*Kidney International, Vol. 38 (1990), pp. 282–288*

# Role of intrinsic antioxidant enzymes in renal oxidant injury

TOSHIMASA YOSHIOKA, TERESA BILLS, TRACY MOORE-JARRETT, HARRY L. GREENE, IAN M. BURR, and IEKUNI ICHIKAWA

*Department of Pediatrics, Vanderbilt University School of Medicine, Nashville, Tennessee, USA*

**Role of intrinsic antioxidant enzymes in renal oxidant injury.** To investigate the functional role of renal intrinsic antioxidant enzymes (AOEs), the levels of AOE activities in isolated glomeruli and the changes in renal function to oxidant insults were assessed in normal control rats (NC, $N = 23$) and rats subjected to 30-minutes of complete renal ischemia for three days (day-3, $N = 20$) or six days (day-6, $N = 23$) prior to study. When compared to NC, the activities of total and manganese (cyanide-insensitive) superoxide dismutase, glutathione peroxidase, and catalase were increased more than twofold in day-6 animals, on average, from 36 ± 4 U/mg protein, 9 ± 1 U/mg protein, 129 ± 21 U/mg protein and 1.32 ± 0.20 k/mg protein, respectively, to 80 ± 5, 27 ± 3, 283 ± 41 and 3.20 ± 0.20, respectively ($P < 0.05$ for all). There were no changes in AOE activities in day-3 animals. In day-6 animals, however, the activities of non-AOEs, LDH and fumarase were found to be unaffected. Separate groups of NC ($N = 12$), day-3 ($N = 5$) and day-6 ($N = 12$) rats were subjected to either 30 minutes of ischemia plus 60 minutes of reperfusion (I/R) or unilateral i.a. infusion of hydrogen peroxide ($H_2O_2$, 35 μmoles in 1 hr). The degree of reduction in inulin and para-amino hippurate clearance rates following I/R were significantly less in day-6 (−21 ± 3% and −12 ± 2, respectively) compared to NC (−69 ± 9% and −59 ± 11, respectively) or day-3 rats (−73 ± 7% and −62 ± 10, respectively). Likewise, whereas urine protein excretion rate increased markedly following $H_2O_2$ administration in NC (from 4 ± 1 μg/min to 309 ± 29), proteinuria did not develop in day-6 (from 5 ± 1 μg/min to 5 ± 3). These findings suggest that renal intrinsic AOE activities can be augmented by the insult of I/R, and the enhanced AOE activities provide kidneys with an effective defense system against ROS-mediated injuries. Thus, the prevailing AOE activity levels within the kidney appear to be an important determinant for renal dysfunction induced by ROS.

Studies in the past demonstrated the pathophysiological importance of the metabolites of partially reduced oxygen molecules, or reactive oxygen species (ROS) in various experimental renal diseases [reviewed in 1], including several animal models of primary glomerulopathy and acute renal failure, both ischemic and nephrotoxic [2–7]. In addition, our recent studies [2, 8] and those by others [3, 4, 7] have shown that ROS per se can compromise renal function, depress glomerular filtration and impair glomerular sieving function. Of note is the widely accepted notion that the expression of injury is determined not only by the nature of offending pathogens, but also by the quality of the host's defense system. Since cells are equipped with various ROS scavenging systems, including antioxidant enzymes (AOEs) [9, 10], it is plausible to speculate that it is when the amount of environmental ROS exceeds the capacity of cell or organ antioxidant systems that normal cell/organ function is disrupted and tissue damage develops [10, 11]. Indeed, it has been shown that experimental manipulation of tissue antioxidant systems achieved by selenium intake restriction (to reduce seleno-dependent glutathione peroxidase), supplementation of AOEs or non-enzymatic antioxidants, resulted in changes in the susceptibility of tissues or cells to injury caused by ROS [2, 4–7, 11]. In addition to these manipulations, several experimental maneuvers have been shown to trigger alterations in tissue/cell AOE levels. In the lung, both in vivo and in vitro experiments demonstrated that intrinsic AOE activities are enhanced following exposure to high oxygen tension or endotoxin and treatment with glococorticoid, as well [12–14].

Given the experimental observations that some forms of acute renal insult which involve ROS generation in kidneys induce a resistance against a recurrent insult of a similar nature [5, 6, 15], we were intrigued by the possibility that this acquisition by the kidney of immunity against ROS may involved enhancement of local levels of AOEs.

In the present study, therefore, we aimed at addressing the following previously-untested issues: 1) Whether the antioxidant enzymes within the kidneys can be modulated and, if so, 2) whether the modulated levels of the AOE activities make a crucial impact on the glomerular dysfunction when ROS insult is imposed. In designing our study, we recognized the experimental obstacle that, currently, there is no known agent or maneuver which specifically manipulates AOEs within the glomerulus or even in any renal cells. Therefore, we simulated experimental protocols employed in other organ experiments, and we found, through a series of pilot studies, that a 30-minute complete renal ischemia results in a subsequent elevation in glomerular AOEs in six days. Using this protocol, in the present study, we focused on testing if the kidney becomes resistant to ROS injury when, and only when, the enhancement of AOEs are achieved, and if such a resistance is related to elevated enzyme activities, specifically, of AOEs, and not to the general well-being status of the tissue.

## Methods

Figure 1 illustrates the experimental protocols employed in the study.

Received for publication August 11, 1989
and in revised form February 20, 1990
Accepted for publication February 22, 1990

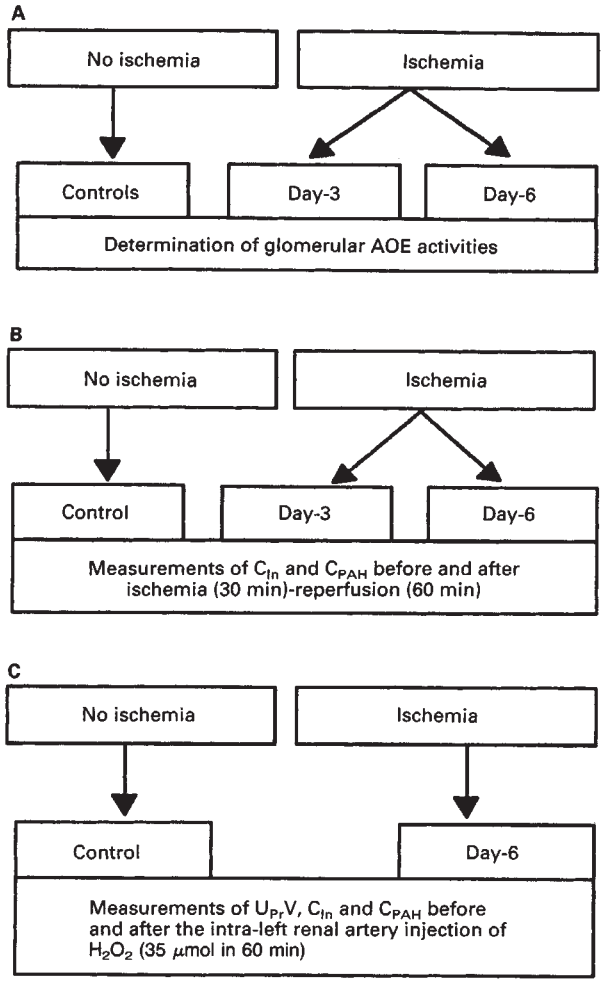


**Fig. 1. A.** *The protocol for the measurements of glomerular antioxidant enzyme (AOE) activities in control and post-30 minute-ischemic kidneys.* Day-3 and Day-6 denote 3 and 6 days after the ischemia, respectively. **B.** The protocols for assessment of susceptibility to ischemia/reperfusion in control and post-ischemic kidneys. $C_{In}$, whole kidney inulin clearance rate; $C_{PAH}$, whole kidney para-amino hippurate clearance rate. **C.** The protocol for assessment of susceptibility to hydrogen peroxide ($H_2O_2$)-induced glomerular dysfunction in control and post-ischemic kidneys. $U_{pr}V$: urine protein excretion rate.

### *Induction of ischemia/reperfusion injury*

Ischemia/reperfusion injury was induced in the left kidney of 60 adult male Munich-Wistar rats (214 to 306 g). Rats were anesthesized by intraperitoneal injection of pentobarbital (50 mg/kg). The abdominal region was shaved and prepped with a 10% providone-iodine solution (Betadine solution, Purdue Frederick Co., Norwalk, Connecticut, USA). The surgical instruments were sterilized by autoclave. A laparotomy was performed using a vertical midline incision; the left renal artery was exposed by a blunt dissection; a hemostatic clamp was applied on the left renal artery for 30 minutes to create a complete renal ischemia. The clamp was then removed, and the abdominal incision closed in two layers using silk sutures. After recovery from anesthesia, animals were returned to cages, and food and water were given ad libitum for either three or six days.

Three or six days after the ischemia, rats were randomly divided into two groups. One group ($N$ = 20, and 23 in day-3 or day-6 post-ischemia, respectively) underwent determination of glomerular antioxidant enzyme activities (Fig. 1A), and the other ($N$ = 5 and 12 in 3- or 6-day post-ischemia, respectively) to functional assessment (Fig. 1B and C).

As a control, 35 rats (203 to 292 g) underwent a surgical procedure which was identical to that performed in the above experimental rats, except that the induction of renal ischemia was omitted. Like experimental rats, these control rats were subjected to glomerular AOE determination ($N$ = 23: Fig. 1A) or functional assessment ($N$ = 12: Fig. 1B and C).

### *Measurement of glomerular AOE activities*

The measurements of glomerular superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase activities (Fig. 1A) were performed as follows. Rats were anesthetized with intraperitoneal pentobarbital (50 mg/kg), and kidneys were harvested following renal perfusion with iced 0.9% NaCl solution. Under cold environment (4°C), glomeruli were isolated using the method previously employed by us [16]. Briefly, minced renal cortex was passed through three consecutive sieves, then glomeruli were collected. Glomerular suspension was washed with 10 mM potassium phosphate, 0.1 mM EDTA, pH 7.8, and centrifuged three minutes × 2000 rpm twice. Preparations consisting of more than 95% with intact isolated glomeruli by light microscopic examination were used. Since glomeruli from one kidney were not sufficient in quantity to perform the assays specified below, glomeruli from two or three kidneys which underwent identical treatment in situ were combined. The glomeruli were then sonicated, centrifuged (1,000 g for 20 min) and supernatants were stored at −70°C until assays of each enzyme activity were performed.

SOD activities were assayed based on the cytochrome c autoxidation method by McCord and Fridovich [17, 18]. In a 1.6 ml cuvette (with a 10 mm light path), 1.0 ml of air-saturated 50 mM potassium phosphate buffer, 0.1 mM EDTA, pH 7.8 was equilibrated at 25°C with the sample (20 μl). The reaction mixture containing 10 μM ferricytochrome c (Sigma, St. Louis, Missouri, USA), 50 μM xanthine (Sigma), 6 μM xanthine oxidase (Sigma), and either 10 μM or 1 mM potassium cyanide was measured for the reduction of cytochrome c at 550 nm using a Hitachi U-600 spectrophotometer (Tokyo, Japan). Low concentrations of potassium cyanide (10 μM) was added to avoid possible interference by cytochrome oxidase [18]. This concentration has been shown not to inhibit Cu/Zn- or Mn-SOD activity considerably, therefore, the activity obtained was considered as a total SOD activity. Mn- (or cyanide insensitive) SOD activity was measured with 10 mM potassium cyanide [18]. The amount of SOD required to inhibit the rate of reduction of cytochrome c by 50% was defined as 1 unit of activity.

GSH-Px activity was measured by the method of Beutler et al, which uses an oxidation reaction of reduced glutathione by glutathione peroxidase coupled to the disappearance of NADPH by glutathione reductase using tert-butyl hydroperoxide as the substrate [19]. The assay mixture contains 730 μl of 0.1 M Tris-HCl, 0.5 mM EDTA buffer, pH 7.6, 100 μl sonicated sample suspension, 20 μl of 2 mM glutathione, 50 μl of 0.2 mM NADPH (Sigma) and 1 U of yeast glutathione reductase (Sigma). After preincubation of the mixture for 15 minutes at room

temperature, the reaction was started by adding 0.35 mM tert-butyl hydroperoxide (Sigma) and was followed at 340 nm for five minutes. The activity was calculated using extinction coefficient of NADPH at 340 nm, 6.22 $mM^{-1}$ $cm^{-1}$.

Catalase activity was measured using the method of Aebi [20]. Thus, in a 1.6 ml quartz cuvette, 25 μl of sample was added to 725 μl of a mixture containing 7.7 mM $H_2O_2$ in 10 mM phosphate buffer, pH 7.0. Change in absorbance at 240 nm was measured. The rate constant of a first order reaction (k) was used as a unit by the equation:

$$k = (1/\Delta t) \times \ln(A1/A2)$$

where Δt is the measured interval (sec); A1 and A2 are the absorbances at initial and final measurement points, respectively.

In some glomerular samples, activities of non-antioxidant enzyme, namely, lactate dehydrogenase (LDH), representing cytosolic enzymes, and fumarase, representing mitochondrial enzymes, were determined to evaluate whether the changes in enzyme activities are specific to AOEs. LDH activity was measured by the method described by Wahlefeld, which measures the amount of L-lactate oxidized at 30°C per unit time by observing a continuous increase in absorbance at 334 nm [21]. Fumarase activity was measured by the method described by Hatch, which measures the formation of malate detected by coupling its production with NADPH reduction by malic enzyme [22].

All enzyme activity values were corrected by the tissue protein content measured by the method of Lowry et al [23].

### *In vivo assessment of susceptibility to ROS-mediated renal injuries*

To evaluate the susceptibility to ROS-mediated dysfunction in post-ischemic kidneys, two forms of insult were imposed. The first was ischemia/reperfusion (Fig. 1B). Based on the previous findings that renal ischemia/reperfusion injury is, at least in part, mediated by ROS [4, 6, 7], control (no previous ischemic episode, $N = 6$), 3-day post-ischemic, ($N = 4$) and 6-day post-ischemic ($N = 6$) rats underwent ischemia/reperfusion, and the degree of glomerular dysfunction induced by this insult was compared. In this experiment, rats were anesthetized with an intraperitoneal injection of Inactin (Byk, Constanze, FRG; 100 mg/kg) and underwent preparatory surgery for whole kidney clearance measurements as previously described [2, 24]. Thus, after tracheostomy, indwelling polyethylene catheters (PE-50, Clay-Adams, Parsippany, New Jersey, USA) were placed into the jugular vein for infusion of plasma, inulin and p-aminohippurate (PAH). The left femoral artery was catheterized to monitor mean systemic arterial pressure (MAP), using an electronic transducer (model P23 ID, Gould, Cleveland, Ohio, USA) which was connected to a recorder (model 2200S, Gould). The left and right ureters were cannulated with PE-10 for the subsequent collection of urine. Fluid loss due to surgical preparation was replaced with rat plasma, infused in a volume equal to 1% of body weight over 30 minutes, followed by a maintenance infusion at the rate of 1.2 ml/hr [24]. After priming dose (0.4 ml) of 0.9% NaCl containing 9% inulin and 1% PAH, a maintenance infusion of this mixed solution was continued throughout each experiment at a rate of 1.2 ml/hr.

Following an equilibration period (30 min), rats underwent baseline measurements of glomerular function. Urine was collected from both ureters for determination of urine flow rate, inulin and PAH concentrations. Arterial blood was collected into capillary tubes (100 μl) for determinations of plasma inulin and PAH concentrations at the mid-point of urine collection. Two consecutive collections with each ≈10 minutes duration were performed. At the completion of these initial measurements and collections, the left renal artery was occluded for 30 minutes.[1] Sixty min after the release of occlusion, the above measurements were repeated. The right kidney, which did not undergo ischemia/reperfusion, served as a control.

To examine the susceptibility to another form of ROS-mediated injury, direct injection of hydrogen peroxide into the left renal artery was performed (Fig. 1C). This protocol has been shown to induce transient massive proteinuria with impairment of glomerular molecular size-selectivity [8]. For this experiment, normal rats (without previous ischemic episode, $N = 6$) and 6-day post-(left-renal)-ischemic rats ($N = 6$) underwent preparatory surgery identical to the above. Following equilibration period and baseline clearance measurements in the left and right kidneys including determination of protein excretion rate, 70 mM hydrogen peroxide in 50 mM phosphate buffer, pH 7.0, was injected for one hour at the rate of 0.5 ml/hr, using the micropipette infusion system previously described [27]. This system consists of a micropipette with an internal diameter ≈50 μm connected to a microinfusion pump, and is manipulated by De Fonbrune micromanipulator (Micro Instrument Co., St. Louis, Missouri, USA). Immediately after the completion of the infusion, the measurements were repeated. The right kidney, which did not receive hydrogen peroxide, served as the control. In our pilot studies, vehicle injection alone was found not to affect any of the parameters measured.

Inulin concentrations in plasma and urine were determined by the macroanthrone method [28]. PAH concentrations in plasma and urine were assayed by the method of Bratton and Marshall, modified by Smith et al [29]. Glomerular filtration rate (GFR) and renal plasma flow rate (RPF) were estimated by inulin and PAH clearance rate, respectively. Urine protein concentration was assessed by the Coomassie brilliant blue method [30], and excretion rate ($U_{pr}V$) was expressed as microgram per minute.

### *Statistics*

All values are expressed as the means ± SE. For comparisons among groups, one-way analysis of variance (ANOVA) followed by modified Tukey's test was performed [31, 32]. For comparison between paired data, a paired *t*-test was performed [31]. $P$ value less than 0.05 was considered to be statistically significant.

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On the basis of comparison between 20 to 60 minutes of complete ischemia in the rat isolated perfused kidneys, others have suggested that the major involvement of the ROS in the pathophysiology of renal injury is less clear in a longer ischemic period (≥45 min) [25, 26]. For this reason, we have chosen the protocol of short term ischemia (that is, 30 min) in the present study.

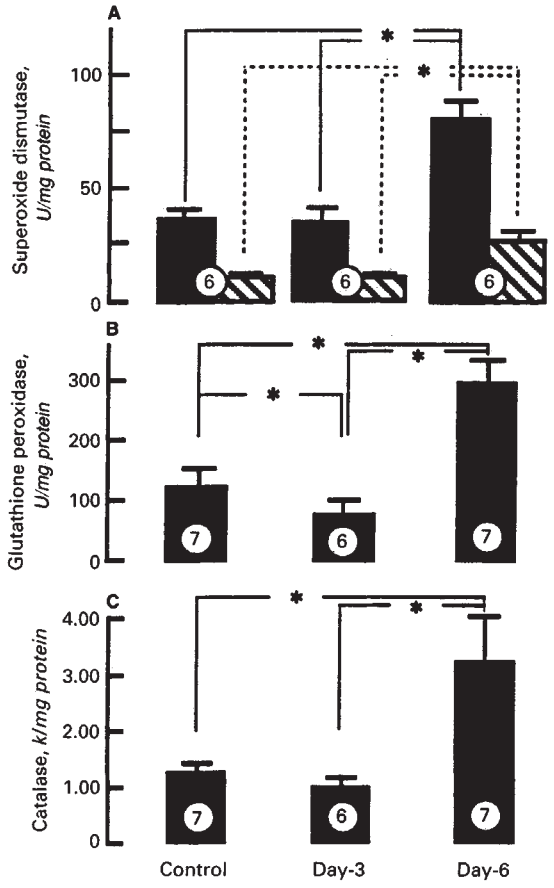


**Fig. 2. A.** *Glomerular total (closed bars) and manganese (cyanide insensitive, hatched bars) superoxide dismutase activities in control and post-ischemic kidneys.* Day-3 and Day-6 denote the activity measured 3 days or 6 days after the 30 minute ischemia. The numbers in circle denote the number of determinations. **B.** Glomerular glutathione peroxidase activities in control and post-ischemic kidneys. **C.** Glomerular catalase activities in control and post-ischemic kidneys. Values are expressed as mean ± SE. * denotes $P < 0.05$.

## Results

### *Effect of ischemia/reperfusion on glomerular AOE activities*

Glomerular AOE activities in control (ischemia/reperfusion episode not imposed), 3-day post-ischemic and 6-day post-ischemic kidneys are shown in Figure 2.

The top panel of Figure 2 depicts SOD activities measured in isolated glomeruli. In control kidneys, the glomerular total and Mn- (or cyanide insensitive-) SOD activities averaged 37 ± 4 and 9 ± 1 U/mg protein, respectively. The respective values in 3-day post-ischemic glomeruli (30 ± 3 and 9 ± 1 U/mg protein) were not significantly different from that of control glomeruli. By contrast, in 6-day post-ischemic kidneys, glomerular total and Mn- SOD activities were significantly elevated, on average, to 80 ± 5 and 27 ± 3 U/mg protein, respectively. The values of total and Mn-SOD activity were increased by some 120 and 190% of the corresponding control values, indicating that the elevation in total SOD activity was mainly attributed to Mn-SOD activity. The levels of glomerular GSH-Px activity are shown in the middle panel of Figure 2. In control glomeruli, the activity averaged 129 ± 21 U/mg protein, and in 3-day post-ischemic glomeruli, 77 ± 11 U/mg protein, a value slightly but significantly below the control. Like that of SOD, GSH-Px activity was significantly elevated in 6-day post-ischemic glomeruli, averaging 283 ± 41 U/mg protein. As shown in the bottom panel of Figure 2, a similar trend was observed in glomerular catalase activity. The control catalase activity averaged 1.32 ± 0.20 k/mg protein, and values in 3-day post-ischemic glomeruli (1.11 ± 0.13 k/mg protein) were similar to the control values. In 6-day post-ischemic glomeruli, catalase activity was elevated more than twofold, on average, to 3.20 ± 0.63 k/mg protein. The glomerular SOD, GSH-Px and catalase activities in contralateral kidneys of experimental rats, that is, kidneys which did not undergo ischemia/reperfusion, were not different from those of control kidneys. Thus, the glomerular total and Mn-SOD activities from contralateral kidneys of 3-day post-ischemia averaged 33.8 ± 5 and 10.6 ± 2.2 U/mg protein (number of determinations = 6), respectively. Those values in the 6-day post-ischemia averaged 43.7 ± 5.6 and 10.2 ± 1.1 U/mg protein ($N = 6$), respectively. Glomerular GSH-Px activities of contralateral kidneys were 125 ± 10 U/mg protein ($N = 6$) and 143 ± 20 ($N = 7$) in 3- and 6-day post-ischemia, respectively; the catalase activities were 1.05 ± 0.12 k/mg protein ($N = 6$) and 1.60 ± 0.22 ($N = 7$) in 3- and 6-day post-ischemia, respectively.

To evaluate whether the observed increases in AOE activities are specific to antioxidant enzymes, the activity of non-antioxidant enzymes, specifically, LDH and fumarase, were measured in control and 6-day post-ischemic glomeruli. The activity of LDH in control glomeruli ($N = 6$) averaged 399 ± 103 mU/mg protein, and that in 6-day post-ischemic glomeruli ($N = 6$) was 291 ± 51 mU/mg protein ($P > 0.10$). Activity of fumarase in control glomeruli averaged 108 ± 17 mU/mg protein, and that in 6-day post-ischemic glomeruli 69 ± 3 mU/mg protein ($N = 7$), a value slightly but significantly below the control. Therefore, increase in enzyme activities following exposure of the glomeruli to ischemia/reperfusion is somewhat specific to antioxidant enzymes.

### *Functional significance of elevated glomerular AOE activities*

To investigate whether the observed changes in glomerular AOE activities are functionally important, kidneys with elevated glomerular AOE activities were challenged with two forms of ROS insult.

In the first set of experiments, control rats (with no previous ischemia/reperfusion episode), 3-day and 6-day post-ischemic rats underwent 30-minute left renal ischemia followed by 60-minute reperfusion. Values for mean systemic arterial pressure, MAP, were not different among study groups, and did not change throughout the experiment. (Values for average MAP during baseline and post-ischemia/reperfusion periods of control, 3-day and 6-day post-ischemic rats were 107 ± 4 mm Hg and 107 ± 3; 104 ± 5 and 102 ± 7; 104 ± 2 and 108 ± 1, respectively). In control rats, both whole kidney inulin and PAH clearances of ipsilateral (left) kidneys were depressed substantially following ischemia/reperfusion, while these values were unaffected in contralateral (right) kidneys (Fig. 3). In 3-day post-ischemic kidneys, baseline values of inulin and PAH clearances were significantly below those in the control kidneys, thus representing incomplete recovery from the initial ischemia/reperfusion episode (Fig. 3). When the second isch-

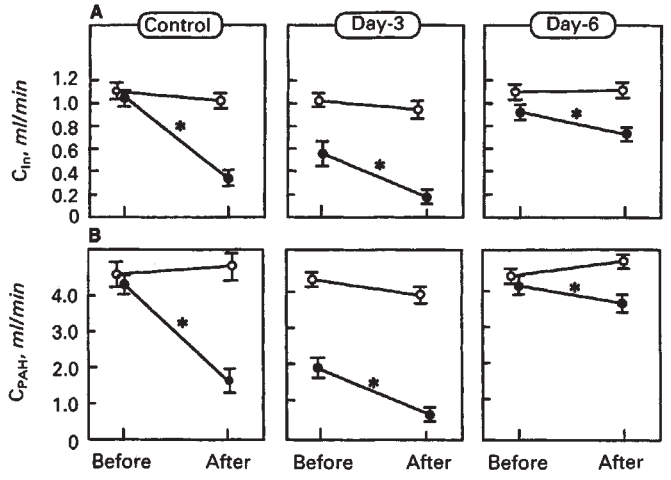


**Fig. 3.** *Whole kidney inulin clearance rate* (**A**) *and PAH clearance rate* (**B**) *during baseline (before) and following left renal 30 minute ischemia + 60 minute reperfusion (after) in control (no previous ischemic episode,* N = *6), 3-day* (N = *5), and 6-day* (N = *6) left renal post-ischemic rats.* Values from the left and right kidneys are given as closed and open circles, respectively. Values are expressed as mean ± SE. * denotes $P < 0.05$.

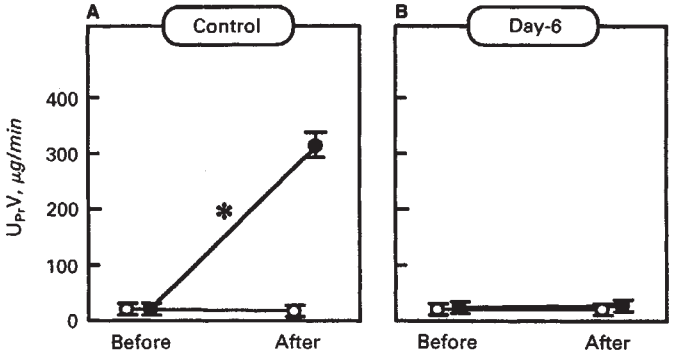


**Fig. 4.** *Urine protein excretion rate during baseline (before) and following left renal arterial hydrogen peroxide (35 mM in 60 min) infusion (after) in control* (**A**, *no previous ischemia,* N = *6) and 6-day* (**B**, N = *6) left renal post-ischemic rats.* Values from the left and right kidneys are given as closed and open circles, respectively. Values are expressed as mean ± SE. * denotes $P < 0.0005$.

emia/reperfusion was induced in the left kidneys, in which glomerular AOE activities were not elevated, a profound reduction in inulin and PAH clearances was observed (Fig. 3). Again, no significant changes were noted in the parameters of contralateral kidneys of the same rats. A markedly different pattern was observed in 6-day post-ischemic rats in which significant elevations of glomerular AOE activities were observed in the ipsilateral kidneys. In their left kidneys, baseline inulin and PAH clearance values were similar to those in control rats, representing a functional recovery from the initial insult (Fig. 3). When the left kidney of these rats was exposed to a second episode of ischemia/reperfusion, the degree of reduction in inulin and PAH clearances was much less than that in control or 3-day post-ischemic kidneys. Thus, as shown in Figure 3, the decrease in inulin and PAH clearances in 6-day post-ischemic kidneys were significantly and substantially less than those achieved by either the other two groups. The degrees of reduction in inulin and PAH clearances, expressed by percent reduction from the baseline values of each group, were −69 ± 3% and −59 ± 11; −73 ± and −62 ± 10; −21 ± 3 and −12 ± 2, in control, 3-day and 6-day post-ischemic rats, respectively (both values of 6-day post-ischemia are significantly less than those in control or 3-day post-ischemic rats). Therefore, these 6-day post-ischemic kidneys, which had elevated AOE activities, were found to be clearly more resistant to dysfunction caused by ischemia/reperfusion.

In some studies, kidneys were harvested at the end of experiments, immersion fixed in 10% buffered formalin, and 3 μ sections were stained with periodic acid Schiff. The light microscopic examination ($N = 2$ for each group) did not reveal appreciable changes in glomerular structures. On the other hand, mild tubular changes including dilatation and desquamation were found in most of 60 tubular profiles per kidney examined in control rats which underwent 30-minute ischemia and 60-minute reperfusion. Day-3 and 6-day post-ischemic kidneys showed moderate tubular regenerative change, dilatation, and loss of brush borders. The effects of the first versus the second ischemia/reperfusion could not be differentiated.

In the second set of experiments, a direct perfusion of kidneys with ROS was performed. Based on our preliminary studies [8] which demonstrated a profound, transient proteinuria following intrarenal injection of hydrogen peroxide (35 μmol in 1 hr), an identical protocol was used to evaluate whether kidneys with elevated AOE activities are protected from ROS-induced injuries. As shown in Figure 4, in control rats, intrarenal injection of hydrogen peroxide resulted in some 80-fold increase in urine protein excretion rate (on average from 4.1 ± 1.0 μg/min to 308.7 ± 29.3 μg/min), without any appreciable effects on the contralateral (right) kidneys (from 5.9 ± 1.2 μg/min to 4.9 ± 0.8 μg/min). Although not shown, GFR and RPF measured in the same rats were unaffected. In the 6-day post ischemic kidneys, the massive proteinuria observed with the hydrogen peroxide infusion in control rats was completely absent (left: from 5.9 ± 1.2 μg/min to 4.9 ± 0.8; right: from 5.1 ± 0.4 μg/min to 5.4 ± 1.2). Thus, only the kidneys with elevated glomerular AOEs were also protected from hydrogen peroxide-induced glomerular permselectivity defect [8].

## Discussion

Among various antioxidant systems equipped within aerobic cells, three antioxidant enzymes, superoxide dismutase, SOD, glutathione peroxidase, GSH-Px, and catalase are major mechanisms to reduce local levels of ROS [9, 10]. Thus, these enzymes distributed in cytosol and/or mitochondria can abase primary ROS, such as superoxide anion (by SOD), and hydrogen peroxide (by GSH-Px and catalase) before they can interact to form more reactive cytotoxic metabolites (hydroxyl radical, hypochlorous acid) [9, 10]. Our measurements of the activities of these enzymes showed that the particular protocol of 30-minute complete ischemia/reperfusion leads to increases in AOE activities in six days. Since the activities of other cytosolic and mitochondrial marker enzymes [13, 21, 22], specifically LDH and fumarase, respectively, were unaffected, the effect of ischemia/reperfusion on enzymes appears to be somewhat specific to these antioxidant enzymes.

While these observations, for the first time, establish an

experimental protocol which induces enhancement of intrinsic AOE activities in renal tissues, the results from our functional studies are indicative that, like those in other organ systems [12, 33], enhanced AOE activities make a crucial impact on the expression of renal dysfunction during ROS stress. In the first set of our functional tests, we subjected the kidneys with elevated AOE activities to ischemia/reperfusion. In this regard, by assessing the glutathione redox ratio or by observing the protective effect of antioxidants given exogenously, others have demonstrated that our protocol of ischemia/reperfusion, indeed, involves ROS stress [4, 6, 7]. In the current first set of in vivo experiments it was shown that when AOE activities are significantly elevated, the hypoperfusion and hypofiltration characteristics of kidneys exposed to post-ischemia/reperfusion are largely prevented.

Our recent study demonstrated that the ROS released from infiltrating polymorphonuclear leukocytes can similarly cause an increase in arteriolar resistances and reduction in ultrafiltration coefficient, which, likewise, can be prevented by exogenous catalase administration [2]. Moreover, recent biochemical and morphometric studies by us [34] and others [35] have shown that ROS are potent inducers of constriction in mesangial cells in culture. Although the reduced inulin and PAH clearances following ischemia/reperfusion may not be attributed solely to the alteration in glomerular hemodynamics, previous controlled studies demonstrated reduced single nephron GFR [4], reduced ultrafiltration coefficient [36], and glomerular ultrastructure changes [37]. Furthermore, other models of renal injuries which involve ROS production in their pathogenesis have been characterized by renal hypofiltration and/or proteinuria [1]. The latter has been also tested in the current study.

The notion that the protection of the 6-day post-ischemic kidney from the subsequent ROS-mediated injury is owed largely to the elevated AOE activities, is supported not only by the above-discussed absence of changes in non-AOE activities in the same glomeruli, but also by two other separate sets of observations. First, in 3-day post-ischemic glomeruli, no elevation in AOE activities was detected, nor was there a development of resistance against glomerular hypoperfusion and hypofiltration following ischemia/reperfusion. The second supporting evidence comes from the experiment using hydrogen peroxide. The origin of ROS is obviously different between ischemia/reperfusion versus hydrogen peroxide, that is, intrinsic versus extra-renal, respectively. Therefore, the site (such as, types of cells) and the nature of injury are different, although the precise reason for the well-recognized difference in the expression of renal dysfunction between the two models, namely, depressed glomerular perfusion and filtration versus impaired glomerular sieving function, is currently unknown [1]. Nevertheless, our experimental findings that 6-day post-ischemic kidneys demonstrated virtually perfect resistance against hydrogen peroxide-induced proteinuria further support the notion that, overall, the kidney with elevated AOE activities has developed a highly efficient immunity against ROS-induced renal dysfunction.

The mechanism underlying this increase in AOE activities remains uncertain. In the enteric bacteria, induction of AOEs by stimulating genes specific to these enzymes has been shown to occur when oxygen-resistant mutants are exposed to oxidative stress, some heat-shock proteins [39], $NO_3^-$, or paraquat in an anaerobic condition [38]. As mentioned earlier, the induction of AOEs takes place also in mammalian cells [12–14, 39]. Studies in rats demonstrated that, depending on host's age and the nature of stimulus, the rate at which regulation of enzyme induction takes place differs at both transcriptional and post-transcriptional (translational) levels [39]. The enzyme induction commonly takes place within hours to a few days after specific stimuli [12, 13, 39], seemingly contrasting to our results that the post-ischemic increase in enzyme activities did not occur for at least three days after the insult. It is conceivable, then, that not only ischemia but also complete reperfusion (that is, normalization of blood flow, which was achieved between three and six days after the ischemic episode) are required for triggering AOE induction.[2] Another possibility is that although the enzyme induction may already have occurred at the transcriptional level, the balance between production and degradation of AOEs is negative in these hypoperfused kidneys due to a large ongoing demand of AOE by the reactive oxygen species.

In summary, the current studies underscore the findings that glomerular antioxidant enzymes levels are modulated. The results also demonstrate that the elevated glomerular antioxidant enzymes protect renal functions against reactive oxygen species-induced injuries. Thus, the glomerular antioxidant enzymes are suggested to play an important role in the functional derangements induced by the reactive oxygen species. The modulation of local antioxidant levels may constitute one of the mechanisms of acquired resistance in acute renal injuries.

## Acknowledgments

Portions of these studies were presented at the Annual Meeting of the American Society of Nephrology, San Antonio, TX, December 12, 1988, and were published in abstract form (*Kidney Int* 35:410, 1989). These studies were supported by the National Institutes of Diabetes and Digestive and Kidney Diseases Grants DK40527 and 26657.

*Reprint requests to Toshimasa Yoshioka, M.D., Division of Pediatric Nephrology, Vanderbilt University Medical Center, C-4204 M.C.N., 1161 Twenty-first Avenue South, Nashville, Tennessee 37232-2584, USA.*

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[2] In keeping with these possibilities, in two models of ROS-mediated renal injuries, namely, puromycin aminonucleoside (100 mg/kg, i.p.) and gentamicin (50 mg/kg, i.p., × 6 days) were found in our pilot studies to lead to depression of glomerular SOD, GSH-Px and catalase measured seven days and one day after the completion of administration, respectively, in association with profound renal hypoperfusion [40].

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