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Editorial

SOD, oxidative stress and human pathologies: a brief history and a future vision

Abstract

Superoxide dismutase (SOD) has now been known for 35 years. While the superoxide radical and SOD have been implicated in many disease states including inflammatory diseases, diseases of ischemia and reperfusion, neurodegenerative diseases, and cancer, as well as more subtle roles in cell signaling and perhaps in immune function, SOD is not yet in widespread usage in human clinical medicine. One obstacle has been that none of the three human SODs possesses attractive pharmacological properties to make it a clinically useful therapeutic agent. These problems may be overcome either by the design of SOD-mimetic drugs or by genetically re-engineering the human SOD genes to produce SODs with more desirable and controllable properties for human clinical usage. A second obstacle has been the fact that a delicate *balance* is involved between superoxide and SOD. Produced in proper amount, superoxide is a normal and useful metabolite, serving important roles as a signaling molecule in processes such as cell division, and even serving to act as a terminator of lipid peroxidation. When flagrantly overproduced, however, the radical can initiate lipid peroxidation, protein oxidation, and DNA damage, leading to cell dysfunction and death by apoptosis or necrosis. It is these paradoxical properties that complicate the precise restoration of optimal balance between superoxide and SOD when that balance has been upset by injury, disease, or aging.

Keywords: Superoxide dismutase; Lipid peroxidation; Dose–response; Oxidative stress

1. Introduction

Since the discovery of the superoxide dismutases (SODs) 35 years ago [1], more than 42,000 publications have appeared on the subjects of the superoxide radical ($O_2^{\bullet-}$) and the enzymes that catalyze its dismutation. The annual rate at which these papers appear continues to accelerate. The radical and/or the SODs have been implicated in a broad range of disease states including inflammatory diseases [2,3], diseases of ischemia and reperfusion injury [4], neurodegenerative diseases [5], diabetes [6], cancer [7], and many others. Despite this explosion of interest in the pathophysiological roles of superoxide, we have not yet seen the widespread clinical use of therapies based on superoxide-scavenging mechanisms, whether by use of SOD as a drug, or by SOD-mimetics, or by stoichiometric scavengers of the radical. There are multiple reasons for the paucity of antioxidant-based therapies. Oxidative stress, per se, is not viewed to be a disease, but rather a component of many diseases. Diabetes, for example, is clearly associated with significant levels of oxidative stress [6], yet few American diabetic patients are advised to supplement with any form of antioxidants; nor are biochemical markers of oxidative stress monitored in such patients. Most hospital laboratories do not provide tests for any markers of oxidative stress, so physicians cannot request these tests. Few, if any, major pharmaceutical companies have drug discovery programs aimed at oxidative stress.

The purpose of the *Third International Conference on Superoxide Dismutases: Recent Advances and Clinical Applications* held at the Pasteur Institute in Paris, June 10–11, 2004, was to assess our current position regarding clinical applications of SOD and SOD-related therapies, and to bring into focus both the advances that have been made and the obstacles that remain. The papers that follow were presented at this Conference, and reflect the great progress that is being made in this arena.

2. SOD: a brief history

The SODs are a family of enzymes that very efficiently catalyze the dismutation of the superoxide radical anion ($O_2^{\bullet-}$):

$$O_2^{\bullet-} + O_2^{\bullet-} + 2H^+ \rightarrow H_2O_2 + O_2$$

The discovery of the enzymatic activity of the SODs was reported in 1968–1969 by McCord and Fridovich [1,8], but the proteins had already been twice discovered before their enzymatic activity was elucidated. Mann and Keilin [9] had purified the protein 30 years earlier from bovine blood and liver as a copper-binding protein of unknown function. The protein was called "erythrocuprein" or "hepatocuprein" or later "cytocuprein." The purification was based solely on cop-

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per content. Superoxide, the substrate for the SODs, had been discovered in the 1930s, by the way, by Linus Pauling [10]. Pauling had no idea that the radical could be produced biologically, or that it could be at the core of so many disease processes. Knowles et al. [11] in 1969 showed that the enzyme xanthine oxidase could indeed produce superoxide. So, McCord and Fridovich showed that the copper protein of Mann and Keilin could catalytically eliminate the Pauling free radical.

It was the third independent discovery of the protein that was the most provocative. Huber et al. [12] isolated the same protein from bovine liver in the 1960s based on its anti-inflammatory activity in animal models. They called the protein *Orgotein*. How did this observation relate to SOD activity? What did superoxide have to do with inflammation? It was a discovery by Bernard Babior [3] in 1973 that linked all the observations together. Babior found that phagocytosing neutrophils produce large amounts of the superoxide radical, which he proposed to be a part of the bactericidal process. Soon it was apparent that some of the tissue damage associated with the inflammatory process could be attributed to neutrophil-generated superoxide, and that SOD could protect cells and extracellular components from damage [2,13].

Another major class of diseases with superoxide-mediated injury was soon discovered: the diseases of ischemia and reperfusion injury [4,14]. The sources of superoxide in conditions such as heart attack, stroke, and transplantation-induced organ failure and rejection include xanthine oxidase [15], inflammatory cells, and injured mitochondria [16].

Clinical trials of SOD have been carried out in conditions involving fibrosis. SOD was used very early on to treat bladder inflammation resulting from irradiation [17]. A frequent sequela of bladder irradiation is fibrosis, a physiological response that often follows inflammation in other tissues, as well. Because the influx of fibroblasts results in the laying down of collagen fibrils to form scar tissue, fibrosis causes loss of elasticity—a property essential to organs such as the bladder or the lungs. The formation of scar tissue is considered irreversible by most medical textbooks, but data suggest that the process is dynamic, and may be reversible in large part [18,19]. Recently, it has been found that the antifibrotic action of Cu,Zn-SOD is mediated by TGF-β1 repression and phenotypic reversion of myofibroblasts [20]. Transforming growth factor beta stimulates the production of the tissue inhibitor of metalloproteinases by human synovial and skin fibroblasts [21]. Thus, as the myofibroblasts revert through the action of SOD, metalloproteinase activity (collagenase) rises and scar tissue is, in effect, broken down.

A study of kidney transplant patients, designed to protect the allograft from ischemia–reperfusion injury, has shed light on apparent roles for SOD and superoxide in graft rejection and immunosuppression [22]. The initial results were not statistically significant, but there was a trend toward improved function, especially in allografts that had been subjected to prolonged cold ischemia. An unanticipated surprise came, however, when the patients were followed out for 4 or 5 years. The results show that SOD caused a significant reduction of first acute rejection episodes (from 33.3% in controls to 18.5% in SOD-treated), as well as early irreversible acute rejection (from 12.5% in controls to 3.7%). The 4-year graft survival rate in SOD-treated patients was 74% (with a projected half-life of 15 years) compared with 52% in controls (with an extrapolated half-life of 5 years). The beneficial effect of SOD observed in this trial is not fully understood, but it appears to have reduced the immunogenicity of the graft. This is particularly intriguing in light of a new hypothesis in which the immune system responds not only to non-self, but also to "danger signals" [23], perhaps including those generated by oxidative stress.

Thus, superoxide radical is being increasingly viewed as a signaling molecule, especially with regard to cell division and proliferation [24,25]. This concept substantially broadens the range of diseases in which $O_2^{\bullet-}$ and SOD may be involved to include the examples mentioned above (reversal of fibrosis and regulation of the immune system), as well as the processes of transformation, metastasis, and angiogenesis [26,27]. Cellular homeostasis may then be upset not only by the chemical insult imposed by substantial overproduction of superoxide causing lipid peroxidation, protein oxidation, and DNA damage, but also by more subtle variations in radical production or disposal, resulting in dysfunctional regulation of essential cellular processes and responses. Sources of $O_2^{\bullet-}$ that must now be considered have expanded beyond the "accidental" overproduction by inflammatory cells or injured mitochondria to include an important new class of enzymes represented by Nox1 [27,28].

3. Practical obstacles to using SOD as a drug

Despite the knowledge summarized above, technical reasons have limited the use of SOD as a drug, even in the laboratory. Typically, proteins make poor drugs, and the SODs are no exception. Rapid renal clearance and slow extravasation due to molecular radius and charge density are factors that affect the pharmacodynamics and pharmacokinetics of enzymes used as drugs. Various attempts at modifying the proteins to improve these properties have been attempted with some success [29], including the delivery of SOD by liposomes [30,31]. Recently, a genetically engineered version of human SOD2 has been described that may overcome some of these problems [32].

A more fundamental problem may be the primary reason that SOD-based antioxidant therapy has not made a greater and more rapid impact on clinical medicine: the problem of oxidant–antioxidant balance.

4. The bell-shaped curve

For a few years after its discovery, SOD appeared to be one of those things that could not possibly have a bad side. It