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COMPARATIVE ANTI-INFLAMMATORY ACTIVITY OF DIFFERENT SUPEROXIDE DISMUTASES AND LIPOSOMAL SOD IN ISCHEMIA

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Comparison of superoxide dismutases from different sources with respect to biological activity in the rat tourniquet poditis model shows that anti-ischemic activity is very variable although all the enzymes have the same specific enzymic activity. Both bovine Cu-SOD and *E. coli* Mn-SOD have excellent properties whereas yeast Cu-SOD and the homologous rat Cu-SOD show zero activity. The results confirm earlier demonstrations that (1) "All superoxide dismutases are equal but some are more equal than others", (2) at the dose levels used (compatible with possible clinical use) homologous enzyme is inefficient and hence human Cu-SOD may not be effective in humans, (3) liposomal encapsulation of bovine Cu-SOD greatly enhances biological efficacity, provides a slow release mechanism of the enzyme and provides a powerful drug for the treatment of ischemic injury.

KEY WORDS: Superoxide dismutase; liposomes; poditis; ischemia; human SOD

INTRODUCTION

We have previously compared the anti-inflammatory activities of superoxide dismutases isolated from different sources using three current models in the rat: carrageenan induced inflammation[1], adriamycin edema[2] and adjuvant polyarthritis[3]. In all cases large differences among different SODs were observed and indeed homologous rat Cu-SOD has zero activity. This and other considerations led us to predict[4-6] that human SOD would not be particularly active in humans but could be useful for veterinary applications[5]. A general mechanism of the anti-inflammatory activity of certain (but not all) SODs has been presented[6]. This mechanism also explains in part the increased efficiency of liposomal bovine Cu-SOD compared with the free enzyme (other considerations such as pharmacokinetics, organ localisation and general distribution are of importance). In the long term (30–90 days) polyarthritis model the liposomal form shows a very marked superiority to the free SOD, and to a lesser extent this is also seen in the adriamycin (5 days) test. With respect to the rapid (5 hr) carrageenan induced inflammation model liposomal SOD had no effect at the dose rates used (33 μg/kg of encapsulated SOD). This is readily explained since liposomal

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forms of SOD provide a slow SOD release mechanism and insufficient enzyme is liberated within the few hours of the test. Direct testing of the liposomes employed in these studies (350 nm diameter) shows only a small percentage of the total SOD activity, essentially due to the outer layers of the multi-lamellar structure[7], and thus the liposomes cannot be directly effective.

The role of free radicals in ischemic and post-ischemic conditions has received considerable attention in recent years. It is therefore of interest to compare the relative efficiencies of certain SODs and of liposomal bovine Cu-SOD in a suitable animal ischemic test. Although many such models for cerebral, cardio, kidney or other organ ischemia exist[7] we have chosen the most simple, tourniquet poditis in the rat[8], to facilitate comparative studies. This model is rapid, easy, highly reproducible and does not lead to death of the animal. In addition the technique provides an excellent approach to treatment of the effects of reduced or arrested circulation in the extremities.

MATERIAL AND METHODS

Male Wistar AF gnotoxenic rats of 300 $\pm$ 20 g were used. Ten rats were employed as control as well as for each test. Since pain level is relatively high, in order to avoid savage internecine attacks the rats should not be grouped.

The tourniquet poditis experimental pedal inflammation model in rats[8], used to test anti-inflammatory drugs such as indomethacin, phenylbutazone or aspirin, depends on the edema produced by ligation of the tibio-tarsal articulation. A moderately tightened double knotted tourniquet (about 10 cms long) us applied just above the articulation of the left hind paw of the rats. This provokes a severe ischemia in the distal part of the leg. The tourniquet is kept in place for 2 hr then removed. Within a few minutes an important edema with swelling develops, reaching a maximum at about 3 hr. This slowly decreases over several days. Two treatment protocols were used. In the first, the SODs at 33 μg/kg were injected intraperitoneal 30 min before removal of the tourniquet whereas in the second, pretreatment at the same dose rates (i.p.) was applied 24 hr before the beginning of ischemia. Plethysmometric measurements at zero time (just before induction of the ischemia) at 3 hr and at 24 hr after removal of the tourniquet were performed as described earlier[1].

The superoxide dismutases and liposomal SOD were prepared as previously described[1].

RESULTS AND DISCUSSION

Studies of experimentally induced intestinal ischemia in cats[9,10], in dogs[11] and in rats[12] has shown that in general SOD protects the bowel against reperfusion damage. In the case of rats, increased survival with decreased necrosis and perforation was observed. Much of the injury occurs during reperfusion probably because xanthine dehydrogenase is converted to xanthine oxidase and hypoxanthine accumulates during ischemia as a consequence of ATP catabolism.

Ischemic damage is reduced by SOD in the hearts of dogs, rabbits and cats[13–16]. Myocardial reperfusion injury in dogs is decreased by infusion of SOD (5 mg/kg)

before reperfusion, suggesting that primary myocardial cell damage due to ischemia is additive to that during the early phase of perfusion. Infarct size (90 minute occlusion of the left circumflex coronary artery) in the SOD group was significantly less than in controls[13]. Similarly, i.v. injection of bovine Cu-SOD (~10 mg/kg) immediately before ligation of the left coronary artery in rats caused increased survival (41% against 25% in controls at one week). If performed 30 min after the operation no effect was observed[17]. It may also be noted that acute myocardial ischemia induced in cats by ligation of the coronary artery causes more than 50% reduction of SOD activity in the ischemic area[18]. In rabbits with experimental myocarditis, SOD levels in the heart were also below normal[19].

Post ischemic renal lesions in rabbits are reduced, and functional impairment improved by SOD[20,21].

Cerebral ischemia in rats, as with ischemia in other organs, is connected with blood flow disturbances associated with altered granulocyte-endothelial interactions in the microvasculature[22]. It is possible that this polymorphonuclear neutrophil-endothelial interaction would be reduced by SOD.

To summarise with respect to free radical mechanisms, at least three factors are involved in ischemic induced injury. These are (1) decreased SOD activity in the ischemic tissue. (2) production of O_2^- by transformation of xanthine dehydrogenase to the oxidase and accumulation of hypoxanthine, (3) increased granulocyte interaction with endothelial membranes with liberation of O_2^- and reduced circulation. Damage occurs during the ischemic phase particularly with respect to lipid peroxidation and during reperfusion. The latter, probably of greater importance in most cases, is more strongly based on a superoxide (and secondary radical) toxicity. A combined superoxide dismutase plus glutathione peroxidase enzymotherapy may resolve these problems to a large extent.

While the above mentioned models are certainly useful, they are not readily applicable to comparative studies (with a statistical significance) of relative efficacity of treatments. Reproducibility is often poor, and with larger animals such as dogs the studies reported in this communication (a total of 110 animals were used) would require a considerable logistic investment. The rat tourniquet poditis model is simple, quantitative and with a relatively small variation in the results. Apart from the value of this test with respect to ischemia of the extremities, it also gives a useful indication of the probable efficacity of similar treatments applied to more complicated models. In a general sense, the tourniquet model is equivalent to clamping circulation in any zone and could thus provide results useful with respect to reduction of reperfusion effects consequent to many surgical interventions.

Results of decrease in edema response by treatment with various SODs are given in Tables I and II. It may be noted that dose rates (33 μg/kg) are far removed from those used in all other studies of ischemia (5–10 mg/kg) and are about 200 times less. Negative effects or reduced efficacity of high doses of SOD have been discussed elsewhere[7] and it is probable that optimal amounts (with respect to dose response effects) are even less than 33 μg/kg for *E. coli* Mn-SOD.

The results of treatment 30 min before the end of ischemia (single i.p. injection) clearly show an order of efficiency for the different SODs studies. Mn-SOD from *E. coli* is totally effective both at 5 hr (i.e. 3 hr after recirculation) and at 24 hr. Bovine Cu-SOD is also highly efficient at both time periods whereas human Cu-SOD shows a significantly lower activity. Yeast Cu-SOD is slightly effective at the first phase but is without action at 24 hr, and the homologous rat Cu-SOD has zero activity both at

TABLE I
% Change in Volume

SOD 33 μg/kg i.p.		% Change in volume at 5 hr ± SD	% Change compared with controls	t	P <
		Treatment 30 min before recirculation			
Controls	5 hrs	+ 36.1 ± 4.3	–	–	–
	24 hrs	+ 33.9 ± 6.2	–	–	–
E. coli Mn-SOD	5 hrs	+ 1.25 ± 2.0	– 96.5	22.2	0.001
	24 hrs	– 5.9 ± 4.2	– 117.4	15.8	0.001
Liposomal bovine Cu-SOD	5 hrs	+ 20.3 ± 3.3	– 43.6	8.8	0.001
	24 hrs	– 3.1 ± 2.2	– 109.2	16.7	0.001
Controls	5 hrs	+ 40.5 ± 3.9	–	–	–
	24 hrs	+ 37.3 ± 5.5	–	–	–
Bovine Cu-SOD	5 hrs	+ 6.3 ± 4.3	– 84.6	17.8	0.001
	24 hrs	+ 2.3 ± 4.3	– 93.9	15.1	0.001
Human Cu-SOD	5 hrs	+ 12.3 ± 4.3	– 69.8	14.6	0.001
	24 hrs	+ 16.5 ± 3.3	– 55.8	9.8	0.001
Yeast Cu-SOD	5 hrs	+ 25.1 ± 4.2	– 38.0	8.1	0.001
	24 hrs	+ 36.2 ± 3.2	– 2.9	0.5	NS
Rat Cu-SOD	5 hrs	+ 35.2 ± 4.4	– 13.1	0.3	NS
	24 hrs	+ 34.2 ± 6.2	– 8.1	1.1	NS
		Pretreatment 24 hrs before ischemia			
Liposomal bovine Cu-SOD	5 hrs	+ 2.3 ± 4.4	– 94.4	19.6	0.001
	24 hrs	+ 3.2 ± 2.5	– 91.4	16.9	0.001
E. coli Mn-SOD	5 hrs	+ 12.2 ± 6.7	– 69.8	11.0	0.001
	24 hrs	+ 30.3 ± 10.1	– 18.7	1.8	0.10
Bovine Cu-SOD	5 hrs	+ 35.3 ± 3.2	– 13.0	0.3	NS
	24 hrs	+ 36.4 ± 4.5	– 2.2	0.4	NS

n = 10 in all cases.

TABLE II
Order of "anti-ischemic" activity. Treatment with SOD 30 min before restoration of the circulation (A) and pretreatment 24 hr before initiation of ischemia (B).

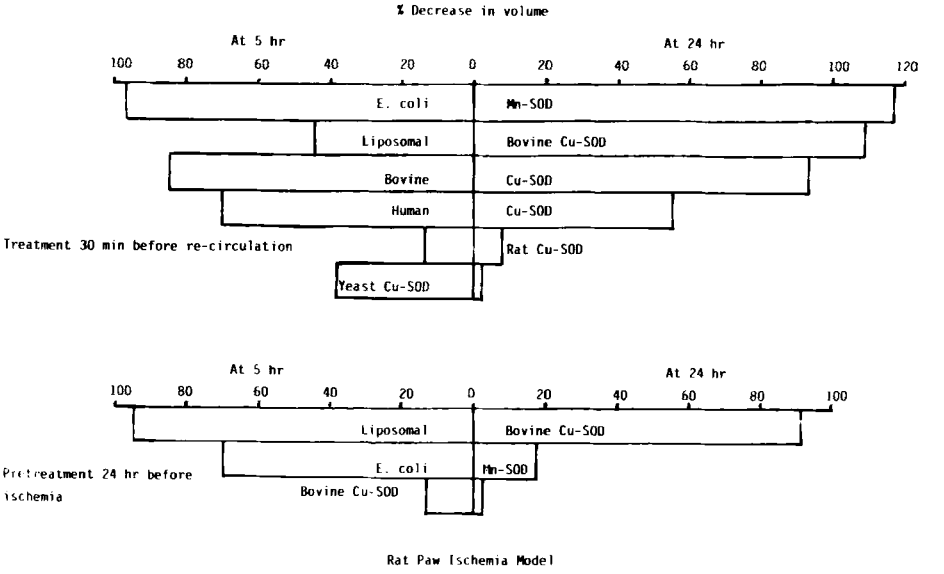
SOD 33 μg/kg i.p.	%Decrease in volume at 5 hr	%Decrease in volume at 24 hr
A. E. coli Mn-SOD	– 96.5	– 117.4
Liposomal bovine Cu-SOD	– 43.6	– 109.2
Bovine Cu-SOD	– 84.6	– 93.9
Human Cu-SOD	– 69.8	– 55.8
Yeast Cu-SOD	– 38.0	– 2.9
Rat Cu-SOD	– 13.1	– 8.1
B. Liposomal bovine Cu-SOD	– 94.4	– 91.4
E. coli Mn-SOD	– 69.8	– 18.7
Bovine Cu-SOD	– 13.0	– 2.2

the early and late stages. Liposomal bovine Cu-SOD is not very active at 5 hr (i.e. 3.5 hr after injection) because of the short time interval and the slow release of SOD, but is highly efficient at 24 hr. Since the five hr results represent a transitory phase and the values at 24 hr a more stable situation resulting from a single injection of SOD a realistic classification is *E. coli* Mn-SOD, bovine Cu-SOD and the liposomal form as excellent, human Cu-SOD as moderate while both yeast and rat Cu-SOD have zero activity.

To test further the relative long term effects of a single i.p. injection a series of animals were pretreated 24 hr before initiation of ischemia. As shown in Figure 1, the effects of bovine Cu-SOD are totally lost at 29 hr (24 plus 5), the effects of *E. coli* Mn-SOD are decreased at 29 hr and are lost at 48 hr whereas liposomal bovine Cu-SOD is highly efficient even 48 hr after administration. The superiority of the liposomal form is thus amply demonstrated.

CONCLUSIONS

Large variations in the "anti-ischemic" activity of SODs from different sources (but all with the same enzymic activity) seen in the rat tourniquet poditis model confirm and are in complete agreement with results using other inflammation models. The exceptional properties of *E. coli* Mn-SOD and of liposomal bovine Cu-SOD are once again evident. Human Cu-SOD is relatively poor as a drug and both yeast and the homologous rat Cu-SODs have zero activity. These results suggest that human Cu-SOD will not be effective for the treatment of ischemic conditions in humans at least at dose rates similar to those used in this work. At very high levels (1000 fold



greater) secondary effects may become of importance as we have discussed elsewhere[6,7] and the dangers of excessive amounts of SOD cannot be neglected.

The possible use of *E. coli* Mn-SOD for the treatment of human pathological conditions is under development. In the meantime the present studies strongly indicate clinical application[23,24] of liposomal bovine Cu-SOD not only to ischemic conditions but also to more general circulatory problems.

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