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INCREASED RESISTANCE AGAINST OXIDATIVE STRESS IS OBSERVED DURING A SHORT PERIOD OF RENAL REPERFUSION AFTER A TEMPORAL ISCHAEMIA

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Reperfusion of rat kidney submitted to temporal ischaemia induces a decrease in glutathione content. Lipid peroxidation is not detected in kidney homogenates but microsomes obtained after periods of reperfusion longer than 60 minutes show increased malondialdehyde values correlated with high oxygen consumption and superoxide free radical generation. Microsomes obtained from kidneys submitted to 15 or 60 minutes of reperfusion are resistant to NADPH-induced lipid peroxidation but after 120 minutes of reperfusion an increased lipid peroxidative response is observed. Although the mechanism of the protection found in microsomes against the induction of oxidative stress in the first 60 minutes of reperfusion is unknown, it is postulated that this subcellular fraction plays an important role in the oxidative stress observed after longer periods of reperfusion.

KEY WORDS: Renal ischaemia, tissue oxidative damage, microsomal free radicals, ischaemia-reperfusion damage.

INTRODUCTION

Renal ischaemia is a consequence of a number of clinical situations and/or surgical procedures. Acute tubular necrosis is a frequent finding as yet not clearly understood, in kidneys removed for renal transplantation. It occurs in 30–60% of the recipients of cadaver donor kidneys and up to 10% of the recipients of living donor kidneys.[1] It seems that reperfusion of ischaemic tissue may produce much more damage than that caused by the ischaemia itself.[2] Reactive oxygen species (superoxide free radicals, $O_2^{\cdot-}$ and hydroxyl free radicals, $\cdot OH$), have been implicated as mediators of the oxidative stress (expressed mainly as lipid peroxidation and tissue damage) observed in organ reperfusion in several experimental models.[3,4] It has been proposed that neutrophils may contribute to injury in ischaemic renal failure through the exacerbation in the production of reactive oxygen species;[5] however, the enzyme xanthine oxidase, formed from the structural modification of its dehydrogenase form,[6] has been implicated as one of the main sources of reactive oxygen free radicals.[7]

Although organ structural and functional damage is observed only after prolonged reperfusion preceded by a temporal ischaemia, little is known about oxygen metabolism and the induction of oxidative stress during the primary events of reperfusion. In

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the present work we assayed the response of renal homogenates and microsomes obtained from rats kidneys submitted to different periods of reperfusion after a temporal ischaemia, to the spontaneous or induced oxidative stress assessed as reduced glutathione (GSH) levels, malondialdehyde formation (MDA), tissue oxygen consumption and superoxide free radicals formation.

MATERIALS AND METHODS

Male Wistar rats (180–220 g) fed with a standard diet and with water ad libitum, were fasted overnight (16 hours) and anaesthesized by an intraperitoneal injection of sodium pentobarbital (60 mg/kg body weight). Following general anesthesia, 80 IU (0.4 ml) of heparin were administered by a femoral vein and through a midline abdominal incision the left renal artery was isolated and occluded with an atraumatic vascular clip for 60 minutes. Tissues were kept moistened with a glucose-saline hypertonic solution to minimize fluid loss by evaporation and dehydration. Rectal temperature of the rats during experimental procedure was maintained at 36–38° C with an infrared lamp. A right nephrectomy was performed immediately after the end of the ischaemic period for the left kidney, which was removed after 15, 60 and 120 minutes of reperfusion. If blood flow was not restored after the first minutes of reperfusion of the left kidney, these animals were not included in the experiment. Controls were obtained from the left kidney of rats submitted to a sham operation (only midline abdominal incision and right nephrectomy) and maintained during the ischaemia and the different periods of reperfusion.

Studies of GSH concentration and MDA formation were performed from 10% (w/v) kidney homogenates in phosphate-saline buffer pH 7.4. GSH was assessed according to Ball[8] and MDA according to Fee and Teitelbaum.[9] Microsomes for oxygen consumption studies, superoxide free radical generation, MDA production and for induction of lipid peroxidation, were obtained according to Albro *et al.*[10] Microsomal oxygen consumption was measured as previously described.[11] Formation of superoxide free radicals by microsomes was assessed according to Boveris *et al.*[12] Induction of microsomal lipid peroxidation by NADPH was performed as described by Devasagayan.[13] Protein was assayed according to Lowry *et al.*[14] All chemicals were reagent grade obtained from Sigma (St. Louis, Mo). Results are expressed as means $\pm$ SD and the significance of the differences between mean values was assessed by Student's t test for unpaired results.

RESULTS AND DISCUSION

Kidney ischaemia induces a depletion of renal GSH concentration which is maintained during the reperfusion time. Moreover, the level of the tripeptide tends to decrease even more during the first minutes after restoration of the blood flow (Figure 1). GSH is considered the most important biomolecule against chemically-induced cytotoxicity[15] and its decrease in tissues is generally associated with the onset of an oxidative injury.[16] This tripeptide acts as a free radical scavenger and/or as a substrate for the enzyme glutathione peroxidase, which destroys H_2O_2 or organic peroxides formed during oxidative damage. The GSH depletion observed in our experimental model is not in accordance with Linas *et al.*,[17] who reported that GSH levels were

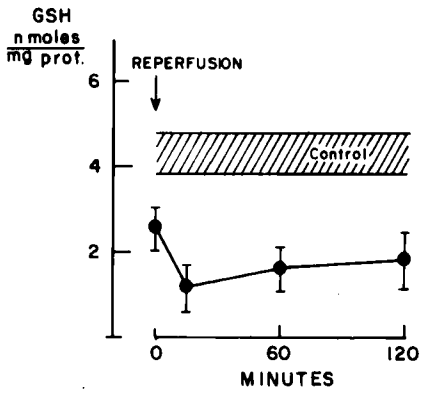

FIGURE 1 Glutathione (GSH) levels obtained during 120 minutes of renal reperfusion after a temporal ischaemia. Each point represents the mean of five experiments ± SD.

decreased in association with renal ischaemia only in the presence of an oxidant. They proposed that the loss of glutathione during ischaemia-reperfusion may reflect a loss of the tripeptide from injured cells rather than oxidative stress.

Lipid peroxidation of kidney homogenate, evaluated as TBA-reactive substances (TBARS), is slightly increased during the first minutes of reperfusion. It tends to decrease during the following minutes, reaching control levels at 120 minutes of reperfusion. This result is in accordance with that obtained by Bird *et al.*,[18] who did not find a significant increase in TBARS after ischaemia and reflow in rat kidney, *in vivo*. TBARS measurement is a good indicator of lipid peroxidation *in vitro*, but because of the transient nature of lipid hydroperoxides and their products and the excretory pathways of urine and blood, it is less reliable *in vivo*.[19] Moreover, TBARS measured in vitro may be formed from non-volatile peroxidic precursors, present in the biological material, which are decomposed under the acid heating conditions of the reaction with TBA. Although the mechanism of this decomposition is not well

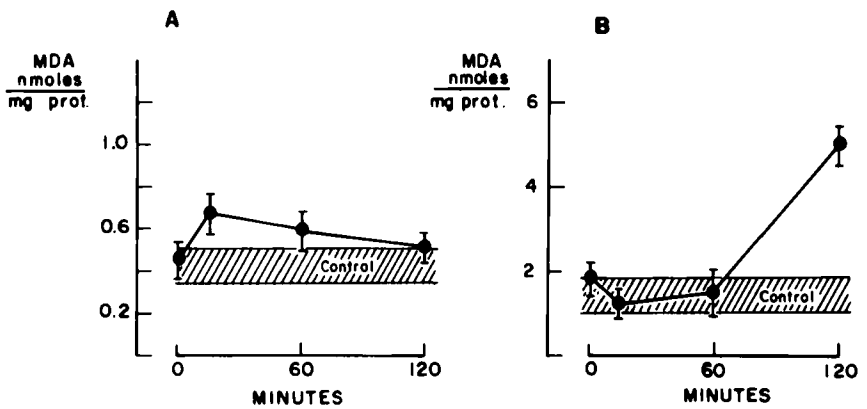

FIGURE 2 TBARS formation during 120 minutes of renal reperfusion after a temporal ischaemia (A) Homogenate (B) Microsomes. Each point represents the mean of six experiments ± S.D.

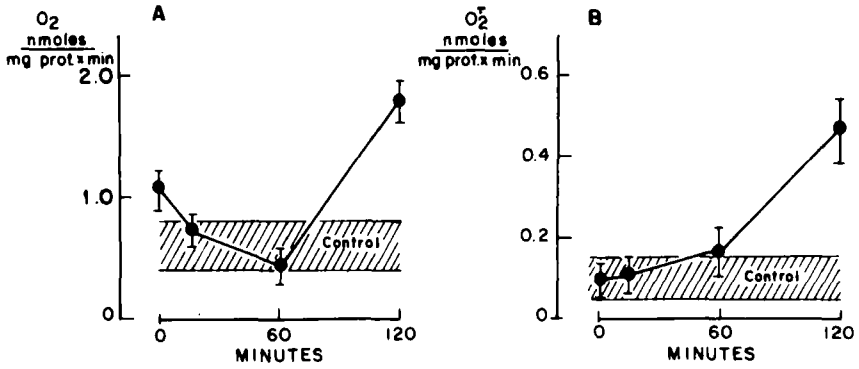

FIGURE 3 Microsomal oxygen consumption (A) and superoxide radical formation (B) during 120 minutes of renal reperfusion after a temporal ischaemia. Each point represents the mean of five experiments ± S.D.

understood it has been suggested that the precursors are mono-cyclic peroxides and bi-cyclic endoperoxides.[20]

Microsomes are highly sensitive to both spontaneous or induced lipid peroxidation.[11] Since the early observation by Hochstein *et al.*[21] of an enzymatic NADPH-dependent oxidation of microsomal lipids, a number of mechanisms have been proposed involving superoxide free radical induced-lipid peroxidation and/or hydroxyl free radicals and singlet oxygen. In this regard we assayed the spontaneous TBARS formation in microsomes obtained from kidneys submitted to different reperfusion times after the ischaemic period. (Figure 2 B). It can be observed that during the first 60 minutes of reperfusion lipid peroxidation is not detectable, the formation of MDA being drastically increased only after 120 minutes of reperfusion. Oxygen consumption which accompanies microsomal oxidation has been related to the redox-cycling of the cytochrome P-450 moiety.[22] As can be seen in Figure 3 A, microsomal oxygen consumption is highly correlated with the increase in TBARS formation (Figure 2 B), augmented consumption being apparent only after 60 minutes of reperfusion. Moreover, an increase in the generation of microsomal superoxide free radicals is also observed only after 60 minutes of reperfusion (Figure 3 B). In this situation, the microsomal lipid peroxidation and the increase in the microsomal oxygen consumption observed after 60 minutes of reperfusion, may reflect a decrease in the activity of the cellular protective mechanisms against oxidative stress and/or an increase in the superoxide free radical generation by the microsomal cytochrome P-450 moiety. It has been postulated that the uncoupling of cytochrome P-450 redox-cycling leads to formation of superoxide free radicals.[23]

NADPH-dependent microsomal lipid peroxidation after different times of reperfusion is shown in Figure 4. Microsomes obtained after 15 minutes of reperfusion appear more resistant to the induction of lipid peroxidation compared to controls (Figure 4 A). This resistance is slightly decreased when lipid peroxidation is induced from microsomes obtained after 60 minutes of reperfusion (Figure 4 B) but, when the blood flow in the reperfused kidney is maintained for 120 minutes a decrease of the protective effect is observed, the microsomal NADPH-induced lipid peroxidation being higher than in controls (Figure 4 C). These results support our hypothesis about a loss of the antilipoperoxidative protection after 60 minutes of reperfusion. We do

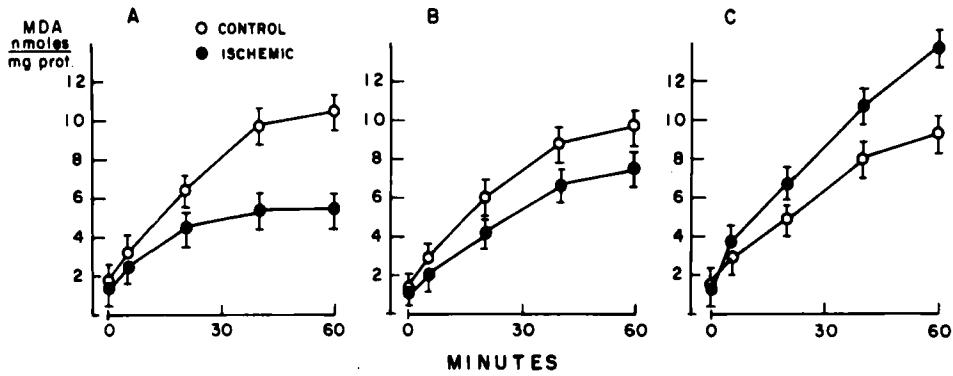

FIGURE 4 Microsomal TBARS formation induced by NADPH (0.4 mM). Microsomes were obtained from kidneys submitted to a temporal ischaemia and reperfused during (A) 15 minutes, (B) 60 minutes and (C) 120 minutes. Each point represents the mean of six experiments ± S.D.

not know however, why microsomes obtained before this time appear more resistant than the controls to the induction of lipid peroxidation. Current work is directed to the elucidation of this phenomenon.

Increased resistance against oxidative stress during the first 60 minutes of renal reperfusion, observed in our experimental model, may be of critical importance in the use of substances which protect against ischaemic renal injury, such as allopurinol,[24] free radical scavengers[25] or calcium channel blocking agents.[26] In addition, although the conversion of xanthine dehydrogenase to an oxidase form is considered as one of the main events in the oxidative stress induced by ischaemic-reperfusion injury, a role for microsomes in this oxidative damage may be important.

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