Superoxide Dismutase Mimetic Preserves the Glomerular Capillary Permeability Barrier to Protein

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ABSTRACT

Overproduction of superoxide (O$_2^-$) occurs in glomerular disease and may overwhelm the capacity of superoxide dismutase (SOD), thereby intensifying oxidant injury by O$_2^-$ and related radical species that disrupt the glomerular capillary permeability barrier to protein. We examined the efficacy of the SOD mimetic tempol in preserving glomerular permeability to protein using 1) a rat model of glomerular immune injury induced by an antiglomerular basement membrane antibody (anti-GBM), and 2) isolated rat glomeruli in which injury was induced by the cytokine tumor necrosis factor-$\alpha$ (TNF-$\alpha$). To induce glomerular immune injury, rats received anti-GBM using a protocol that results in prominent infiltration of glomeruli by macrophages and in which macrophage-derived TNF-$\alpha$ has been shown to mediate albuminuria. To increase glomerular capillary permeability to albumin (Palb) ex vivo, isolated glomeruli were incubated with TNF-$\alpha$ at concentrations (0.5–4.0 $\mu$g/ml) known to stimulate O$_2^-$ production. Increments in P$_{\text{lab}}$ were detected by measuring changes in glomerular volume in response to an applied oncotic gradient. Significant increases in the urine excretion of albumin and F$_{2\alpha}$-isoprostane were observed in rats with glomerular immune injury without a significant change in systolic blood pressure. Tempol treatment significantly reduced urinary isoprostane and albumin excretion. In isolated glomeruli, TNF-$\alpha$ increased P$_{\text{lab}}$ and tempol abrogated this effect, both in a dose-dependent manner. These observations indicate that SOD mimetics can preserve the glomerular permeability barrier to protein under conditions of oxidative stress from O$_2^-$ production.

In various forms of glomerular disease, there is an overproduction of superoxide (O$_2^-$) in glomeruli while the activity of the principal O$_2^-$ scavenger superoxide dismutase (SOD) is reduced (Shah, 1989; Gaertner et al., 2002). This imbalance may intensify oxidative injury mediated by O$_2^-$ and/or by O$_2^-$-driven formation of more potent reactive oxygen species, including hydrogen peroxide (H$_2$O$_2$), hydroxyl radicals, and peroxynitrite. Therefore, administration of SOD to attenuate O$_2^-$-mediated injury is a logical therapeutic approach that has been explored, principally by using the cytosolic copper and zinc-containing native (bovine) SOD, in various experimental models of glomerular disease for over 20 years (Shah, 1989).

However, the negative charge, short half-life, 32,000 molecular weight, and the antigenicity of native SOD may prevent its distribution to the mitochondria, the major site of O$_2^-$ generation. Further limiting the application of native SOD is its proinflammatory effect at high doses (Dowling et al., 1993), mediated via Fenton-type reactions (Mao et al., 1993), although low doses may have an anti-inflammatory action. These factors may contribute to the conflicting results of early studies with native SOD treatment to attenuate O$_2^-$-induced glomerular injury. For example, in the rat model of glomerular injury induced by an antibody against the basement membrane of the glomerular capillary, several investigators reported an attenuation of the increase in glomerular capillary permeability to protein (proteinuria) (Adachi et al., 1986; Birtwistle et al., 1989), whereas others observed little or no effects (Rehan et al., 1984; Webb et al., 1985).

The number of pathophysiological conditions associated with overproduction of O$_2^-$, including degenerative central nervous system diseases, is rapidly increasing. For example, peroxynitrite has been implicated in the pathogenesis of Alzheimer’s disease and Parkinson’s disease (Stadtman et al., 2004), as well as in sepsis (Beckman et al., 2000), diabetic retinopathy, and atherosclerosis (Wang and Beckman, 1997). In various forms of glomerular disease, there is an overproduction of superoxide (O$_2^-$) in glomeruli while the activity of the principal O$_2^-$ scavenger superoxide dismutase (SOD) is reduced (Shah, 1989; Gaertner et al., 2002). This imbalance may intensify oxidative injury mediated by O$_2^-$ and/or by O$_2^-$-driven formation of more potent reactive oxygen species, including hydrogen peroxide (H$_2$O$_2$), hydroxyl radicals, and peroxynitrite. Therefore, administration of SOD to attenuate O$_2^-$-mediated injury is a logical therapeutic approach that has been explored, principally by using the cytosolic copper and zinc-containing native (bovine) SOD, in various experimental models of glomerular disease for over 20 years (Shah, 1989).
system diseases, diabetes, hypertension, and inflammation, and the limitation of the therapeutic use of native SOD stimulated the synthesis of low molecular weight, cell membrane-permeable SOD mimetics such as the nitroxide tempol (4-hydroxy-2,2,6,6-tetramethylpiperidine-N-oxyl; mol. wt., 172), a stable, water-soluble, metal-independent SOD mimetic that has been widely used in models of oxidant-induced injury (Salvemini et al., 2002). The present studies explored the effect of tempol on the permeability of the glomerular capillary to protein using a rat model of glomerular capillary injury induced by the administration of an antibody against the glomerular capillary basement membrane (anti-GBM). In this model, increased capillary permeability to protein occurs due to immune injury initiated following binding of the antibody to the GBM, complement activation, recruitment of macrophages, and release of macrophage-derived proinflammatory cytokines. Of the various cytokines released, tumor necrosis factor-α (TNFα) has been shown to mediate proteinuria (Khan et al., 2005). In addition, we used an ex vivo model of isolated rat glomeruli in which permeability of the capillary wall to protein was increased by exposure to TNFα. In this model, TNFα has been shown to induce O2- production in glomerular cells (Radeke et al., 1990) and to increase glomerular permeability to protein via an O2- dependent mechanism (McCarthy et al., 1998).

**Materials and Methods**

**Glomerular Immune Injury.** This was induced by administration of immune serum raised in rabbits against rat particulate glomerular basement membrane (GBM), as previously described (Lianos et al., 1983). Briefly, Lewis rats (180–200 g) were immunized with 1 mg of rabbit IgG mixed in complete Freund’s adjuvant and given as a single intraperitoneal injection. Six days later, the animals received two intravenous injections (0.3 ml/animal given 24 h apart) of heat-inactivated rabbit anti-rat GBM serum. This protocol results in infiltration of glomeruli by activated macrophages, which peaks 18 to 24 h following administration of the second anti-GBM injection and causes glomerular capillary injury characterized by an increased permeability to protein (proteinuria). This response is mediated by the release of cytokines, in particular TNFα (Radeke et al., 2005), which also promotes generation of O2- in glomerular cells (Radeke et al., 1990). Studies were performed early (18–24 h) and on day 6 following the second anti-GBM injection.

The animals were grouped (n = 6/group) and treated with 1 anti-GBM serum only as described above; 2) anti-GBM serum and tempol at 230 mg/kg intraperitoneally, as described elsewhere (Chatterjee et al., 2000) for a rat model of renal ischemia-reperfusion injury, beginning 1 h before each injection of anti-GBM serum; and 3) two injections of nonimmune rabbit serum (as a control) and tempol treatment as in group 2. Following completion of the injections, animals were placed in metabolic cages for an 18-h urine collection (Radeke et al., 1990). Studies were performed early (18–24 h) and on day 6 following the second anti-GBM injection.

To determine whether glomerular immune injury was associated with changes in blood pressure (BP), systolic BP was measured in rats with immune injury (day 6) and in parallel controls. Tail systolic BP was measured based on the plethysmometry principle. The instrument used (monitor XBP/1000, Kent Scientific, Torrington, CT) allows BP monitoring every 1 min. Individual animals were placed in a retainer and allowed to become acclimated for 15 min before systolic BP readings were recorded. Eight independent readings, 1 min apart, were recorded for each blood pressure recording session, and the mean value was taken to represent the systolic BP pressure for the particular recording session. BP measurements were performed before animals were placed in the metabolic cages (see above).

**Isoprostane Excretion.** Isoprostanes are formed nonenzymatically by the attack of reactive oxygen species, including O2-, on arachidonic acid, and their levels have been shown to provide a highly accurate index of oxidative stress occurring in vivo (Morrow and Roberts, 1997; Morrow, 2005). An advantage of measuring urinary isoprostanes is that neither 15-F2-isoprostanes (reflecting systemic rather than renal formation) nor 5-F2-isoprostanes are formed ex vivo by autooxidation in urine. Isoprostanes were measured in duplicate by gas chromatography/mass spectrometry after isolation using high-pressure liquid chromatography, silylated, and analyzed by gas chromatography/mass spectrometry. Isoprostanes were separated on an Agilent DB 1701 (30 mm × 0.25 mm column; Agilent Technologies, Palo Alto, CA) and identified with a mass selective detector, run in negative chemical ionization mode.

**Glomerular TNFα Production and Assessment of Its Cell Origin.** TNFα production was assessed in glomeruli isolated 24 h following immune injury. Glomeruli, isolated by an established sieving method, were suspended in RPMI-1640 containing 15% fetal calf serum, 15 mM HEPES, insulin (0.66 U/ml), and an antibiotic-antimycotic solution consisting of penicillin (60 μg/ml), streptomycin (60 μg/ml), and amphotericin (0.25 μg/ml). Glomeruli were subsequently seeded in 60-mm tissue culture plates at 5 × 105 glomeruli/plate and incubated at 37°C in 5% CO2 for 24 h. TNFα was measured by enzyme-linked immunosorbent assay in incubation media using a hamster monoclonal antibody against murine TNFα (Genzyme, Cambridge, MA). Briefly, 96-well plates were coated with 0.1 ml of hamster anti-murine TNFα Ab, 2.0 μg/ml in 0.05 M PBS, under shaking for 2 h at room temperature. Media were decanted, and 0.3 ml of casein-bovine serum albumin (BSA) block solution (0.5% casein-in/0.5% BSA/Tween/thiomerosal) was added to each well for 1 h at 37°C. Wells were washed in 0.05 M PBS-0.05% Tween. Serial dilutions of standard recombinant murine TNFα (Genzyme) in 0.05 M PBS or 50 μl of glomerular incubation media samples were subsequently added to the wells with 50 μl of blocking buffer, and binding was allowed to occur overnight at 4°C. Wells were washed five times in PBS-Tween. A rabbit anti-murine TNFα Ab (Genzyme) diluted in blocking buffer (secondary antibody) was subsequently added (0.5 ml) and incubated for 2 h at 37°C. Wells were then washed, and 0.1 ml of peroxidase-conjugated goat anti-rabbit Ab, diluted in blocking buffer, was added and incubated for 2 h at 37°C. The plates were again washed, and 0.1 ml of substrate solution (1 mg/ml orthophenylenediamine) was added for 20 min at room temperature under shaking. The reaction was stopped with 0.025 ml of 0.1 M NaF and read at absorbance 460 nm using a MR7000 Dynateck plate reader (Dynatech Laboratories, Inc., Alexandria, VA).

Since macrophage infiltration in glomeruli is a prominent feature in the glomerular immune injury model used, we assessed whether these cells were a significant source of glomerular TNFα production following the onset of immune injury. Whole-body X-irradiation was used to deplete animals of circulating macrophages using a protocol that has no effect on glomerular antibody binding (Lianos et al., 1991). Rats received 250-kV X-ray orthovoltage X-rays with a half-value of 1 mm Cu at a dose rate of 133 rad/min (total dose 900-1100 rad). Kidneys were protected with 6-mm lead shields covering margins within 5 mm; positioning was verified with diagnostic X-rays. Dosimetry was done in a Plexiglas phantom using a Farmer-type ionization chamber. The effect of X-irradiation on peripheral leukocyte counts was assessed in peripheral blood smears before induction of
immune injury by an automated hematology analyzer (Coulter Electronics, Hialeah, FL).

**Glomerular Injury by TNFα-Mediated O₂⁻ Release.** Exposure of isolated glomeruli to TNFα increases their permeability to albumin (McCarthy et al., 1998). Changes in glomerular capillary permeability to albumin were measured by determining changes in glomerular volume in response to an applied oncotic gradient (Savin et al., 1992). This method detects rapid and subtle changes in albumin permeability without the influence of hemodynamic or circulating factors. Briefly, glomeruli were isolated from healthy rats anesthetized with halothane. After the kidney capsule was removed, the outer cortex was minced in 1- to 2-mm fragments and passed through consecutive 80- and 120-mesh sieves and recovered from the 200-mesh sieve. Isolation of glomeruli was carried out at room temperature in a medium containing 115 mM sodium chloride, 5.0 mM potassium chloride, 1.0 mM calcium chloride, 5.5 mM glucose, 6.0 mM L-alanine, 1.0 mM calcium chloride, 5.5 mM magnesium sulfate, 1.0 mM sodium citrate, and 4.0 mM sodium lactate. BSA (5.0 g/dl) was included in the medium as an oncotic agent (isolation/incubation buffer). The pH of the medium was adjusted to 7.4. The oncotic pressure was measured using a membrane colloid osmometer (model 4100, Wescor Inc., Logan, UT).

Changes in glomerular permeability to albumin (P\text{ab}) were measured by calculating the glomerular volume response to an applied oncotic gradient generated by defined concentrations of albumin. Glomeruli were incubated in control media or in media containing oncotic gradient generated by defined concentrations of albumin. The perfusion pressure was measured using a membrane colloid osmometer (model 4100, Wescor Inc., Logan, UT).

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**Convexional Permeability to Albumin (P\text{ab}).** Convexional albumin permeability (P\text{ab}) was defined as (1-\delta\text{ab}) to describe the movement of albumin consequent to water flow. When \delta\text{ab} is zero, albumin moves at the same rate as water and P\text{ab} is 1.0. When \delta\text{ab} is 1.0, albumin cannot cross the membrane with water and P\text{ab} is zero (Savin et al., 1992).

To assess the effect of tempol on changes in P\text{ab} induced by TNFα, dose-response and time course experiments were performed. Isolated glomeruli were incubated for 10 min at 37°C with TNFα at 0.5 to 4.0 ng/ml, concentrations consistent with tissue levels attained in inflammatory states (Nakamura et al., 2003) and reported to increase P\text{ab} in isolated glomeruli via an O₂-dependent mechanism (McCarthy et al., 1998). The effect of 5 mM tempol on TNFα-induced changes in P\text{ab} was determined in glomeruli incubated for 10 min at 37°C with 4 ng/ml TNFα.

**Statistical Analyses.** Values of urinary protein and isoprostane were adjusted for urinary creatinine and expressed as mean ± S.D. Values between groups were compared using unpaired t test statistics. P\text{ab} values in isolated glomeruli were expressed as mean ± S.D., and values between TNFα- and tempol-treated groups of glomeruli were compared using analysis of variance.

**Results**

**Effect of Tempol on Proteinuria.** The effect of tempol treatment on urine protein excretion following the onset of glomerular immune injury is shown in Fig. 1 and Table 1. Administration of anti-GBM increased urine protein excretion (expressed as the ratio of urine albumin and creatinine concentration U\text{alb}/U\text{creat} in Fig. 1 or as the amount of albumin per total urine volume collected in Table 1) compared with control animals that received nonimmune rabbit serum. Systolic blood pressure (mm Hg) did not change in animals that received anti-GBM serum compared with control [values were: controls = 113.7 ± 31, n = 4; glomerular immune injury (GII)-24 h = 108.0 ± 38, n = 7; GII-day 6 = 110.9 ± 35, n = 4]. In animals treated with tempol, urine protein excretion was significantly reduced at 24 h and on day 6 following...
the onset of immune injury (Fig. 1 and Table 1). Tempol treatment also reduced urine excretion of total isoprostanes [expressed as the ratio of urine total isoprostane to creatinine concentration $U_{\text{tp}}/U_{\text{creat}}$ and assessed at 24 h following the onset of injury (Fig. 1, left panel)].

**TNFα Synthesis and Cell Origin in Glomerular Immune Injury.** In anti-GBM antibody-induced glomerular injury, TNFα mediates proteinuria (Khan et al., 2005) and increases glomerular $O_2^-$ production (Radeke et al., 1990), whereas superoxide directly increases the glomerular capillary permeability to albumin (Dileepan et al., 1993). As shown in Fig. 2, production of TNFα by glomeruli isolated from rats with glomerular immune injury was markedly increased. In glomeruli isolated from rats subjected to X-irradiation prior to induction of immune injury, TNFα production was not different from that in controls, indicating that infiltrating macrophages were a major source of TNFα, which likely acted on glomerular capillaries in a paracrine manner to increase permeability to protein, possibly via $O_2^-$ production.

**Effect of Tempol on Glomerular Permeability to Albumin Induced by TNFα.** Although changes in urine albumin excretion in glomerular injury generally reflect changes in glomerular capillary permeability to protein, the tubules may significantly contribute to the processing (uptake and degradation) of protein filtered by glomeruli (Russo et al., 2002). Because the excreted albumin peptide fragments are not detected by conventional urine protein assays, measurement of protein in the urine as an indicator of changes in glomerular protein filtration can be seriously flawed. Therefore, the reduction in urine protein excretion observed in tempol-treated animals with glomerular immune injury (Fig. 1) does not permit a direct conclusion regarding the mechanism of action. Thus, after having found that in anti-GBM-induced glomerular immune injury TNFα originates almost entirely from infiltrating leukocytes (Fig. 2), we explored the effect of tempol on TNFα-induced changes in glomerular capillary permeability to albumin using an ex vivo system of isolated glomeruli. TNFα was used at concentrations based on production rates by glomeruli isolated from animals with glomerular immune injury (Fig. 2) and also on TNFα concentrations attained in inflammatory states (Suranyi et al., 1993). TNFα induced a concentration-dependent increase in $P_{\text{alb}}$ in the isolated glomeruli (Fig. 3). In glomeruli incubated with 4 ng/ml TNFα, the increase in $P_{\text{alb}}$ was abrogated in the presence of tempol (Table 2).

**Discussion**

Although native SOD had shown some efficacy in treating osteoarthritis and rheumatoid arthritis, it has been withdrawn from the market and replaced with synthetic, low-molecular weight SOD mimetics like tempol, an analog of the spin label 2,2,6,6-tetramethylpiperidine-N-oxyl (TEMPO) used in electron spin resonance spectroscopy. Tempol has been shown to have beneficial actions in animal models of diabetes, endothelial cell dysfunction, hypertension, inflammation, ischemia-reperfusion injury, and shock (Thiemermann, 2003). Although there is some controversy as to whether tempol and other stable nitroxides are SOD mimetics or act instead as stoichiometric scavengers of $O_2^-$ (Kashihara et al., 1992), ample evidence indicates that tempol does attenuate the effect of $O_2^-$ in vivo (Samuni et al., 1991; Reddan et al., 1992; Laight et al., 1998).

There is conflicting evidence regarding the efficacy of native SOD in attenuating proteinuria in animal models of glomerular injury with positive (Adachi et al., 1986; Birtwistle et al., 1989) to null outcomes (Rehan et al., 1984; Webb et al., 1985). Such discrepancies may be due to the use

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**Table 1**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>$n$</th>
<th>$U_{\text{alb}}$ (mg)</th>
<th>$U_c$ (μl/min)</th>
<th>$C_{\text{Cr}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4</td>
<td>36.75 ± 2.80</td>
<td>4.48 ± 0.49</td>
<td>780 ± 82</td>
</tr>
<tr>
<td>GII-24 h</td>
<td>7</td>
<td>141.37 ± 100.10</td>
<td>5.63 ± 0.81</td>
<td>not done</td>
</tr>
<tr>
<td>GII-24 h + tempol</td>
<td>7</td>
<td>58.88 ± 25.54</td>
<td>5.85 ± 1.17</td>
<td>not done</td>
</tr>
<tr>
<td>GII-6 day</td>
<td>4</td>
<td>115.91 ± 100.55</td>
<td>5.72 ± 0.39</td>
<td>237 ± 67</td>
</tr>
<tr>
<td>GII-6 day + tempol</td>
<td>4</td>
<td>35.42 ± 19.26</td>
<td>6.60 ± 2.96</td>
<td>281 ± 92</td>
</tr>
</tbody>
</table>

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**Fig. 2.** TNFα production by glomeruli isolated from control rats, rats with GII, and rats subjected to X-irradiation and GII.

**Fig. 3.** Effect of TNFα on $P_{\text{alb}}$ in isolated glomeruli. Each bar reflects the mean ± S.E.M. obtained from $P_{\text{alb}}$ measurements performed in 15 individual glomeruli isolated from three rats (five glomeruli/rat). Number corresponding to each bar reflects TNFα concentration (0.5, 1, 2, 3, and 4 ng/ml).
of native SOD, which has low membrane permeability and high antigenicity. However, in addition, the evaluative criteria of these studies were based solely on changes in urine protein excretion without an attempt to directly examine the effect of SOD on changes in protein filtration at the glomerular capillary level. This is an important consideration since it is now recognized that filtered proteins undergo rapid degradation during passage through nephron segments distal to the glomerular capillary. Thus, conventional assays used to measure urine protein excretion can substantially underestimate such changes.

We assessed the efficacy of tempol to reduce proteinuria in an animal model of anti-GBM glomerular immune injury and in an ex vivo model of TNFα-induced glomerular permeability to albumin. The rat model was used to address the discrepancy of results reported in earlier studies using native SOD. One could argue that the ideal design would be the use of anti-GBM antibody to cause glomerular immune injury both in vivo and in isolated glomeruli. However, immune injury of the glomerular capillary using anti-GBM antibody cannot be reproduced in preparations of isolated glomeruli since the development of injury requires complement activation, the presence of activated macrophages and T lymphocytes, and the presence of specific cytokines (Cattell, 1994). We therefore simplified the ex vivo model by using the cytokine TNFα, which in the model of anti-GBM antibody-induced glomerular injury was shown to mediate proteinuria (Khan et al., 2005), whereas in isolated glomeruli it increases permeability to protein via a superoxide-dependent mechanism (McCarthy et al., 1998). Further support for this experimental approach comes from studies showing 1) in the model of anti-GBM antibody-induced injury used in the present studies, infiltrating macrophages are a major source of TNFα (Tipping et al., 1991; Yoshioka et al., 1993), and 2) in the same model, the TNFα R2 receptor is required for the development of glomerular capillary injury (proteinuria), and this receptor is induced mainly on glomerular endothelial cells while being nearly undetectable in infiltrating macrophages (Vielhauer et al., 2005). These observations, combined with our data shown in Fig. 2, support the contention that macrophage-derived TNFα can act on the glomerular capillary in a paracrine manner to increase permeability to protein.

At doses sufficient to reduce in vivo oxidative stress, i.e., decrease urine isoprostanate excretion, tempol reduced urine albumin excretion in rat anti-GBM injury (Fig. 1 and Table 1) without a significant effect on the clearance of endogenous creatinine (Table 1). However, since glomerular albumin filtration is associated with tubular reabsorption and degradation, albumin excretion is not an accurate measure of changes in glomerular albumin permeability. Thus, the effect of tempol could be due partly to changes in the extent of reabsorption and degradation of filtered albumin at nephron sites distal to the glomerular capillary. To assess the direct effect of tempol on glomerular albumin permeability, we performed the experiments shown in Fig. 3 and Table 2. TNFα increased Pab in a dose-dependent manner (Fig. 3), and this effect was abrogated by tempol (Table 2).

Superoxide-mediated oxidant injury has been shown to degrade the glomerular basement membrane (Shah et al., 1987), decrease de novo synthesis of proteoglycans (Kishihara et al., 1992), and enhance gelatinase synthesis by glomerular cells (Kakita et al., 1993). Thus, SOD mimetics in renal disease secondary to glomerular inflammatory injury may attenuate the rate of disease progression toward irreversible stages. In promoting O2− dismutation, SOD mimetics could facilitate formation of H2O2, another reactive oxygen species capable of inducing cell injury at concentrations >100 μM. However, in the presence of adequate catalase activity and with low concentrations of free, reduced iron (Fe2+) available for participation in Fenton-type reactions (Koppel, 1998), this potential for toxicity appears small.

The structural elements of the glomerular capillary permeability barrier altered by O2− have yet to be identified. This barrier consists of a fenestrated endothelial layer, a glomerular basement membrane, and an epithelial cell layer. The contribution of each layer to the barrier function differs with the epithelial layer contributing most to this function. The endothelial layer contributes to a lesser but significant degree, whereas the contribution of the GBM is debatable (Deen, 2004). Cells of the epithelial layer (podocytes) express the receptor for the NADPH assembly (Chabrashvili et al., 2002) and could be a major site of O2− generation. Thus, these cells are a likely site of action for SOD mimetics. A major structural component of the epithelial cell barrier to protein movement is the slit diaphragm. Epithelial cell proteins such as nephrin and podocin play a key role in the structural integrity of this diaphragm (Pavenstadt et al., 2003). It is conceivable that the superoxide-mediated increase in Pab observed in response to TNFα might involve changes in synthesis of these proteins. However, the changes in Pab in our experiments occurred within 10 min following incubation of glomeruli with TNFα in the presence or absence of tempol (Fig. 3 and Table 2). This makes it unlikely for changes in synthesis (i.e., reduced synthesis) of slit diaphragm proteins to occur. Recent studies by our group have demonstrated that nitric oxide (NO) plays a key role in preserving the glomerular permeability barrier to protein by antagonizing superoxide (Sharma et al., 2005). This raises the possibility that the salutary effect of tempol on this barrier could, in part, be mediated by scavenging of superoxide, thereby preventing this radical from complexing NO and causing NO depletion.

In summary, our observations establish the efficacy of tempol, a SOD mimetic, in reducing proteinuria in a model of glomerular immune injury and in preserving the glomerular permeability barrier to protein against TNFα, a cytokine that induces O2− production. Long-term studies are now needed to establish the efficacy of SOD mimetics in preserving glomerular capillary permeability to protein in the course of glomerular disease.

### Table 2

The superoxide scavenger tempol abrogates the effect of TNFα on glomerular albumin permeability (Pab).

<table>
<thead>
<tr>
<th>Group</th>
<th>Pab ± S.E.M.</th>
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<tbody>
<tr>
<td>Untreated control</td>
<td>0.002 ± 0.08 (15 glomeruli from three rats)</td>
</tr>
<tr>
<td>TNFα (4 ng/ml)</td>
<td>0.81 ± 0.12 (P &lt; 0.001 vs. control)</td>
</tr>
<tr>
<td>TNFα (4 ng/ml) + tempol (2 mM)</td>
<td>0.21 ± 0.13 (P &lt; 0.001 vs. control; P &lt; 0.005 vs. TNFα)</td>
</tr>
<tr>
<td>TNFα (4 ng/ml) + tempol (5 mM)</td>
<td>−0.027 ± 0.065 (not significant vs. control)</td>
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<tr>
<td>Tempol (3 mM)</td>
<td>0.027 ± 0.1 (not significant vs. control)</td>
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</table>
References


