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The role of oxygen free radicals and prostaglandins in reperfusion injury to warm ischemic kidneys

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Summary. The present study was designed to determine whether the administration of superoxide dismutase (SOD) can alleviate ischemic kidney damage and whether there is a relationship beween oxygen free radicals and thromboxane (Tx). In 17 dogs, the right kidney was removed and the vascular pedicle of the left kidney was clamped for 75 min. Prior to reperfusion, the ischemic kidney was rinsed with 5 mg SOD and an additional 20 mg SOD was infused systemically. Blood samples were drawn from the renal vein before ischemia and after reperfusion to determine serum levels of thromboxane B_2 (TxB_2). All eight untreated dogs died within 1 week of renal failure, and the nine treated dogs demonstrated transient renal failure, with a significant difference ($P<0.001$) being found beween the two groups. A significant difference ($P<0.001$) in TxB_2 levels was found in the untreated dogs before and after ischemia and beween the two groups following reperfusion. Animals that are treated with SOD after the ischemic event has occured but before reperfusion exhibit a favorable clinical course in terms of survival and renal function. Tx synthesis in the kidney can be blocked by the administration of SOD.

Key words: Renal failure – Reperfusion renal injury – Oxygen free radicals – Prostaglandins – Superoxide dismutase

Oxygen free radicals (OFRs) generated at reperfusion following ischemia cause a substantial proportion of tissue injury. Other hormonal mediators such as prostaglandins (PGs), leukotrienes (LKs), and thromboxane (Tx) have also been found to participate in the mechanism of reperfusion injury [6]. The present study was designed to evaluate the interrelationship among these mediators in reperfusion injury. We addressed two questions:

1. Can tissue damage caused by kidney ischemia be diminished by blocking the generation of OFRs?

2. Does a relationship exist between OFRs and synthesis of Tx?

Materials and methods

Model of renal ischemia

Mongrel dogs weighing 25–35 kg were anesthetized using sodium thiopental (2 mg/kg) and were ventilated with a mixture of room air and halothane. Through a midline incision, the right kidney was removed and the vascular pedicle of the left kidney was skeletonized. After 30 min, during which the dog was left to stabilize, the vascular pedicle and the ureter of the oft kidney were closed with a vascular clamp for 75 min. In the meantime, the left renal artery and vein were cannulated with a 21-gauge plastic (Venflon) needle and the vascular clamp was then removed. The abdomen was closed using metal sutures and the dogs were provided with food and water ad libitum. The animals were followed for 14 days and then killed by the injection of an overdose of sodium thiopental.

Superoxide dismutase

The SOD used in the present study was a human recombinant form derived from *Escherichia coli* and manufactured by Biotechnology General Corporation (Weizmann Institute of Science, Rehovot, Israel).

Study protocol

Animals were randomly assigned to a control group (nine dogs) or to a treatment group (eight dogs). For use in the treatment group, 5 mg SOD was dissolved in 200 cc cold Euro-Collins solution. At 20 min prior to reperfusion, the ischemic kidney was rinsed with this solution using the needle located in the renal artery. The rinsing fluid was drained through the needle placed in the renal vein. An additional 20 mg SOD dissolved in 200 cc Euro-Collins solution at room temperature was infused intravenously for 20 min starting at 1 min before removal of the vascular clamp from the ischemic kidney. Thus, by the time the blood flow to the kidney had been reestablished, the blood was loaded with SOD. Using the needle placed in the artery, the ischemic kidney in the control group was

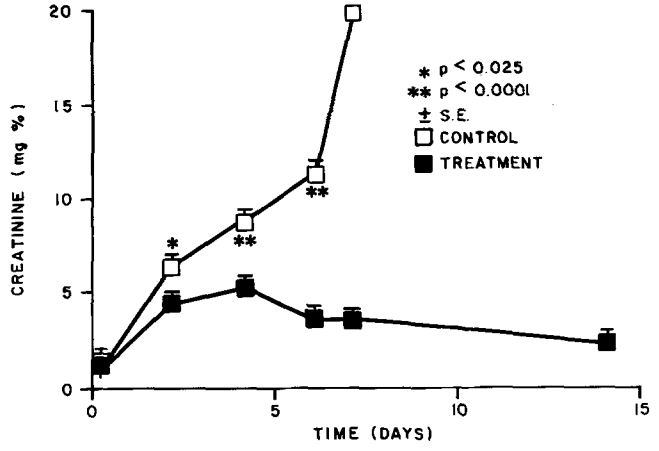

Fig. 1. BUN values obtained during the study

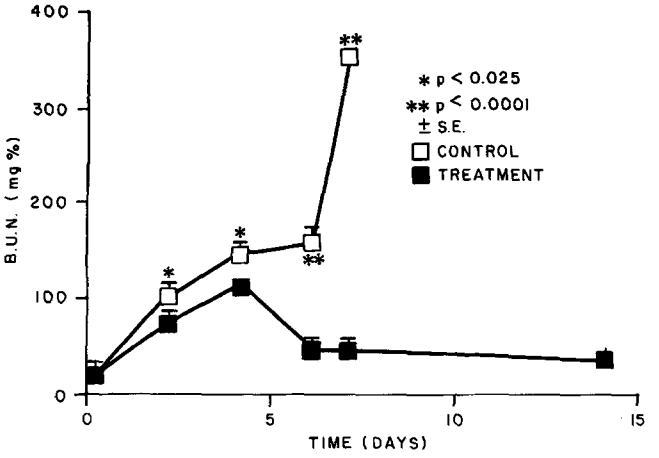

Fig. 2. Creatinine levels measured during the study

Table 1. TxB_2 levels in blood samples obtained from the renal vein

	Controls	Treated dogs
Before ischemia	613 ± 38 µg/ml	493 ± 28 µg/ml
After ischemia	1,642 ± 285 µg/ml*	492 ± 52 µg/ml*

* $P<0.001$

rinsed with 200 cc Euro-Collins solution at 4°C. The rinsing fluid was drained through the needle in the renal vein. An additional 200 cc Euro-Collins solution was infused intravenously for 20 min at room temperature starting at 1 min before removal of the vascular clamp.

Peripheral venous blood was sampled for determination of blood urea nitrogen (BUN) and creatinine levels before the operation and on days 2, 4, 6, 7, and 14 postsurgery. Levels of TxB_2, the stable degradation product of TxA_2, were measured in blood samples obtained from the renal vein at 2 min prior to ischemia and 5 min following reperfusion. The blood was drawn directly into a syringe containing ethylenediaminetetraacetic acid (EDTA) an aspirin. Each blood sample was centrifuged and stored. The results obtained in both groups were evaluated using Student's t-test. Mortality was analyzed using Fisher's exact test.

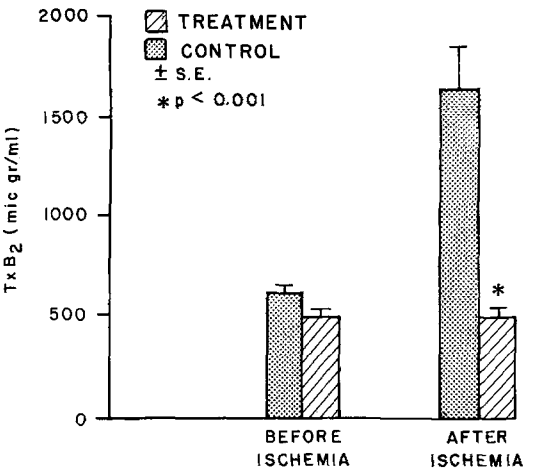

Fig. 3. TxB_2 levels measured during the study

Results

In the control group, all dogs died of acute renal failure within 1 week. In the treated group, only one dog died of renal failure on day 7; all other treated animals survived the 14 days of the study and were then killed. The difference in mortality between the two groups was found to be statistically significant ($P<0.0004$).

Deterioration of renal function was noted in both groups over the first 4 days after surgery. The rise in BUN and creatinine levels in the control dogs was significantly higher than that in the treated animals ($P<0.025$ over the first few days following surgery; $P<0.0001$ after the 4th day.) In the treated dogs, there was a significant improvement in BUN (Fig. 1) and creatinine values after day 5 (Fig. 2). Although renal function in the treated animals never returned to preoperative levels, the difference in BUN and creatinine levels between the two groups was statistically significant ($P<0.025$ and $P<0.0001$). In the control group, the blood levels of creatinine and BUN continued to rise until death; following reperfusion, a significant increase in the TxB_2 level in venous renal blood was found ($P<0.001$) as compared with the values obtained before and after ischemia (Table 1, Fig. 3). In the treated group, the TxB_2 concentration following reperfusion was maintained at the baseline level (Table 1, Fig. 3). A comparison of the two groups after reperfusion revealed that the TxB_2 level in venous renal blood was significantly elevated ($P<0.001$) in the untreated dogs (Table 1, Fig. 3).

Discussion

Renal ischemia is a major problem in surgery and trauma [5]. Research in this field is devoted to understanding the pathophysiology of the ischemic/reperfusion renal injury and to prolonging the period of organ ischemia and avoiding irreversible tissue damage.

Ischemic renal damage has been found to start within a few minutes after reperfusion [6]. The noxious effect of

ischemia itself cannot explain the extent of the resulting damage. A search for other mechanisms revealed that ischemia also causes the generation of different humoral mediators, including TxA_2, PGs, and LT [6]. OFRs are regularly formed during the process of normal cell respiration. However, their development is kept at physiologically low levels by intracellular free-radical scavengers (catalase, glutathione, glutathione peroxidase) [9].

During ischemia, the process of oxidation ceases and hypoxanthine is accumulated in high quantities inside the cell. When reperfusion is reestablished, a large proportion of the hypoxanthine is converted to xanthine by xanthine oxidase, which results in the regeneration of free radicals [2]. Under these circumstances, excess generation of OFRs (combined with calcium) can destroy lipids and may lead to cellmembrane disruption [6]. Membrane phospholipids are precursors of arachidonic acid from which TxA_2 is synthesized. This vasoactive mediator is a potent vasoconstrictor and platelet proaggregator whose levels increase after ischemia to different organs including the limbs and the kidney. Blockade of TxA_2 has been found to improve kidney performance after ischemia [5].

The present study was disigned to evaluate the interrelationship of these mediators in renally, induced reperfusion damage following a long period of warm ischemia. The animal model was used to simulate clinical situations such as aeortic surgery, partial nephrectomy, or clamping of the renal pedicle in cases of acute bleeding from the kidney. To avoid any technical surgical influence on the results, vascular anastomoses were not performed. In patients in whom the ischemic event has occurred, the teratment must be introduced following the ischemic insult. This challenging clinical situation was reproduced in our model. Neither continuous cooling of the kidney throughout the ischemic period nor the administration of mannitol, heparin, furosemide, or allopurinol was initiated prior to clamping of the renal vessels.

Our study clearly demonstrates that animals treated with free-radical scavengers exhibit an improved clinical course in terms of survival and renal function. Our findings are in accordance with the results reported by others investigators. Using a rat model, Kaufman et al. [3] demonstrated that SOD administration can inhibit TxA_2 synthesis. Using a similar surgical procedure, we performed unilateral nephrectomy, clamping of the contralateral renal pedicle, and renal-vein blood sampling. However, in the study by Kaufman et al. [3], SOD was infused both before and after the establishment of ischemia; moreover, the rats were killed at 24 h postsurgery and a longer follow-up was not undertaken. Ouriel et al. [7] demonstrated less formation of edema in the kidneys of dogs treated with SOD after the ischemic period. In a rat model, Paller and co-workers [8] reported a ca. 50% reduction in the rise in serum creatinine levels. These authors also found a more rapid return of renal function when SOD was applied before and after ischemia. Baker et al. [1] reported better survival for rats treated with SOD as compared with untreated controls; they also found a transient elevation in blood creatinine levels in treated rats that lasted until the 3rd postoperative day and returned to normal thereafter.

We observed transient renal failure in the treated dogs until the 5th postoperative day. Using SOD infused both into the renal artery and systematically, we interceded with the PG cascade system, as was demonstrated by the lower TxB_2 levels found in the treated group, and thus eliminated the noxious effect of the TxA_2 on renal failure. The deleterious effect of TxA_2 on renal function, mainly involving vasoconstriction, can itself cause renal damage and may be additive to the damage caused by the OFRs. Based on our results, it seems that the PG cascade may be blocked using OFR scavengers.

We recommend the use of SOD in renal transplantation and other clinical situations in which renal perfusion and reperfusion damage is encountered. Further research is needed to address the question as to whether SOD infused either systemically or into the renal artery is more effective than the combination (systemic and renal arteryial administration) in preventing renal damage.

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