Title: THE EFFECT OF SUPEROXIDE DISMUTASE AND CATALASE ON FOCAL CEREBRAL ISCHEMIA.

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Introduction. Stroke affects 500,000 Americans per year, is the third leading cause of death and complicates their anesthetic and surgical management. Excessive free radical production may play a part in exacerbating cerebral injury. During ischemia, it is hypothesized that the enzyme xanthine dehydrogenase (XDH) is converted to xanthine oxidase (XO) by proteolysis or by intramolecular sulfhydryl oxidation. Concurrently, ATP is catabolised to hypoxanthine during the period of ischemia. At the time of reperfusion, XO in combination with molecular oxygen and hypoxanthine generates toxic oxygen metabolites which cause further injury. We investigated the protective effects of the free radical scavenging enzymes - superoxide dismutase (SOD) and catalase - in a model of focal cerebral ischemia in the rat. To increase the circulating half-lives of SOD and catalase beyond the 6 min half-life of the native proteins, they were covalently conjugated to polyethylene glycol (PEG).

Method. Male Long-Evans rats (250-300 g) were anesthetized with ketamine (100 mg/kg IP) plus xylizine (2 mg/kg, IP) and randomly assigned to receive either PEG-SOD plus PEG-catalase (10,000 U each/kg) or equivalent amounts of inactive enzymes, given intravenously. The right middle cerebral artery (MCA) was exposed at the level of the rhinal fissure and then ligated with 10/0 silk suture. Common carotid arteries were occluded for 90 min with atraumatic clamps. Infarct volume was calculated from the cross-sectional areas of eight 2 mm thick coronal sections that remained unstained after incubation with 2% triphenyltetrazolium chloride (TTC) in phosphate buffered saline (pH 7.4).

Results. Occlusion of the right MCA together with 90 min carotid occlusion, yields a cortical infarct restricted to the MCA territory with a volume of 189 ± 18 mm^3 ($n = 9$). Infarct volume was reduced by 30% in rats receiving active PEG-superoxide dismutase and PEG-catalase (132 ± 9 mm^3; mean ± SEM; $n = 18$) when compared to the inactive enzyme group (188 ± 9 mm^3; $p < 0.01$, $n = 18$).

We examined the time course of conversion of xanthine dehydrogenase to oxidase following occlusion of the MCA and carotid arteries (Table 1). Using a sensitive flurometric assay, total xanthine dehydrogenase and xanthine oxidase activity was 0.65 ± 0.07 mU g^{-1} cortex in control and ischemic rat brain. By 3 hr after release of carotid arterial clamps, the fraction of xanthine dehydrogenase converted to the free radical-producing xanthine oxidase increased by 12% in the MCA territory of the right cortex and 5% in the left cortex. This increased by 15% in the right cortex after reperfusion for 24 hrs. Rat plasma contained 6.5 ± 0.8 mU ml^{-1} of XDH and XO activity of which 92% was in the free radical-producing oxidase form.

Table 1. Comparison of xanthine oxidase and dehydrogenase with time in ischemic rat brain following removal of arterial clamps. Total xanthine dehydrogenase plus oxidase is shown as **XO + XDH**,(mU g^{-1} cortex)while the fraction of total activity in the free radical-producing form is shown as **% XO**.

	% XO		XO + XDH	
	Right Cortex	Left Cortex	Right Cortex	Left Cortex
Control	20%	23%	0.60	0.53
ReperfusionTime				
0 hr	25%	25%	0.67	0.66
3 hr	32%	26%	0.65	0.65
24 hr	35%	25%	0.48	0.50
Plasma	92%		5.68	

Discussion. PEG-SOD plus PEG-catalase reduced infarct volume in rats undergoing MCA-carotid occlusion. Since the catalytically inactive enzymes provided no protection, this protection must have resulted from scavenging of O_2- and H_2O_2. Proteins do not readily cross the blood-brain barrier, though we have shown that PEG-SOD and PEG-catalase are taken up slowly by endothelium. Thus, PEG-SOD and PEG-catalase would be expected to principally interact with the brain microvasculature, suggesting that the microvasculature may be both a key site of injury as well as a source of oxygen radicals.

The increased XO activity in the right cortex may be due to infiltration of plasma XO as the blood brain barrier becomes more permeable. Normally plasma concentrations of hypoxanthine are too low to produce toxic oxygen metabolites, but during cerebral ischemia, hypoxanthine formed by ATP degradation is released into microvessels. The amount of xanthine oxidase formed in microvessels of ischemic right cortex can generate 10 and 24 μM min^{-1} of O_2- and H_2O_2 respectively, more than sufficient to destroy barrier function in cultured endothelial cells.