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Renal protective effect of liposomed superoxide dismutase in an experimental warm ischemia model

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Abstract Superoxide dismutase (SOD) is a potent scavenger of superoxide radicals produced during normothermic ischemia-reperfusion. Since it has a short half-life, its optimal effect is achieved when it is given prior to reperfusion. The inclusion of SOD in liposomes (lipo-SOD) prolongs its half-life (free SOD: 6 min; lipo-SOD: 4 h). The protective effect of lipo-SOD in a 60-min bilateral renal warm ischemia model was studied. We divided 60 male Wistar rats between two control groups and five study groups according to the drug used (SOD or lipo-SOD) and to the time of SOD administration (prior to ischemia or prior to reperfusion). SOD and lipo-SOD were both given at 20 mg/kg endovenously. Weight, diuresis, creatinine per 100 g (Cr/100 g), and creatinine clearance per 100 g (CrCl/100 g) were studied. Conventional renal histology was performed after reperfusion and on day 7. Renal malondialdehyde, 6 keto PGF 1 alpha, and TxB2 tissue levels were studied after reperfusion. Results showed that the renal protective effect of free SOD on warm ischemic-reperfusion injury depended on the time of administration, being more effective when given before reperfusion. On the other hand, the renal protective effect of liposomed SOD did not depend on the time of administration since efficacy was similar when given before reperfusion or before ischemia. The functional protective effect of liposomed SOD was similar to that of free SOD when they were given prior to reperfusion. Nevertheless, since histological damage observed with liposomed SOD was less than with free SOD, it is suggested that the liposomed galenic form may offer better protection against renal warm ischemia. In addition, liposomed SOD was better at preventing tissue prostanoid generation after renal warm ischemic-reperfusion injury than free SOD. We concluded that liposomed SOD shows a higher renal protective effect against warm ischemia than free SOD.

Key words SOD · Liposomed SOD · Renal warm ischemia · Renal malondialdehyde · Renal 6 keto PGF1 alpha · Renal TxB2

Introduction

Generation of oxygen free radicals (OFR) during reperfusion of the ischemic kidney causes lipoperoxidation of cellular membranes resulting in changes in cellular membrane permeability and altered mitochondrial function [8]. The main source of OFR is the endothelial cell but it has been suggested that leukocytes play an important role in ischemic-reperfusion injury through their adherence to endothelial cells induced by toxic mediators and also by OFR, followed by further production of OFR by these adherent leukocytes [2].

Superoxide dismutase (SOD) is a potent scavenger of superoxide radicals and its beneficial effect in renal ischemic injury has been demonstrated in animal experimental models [1, 7, 14]. Since SOD rapidly undergoes renal glomerular filtration, its half-life is short (6 min) and its maximal protective effect on renal damage induced by warm ischemia is obtained when it is given prior to reperfusion. The inflammatory response due to ischemia lasts several hours after reperfusion and OFR are released during all this time. Liposomed entrapped SOD has a half-life of 4 h and, therefore, theoretically, could offer better protection than free SOD does. In this study, the ability of liposomed entrappped SOD (lipo-SOD) to prevent organ damage due to renal warm ischemia was studied and compared with free SOD.

Materials and methods

Male Wistar rats weighting 250–325 g were used. Animals were acclimated in metabolic cages for 1 week prior to experimentation and fed standard rat chow and water ad libitum. Under intramuscular Ketamine anesthesia (75 mg/kg BW) laparotomy was performed and aorta, cava, and renal vessels were widely dissected. After the administration of n 500 units heparin, both renal pedicles were occluded using bulldog clamps, the abdominal cavity was closed, and the animal was placed in a warm cage for 60 min. After ischemia, the renal clamps were removed. After 15 min of reperfusion, right nephrectomy was performed and the kidney processed for conventional histology and for quantification of renal tissue levels of malondialdehyde (MDA), 6 keto PGF1 alpha, and TxB2. SOD and lipo-SOD were both given at 20 mg/kg endovenously through the vena cava or intracardiacly.

Sixty rats were studied and divided between two control groups: SALINE, isovolumetric saline ($n = 10$); LIPOSOME, isovolumetric liposome ($n = 7$), and five study groups: ISC SOD, SOD prior to ischaemia ($n = 8$); REP SOD, SOD prior to reperfusion ($n = 10$); ISC LSOD, lipo-SOD prior to ischaemia ($n = 9$); REP LSOD, lipo-SOD prior to reperfusion ($n = 9$); I-C LSOD, lipo-SOD 1 h prior to ischemia by intracardiac puncture ($n = 7$).

Prior to surgery and on days 1, 2, 3, and 7 after surgery, weight and serum creatinine levels per 100 g body weight (Cr/100 g) were determined. Also, prior to surgery and on days 2 and 7 after surgery, 24-h urine was measured and creatinine clearance per 100 g body weight (CrCl/100 g) was calculated. Blood was obtained from retroocular vessels throughout micropuncture. Creatinine levels were measured using Kodak Ektachem DT slides (Kodak Ektachem DTSC Module, Kodak, Inc.). On the seventh day post-surgery, rats were killed under ether anesthesia and the left kidney was processed for conventional histology.

Light microscopic study was blindy reviewed by a pathologist and eight tubulo interstitial parameters were considered. Abnormalities were graded using a semiquantitative scale graded from 0 to 3+. The histological score for each kidney was obtained from the sum of all these parameters. Polyunsaturated fatty acid peroxidation was determined by the thiobarbiturate reaction measuring the formation of MDA [13]. Tissular prostanoids were determined as 6 keto PGF1 alpha and TxB2, which are stable metabolites of PGI2 (prostacyclin) and TxA2 (thromboxane A2), respectively [5]. Results are expressed as mean ±SE. Statistical analysis was made using an analysis of variance test. Individual comparisons were made using the Fisher test.

Results

One rat from the SALINE group died of uremia. Initial and follow-up weight, initial diuresis, and initial renal function showed no significant differences between groups. Renal histology at 15 min was not different between groups.

On day 1, 24-h urine volume was significantly lower in both control groups than in the REP SOD, ISC LSOD, and REP LSOD groups. The REP LSOD group showed the highest 24-h urine volume compared with the remaining groups. On day 7, all groups showed similar 24-h urine volume. On day 2, the Cr/100 g was significantly higher in both control groups and in the ISC SOD and I-C LSOD groups than in the remaining groups. On day 7, all groups showed similar Cr/100 g. On day 2, CrCl/100 g was significantly lower in both control groups and in the ISC

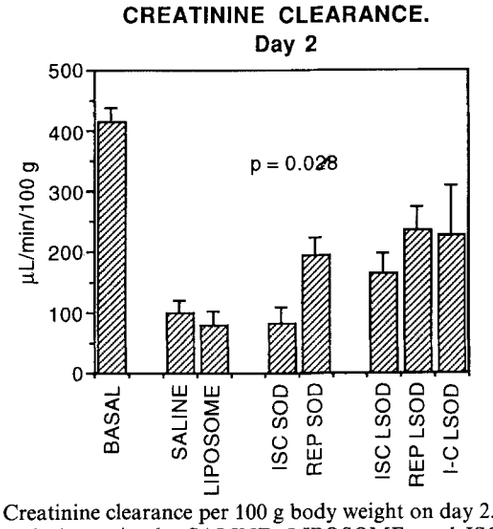

Fig. 1 Creatinine clearance per 100 g body weight on day 2. It was significantly lower in the SALINE, LIPOSOME, and ISC SOD groups than in the remaining groups

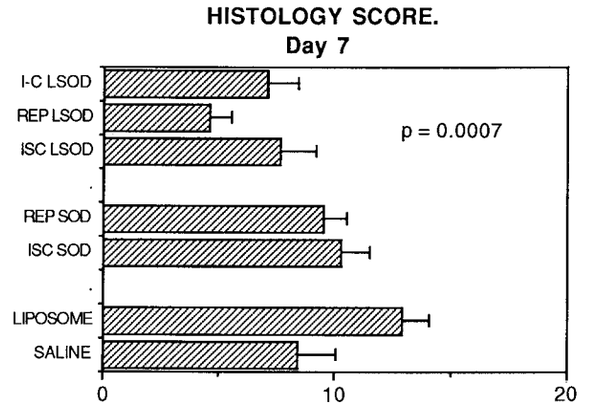

Fig. 2 Renal histological score on day 7. It was significantly lower in the REP LSOD group than in the remaining groups, and significantly higher in the LIPOSOME group than in the remaining groups

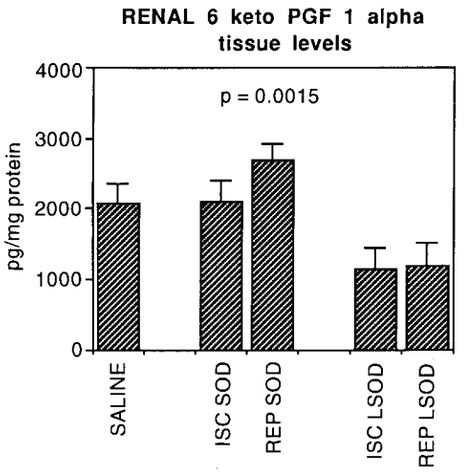

Fig. 3 Renal 6 keto PGF1 alpha tissue levels after 15 min of reperfusion. It was significantly lower in the ISC LSOD and REP LSOD groups than in the remaining groups

SOD group than in the remaining groups (Fig. 1). On day 7, CrCl/100 g was significantly lower in both control groups than in the remaining groups, and significantly higher in the REP SOD group than in the remaining groups.

Morphological studies showed a significantly lower mean score in the REP LSOD group than in the remaining groups. The LIPOSOME control group showed a significantly higher mean score than the remaining groups (Fig. 2). These differences were more pronounced with respect to tubular dilation, cellular necrosis, intratubular cell detachment, tubular cell brush

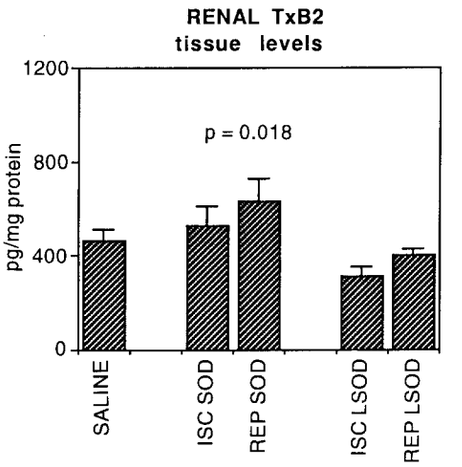

Fig. 4 Renal TxB2 tissue levels after 15 min of reperfusion. It was significantly lower in the ISC LSOD group than in the SALINE, ISC SOD, and REP SOD groups, and significantly lower in the rep LSOD than in the ISC SOD and REP SOD groups. The ISC LSOD and REP LSOD groups showed similar values (*P*, NS)

border integrity, edema, cellular infiltrate, and fibrosis (data not shown).

MDA tissue levels showed no differences between groups. Renal 6 keto PGF 1 alpha and TxB2 tissue levels in the ISC SOD and REP SOD groups were both similar to the SALINE group but were significantly higher than in the ISC LSOD and REP LSOD groups (Figs. 3 and 4).

Discussion

The superoxide anion is one of the most important oxygen free radicals produced at the time of reperfusion. SOD, a natural intracellular scavenger, accelerates the spontaneous dismutation of superoxide to hydrogen peroxide and oxygen and protects against OFR injury. The provision of exogenous SOD in preservation or reperfusion solutions has been evaluated in a number of experimental animal models including renal ischemia and transplantation with success [1, 7, 14].

Recently, some trials have been undertaken in human renal transplantation utilizing bovine SOD [11] or human recombinant SOD [9, 12] with poor results. Some reasons for the lack of efficacy of SOD in improving early posttransplant renal alograft function in these studies have been proposed. Too low dosage, too short intravenous infusion, or too long cold ischemia times have been considered as theoretical possibilities. Recently, SOD has been conjugated to polyethylene glycol [10] or entrapped in liposomes [4] in order to prolong its half-life with good results in preventing ischemic-reperfusion

injury. We hypothesized, therefore, that the use of liposomed SOD would result in improved renal function after warm ischemia.

Our results showed that the renal protective effect of free SOD on warm ischemic-reperfusion injury depends on the time of administration, being more effective when it is given just prior to reperfusion, as was expected. The renal protective effect of liposomed SOD on warm ischemic-reperfusion injury did not depend on the time of administration since it showed similar efficacy when it was given prior to reperfusion or prior to ischemia. Moreover, functionally, the protective effect of liposomed SOD was similar to that of free SOD when given prior to reperfusion. Nevertheless, since histological damage observed with liposomed SOD was less than with free SOD, we suggest that this galenic presentation could offer better protection for the kidney during warm ischemia.

During ischemic-reperfusion injury, phospholipase A2 becomes activated by lipid peroxidation and this enzyme preferentially removes oxidized fatty acids from membrane phospholipids but also promotes release of prostanoids from membranes [6]. It has been shown that SOD prevents both events in pancreas transplantation [6]. Our results failed to demonstrate differences in lipid peroxidation among study groups ascertained through MDA tissue levels. This was expected to some extent, since it has been pointed out that MDA formation is a late event in the lipoperoxidation of cell membranes [3], and our renal tissue was studied early in the reperfusion period. On the contrary, our results showed that liposomed SOD was better at preventing tissue prostanoid generation after renal warm ischemic-reperfusion injury than free SOD. We concluded that liposomed SOD shows a higher renal protective effect against warm ischemic-reperfusion damage than free SOD.

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