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THERAPEUTIC USAGES OF OXYGEN RADICAL SCAVENGERS IN HUMAN DISEASES: MYTHS AND REALITIES

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Some distinct advances in pharmacologic manipulation of oxygen radical scavengers have been made which could ultimately greatly enhance the use of these reagents as drugs, as well as some innovative techniques for drug delivery. Unfortunately, most of the therapeutic reports in the literature, almost all of which are based on usage of standard (native) SOD and/or catalase, are still anecdotal and/or uncontrolled. A review of the human disease/treatment literature suggests that further tightening of the scientific design of such trials is still badly needed; hopefully better experimental design will be applied when products such as PEG conjugates or genetically engineered polymers are ready for testing.

KEY WORDS: Superoxide dismutase, catalase.

INTRODUCTION

The list of mammalian disease processes in which oxygen radicals are being implicated continues to grow rapidly; my tabulation published in 1983[1] of nearly such 30 pathologic processes has been supplanted by new lists more than twice as long.[2] The widening scope of potential therapeutic usages for oxygen radical scavengers, along with new developments in the technology for mass production of human proteins, methods for manipulation of enzyme pharmacology, and identification of new classes of scavengers, has continued to fuel the search for evidence of therapeutic usefulness in human diseases.

In a prior review,[3] I discussed the problem of an appropriate control for studies of SOD therapeutic efficacy. It was suggested that inactivated SOD (exposure to hydrogen peroxide at alkaline pH) would be an ideal control substance for the placebo arm of a study, since this would unequivocally distinguish oxygen scavenging from any other possible non-specific protein effect. Flohe has attacked this idea[4] on the grounds that inactivated SOD "proves notoriously unstable" and that "[it] would never survive the obligatory stability testing and subchronic animal safety studies." Although it would indeed take a great deal of time and effort to get preliminary approval to use inactive SOD in humans, this should not preclude such controls in animals studies or in vitro experiments, nor does it justify anecdotal studies with no controls at all. Alternatively, the control or placebo groups could be treated with extremely low doses of the scavenger being tested, e.g. conjugated SOD; it is generally accepted that a dose below 5% of the active treatment arm is equivalent to a placebo. Even albumin, readily available for human use, is a better control than saline.

PHARMACOLOGY OF SOD AND CATALASE

SOD continues to be sold in health food stores in tablet form despite the florid irrationality of such a preparation. When ^{65}Zn-labelled SOD was fed to mice by oral gavage, 90% of the ion came out in the feces, and the remainder was almost certainly free metal which became separated from the protein.[5] No discernible rise in blood or hepatic SOD activity could be demonstrated after feeding the tablets. The medical use of SOD continues to depend on either local instillation, controlled parenteral systemic delivery, and/or development of conjugates with enhanced survival features.

The use of PEG-conjugation to lengthen the half-life of scavenging enzymes has attracted substantial attention. Mossman *et al.*[6] administered PEG-coupled catalase to rats using an osmotic pump implanted subcutaneously. Both lung and serum levels of catalase increased in a dose-related manner without altering the normal physiology of the tissues and was not in and of itself inflammatory. Beckman *et al.*[7] added radiolabelled PEG conjugates of SOD or catalase to cultures of porcine endothelial cells; enzyme activity as well as radioactivity were readily assimilated by the cells in sufficient concentration to afford the cells protection against xanthine oxidase induced damage. The authors proposed that the PEG conjugation reaction protected the proteins from proteolysis in the medium.

Another approach to half-life extension was reported by Schalkwijk *et al.*[8] The proteins were made cationic by amidation of free carboxyl groups; they retained their catalytic activity, had the same molecular weight as the parent protein, had cationic IEPs, and when injected into mouse joints, were cleared more slowly than native molecules. Amidated catalase and horseradish peroxidase were found to have measurable anti-inflammatory effects in a mouse arthritis model, whereas amidated SOD was inactive. The cationic proteins were presumed to stick tightly to the fixed anions of cartilage and other joint tissues, which might have benefits with regard to local treatment of joint disorders.

A third approach to SOD half-life prolongation was reported by Hallewell *et al.*[9] using techniques of molecular biology. These authors genetically engineered a series of SOD polymers; one consisted of two SOD single chains joined end-to-end, and the other was constructed of two SOD monomers linked by an IgA hinge sequence. Both preparations showed high specific activity, and the latter formed large (up to 750,000 MW) polymers which were thermostable and soluble, had well preserved enzymatic function, and showed an extended half-life in rats of 145 min. Obviously, this approach offers the most exciting (as well as the most elegant) potential for development of oxygen scavenger protein preparations for human use.

Finally, a fourth method of conjugating SOD by formation of a copolymer with pyran (divinylether maleic acid) was reported.[10] The resultant polymer showed a very prolonged serum half life and protected mice from potentially fatal influenza virus infection.

Fortunately, the safety data for SOD continues to be quite favorable. The agent has been administered safely to human neonates with minimal evidence of local allergic hypersensitivity, systemic allergy, or toxicity towards hematologic, hepatic, or renal systems.[11] In a recent study involving joint injections, several patients experienced minor local irritation and/or itching.[12]

PULMONARY DISEASE AND OXYGEN TOXICITY

Bronchopulmonary dysplasia (BPD) is a complication of intensive respiratory therapy in low birth weight neonates receiving high concentrations of oxygen under pressure; such infants are also subject to retinopathy, nervous system hemorrhage, and bowel disorders. Rosenfeld *et al.* conducted a randomized, blinded trial of subcutaneous bovine SOD in 45 neonates.[13] By a variety of parameters, the SOD trial appeared to have been most effective. Detectable serum SOD levels were noted 2.5 hr after injection, persisting for several hours, and no toxicity was observed. Overall, the trial seemed to be quite encouraging.

It is somewhat surprising that SOD treatment of BPD has not gained greater acceptance. The major trend amongst neonatologists, of course, is prevention by avoidance of excessive oxygen exposure rather than drug prophylaxis and/or treatment. However, there are also some conceptual problems in the Rosenfeld approach. One is the disparity between their observations of detectable circulating SOD for several hours vs. the very short half-life of SOD expected from the rat studies. Secondly, it is hard to visualize SOD in serum effectively trapping radicals at the level of pulmonary epithelial (or endothelial) cell, let alone intracellularly.

Two other pulmonary conditions with pathophysiologic features suggesting a potential therapeutic role for SOD have been so treated. Decompression sickness is characterized by air embolism to the lungs, and superoxide generation from PMNs has been implicated in the disorder. Disappointingly, neither SOD nor SOD plus catalase proved effective in dogs subjected to simulated diving conditions.[14] Infusion of SOD combined with high doses of ascorbic acid and vitamin E failed to save a young woman's life after suicidal paraquat poisoning.[15]

CENTRAL NERVOUS SYSTEM

Since hyperbaric oxygen toxicity often causes seizures prior to death, SOD was tested as a potential protective agent. No beneficial effect was noted in rats pretreated with intraperitoneal SOD and then exposed to 100% oxygen at 5 atmospheres.[16] In another study from a different group,[17] SOD again failed, although a weak effect from catalase was noted. Since systemic administration of native proteins would not be expected to substantially enhance brain tissue levels, Yusa *et al.* [18]used liposome encapsulation to augment tissue levels, with a much more impressive effect. SOD delivered via liposomes also protected rats against brain edema and vascular permeability changes induced by cold injury to rat brains,[19] a situation in which free SOD once again failed.

With the advent of the hypotheses invoking ischemia as a cause of free radical mediated tissue damage, a new viewpoint on diseases of many organ systems, especially the CNS, becomes apparent. Using a rabbit model of ischemic damage to the spinal cord, Cuevas *et al.*[20] reported a decreased incidence of paralysis and motor neuron damage when SOD was present during reperfusion. As with the cardiac studies, applicability of these findings to human disease, especially traumatic spinal cord injury, will depend on clarification of the true role of ischemia and on the ability to deliver the proper scavenger to the affected site within the time frame of maximal tissue damage, a set of obstacles not readily overcome.

Treatment of human neurologic disease with SOD has been quite limited. Put[21] claimed to have treated 684 patients in one year with daily or thrice weekly IM

injections of catalase for a variety of ill defined syndromes characterized by cervical or lumbar pain attributed to chronic spinal disk degeneration. There is no known pathophysiologic justification for invoking a role for H_2O_2 in such disorders. Although the study was supposedly randomized, it was unblinded, a treacherous flaw when studying disorders exceedingly prone to the placebo effect of daily injections. The author concluded that IM catalase injections accelerated the recovery from these disorders.

Lund-Olesen, who by his own admittance has injected over 4000 ampules of SOD into skin, joints, spinal canal, veins, arteries, and muscles, reported non-scientifically that intrathecal SOD was beneficial for multiple sclerosis.[22] The only scientifically valid study of SOD treatment for neurologic disease is that of Stern *et al.*[23] in subjects with Duchenne's muscular dystrophy. In this multicenter, randomized, double-blind trial, 51 patients were studied for 18 months using the best available objective criteria for that disorder. No consistent improvement in muscle strength, functional status, or biochemical markers of the disease could be proved. As usual, the treatment proved innocuous.

NON CARDIAC ISCHEMIA

Sagi *et al.*[24] created standardized flaps in rats, allowed them to remain ischemic for 10 hr, and then reimplanted them using treatment regimens that included either perfusion with heparinized Ringer's lactate and/or SOD. The scavenger treatment significantly enhanced and prolonged the survival of the flaps, above and beyond a mild effect seen from buffer perfusion alone. Once again, the potential for pretreatment of previously ischemic tissues with SOD before restoration of circulation gains some validity.

Another target organ of great importance with regard to human disease is the vascular supply of the intestinal tract, e.g. mesenteric and splanchnic arteries. Bitterman *et al.*[25] produced a vascular occlusion model in rats (40 min of ischemia) such that severe shock developed. Groups of animals were treated with recombinant human SOD (Grunenthal) at the time of reperfusion. A variety of parameters were assessed (postreperfusion blood pressure, various plasma factors, survival), and the SOD treatment once again proved to be beneficial.

Three French patients[26] were found at surgery to have severe abdominal vascular occlusion; prior to planned surgical resection, SOD was injected intramuscularly. In all 3 cases, the authors report return of intestinal viability (normal color, peristalsis, etc.) without the need for resection.

A model of disseminated intravascular coagulation was established in rats using endotoxin infusion,[27] and the potential effects of subcutaneous SOD or catalase injections were tested. At extremely high doses, both scavengers were effective in mitigating not only the changes in the coagulation proteins, but also measurable tissue damage (fibrin deposition in glomeruli).

NON-VASCULAR GASTROINTESTINAL DISORDERS

Only two anecdotal, European case report studies have been published. In an abstract, Emerit *et al.*[28] reported successful therapy of several cases of Crohn's disease

of the intestine or post-radiation bowel necrosis using lipsome encapsulated SOD. In a report from Romania,[29] 12 patients with duodenal ulcer said to be resistant to conventional therapy were treated with a hitherto unused regimen, *viz.* direct injection of scavenger into the tissue surrounding their ulcers via an endoscope. The product used was Epurox, a mixture of human SOD and catalase. The results varied: 4 patients were dramatically improved, 5 had partial relief, and three needed surgery within a few days. The authors used their results to suggest that free radicals play a role in causing the pain of ulcers. If ever there were a disease (other than arthritis) that needs carefully designed and controlled clinical trials, it is ulcer pain.

GENITOURINARY TRACT DISORDERS

The use of SOD for chronic cystitis has a long history, going back to at least 1974. In a preliminary review of human treatment regimens with SOD, Beckmann and Flohe[30] stated that chronic cystitis was especially amenable to SOD trials for two reasons: the ability to design controlled clinical trials, and the ease with which the active agent can be injected into the site of pathology, in this case meaning the wall of the bladder as visualized through the cystoscope. They then report uncontrolled data from 32 patients, assessed by various objective parameters but without any placebo or cross-over treatments, indicating a generally beneficial effect. Similar results were reported from Germany.[31] Flohe[4] contends that controlled trials would be unethical in that an effective therapy would be denied to the placebo group; crossover designs are readily available which obviate this concern.

The rationale for treatment of human disease with SOD is sometimes based solely on the fact that certain disorders are resistant to all known therapies, hence SOD is offered a chance. There is no better example than Peyronie's disease, a chronic inflammatory condition of the penis characterized by induration and fibrosis whose etiology and pathogenesis are most perplexing. Naturally, a *post hoc* hypothesis based on inflammatory processes can be constructed to justify such trials. SOD has been extensively used for this disorder, either by direct local injection into fibrotic plaques[32] or by an innovative iontophoresis technique,[33] and once again, encouraging results from uncontrolled trials are reported.

OCULAR DISORDERS

Three types of eye disease in animals have been treated with SOD: canine cataract, alkali burns, and immune mediated degeneration. Although there had been some initial enthusiasm for the use of SOD in senile canine cataract, especially since SOD (as Orgotein) is well known to veterinarians, two studies involving multiple animals treated with injection into the anterior chamber of the affected eye were negative.[34,35]

The study by Nirankari *et al.*[36] on alkali burns in rabbits is notable for the fact that heat inactivated SOD was used for the control animals. Rabbits were subjected to a standardized corneal lesion and then treated with subconjunctival injections of native or inactivated SOD; additional animals received ascorbic acid or glutathione. Ascorbate and native SOD were both beneficial in this model.

Guy *et al.*[37] sensitized guinea pigs by injection of homologous spinal cord, thereby producing an immune optic neuritis. Catalase, but not SOD, given systemically, had

a mild protective effect on disk edema measured morphometrically. In a somewhat analogous model, rats were injected with an extract of normal lens combined with adjuvant, such that an inflammatory uveitis developed.[38] Systemic treatment with SOD was reported to have lessened the severity of the inflammatory and vasculitic changes.

MISCELLANEOUS CONDITIONS

Treatment with liposome encapsulated SOD was reported to be of substantial benefit in Kawasaki's disease, even reducing the incidence of coronary artery complications.[39] Behcet's disease is even more mysterious, and not surprisingly, IM SOD has been tried there as well, with supposed good results;[40] a rationale for the treatment based on increased radical generation by neutrophils from such patients has been adduced as well.

SOD has been invoked in the oncology literature in two connotations: as a potential ameliorator of the side effects of anti-cancer treatments, either radiation or chemical, and as a potential direct anti-neoplastic agent. In the former area, there have been no major new developments since Petkau reviewed the field in 1986.[41] In the latter regard, Oberley *et al.*,[42] responding to the observation that SOD had no effect in rodent tumors which might have been attributed to lack of cell penetration, tested a variety of low molecular SOD mimics for antineoplastic activity; none was found.

CONCLUSION

The major developments in the SOD therapy field have been pharmacologic; documentation of clinical efficacy with the preparations already approved for use, primarily native bovine SOD, has lagged far behind. If the polymers and genetically engineered proteins can provide greater tissue penetration for longer periods with no loss of scavenging effectiveness, then greater therapeutic potential may someday be realized. The ultimate limitation, however, depends on whether or not oxygen derived free radicals actually are pathologic in human diseases, and this has yet to be proven by a means independent of what happens when scavengers are administered.

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