Title: CHARACTERIZATION OF XANTHINE DEHYDROGENASE CONVERSION TO XANTHINE OXIDASE DURING RENAL ISCHEMIA

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Introduction. Acute tubular necrosis after cadaveric renal transplantation jeopardizes graft survival and prolongs hospital stay. Acute tubular necrosis after warm ischemia (aortic crossclamping, severe hypotension) increases patient mortality. Excessive oxygen free radical production during or after ischemic episodes may play a role in each of these injuries. During ischemia, we hypothesized that the enzyme xanthine dehydrogenase (XDH) converted to xanthine oxidase (XO) by proteolysis. Concurrently, ATP is metabolized to hypoxanthine during this period of ischemia. At the time of reperfusion, XO in combination with molecular oxygen and its substrate hypoxanthine can generate superoxide radicals and hydrogen peroxide, which react with cell protein, lipid and DNA to cause cellular and organ injury. Other investigators have shown that superoxide dismutase, a scavenger of superoxide, and allopurinol, an inhibitor of XO, are protective after renal ischemia.(1)

Methods. After induction of anesthesia with IP pentobarbital, a midline incision was performed to allow occlusion of left and right renal arteries in 350-450 g, Sprague-Dawley rats. Ventilation was controlled and rectal temperature maintained after 37°C during the occlusion phase. Kidneys were harvested at 0, 30, 60, and 90 minutes of ischemia and assayed for XO and XDH enzyme activities by measuring the conversion of xanthine to uric acid spectrophotometrically at 295 nm.(2)

Results. Progressive duration of ischemia (0, 30, 60, 90 min) resulted in a significant increase ($p < 0.05$) in the percentage of XO compared to total (XO + XDH) enzyme activity (figure 1). Control kidney had 17% XO activity, compared to 28% after 90 minutes of ischemia. An apparent decrease in total (XO + XDH) enzyme activity with time was not statistically significant (figure 2). To ensure that all measured enzyme activities were due to XO and XDH, allopurinol, an inhibitor of both forms of the enzyme, was added to the assays. The addition of allopurinol resulted in complete inhibition of enzyme activity. Samples were also chromatographed over Sephadex G-25 to remove endogenous purines without an effect on total activity.

Conclusions. During 90 minutes of renal ischemia, the percentage of the free radical producing XO increased almost linearly with the duration of ischemia. However, total XO + XDH enzyme activity did not change significantly. Thus, after a period of renal ischemia, xanthine oxidase can combine with hypoxanthine and molecular oxygen to produce hydrogen peroxide and superoxide. We calculate that an increase in H_2O_2 and O_2^- production of 13 μM/min and 5.3 μM/min, respectively, can occur in the kidney following 90 minutes of ischemia. This represents a 65% increase in the tissue rate of production of these species. Actually, further increases in H_2O_2 and superoxide generation rates could be expected because hypoxanthine also increases during ischemia, providing more substrate for xanthine oxidase. Some investigators have shown protection against renal ischemia with the oxygen radical scavengers, superoxide dismutase, catalase and mannitol. These agents scavenge partially reduced oxygen species that may not be neutralized by naturally occurring scavengers. We are currently investigating the role of reperfusion on the conversion of XDH to XO, which may lead to a further increase in H_2O_2 and O_2^- production. We have measured the conversion of XDH to XO during renal ischemia, thus identifying a source of toxic oxygen species.

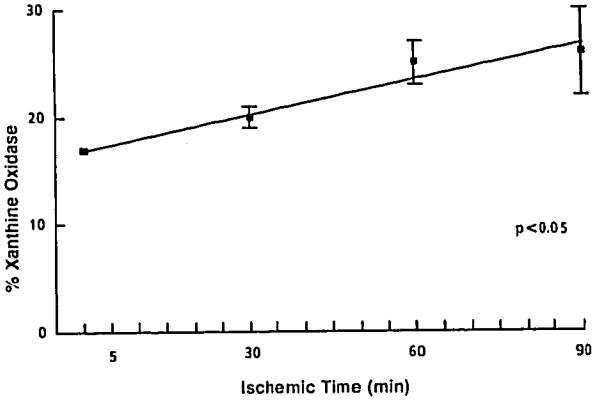

Figure 1. The percentage of total XDH + XO activity present in the free radical generating form XO is shown for kidneys subjected to varying periods of ischemia. Each time point represents the mean ± S.E.M. of five animals.

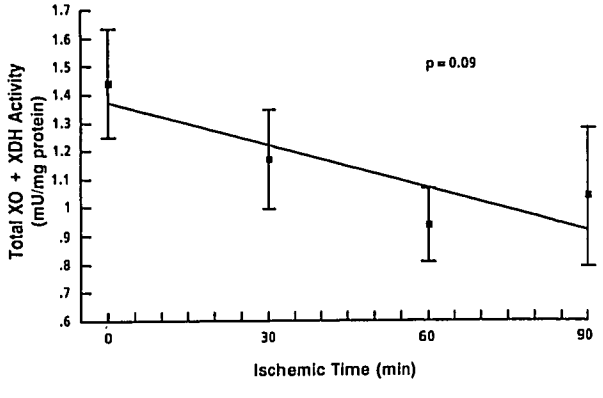

Figure 2. The total activity of XO +XDH in terms of milli-International Units per mg protein is shown for the same kidney data as in Figure 1.

References.

1. Hansson R, et al: Kidney protection by pretreatment with free radical scavengers and allopurinol: Renal function at recirculation after warm ischaemia in rabbits. Clin Sci 71:245-251, 1986.
2. Krenitsky TA, et al: A comparison of the distribution and electron acceptor specificities of xanthine oxidase and aldehyde oxidase. Comp Biochem Physiol 49B:687-703, 1974.