Hypoperfusion-Induced Acute Renal Failure in the Rat: An Evaluation of Oxidant Tissue Injury

Richard A. Zager

Reactive oxygen species (ROS) have been reported to be critical cellular mediators of experimental ischemic acute renal failure (ARF). This conclusion is based on observations that in the renal artery occlusion (RAO) model of ARF, antioxidant drugs confer protection and that renal malondialdehyde (MDA) concentrations, an index of lipid peroxidation, rise in the postischemic period. Human ischemic ARF is most often due to hypoperfusion, not to total blood flow interruption. Therefore, the goal of this study was to determine whether ROS also mediate hypoperfusion-induced renal injury. Renal hypoperfusion was induced in rats by suprarenal partial aortic ligation, lowering renal perfusion pressure to 20–25 mm Hg for 45 minutes. Renal MDA concentrations were measured 15 minutes after ligation release. Renal function and morphology were assessed 24 hours after hypoperfusion in control rats and in rats pretreated with antioxidant agents (allopurinol, superoxide dismutase, dimethylthiourea, glutathione, and catalase), a majority of which have been shown to lessen RAO-induced ARF. Hypoperfusion caused no rise in renal MDA concentrations ($p = 0.54$). Control ARF rats developed significant azotemia (blood urea nitrogen 119 ± 6 mg/dl; creatinine 3.3 ± 0.37 mg/dl) and widespread tubular necrosis by 24 hours after surgery. None of the antioxidants, administered singly or in combination, lessened the ischemic damage. Therefore, renal MDA concentrations do not rise in hypoperfusion ARF, and antioxidants do not confer protection. This indicates that previous evidence for ROS as mediators of ischemic renal injury is restricted to the RAO model of ARF, which does not closely simulate most human ischemic renal injury. (*Circulation Research* 1988;62:430–435)

In recent years, a number of publications have appeared that suggest that reaction oxygen species (ROS) such as superoxide, hydrogen peroxide, and hydroxyl radicals are important cellular mediators of ischemic acute renal failure (ARF). The most compelling argument for this conclusion are studies by Paller et al, which demonstrate that the intravenous infusion of antioxidant agents including allopurinol (allo), superoxide dismutase (SOD), dimethylthiourea (DMTU), and reduced glutathione (GSH) can each ameliorate the severity of ARF in rats induced by 60 minutes of renal ischemia.[1,2] These workers further supported a role for ROS in ischemic renal injury by demonstrating that allo, SOD, and DMTU each decrease the generation of malondialdehyde,[1] a product of ROS-triggered lipid peroxidation.[1-4] More indirect evidence of oxidant tissue injury following renal ischemia has come from other laboratories that have documented renal GSH loss in the aftermath of an ischemic event.[5-7] Since GSH is a substrate for glutathione peroxidase, an important inactivator of H_2O_2 and lipid hydroperoxides,[8] these findings are also suggestive of renal oxidant stress.

Each of the above studies was performed using the renal artery occlusion (RAO) model of ischemic ARF, which is characterized by a total cessation of renal blood flow. Ischemic ARF in humans most often results from hypoperfusion, not from total blood flow interruption (notable exceptions being that due to aortic cross clamp and kidney transplant surgery). Thus, conclusions about a pathogenetic role for ROS in ischemic ARF based on the RAO model might not have widespread clinical relevance if this model fundamentally differs from hypoperfusion-induced ischemic renal injury. Recent experiments completed in this laboratory suggest that this is the case.[9] We developed a hypoperfusion ARF model in rats in which severe azotemia and proximal tubular necrosis are induced by temporary (30–45-minute) partial aortic ligation (PAL) sufficient to reduce renal perfusion pressure to 20 mm Hg and renal blood flow to 0.1–0.2 ml/min. In comparison to RAO, PAL induces far less adenosine triphosphate (ATP) depletion, minimal medullary vascular congestion, less cast formation, and less renal functional impairment.[9] These differences underscore the possibility that RAO may not closely simulate human hypoperfusion renal injury.

There are a number of reasons to suspect that in addition to the above differences, differences in degrees of oxidant tissue damage might also exist between hypoperfusion and total blood flow cessation ARF models, such as RAO. For example, continuous oxygen delivery during a hypoperfusion period could theoretically exacerbate ROS formation by supplying a continuous, albeit limited, supply of oxygen to the injured organ. Blood flow interruption with RAO precludes this possibility. Alternatively, at least two factors suggest that renal hypoperfusion might induce less ROS formation than RAO. First, hypoperfusion causes much less ATP breakdown than does RAO.[9] Since ATP degrades to hypoxanthine,[10,11] a critical substrate for ROS formation,[12] greater oxidant damage might result from RAO than from renal hypoperfusion.

From the Department of Medicine, University of Washington, Seattle, Washington.

Supported by grants from the National Institutes of Health (AM 36811) and from the Northwest Kidney Foundation.

Address for reprints: Richard A. Zager, MD, Harborview Medical Center, ZA-67, 325 Ninth Avenue, Seattle, WA 98104.

Received March 26, 1987; accepted September 18, 1987.

Downloaded from http://ahajournals.org by on March 14, 2022

In support of this notion are recent demonstrations in this laboratory that show that renal hypoxanthine concentrations are 75% lower under conditions of hypoperfusion compared with RAO (Zager and Altschuld[5] and unpublished data). A second reason to suspect less oxidant injury with PAL than with RAO is that RAO traps blood within the kidney, a phenomenon that persists even with vascular reflow as evidenced by severe medullary vascular congestion in the reperfusion period.[9] This occurs to a far lesser degree with renal hypoperfusion.[9] Since polymorphonuclear leukocytes (PMNs) are also sequestered, they could participate in ischemic tissue damage,[13,14] possibly by exacerbating oxidant injury. Support for this concept comes from a recent study by Linas et al, which indicates that anoxic injury in an isolated perfused kidney is worsened by including PMNs in the kidney perfusate.[15]

In light of these questions and because of the current intense interest in the role of ROS in ischemic ARF, the present study was undertaken to assess whether ROS are important mediators of hypoperfusion-induced renal injury. To achieve this goal, we tested whether the available evidence for oxidant injury in the RAO model of ARF (protection with antioxidants and increments in MDA) also applies to a non–blood flow cessation model of acute ischemic renal injury.

Materials and Methods

Partial Aortic Ligation Model

Female Sprague-Dawley rats (Batum and Kingsman, Fremont, California) were anesthetized with pentobarbital (40 mg/kg) and placed on a heated surgical table to maintain body temperature at 37° C. A 200-μl tail vein plasma sample was obtained for blood urea nitrogen (BUN) and creatinine (Cr) determinations, and PE 50 jugular vein and femoral artery catheters were inserted for therapeutic agent infusion and direct mean arterial blood pressure monitoring,[6] respectively. A right nephrectomy was performed through a midline abdominal incision. This was done because previous studies demonstrating oxidant damage in the RAO model of ARF were performed in uninephrectomized rats.[1,2] After nephrectomy, a loose silk ligature was placed around the abdominal aorta between the superior mesenteric and left renal artery orifaces. A stiff wire was inserted into the noose, and it was slowly turned in a clockwise direction, progressively constricting the aorta until a femoral artery blood pressure of 20–25 mm Hg was achieved. The PAL was left in place for 45 minutes, maintaining the 20–25 mm Hg perfusion pressure throughout. The abdominal incision was kept covered with a warm saline-soaked gauze. During this 45-minute period, 5 ml of 0.9% saline was infused intravenously to replace surgical fluid losses. Upon completion of the 45 minutes of PAL, the aortic ligature and the vascular catheters were removed, and all incision sites were sutured. The rats were allowed to recover from anesthesia without further interventions. Free access to food and water were provided. Twenty-four hours later, the rats were reanesthetized and killed by phlebotomy. The severity of ARF was assessed by terminal BUN and Cr concentrations and by renal histologic evaluation (see below).

Experimental Groups

1) *Allopurinol treatment.* The xanthine oxidase inhibitor allo prevents oxygen free radical formation by inhibiting the metabolism of hypoxanthine to uric acid, a process that gives rise to superoxide,[16] and, hence, hydroxyl ion and H_2O_2.[1] To assess whether such inhibition protects against hypoperfusion renal injury, five rats were subjected to the above PAL protocol, receiving allo (40 mg/kg i.v.) 8 minutes before inducing aortic constriction. The allo was dissolved in 0.7 ml of 0.1N NaOH, as suggested by the manufacturer (Sigma Chemical, St. Louis, Missouri). The allo dosage, source, timing of administration, and vehicle were all identical to those employed by Paller et al in their study of allo-induced protection against RAO ARF in the rat.[1]

2) *Superoxide dismutase treatment.* The superoxide scavenger SOD (from bovine blood; Sigma) was administered intravenously to five rats in a dosage of 8 mg/kg (in 0.5 ml of 5% dextrose) 8 minutes before inducing PAL and 8 minutes before releasing the aortic constriction. This dosage schedule was identical to that which has been shown to protect against RAO in the rat.[1]

3) *Dimethylthiourea treatment.* The hydroxyl radical scavenger DMTU,[17] 500 mg/kg in 1 ml of 5% dextrose, was administered intravenously to five rats 8 minutes before inducing PAL. This dosage schedule was identical to that which has been reported to protect against RAO in the rat.[1] Additional rats were pretreated with 250 mg/kg ($n = 1$) or 150 mg/kg ($n = 2$) of DMTU intraperitoneally 15 minutes before performing PAL. The latter dosages and route of administration were comparable to those used by Shah, who demonstrated that DMTU is protective against passive Heymann nephritis.[18]

4) *Catalase treatment.* The hydrogen peroxide scavenger catalase (Sigma) was given intravenously to four rats in a dosage of 8 mg/kg (in 0.5 ml of 5% dextrose) 8 minutes before PAL and 8 minutes before PAL release. This dosage schedule was identical to that used by Paller et al.[1]

5) *Glutathione treatment.* Reduced glutathione (GSH) is actively extracted from peritubular capillary blood and concentrated in proximal tubular cells.[19,20] To assess whether enhancement of endogenous GSH stores protects against PAL, five rats received 2 mmol GSH/kg i.v. over 10 minutes in 1.0 ml saline. The rats were then prepared for PAL, which was commenced 1 hour after completing the GSH infusion. The timing and dosage of GSH employed was based on the protocol of Paller,[2] who demonstrated that 1 mmol/kg GSH administered to uninephrectomized rats protects against RAO. The 2 mmol/kg dosage employed in the current experiments was used because the rats had not yet undergone right nephrectomy at the time of GSH

Downloaded from http://ahajournals.org by on March 14, 2022

infusion. Therefore, twice the amount was used to keep the dose per kidney the same as Paller employed.

6) *Combined treatment.* To assess whether a combination of antioxidant agents could provide greater protection than any of the above agents used alone, five rats received SOD, allo, catalase, and GSH, each administered as separate infusions as described in the above individual protocols, and the PAL protocol was performed. DMTU was not infused because of the high mortality rate associated with its administration (see "Results").

7) *Controls.* Eleven rats subjected to the PAL protocol without administration of antioxidant agents served as controls. Four received the 0.1N NaOH vehicle as described under the allo experiments, three received the saline vehicle as described for GSH, and four rats received 5% dextrose as described for the SOD experiments. Since the results obtained with these three vehicles did not significantly differ, the data from these 11 rats were combined to form a single control group.

Renal Histology

The left kidneys were removed 24 hours after PAL and were immersion fixed in 10% buffered formaldehyde. Four-micron frontal sections running from outer cortex through the papilla were stained with hematoxylin and eosin. The sections were coded and evaluated in a blinded fashion. The extent of tubular necrosis was graded semiquantitatively in both the cortex and the outer medullary stripe using a 1+ to 10+ scale. A 1+ equalled tubular cell necrosis in 1 of every 10 high powered fields (100×). A 10+ equalled necrosis in 10 consecutive high powered fields. Scores of 2+ to 9+ represented intermediate values. At least 30 high powered fields were examined per kidney.

Malondialdehyde Tissue Assay

A biochemical marker of ROS-induced tissue injury is the production of malondialdehyde (MDA), a product of lipid peroxidation. To assess whether hypoperfusion ARF induces a rise in MDA, seven rats underwent the PAL protocol. The right (nonischemic) kidney was harvested immediately before PAL, and the left kidney was resected 15 minutes after completing PAL. A 20% homogenate was prepared in cold 1.15% KCl, and the supernatants were assayed for MDA by the thiobarbituric acid method adapted from that of Uchiyama and Mihara.[4] Values were expressed as nanomoles per gram tissue wet weight. Since renal ischemia induces an approximate 10% increase in wet weight due to edema, the MDA values of the left kidneys were also expressed after correction for this weight change. This was done by assuming that the preischemia wet weight of the left kidney (which could not be determined) equalled that of the right kidney from the same rat, as previously done by Paller.[1] For each rat, the uncorrected and corrected wet weight left renal MDA concentrations were compared with the right kidney value to assess whether an increase had occurred.

Effects of Exogenous GSH on Renal GSH Concentrations and on RAO-Induced ARF

Ten rats were anesthetized, BUN and Cr concentrations were determined, and then half of the rats were treated with either 2 mmol/kg i.v. GSH or an equal volume of saline (1.0 ml). One hour later, all rats underwent right nephrectomy, and the kidneys were assayed for GSH by thiol assay.[5,6] The left kidneys were subjected to 40 minutes RAO. The rats were then sutured and allowed to recover from anesthesia. The severity of ARF was assessed 24 hours later by repeat BUN and Cr assay.

Calculations and Statistics

All values are given as means ± SEM. BUN and Cr concentrations and histological scores for the PAL groups were compared by analysis of variance. The left versus right kidney MDA concentrations were analyzed by paired Student's t test. Comparisons between the RAO rats were by unpaired t test. Significance was judged by a p value of <0.05.

Results

Renal Functional Assessments After Partial Artery Ligation

Baseline BUN and Cr values for all rats were 16 ± 1 and 0.59 ± 0.02 mg/dl, respectively. By 24 hours after PAL, the control rats manifested severe azotemia (BUN 119 ± 6 mg/dl; Cr 3.3 ± 0.37 mg/dl). None of the groups treated with antioxidants, either singly or together, showed a significantly different degree of azotemia from that observed in the control group, indicating a lack of functional protection (see Table 1).

During the period of PAL, the femoral mean arterial pressure (MAP) had remained stable in the 20–25 mm Hg range in all treatment groups. MAP returned to normal values within 5 minutes after discontinuing the PAL (>105 mm Hg). All five rats treated with 500

Table 1. Severity of Renal Injury 24 Hours After Renal Hypoperfusion

Group	n	BUN	Cr	Necrosis	
				OMS	Cortex
Controls	11	119 ± 6	3.3 ± 0.37	8.1 ± 0.4	3.3 ± 0.6
Allopurinol	5	135 ± 17	2.9 ± 0.20	8.4 ± 0.6	2.6 ± 0.5
SOD	5	129 ± 8	3.1 ± 0.40	8.2 ± 0.5	2.8 ± 0.6
Catalase	4	124 ± 8	3.0 ± 0.21	7.3 ± 0.3	2.3 ± 0.6
Glutathione	5	141 ± 9	3.4 ± 0.14	8.6 ± 0.4	2.8 ± 0.9
DMTU*	3	142 ± 15	3.2 ± 0.8	6.7 ± 1.4	3.0 ± 0.6
Combined therapy†	5	137 ± 7	3.7 ± 0.24	9.2 ± 0.6	4.4 ± 1.4

All values are mean ± SEM. No significant differences existed between any of the treatment groups (ANOVA). BUN, blood urea nitrogen; Cr, creatinine; OMS, outer medullary stripe; SOD, superoxide dismutase; and DMTU, dimethylthiourea.

*DMTU rats were those treated with 150–250 mg/kg i.p. All five DMTU rats treated with 500 mg/kg DMTU died. †Combined therapy = allopurinol, SOD, catalase, and glutathione. Baseline BUN and Cr values for all rats were 16 ± 1 and 0.59 ± 0.02 mg/dl, respectively. Histological scores as explained in text.

Downloaded from http://ahajournals.org by on March 14, 2022

In support of this notion are recent demonstrations in this laboratory that show that renal hypoxanthine concentrations are 75% lower under conditions of hypoperfusion compared with RAO (Zager and Altschuld[5] and unpublished data). A second reason to suspect less oxidant injury with PAL than with RAO is that RAO traps blood within the kidney, a phenomenon that persists even with vascular reflow as evidenced by severe medullary vascular congestion in the reperfusion period.[9] This occurs to a far lesser degree with renal hypoperfusion.[9] Since polymorphonuclear leukocytes (PMNs) are also sequestered, they could participate in ischemic tissue damage,[13,14] possibly by exacerbating oxidant injury. Support for this concept comes from a recent study by Linas et al, which indicates that anoxic injury in an isolated perfused kidney is worsened by including PMNs in the kidney perfusate.[15]

In light of these questions and because of the current intense interest in the role of ROS in ischemic ARF, the present study was undertaken to assess whether ROS are important mediators of hypoperfusion-induced renal injury. To achieve this goal, we tested whether the available evidence for oxidant injury in the RAO model of ARF (protection with antioxidants and increments in MDA) also applies to a non–blood flow cessation model of acute ischemic renal injury.

Materials and Methods

Partial Aortic Ligation Model

Female Sprague-Dawley rats (Batum and Kingsman, Fremont, California) were anesthetized with pentobarbital (40 mg/kg) and placed on a heated surgical table to maintain body temperature at 37° C. A 200-μl tail vein plasma sample was obtained for blood urea nitrogen (BUN) and creatinine (Cr) determinations, and PE 50 jugular vein and femoral artery catheters were inserted for therapeutic agent infusion and direct mean arterial blood pressure monitoring,[6] respectively. A right nephrectomy was performed through a midline abdominal incision. This was done because previous studies demonstrating oxidant damage in the RAO model of ARF were performed in uninephrectomized rats.[1,2] After nephrectomy, a loose silk ligature was placed around the abdominal aorta between the superior mesenteric and left renal artery orifaces. A stiff wire was inserted into the noose, and it was slowly turned in a clockwise direction, progressively constricting the aorta until a femoral artery blood pressure of 20–25 mm Hg was achieved. The PAL was left in place for 45 minutes, maintaining the 20–25 mm Hg perfusion pressure throughout. The abdominal incision was kept covered with a warm saline-soaked gauze. During this 45-minute period, 5 ml of 0.9% saline was infused intravenously to replace surgical fluid losses. Upon completion of the 45 minutes of PAL, the aortic ligature and the vascular catheters were removed, and all incision sites were sutured. The rats were allowed to recover from anesthesia without further interventions. Free access to food and water were provided. Twenty-four hours later, the rats were reanesthetized and killed by phlebotomy. The severity of ARF was assessed by terminal BUN and Cr concentrations and by renal histologic evaluation (see below).

Experimental Groups

1) *Allopurinol treatment*. The xanthine oxidase inhibitor allo prevents oxygen free radical formation by inhibiting the metabolism of hypoxanthine to uric acid, a process that gives rise to superoxide,[16] and, hence, hydroxyl ion and H_2O_2.[1] To assess whether such inhibition protects against hypoperfusion renal injury, five rats were subjected to the above PAL protocol, receiving allo (40 mg/kg i.v.) 8 minutes before inducing aortic constriction. The allo was dissolved in 0.7 ml of 0.1N NaOH, as suggested by the manufacturer (Sigma Chemical, St. Louis, Missouri). The allo dosage, source, timing of administration, and vehicle were all identical to those employed by Paller et al in their study of allo-induced protection against RAO ARF in the rat.[1]

2) *Superoxide dismutase treatment*. The superoxide scavenger SOD (from bovine blood; Sigma) was administered intravenously to five rats in a dosage of 8 mg/kg (in 0.5 ml of 5% dextrose) 8 minutes before inducing PAL and 8 minutes before releasing the aortic constriction. This dosage schedule was identical to that which has been shown to protect against RAO in the rat.[1]

3) *Dimethylthiourea treatment*. The hydroxyl radical scavenger DMTU,[17] 500 mg/kg in 1 ml of 5% dextrose, was administered intravenously to five rats 8 minutes before inducing PAL. This dosage schedule was identical to that which has been reported to protect against RAO in the rat.[1] Additional rats were pretreated with 250 mg/kg ($n = 1$) or 150 mg/kg ($n = 2$) of DMTU intraperitoneally 15 minutes before performing PAL. The latter dosages and route of administration were comparable to those used by Shah, who demonstrated that DMTU is protective against passive Heymann nephritis.[18]

4) *Catalase treatment*. The hydrogen peroxide scavenger catalase (Sigma) was given intravenously to four rats in a dosage of 8 mg/kg (in 0.5 ml of 5% dextrose) 8 minutes before PAL and 8 minutes before PAL release. This dosage schedule was identical to that used by Paller et al.[1]

5) *Glutathione treatment*. Reduced glutathione (GSH) is actively extracted from peritubular capillary blood and concentrated in proximal tubular cells.[19,20] To assess whether enhancement of endogenous GSH stores protects against PAL, five rats received 2 mmol GSH/kg i.v. over 10 minutes in 1.0 ml saline. The rats were then prepared for PAL, which was commenced 1 hour after completing the GSH infusion. The timing and dosage of GSH employed was based on the protocol of Paller,[2] who demonstrated that 1 mmol/kg GSH administered to uninephrectomized rats protects against RAO. The 2 mmol/kg dosage employed in the current experiments was used because the rats had not yet undergone right nephrectomy at the time of GSH

Downloaded from http://ahajournals.org by on March 14, 2022

ischemic period was used. It could be argued that the present protocol, which employed 45 minutes of hypoperfusion, induced a less severe tubular lesion than did 60 minutes of RAO, explaining the failure of MDA concentrations to rise. However, this explanation is untenable because the severity of azotemia in the present study is comparable to that which resulted from Paller's 60-minute RAO protocols (serum Cr of 3–4 mg/dl).[1,2] Furthermore, renal histology in the present study revealed widespread and often confluent tubular necrosis. Thus, rather than attributing a lack of rise in renal MDA concentrations to insufficient tubular damage, a far more compelling explanation is that significant lipid peroxidation did not occur in response to the hypoperfusion-induced renal injury.

The third principal argument made for oxidant injury in ischemic ARF is that several laboratories have documented reduced GSH depletion in kidneys in an early vascular reflow period.[5-7] The assumption has been that with redelivery of molecular oxygen, ROS form, which oxidize GSH to oxidized GSH (GS-SG). However, to our knowledge, no laboratory has reported finding an increase in GS-SG to account for the GSH depletion. Indeed, although this laboratory has also reported GSH depletion with both PAL and RAO,[5,6,9] in subsequent experiments we have not been able to document a rise in GS-SG by specific analysis[9] in either model. This suggests that GSH is being lost by mechanisms other than by oxidation to GS-SG (e.g., leakage out of damaged tubular cells and/or catabolism). Furthermore, a rise in GS-SG, even if it did occur, would only indicate oxidant stress, not oxidant injury. MDA would be a far better marker of this phenomenon, and as the present data indicate, no such rise occurs with our hypoperfusion ischemic renal injury protocol.

As noted previously, there are at least two theoretical explanations for why ROS might play a pathogenic role in RAO but not in hypoperfusion-induced ischemic ARF. First, renal hypoxanthine accumulation, an important determinant of ROS production,[12] is approximately four times greater with RAO than with PAL. This is because greater adenosine triphosphate breakdown occurs with RAO than with PAL[9] and possibly because continued blood flow with PAL permits renal hypoxanthine washout into renal venous blood, not possible during RAO. A second possible reason for greater oxidant damage with RAO than with PAL is that greater medullary vascular congestion with the former model could give rise to greater intrarenal PMN sequestration, leading to more ROS production.[15] These two possibilities might explain the divergent findings of the present study with those of Paller.[1,2] A third possibility we cannot exclude is that oxidant injury might not even be a consistent pathophysiologic mechanism in the RAO model. For example, this laboratory has previously failed to document a significant rise in whole kidney MDA concentrations after 25 or 60 minutes of RAO.[6] McCoy et al could not document a rise in renal MDA after 40 minutes of RAO unless rats were first rendered selenium deficient, a maneuver that enhances oxidant tissue injury.[7] Fuld et al[21] found an increase in MDA after 60 minutes of RAO but felt that this was due to a hemoglobin-induced artifact in the routinely used thiobarbituric acid MDA assay method. The present finding that exogenous GSH failed to protect against 40 minutes of RAO further raises the question as to whether oxidant injury is a consistent and major mediator of RAO-induced ARF or whether interlaboratory variations or differences in rats underlie these discrepant results.

In conclusion, the present study has evaluated a possible role for oxidant tissue injury in a hypoperfusion model of ischemic ARF. Despite continued oxygen delivery during the period of hypoperfusion, no evidence for oxidant tissue damage could be obtained either by renal MDA assay or by trials with antioxidant agents. These findings stand in contrast to previous evidence obtained, using the RAO model, that oxidant injury is a critical cellular mediator of ischemic renal damage. Since human ischemic ARF typically results from hypoperfusion and not from total blood flow interruption, it appears that no compelling experimental data exist to support the notion that ROS mediate most forms of clinical ischemic renal injury.

Acknowledgments

The author thanks Ms. D. Stein and Ms. E. Teubner for their technical support and Ms. C. Bisaillon for her secretarial assistance.

References

1. Paller MS, Hoidal JR, Ferris TF: Oxygen free radicals in ischemic acute renal failure in the rat. *J Clin Invest* 1984; 74:1156–1164
2. Paller MS: Hypothyroidism protects against free radical damage in ischemic acute renal failure. *Kidney Int* 1986; 29:1162–1166
3. Freeman BA, Crapo JD: Free radicals and tissue injury. *Lab Invest* 1982;47:412–426
4. Uchiyama M, Mihara M: Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. *Ann Biochem* 1978;86:271–276
5. Zager RA, Altschuld R: Body temperature: An important determinant of severity of ischemic renal injury. *Am J Physiol* 1986;251:F87–F93
6. Zager RA, Mahan J, Merola AJ: Effects of mannitol on the post-ischemic kidney: Biochemical, functional and morphologic assessments. *Lab Invest* 1985;53:433–442
7. McCoy RM, Ayon MA, Hill KE, Stein JH, Burk RF: Oxidant stress following renal ischemia (abstract). *Kidney Int* 1986;29:307A
8. Chance B, Boveris A, Nakase Y, Sies H: Hydroperoxide metabolism. An overview, in Sies H, Wendel A (eds): *Functions of Glutathione in Liver and Kidney.* Berlin, Springer-Verlag, 1978, pp 95–106
9. Zager RA: Partial aortic ligation: A hypoperfusion model of ischemic acute renal failure and a comparison to renal artery occlusion. *J Lab Clin Med* (in press) (NB: Previously listed as *Kidney Int* 1987;31:378A)
10. Oswald H, Schmitz HJ, Kemper R: Tissue content of adenosine, inosine, and hypoxanthine in the rat kidney after ischemia and post-ischemic recirculation, *Pflugers Arch* 1977;371: 45–49
11. Miller WL, Thomas RA, Berne RM, Rubio R: Adenosine production in the ischemic kidney. *Circ Res* 1978;43:390–397
12. McCord JM: Oxygen-derived free radicals in post ischemic tissue injury. *N Engl J Med* 1985;312:159–163

Downloaded from http://ahajournals.org by on March 14, 2022

13. Romson JL, Hook BG, Kunkel SL, Abrams GD, Schork MA, Lucchesi BR: Reduction of the extent of ischemic myocardial injury by neutrophil depletion in the dog. *Circulation* 1983;67:1016–1023
14. Engler RL, Dahlgren MD, Morris DD, Peterson MA, Schmid-Schonbein GW: Role of leukocytes in response to acute myocardial ischemia and reflow in dogs. *Am J Physiol* 1986;251:H314–H323
15. Linas SL, Whittenburg D, Berger E, Repine JE: Toxic O_2 metabolites from neutrophils accentuate renal ischemia-reperfusion injury in the isolated perfused rat kidney (abstract). *Kidney Int* 1987;31:396A
16. Fridovich I. Quantitative aspects of the production of superoxide anion radical by milk xanthine oxidase. *J Biol Chem* 1970;245:4053–4057
17. Dorfman LM, Adams GE: Reactivity of the hydroxyl radical in aqueous solution, in: *National Standard Reference System.* Washington, DC, National Bureau Standards, 1973, p 47–48
18. Shah SU, Baricos WH, Schwartz S, Walker PD: Dimethylthiourea (DMTU), a hydroxyl radical scavenger, reduces proteinuria in passive Heymann nephritis in rats (abstract). *Kidney Int* 1987;31:330A
19. Lash LH, Jones DP: Transport of glutathione by renal basal lateral membranes vesicles. *Biochem Biophys Res Commun* 1983;112:55–69
20. Lash LH, Jones DP: Renal glutathione transport: Characteristics of the sodium-dependent system in the basal lateral membrane. *J Biol Chem* 1984;259:14505–14514
21. Fuld R, Spar B, Urbaitus BK: Measurement of malonaldehyde in kidney following ischemia and reflow (abstract). *Kidney Int* 1986;29:301A

KEY WORDS • ischemia • reactive oxygen species • acute renal failure • antioxidants

Downloaded from http://ahajournals.org by on March 14, 2022