in saline, IV), or tempol and DEF in combination (30 and 40 mg/kg/h, respectively, in saline, IV). To elucidate the effects of tempol or DEF on cardiovascular hemodynamics and organ function in sham-operated rats, respective groups of animals received the treatments described previously in this article and as outlined in Table 1. The concentration of tempol administered in vivo was based on those previously demonstrated by us to provide significant protection against myocardial ischemia/reperfusion injury in an in vivo rat model [21]. Similarly, the concentration of DEF used was identical to that which we have previously used to provide significant protection against hepatic ischemia/reperfusion injury in in vivo rat and rabbit models [22].

In all of the groups of animals, hemodynamic parameters (MAP and HR) were recorded for the duration of the experiment.

Measurement of biochemical parameters

At the end of the reperfusion period, blood (1 mL) samples were collected via the carotid artery into S1/3 tubes containing serum gel. The samples were centrifuged (6000 r.p.m. for 3 min) to separate plasma. All plasma samples were analyzed for biochemical parameters within 24 hours after collection (Vetlab Services, Sussex, UK). Plasma concentrations of urea and creatinine were measured as indicators of impaired glomerular function [23, 24]. Plasma concentrations of γ-glutamyl transferase (γGT) and aspartate aminotransferase (AST), enzymes that are both located in the PT [25], were used as indicators of renal reperfusion-injury (Discussion section). Total plasma bilirubin (BIL) and alanine aminotransferase (ALT; a specific marker for hepatic parenchymal injury) were also measured and used as indicators of liver function and injury, respectively (Discussion section) [24, 26].

Urine samples were collected during the reperfusion period, and the volume of urine produced was recorded. Urine concentrations of Na⁺ were measured (Vetlab Services) and were used in conjunction with plasma Na⁺ concentrations to calculate fractional excretion of Na⁺ (FENa) using standard formulae, which was used as an indicator of tubular function. Concentrations of urinary N-acetyl-β-d-glucosaminidase (NAG), an indicator of tubular damage [27], were also measured (Laboratory of Pharmacology, University of Lisbon, Lisbon, Portugal).

Determination of myeloperoxidase activity

Myeloperoxidase (MPO) activity in kidneys was used as an indicator of polymorphonuclear (PMN) cell infiltration using a method previously described [28]. Briefly, at the end of the experiments, kidney tissue was weighed and homogenized in a solution containing 0.5% (wt/vol) hexadecyltrimethylammonium bromide dissolved in 10 mmol/L potassium phosphate buffer (pH 7.4) and centrifuged for 30 minutes at 20,000 g at 4°C. An aliquot of supernatant was then removed and added to a reaction mixture containing 1.6 mmol/L tetrathionate solution and 0.1 mmol/L hydrogen peroxide (H₂O₂). The rate of change in absorbance was measured spectrophotometrically at 650 nm. MPO activity was defined as the quantity...
of enzyme required to degrade 1 mmol of H$_2$O$_2$ at 37°C and was expressed in mU/100 mg wet tissue.

**Determination of malondialdehyde levels**

Levels of malondialdehyde (MDA) in kidneys were determined as an indicator of lipid peroxidation following a protocol described previously [29]. Briefly, kidney tissue was weighed and homogenized in a 1.15% (wt/vol) KCl solution. A 100 mL aliquot of homogenate was then removed and added to a reaction mixture containing 200 mL 8.1% (wt/vol) lauryl sulfate, 1.5 mL 20% (vol/vol) acetic acid (pH 3.5), 1.5 mL 0.8% (wt/vol) thiobarbituric acid, and 700 mL distilled water. Samples were then boiled for one hour at 95°C and centrifuged at 3000 × g for 10 minutes. The absorbance of the supernatant was measured spectrophotometrically at 560 nm. MDA levels were expressed as mmol/L MDA/100 mg wet tissue.

**Histologic evaluation**

At postmortem, a 5 mm section of kidney was removed and placed in formalin and processed through to wax. Five millimeter sections were cut and stained with hematoxylin and eosin. Histologic assessment of tubular necrosis was determined semiquantitatively using a method modified from McWhinnie et al [30]. Random cortical fields were observed using a ×20 objective. A graticule grid (25 squares) was used to determine the number of line intersects involving tubular profiles. One hundred intersections were examined for each kidney, and a score from 0 to 3 was given for each tubular profile involving an intersection: 0 = normal histology (Fig. 5A); 1 = tubular cell swelling, brush border loss, nuclear condensation, with up to one third of tubular profile showing nuclear loss (Fig. 5B); 2 = same as for score 1, but greater than one third and less than two thirds of tubular profile shows nuclear loss (Fig. 5C); 3 = greater than two thirds of tubular profile shows nuclear loss (Fig. 5D). The total score for each kidney was calculated by addition of all 100 scores.

**Immunohistochemical localization of nitrotyrosine and poly(ADP-ribose)**

Rat kidneys fixed in 10% (wt/vol) neutral buffered paraformaldehyde and 8 mm sections were prepared from paraffin-embedded tissues. After deparaffinization, endogenous peroxidase was quenched using 0.3% (vol/vol) H$_2$O$_2$ in 60% methanol for 30 minutes. The sections were permeabilized using 0.1% (wt/vol) Triton X-100 in phosphate-buffered saline (PBS; 0.01 mol/L, pH 7.4) for 20 minutes. Nonspecific adsorption was minimized by incubating sections in 2% (vol/vol) normal goat serum in PBS for 20 minutes. Endogenous avidin- and biotin-binding sites were blocked by sequential incubation for 15 minutes with avidin (DBA, Milan, Italy) and biotin (DBA, Milan, Italy), respectively. Kidney sections were then used for the immunohistochemical localization of nitrotyrosine and poly(ADP-ribose) synthetase (PARS). Tyrosine nitration, which was used as an index of the nitrosylation of protein by peroxynitrite and/or ROS, was determined using immunohistochemistry as previously described [31]. Sections were incubated overnight with a 1:100 dilution of primary antinitrotyrosine antibody (DBA). PARS activation was assessed using a protocol previously described [32] in which sections were incubated overnight with a 1:500 dilution of primary anti-PARS antibody (DBA). Separate sections were also incubated with control solutions consisting of PBS alone or a 1:500 dilution of nonspecific purified rabbit IgG (DBA). Specific labeling was detected using a biotin-conjugated goat antirabbit IgG (DBA) and avidin-biotin peroxidase (DBA). Samples were then viewed under a light microscope.

**Isolation and culture of rat proximal tubule cells**

Proximal tubular cells were isolated from kidneys obtained from 18 male Wistar rats (weighing 260 to 350 g) using collagenase digestion, differential sieving, and Percoll density centrifugation as described previously [33–35]. Briefly, kidney cortex was separated and washed with Hank’s buffered salt solution (GIBCO BRL/Life Technologies, Paisley, Scotland, UK) to remove blood and urine and was chopped into small pieces. These were digested in medium consisting of a 1:1 mixture of Dulbecco’s modified Eagle’s medium and Ham’s F12 nutritional mixture (DMEM/Ham’s F12; GIBCO BRL/Life Technologies) containing collagenase A (0.1% wt/vol; Boehringer Mannheim, Sussex, UK) for 20 minutes at 37°C. The resulting suspension was filtered through metal sieves of decreasing pore size (300, 150, and 75 mm; Endecotts, London, UK) to remove glomeruli and larger undigested fragments, after which the filtrate was washed with DMEM/Ham’s F12. The cell pellet was then resuspended in a Percoll solution (30% vol/vol Percoll prepared in DMEM/Ham’s F12, starting density 1.044 g/mL; Sigma Chemical Co., Dorset, UK) and centrifuged at 13,000 r.p.m. for 30 minutes at 4°C. Percoll density centrifugation produced three distinct bands at densities 1.040, 1.060, and 1.065 g/mL from which the 1.060 g/mL band has previously been shown to contain single and small clumps of PT cells at a purity of greater than 96% [33]. This band was removed, and the cells were washed with DMEM/Ham’s F12 before the cell pellet was resuspended in minimum essential medium (MEM; containing D-valine substituted for L-valine; GIBCO BRL/Life Technologies) and an aliquot removed for determination of cell number and viability using the Trypan blue exclusion method. The remaining suspension was diluted with MEM containing 10% (vol/vol) fetal calf serum (FCS; Sigma Chemical Co.) and seeded at a density of
1 × 10^5 cells/mL. Rat PT cells were cultured in MEM containing 10% (vol/vol) FCS in a humidified 5% CO\textsubscript{2}/95% air atmosphere at 37°C. Medium was changed every 48 hours until the cells reached confluence.

**Experimental design**

To investigate the effect of tempol, DEF, and DEF coadministered with tempol on H\textsubscript{2}O\textsubscript{2}-mediated cell injury and death, confluent cultures of PT cells were preincubated (10 min at 37°C) with tempol (0.03 to 10 mmol/L), DEF (0.03 to 10 mmol/L), or DEF (3 mmol/L) in combination with tempol (3 mmol/L). The ranges of concentrations of tempol and DEF were based on those previously shown in this laboratory to reduce on H\textsubscript{2}O\textsubscript{2}-mediated cell injury and death in (I) cultured rat cardiac myoblasts (tempol [21]) and (2) primary cultures of rat PT cells (DEF [35]). PT cell cultures were then incubated with H\textsubscript{2}O\textsubscript{2} (1 mmol/L) for four hours, after which cellular injury and death were assessed as described later in this article, which we have previously shown to produce a significant but submaximal inhibition of mitochondrial respiration and subsequently, the release of the cytosolic enzyme LDH into the supernatant [35]. Upon completion of incubations, cellular injury and death were assessed using the spectrophotometric assays described later in this article.

**Measurement of cellular injury**

*MTT assay.* As previously described [35], cellular injury was determined indirectly by measurement of the mitochondrial-dependent conversion of MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide; Sigma Chemical Co.) into a formazan, which unlike the tetrazolium salt, can be measured spectrophotometrically at 550 nm. The reduction of MTT, mainly by the mitochondrial complexes I and II, is a useful indicator of the general metabolic status of the cell, which is dependent on mitochondrial respiration. This measurement was therefore used as an indicator of mitochondrial respiration and hence cellular viability. Although the reduction of MTT appears to be mainly via the mitochondrial complexes I and II, it may also involve NADH- and NADPH-dependent energetic processes, which occur outside of the mitochondrial inner membrane [36]. Thus, this method cannot be used to separate the effect of free radicals, oxidants, or other factors on the individual enzymes in the mitochondrial respiratory chain, but is nevertheless a useful assay for monitoring changes in the general energetic status of the cells.

Briefly, cells were incubated with MTT (0.2 mg/mL in PBS) for one hour at 37°C, after which the MTT solution was removed by aspiration. The cells were then solubilized in dimethyl sulfoxide, and an aliquot was transferred into a 96-well plate, after which the reduction of MTT to formazan was quantitated spectrophotometrically by measurement of absorbance at 550 nm using an Anthos Labtec microplate reader (Labtec, Uckfield, Sussex, UK). Results were expressed as mitochondrial respiration as a percentage of that of control cells (that is, those not exposed to H\textsubscript{2}O\textsubscript{2}), which was taken as 100%.

**Measurement of cytotoxicity**

*Lactate dehydrogenase assay.* Cell death was determined by measurement of lactate dehydrogenase (LDH) released into the incubation medium due to the loss of membrane integrity using a commercially available cytotoxicity detection kit (Boehringer Mannheim) as previously described [35]. The assay operates on the principle that LDH released from damaged cells reduces NAD to NADH and H\textsuperscript{+} by oxidation of lactate to pyruvate, followed by the transfer of 2 H from NADH and H\textsuperscript{+} to a yellow tetrazolium salt (2-[4-iodophenyl]-3-[4-nitrophenyl]-5-phenyltetrazolium chloride) in the presence of a catalyst, forming a formazan. Thus, an increase in LDH in the medium correlates directly with increased formazan production, which unlike the tetrazolium salt, can be measured spectrophotometrically at 492 nm.

Briefly, an aliquot of incubation medium was transferred into a 96-well plate, and reaction buffer was added to each well. The mixture was incubated for 30 minutes at room temperature. The reaction was stopped by the addition of 2 mol/L HCl, and absorbance was measured spectrophotometrically at 492 nm using an Anthos Labtec microplate reader (Labtec). Results were expressed as percentage of the total LDH released from cells incubated with 1% (wt/vol) Triton X-100.

**Materials**

Unless otherwise stated, all compounds used in this study were purchased from Sigma-Aldrich Company Ltd. (Poole, Dorset, UK). All solutions used for in vivo infusions were prepared using nonpyrogenic saline (0.9% wt/vol NaCl; Baxter Healthcare Ltd., Thetford, Norfolk, UK).

**Statistical analysis**

All values are expressed as mean ± SEM for N observations. For the in vivo studies, each data point represents biochemical measurements obtained from 6 to 12 separate animals (Table 1). For histologic scoring, each data point represents an analysis of kidneys taken from 6 to 12 individual animals. For immunohistochemical analysis, the figures shown are representative of at least six experiments performed on different experimental days. For in vitro studies involving PT cell cultures, measurements were taken from cultures obtained from 18 separate isolations. For the determination of cellular injury (MTT assay) and cell death (LDH assay), experiments were performed in triplicate. Statistical analysis was carried out using GraphPad Prism/Instat 1.1 (GraphPad Software, San Diego, CA, USA). Data were ana-
lyzed using one-way analysis of variance followed by Dunnett’s post hoc test, and a $P$ value of less than 0.05 was considered to be significant (NS is nonsignificant).

**RESULTS**

**Renal ischemia/reperfusion model**

This study was carried out using 83 male Wistar rats, 4 of which died during the course of the experiments, giving an overall mortality of 4.8%. All results from rats that died were excluded from the data analysis, and $N$ provided in the text and in Table 1 refers to “survivors” of the entire experimental period only. Administration of tempol or DEF or the combination of DEF and tempol, prior to and continued throughout reperfusion, did not have a significant effect on MAP or HR in comparison with those obtained from sham-operated animals or control animals (data not shown).

**Effect of tempol and DEF on ischemia/reperfusion-mediated glomerular dysfunction**

Animals that underwent 45-minute renal pedicle clamping followed by six-hour reperfusion exhibited significant increases in the plasma concentrations of urea and creatinine compared with sham-operated animals (Fig. 1), suggesting a significant degree of glomerular dysfunction. During reperfusion, plasma levels of urea increased by approximately fourfold from $7 \pm 1$ mmol/L (sham-operated) to $33 \pm 2$ mmol/L (controls; $N = 11$ to 14, $P < 0.05$; Fig. 1A). Plasma creatinine concentrations increased by approximately sixfold from $34 \pm 2$ (sham-operated) to $212 \pm 10$ mmol/L (controls; $N = 11$ to 14, $P < 0.05$; Fig. 1B).

Tempol, administered prior to and continually throughout reperfusion, produced significant reductions in plasma urea and creatinine concentrations compared with values obtained from control animals (Fig. 1), reducing urea levels by approximately 37% ($P < 0.05$, $N = 12$ to 14; Fig. 1A) and creatinine concentrations by approximately 40% ($P < 0.05$, $N = 12$ to 14; Fig. 1B). The administration of DEF produced similar, significant reductions in plasma urea and creatinine concentrations of approximately 32% ($P < 0.05$, $N = 12$ to 14) and 36% ($P < 0.05$, $N = 12$ to 14), respectively, compared with control plasma urea and creatinine concentrations (Fig. 1). The administration of DEF with tempol also produced significant reductions in plasma urea and creatinine concentrations (Fig. 1). The combined administration of DEF and tempol significantly reduced plasma urea from $33 \pm 2$ (controls) to $21 \pm 1$ mmol/L (DEF/tempol; $N = 12$ to 14, $P < 0.05$; Fig. 1A). Plasma creatinine concentrations were also significantly reduced from $212 \pm 10$ (controls) to $155 \pm 6$ mmol/L (DEF/tempol; $N = 12$ to 14, $P < 0.05$; Fig. 1B). However, the degree of reduction in either plasma urea or creatinine afforded by the coadministration of DEF and tempol was not significantly different from those obtained upon administration of tempol or DEF alone.

**Effect of tempol and DEF on ischemia/reperfusion-mediated tubular dysfunction and injury**

Fractional excretion of sodium, calculated using plasma $Na^+$ concentrations, urine production (urine flow, mL/min) and urinary concentrations of $Na^+$, was used as an indicator of PT function (Fig. 2A). $FE_{Na}$ remained at approximately 1% when calculated from sham-operated rats (0.99 ± 0.35%, $N = 11$; Fig. 2A). However, ischemia for 45 minutes followed by 6 hours of reperfusion produced an approximately 38-fold increase in $FE_{Na}$ to 38 ±
Administration of tempol prior to and during reperfusion significantly decreased FE\textsubscript{Na} by approximately 69\% compared with control values, from 38 ± 3\% (controls; \(N = 14\)) to 12 ± 2\% (tempol; \(N = 12\), \(P < 0.05\); Fig. 2A). Tempol also significantly decreased urinary NAG levels by approximately 60\% compared with control values, from 43 ± 5 (controls; \(N = 14\)) to 18 ± 2 U/L (tempol; \(N = 12\), \(P < 0.05\); Fig. 2B). The administration of DEF produced similar effects on FE\textsubscript{Na} and NAG; DEF produced a significant decrease in FE\textsubscript{Na} of 65\% from 38 ± 3\% (controls; \(N = 14\)) to 13 ± 2\% (DEF; \(N = 12\), \(P < 0.05\); Fig. 2A) and in NAG of 80\% from 43.5 ± 4.5 (controls; \(N = 14\)) to 9 ± 2 U/L (DEF; \(N = 12\), \(P < 0.05\); Fig. 2B). Coadministration of DEF and tempol also produced significant reductions in FE\textsubscript{Na} and urinary NAG concentrations (Fig. 2). This combination of DEF and tempol significantly reduced FE\textsubscript{Na} from 38 ± 3\% (controls) to 19 ± 2\% (DEF/tempol; \(N = 12\) to 14, \(P < 0.05\); Fig. 2A). Urinary NAG levels were also significantly reduced from 43 ± 5 (controls) to 26 ± 1 U/L (DEF/tempol; \(N = 12\) to 14, \(P < 0.05\); Fig. 2B). However, the reduced values for FE\textsubscript{Na} and NAG obtained using DEF and tempol in combination were not significantly different from those obtained upon administration of tempol or DEF alone.

**Effect of tempol and DEF on ischemia/reperfusion-mediated renal reperfusion injury**

Plasma concentrations of γGT and AST were both used as markers of renal reperfusion injury in this model. γGT increased by approximately ninefold from 0.6 ± 0.3 (sham-operated) to 5.3 ± 0.6 U/L (controls; \(N = 11\) to 14, \(P < 0.05\); Fig. 3A). Plasma AST also increased by ninefold from 170 ± 12 (sham-operated) to 1436 ± 178 U/L (controls; \(N = 11\) to 14, \(P < 0.05\); Fig. 3B). However, renal ischemia/reperfusion did not have a significant effect on plasma BIL or ALT concentrations, respective markers of hepatic function and injury (data not shown).

The administration of tempol or DEF produced significant reductions in plasma γGT and AST compared with values obtained from control animals (Fig. 3). Tempol significantly reduced plasma γGT and AST levels by approximately 87 and 63\%, respectively (\(P < 0.05\), \(N = 12\) to 14; Fig. 3). DEF also significantly reduced plasma γGT and AST levels by approximately 70 and 56\%, respectively (\(P < 0.05\); Fig. 3). Coadministration of DEF and tempol also produced significant reductions in both plasma γGT and AST concentrations (Fig. 3). This combination of DEF and tempol significantly reduced γGT from 5.3 ± 0.6\% (controls) to 0.7 ± 0.2 U/L (DEF/tempol; \(N = 12\) to 14, \(P < 0.05\); Fig. 3A) and AST from 1436 ± 178 (controls) to 603 ± 58 U/L (DEF/tempol; \(N = 12\) to 14, \(P < 0.05\); Fig. 3B). However, the reduced values for plasma γGT and AST obtained using DEF and tempol in combination were not significantly differ-
ent from those obtained upon administration of tempol or DEF alone.

Administration of tempol or DEF to sham-operated animals did not have any effect on any plasma or urinary biochemical parameters that were similar to those obtained from sham-operated animals (data not shown).

**Effects of tempol and DEF on kidney MPO activity and MDA levels**

Rats subjected to renal ischemia/reperfusion exhibited a substantial increase in MPO activity and MDA levels in the kidney (Fig. 4). Renal ischemia/reperfusion produced a significant increase in kidney MPO activity from 60 ± 3 (sham-operated) to 354 ± 30 (controls) μU/100 mg wet tissue (N = 11 to 14, P < 0.05; Fig. 4A). However, treatment of rats with tempol produced a significant attenuation of MPO activity caused by ischemia/reperfusion of the kidney, from 354 ± 30 (controls) to 118 ± 10 (tempol) μU/100 mg wet tissue (N = 12 to 14, P < 0.05; Fig. 4A). DEF produced a similar significant reduction in MPO activity from 354 ± 30 (controls) to 104 ± 7 (DEF) μU/100 mg wet tissue (N = 12 to 14, P < 0.05; Fig. 4A). The administration of the combination of DEF and tempol also produced a significant attenuation of MPO activity from 354 ± 30 (controls) to 146 ± 11 (DEF/tempol) μU/100 mg wet tissue (N = 12 to 14, P < 0.05; Fig. 4A). However, this was not significantly
different from the reduction in MPO activity obtained upon administration of tempol or DEF alone.

Renal ischemia/reperfusion also produced a significant increase in kidney MDA levels from $77 \pm 15$ (sham operated) to $260 \pm 23$ (controls) mmol/L/100 mg wet tissue ($N = 11$ to $14$, $P < 0.05$; Fig. 4B). Treatment of rats with tempol produced a significant reduction in MDA levels produced by ischemia/reperfusion, from $260 \pm 23$ (controls) to $105 \pm 6$ (tempol) mmol/L/100 mg wet tissue ($N = 12$ to $14$, $P < 0.05$; Fig. 4B). DEF also produced a similar significant reduction in MDA levels from $260 \pm 23$ (controls) to $120 \pm 17$ (DEF) mmol/L/100 mg wet tissue ($N = 12$ to $14$, $P < 0.05$; Fig. 4B). The administration of the combination of DEF and tempol also produced a significant attenuation of MDA levels, from $260 \pm 23$ (controls) to $154 \pm 6$ (DEF/tempol) mmol/L/100 mg wet tissue ($N = 6$ to $12$, $P < 0.05$; Fig. 4B). This reduction in MDA was not, however, significantly different from the reduction obtained upon administration of tempol or DEF alone.

The administration of tempol or DEF to sham-operated animals did not have any effect on kidney MPO activity or MDA levels, which were similar to those obtained from sham-operated animals (data not shown).

**Effects of tempol and DEF on ischemia/reperfusion-mediated renal histopathology**

On comparison with normal tubular histology in sham-operated rats (Fig. 5A), animals that underwent renal ischemia/reperfusion demonstrated the recognized features of severe acute tubular damage (Fig. 5D) [37]. These features included brush border loss, nuclear condensation, cytoplasmic swelling, and consistent loss of significant numbers of nuclei from tubular profiles (Fig. 5D).

Total severity score was significantly reduced in rats administered tempol from $223 \pm 6$ (controls) to $109 \pm 26$ (tempol; $N = 12$, $P < 0.05$; Fig. 5E). The administration of DEF had a similar significant effect on total severity score, reducing it from $223 \pm 6$ (tempol) to $133 \pm 23$ (DEF; $N = 12$, $P < 0.05$; Fig. 5E), as did coadministration of DEF and tempol [from $223 \pm 6$ (controls) to $145 \pm 31$ (DEF/tempol), $N = 12$, $P < 0.05$; Fig. 5E]. Coadministration of DEF and tempol did not have any additive effects on reduction of total severity score on comparison with that obtained after tempol or DEF administration, respectively.

**Immunohistochemical localization of nitrotyrosine and PARS formation**

Immunohistochemical analysis of renal sections obtained from rats subjected to renal ischemia/reperfusion injury revealed positive staining for nitrotyrosine, which was primarily localized to the PT, although some staining was also observed in the macula densa structures (Fig. 6A). In contrast, substantially reduced staining was observed in the kidney sections obtained from rats administered tempol or DEF (Fig. 6 B, C).

Kidneys obtained from rats subjected to ischemia/reperfusion also demonstrated marked staining for PARS, which was also localized to the PT (Fig. 7A). Kidneys obtained from rats administered tempol or DEF demonstrated markedly reduced staining for PARS upon comparison with kidneys obtained from control animals (Fig. 7 B, C), suggesting a reduction in the activation of PARS after six hours of reperfusion.

No evidence of staining for either nitrotyrosine or PARS was observed in kidney tissues obtained from sham-operated rats administered saline, tempol, or DEF (data not shown).
Isolation, culture, and characterization of rat PT cells

On removal from Percoll and observation under light microscope, the 1.060 g/mL band was found to consist of both single and small two- to four-cell clumps of PT, in keeping with the findings of others [33, 34]. Once confluent, cultures of PT cells demonstrated the typical “cobblestone” morphology associated with epithelial cells. These cultures did not exhibit any evidence of fibroblast contamination, which was prevented by the use of MEM throughout culture in which l-valine was substituted with d-valine [38]. As discussed previously [35], suspensions and cultures of rat PT cells obtained using the isolation method described here have been previously characterized using phase-contrast and electron-scanning microscopy, enzyme histochemistry, and the demonstration of PT-specific transport mechanisms (for example, d-glucose and anionic/cationic transport).
These studies have demonstrated that the cells isolated using this technique are of PT origin and maintain PT specific properties throughout culture and during experiments performed using primary cultures.

**H$_2$O$_2$-mediated rat PT cell injury and necrosis**

Incubation of rat PT cell cultures with 1 mmol/L H$_2$O$_2$ for four hours produced a significant decrease in mitochondrial respiration, as determined by using the MTT assay (Fig. 8) and a significant increase in the release of LDH into the cell supernatant (Fig. 9). In the presence of 1 mmol/L H$_2$O$_2$ for four hours, mitochondrial respiration decreased from 100 (untreated controls) to 24 ± 1% of control ($N = 12$, $P < 0.05$; Fig. 9A) and caused a significant release of LDH into the incubation medium, as demonstrated by using the LDH assay from 0 (untreated controls) to 65 ± 2% Triton X-100 control ($N = 12$, $P < 0.05$; Fig. 9A).

**Attenuation of H$_2$O$_2$-mediated cellular injury by tempol and DEF**

As described earlier in this article and previously [35], incubation of rat PT cells with 1 mmol/L H$_2$O$_2$ for four hours produced a significant, but submaximal, cellular injury and cell death. The effects of tempol and DEF were therefore investigated after incubation with 1 mmol/L H$_2$O$_2$ for this time period. Furthermore, the effects of coinubation of DEF and tempol, each at concentrations that provided maximal reduction in cellular injury and death when used individually, were also investigated.

Incubation with increasing concentrations of tempol (0.03 to 10 mmol/L) resulted in a reduction in H$_2$O$_2$-mediated decrease in mitochondrial respiration (Fig. 8A), which was significant at concentrations of 1 and 3 mmol/L [from 25 ± 1 (H$_2$O$_2$ control) to 37 ± 3 (3 mmol/L tempol) percentage of control; $N = 6$, $P < 0.05$; Fig. 8A]. Incubation with increasing concentrations of DEF (0.03 to 10 mmol/L) also attenuated the fall in mitochondrial respiration mediated by H$_2$O$_2$ (Fig. 8B). Incubation with DEF produced a significant reduction in H$_2$O$_2$-mediated inhibition of mitochondrial respiration at 1, 3, and 10 mmol/L DEF [25 ± 1 (H$_2$O$_2$-control) to 49 ± 6 (3 mmol/L DEF) percentage of control; $N = 6$, $P < 0.05$; Fig. 8B]. Incubation of PT cell cultures with DEF and tempol in combination also significantly reduced the H$_2$O$_2$-mediated reduction in mitochondrial respiration [22 ± 1 (H$_2$O$_2$-control) to 45 ± 3 (3 mmol/L DEF + 3 mmol/L tempol) percentage of control; $N = 6$, $P < 0.05$; Fig. 8C]. However, this attenuation of H$_2$O$_2$-mediated reduction in mitochondrial respiration was not significantly different from that obtained using 3 mmol/L tempol or 3 mmol/L DEF (Fig. 8C).
Attenuation of H$_2$O$_2$-mediated cellular necrosis by tempol and DEF

Incubation with increasing concentrations of tempol (0.03 to 10 mmol/L) produced a decrease in H$_2$O$_2$-mediated LDH release (Fig. 9A). A significant reduction in LDH release was obtained using tempol at concentrations of 1 and 3 mmol/L [from 65 ± 3 (H$_2$O$_2$-control) to 44 ± 3 (3 mmol/L tempol) percentage Triton X-100 control; $N = 6$, $P < 0.05$; Fig. 9A]. DEF (0.03 to 3 mmol/L) also attenuated LDH release produced by incubation with 1 mmol/L H$_2$O$_2$ for four hours (Fig. 9B). This reduction was found to be significant using DEF concentrations of 1, 3, and 10 mmol/L [65 ± 3 (H$_2$O$_2$-control) to 39 ± 3 (3 mmol/L DEF) percentage Triton X-100 control; $N = 6$, $P < 0.05$; Fig. 9B]. Incubation of PT cell cultures with DEF and tempol in combination also significantly reduced the H$_2$O$_2$-mediated increase in LDH release [69 ± 4 (H$_2$O$_2$-control) to 39 ± 5 (3 mmol/L DEF + 3 mmol/L tempol) percentage Triton X-100 control; $N = 6$, $P < 0.05$; Fig. 9C]. However, this reduction of H$_2$O$_2$-mediated increase in LDH release was not significantly different from that obtained using 3 mmol/L tempol or 3 mmol/L DEF (Fig. 9C).

DISCUSSION

There is good evidence from both in vivo and in vitro studies that ROS plays an important role in the pathophysiology of renal ischemia/reperfusion injury [5]. We demonstrate here, to our knowledge for the first time, that administration prior to and during reperfusion of the stable nitroxide radical tempol reduces the renal dysfunction and injury caused by ischemia/reperfusion of the rat kidney. We also report that tempol reduces the cellular injury and death of PT cell cultures caused by H$_2$O$_2$-mediated oxidant stress. These conclusions are supported by the following key findings that in an in vivo rat model of renal ischemia/reperfusion injury, administration of tempol significantly reduced the ischemia/reperfusion-mediated increases in: (1) plasma levels of urea and creatinine; (2) FE$_{Na}$, and urinary NAG concentrations; (3) plasma levels of γGT and AST; and (4) MPO activity and the levels of MDA in the kidney. Tempol significantly reduced the histologic evidence of ischemia/reperfusion-mediated tubular injury and reduced the immunohistochemical evidence of nitrotyrosine production and the activation of PARS. Using primary cultures of rat PT cells, this study also demonstrated that tempol attenuated both the impairment in mitochondrial respiration as well as the release of LDH caused by H$_2$O$_2$.

Since it is not valid to use clearance methods for the assessment of glomerular filtration rate (GFR) in renal models that involve extensive tubular injury such as the one used here, GFR, estimated using creatinine clearance, was not measured in this study. However, indica-
tors of glomerular function in the forms of plasma concentrations of urea and creatinine were measured, and the significant increase in both subsequent to ischemia/reperfusion suggests moderate but significant impaired glomerular function [23, 24]. Furthermore, ischemia/reperfusion resulted in significant increase in both FE\(_{\text{S}}\) and urinary NAG (a specific marker for tubular injury and possibly function) [27], suggesting a reduction in tubular function and increased tubular injury, respectively. Renal ischemia/reperfusion also produced significant increases in plasma concentrations of γGT and AST, which were used in this study as markers of renal reperfusion injury. Both enzymes are present within the PT [25] and can be released into the urine subsequent to renal injury [39, 40]. However, it is also feasible that their increased plasma levels subsequent to renal ischemia/reperfusion originated from the kidney after damage to the tubular architecture. Both enzymes are regarded as nonspecific markers of extensive cellular disruption or necrosis [24], and one could argue that these enzymes are also released from the liver after hepatic injury subsequent to renal ischemia/reperfusion, presumably caused by circulating mediators. However, plasma levels of total BIL (a marker of hepatocellular injury and dysfunction) [26] and AST (a specific marker of hepatic parenchymal injury) [26] were also measured, and in this study, these did not show a significant increase subsequent to renal ischemia/reperfusion. Furthermore, plasma concentrations of γGT have been shown not to rise in primary hepatocyte necrosis [24].

Renal ischemia/reperfusion also caused a significant increase in MPO activity, indicating PMN accumulation, and in MDA levels, indicating increased lipid peroxidation and, therefore, increased oxidative stress. Histologic examination of kidney sections showed increased evidence of tubular injury subsequent to ischemia/reperfusion, and this was reflected by a significant increase in the total severity score obtained from sections of kidney from rats that underwent ischemia/reperfusion. Renal ischemia/reperfusion also increased the nitration of proteins (as determined by measurement of nitrotyrosine formation using immunohistochemistry). Nitrotyrosine formation, along with its detection using immunohistochemical staining, was initially proposed as a relatively specific marker for the detection or “footprint” of the endogenous formation of peroxynitrite [41]. There is, however, recent evidence that certain other reactions can also induce tyrosine nitration. For example, the reaction of nitrite with hypochlorous acid and the reaction of MPO with H\(_2\)O\(_2\) can lead to the formation of nitrotyrosine [42]. Therefore, increased nitrotyrosine staining is now considered to act as an indicator of “increased nitrosative stress” rather than a specific marker of the generation of peroxynitrite.

Reactive oxygen species also causes strand breaks in DNA, which trigger energy-consuming DNA repair mechanisms and activate the nuclear enzyme PARS. As described previously [32, 35], activation of PARS results in the depletion of its substrate NAD and also in a reduction in the rate of glycolysis. As NAD functions as a cofactor in glycolysis and the tricarboxylic acid cycle, NAD depletion leads to a rapid fall in intracellular ATP. The substantial decrease in NAD and ATP concentrations lead to cellular dysfunction and death, and the overall process has been termed “the PARS Suicide Hypothesis” [43]. We have recently discovered that the activation of PARS plays an important role in both ischemia/reperfusion dysfunction and injury of the kidney in vivo [32] and in the cellular injury and death caused by H\(_2\)O\(_2\) in rat PT cell cultures [35]. Using immunohistochemistry, we demonstrate in this study that ischemia/reperfusion of the rat kidney in vivo leads to an increase in PARS activity.

Taken together, the evidence presented in this study suggests that ischemia/reperfusion of rat kidneys produces moderate glomerular dysfunction, but a substantial increase in tubular dysfunction and injury. This is in keeping with the notion that ischemia/reperfusion of the kidney causes both glomerular and tubular dysfunction [44]. There are several reports describing the vulnerability of the outer medulla and thick ascending limb cells to structural damage during renal ischemia/reperfusion, which has been attributed to the imbalance in metabolic demand of this region of the kidney and oxygen delivery to these cells [45–47]. Although it is important to consider the role of glomerular dysfunction and injury as well as the pathophysiology of the renal medulla during episodes of renal ischemia/reperfusion, early observations, among several others, have suggested that the damage to kidney during ischemia/reperfusion is primarily to the S3 segment of the PT [48]. This was further reinforced by the demonstration in this study that (1) renal ischemia/reperfusion in vivo caused relatively greater tubular dysfunction and injury than glomerular dysfunction, and that (2) oxidant stress in the form of H\(_2\)O\(_2\) produced significant levels of cellular injury and death in primary cultures of rat PT cells in vitro.

Our study demonstrates, to our knowledge for the first time, that tempol (I) reduces the degree of renal dysfunction and injury caused by ischemia/reperfusion of the rat kidney in vivo, and (2) reduces the cellular injury and death caused by H\(_2\)O\(_2\) in primary cultures of rat PT cells in vitro. Tempol is a stable piperidine nitroxide and a water-soluble analogue of the spin label tempo that has a relatively low molecular weight (172), permeates biological membranes [13], and accumulates in the cytosol [16]. Tempol has a very short half-life and is rapidly bioreduced in the rat in vivo [49], and this was
the basis for the infusion protocol used for the administration of tempol in this study. Tempol is reduced by ascorbic acid but not by reduced glutathione into its inactive hydroloxamine metabolite [17]. However, tempol, along with other nitroxides, can also be recycled back into its oxidized form, and their antioxidative activity is associated to their ability to switch between oxidized and reduced forms [50, 51]. Furthermore, tempol can also be produced in situ in rat liver upon metabolism of tempone [52]. Tempol has been shown to cause tyrosine phosphorylation, activation of Raf-1 protein kinase and an increase extracellular signal-regulated kinase 1 activity in human MDA-MB-231 breast cancer cells [53]. In a rat model of cerebral injury, reducing tumor necrosis factor-α toxicity is possible by interfering with the activation of transcription factor nuclear factor-κB [54].

There is some evidence of toxicological actions associated with administration of tempol. Tempol has been reported to cause dose-related hypotension accompanied by reflex tachycardia [55], presumably by scavenging of superoxide and thereby prolonging the presence of systemic nitric oxide (NO) levels leading to vasodilation. However, this hypotensive effect was used to combat the hypertension characteristic of SHR in a study where tempol was administered to rats in their drinking water over a two week period [18]. In the same study, tempol did not have any effect on the MAP when administered to normal (Wistar-Kyoto) rats in a similar fashion [18], and at the concentrations used in this study, tempol did not have a significant effect on cardiovascular parameters (MAP/HR). Tempol has been reported to inhibit the growth of neoplastic human and rodent cell lines by apoptosis [56] and to increase skin temperature [55], although the relevance of these observations is not known.

It is intriguing to consider the mechanisms by which tempol reduces renal dysfunction and injury, and more specifically, PT cell injury/death mediated by oxidative stress-mediated renal injury. There is good evidence that ROS contributes to renal injury [3, 5, 57]. Several studies have demonstrated how tempol scavenges superoxide anions in vitro [11, 13, 58, 59] and how it may also act as a genuine “SOD-mimetic” [14]. Tempol has also been reported to reduce the formation or the effects of hydroxyl radicals by scavenging superoxide anions or by reducing intracellular Fe2+ concentrations or even by directly scavenging hydroxyl radicals [15–17]. In our study, the renal dysfunction and injury produced during ischemia/reperfusion appears to be secondary to the generation of hydroxyl radicals, as it was also attenuated to a similar degree by DEF, an iron chelator that prevents the formation of hydroxyl radicals from H2O2 via inhibition of the Fenton and Haber-Weiss reactions [60]. This was reflected in the finding that cellular injury and death caused by H2O2 in PT cell cultures was also attenuated by DEF to a similar degree. There were no significant differences between the reduction in oxidative stress-mediated renal or PT cell dysfunction and injury obtained using tempol and DEF. As tempol has been shown not to scavenge H2O2 [58, 61, 62], it is likely that tempol prevented H2O2-mediated cell injury/death by reducing the generation, or the effects, of hydroxyl radicals. This is supported by the finding that coadministration of DEF and tempol together did not provide any additional beneficial actions compared with that obtained using either tempol or DEF alone. Furthermore, it is unlikely that tempol affects the activity of xanthine oxidase, as similar compounds, including tempo and the SOD-mimetic tiron, do not affect the activity of this enzyme [63].

As tempol attenuated the nitration of proteins (as determined using immunohistochemical localization of nitrotyrosine formation) in the kidney of rats subjected to ischemia/reperfusion injury, one could argue that the prevention of “nitrosative stress” plays an important part in the reduction by tempol of renal ischemia/reperfusion injury. This is unlikely to be a major factor in our study, as the protection of the kidney caused by coadministration of DEF and tempol was not greater than the protection caused by either DEF or tempol alone, and it is therefore likely that a greater degree of tissue injury is mediated by hydroxyl radicals. Hydroxyl radicals (and other ROS and peroxynitrite) cause strand breaks in DNA, which trigger energy-consuming DNA repair mechanisms and activate the nuclear enzyme PARS. We have previously demonstrated that H2O2, via the production of hydroxyl radicals, leads to DNA strand breakage and the activation of PARS in PT cell cultures [35]. We demonstrate here that ischemia/reperfusion of the rat kidney in vivo leads to an increase in PARS activity (as demonstrated using immunohistochemistry), which is significantly reduced by tempol. Thus, we propose that the prevention of the activation of PARS also contributes to the beneficial effects of tempol observed in our study.

In conclusion, this article demonstrates, to our knowledge for the first time, that tempol reduces the degree of renal dysfunction and injury caused by ischemia/reperfusion of the rat kidney in vivo, and reduces the cell injury/death caused by H2O2 in rat PT cells in vitro. We propose that the beneficial effects of tempol observed in our study are due to its ability to reduce the generation or the effects of hydroxyl radicals.

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