Superoxide Dismutase as a Potential Therapeutic Agent

Abstract
Superoxide dismutase (SOD) is a major antioxidative enzyme and the only one that neutralizes superoxide ion, a precursor of reactive oxygen species (ROS) and their reactive derivatives. ROS can cause considerable damage to biological systems and are implicated in many pathologies, e.g. neurodegenerative diseases, tumors, atherosclerosis, hypertension, and diabetes. This paper presents a history of the discovery of superoxide dismutase and the functions of particular SOD isoforms in the organism. It also describes the possibilities of their use in the treatment of free-radical-based diseases (Adv Clin Exp Med 2007, 16, 4, 561–568).

Key words: superoxide dismutase, oxidative stress, enzymatic therapy, SOD mimetics.

Streszczenie

Słowa kluczowe: dysmutaza ponadtlenkowa, stres oksydacyjny, terapia enzymatyczna, mimetyki SOD.

The copper-containing superoxide dismutase (SOD) was first isolated from bovine erythrocytes in 1938 [1]. In the 1960s, SOD was also isolated from brain and liver and it was observed that under inflammatory conditions, SOD protects cells against inflammatory injury, which indicated the anti-inflammatory properties of this protein [2, 3]. The exact mechanism of the reaction catalyzed by SOD was finally explained in 1968–1969 by McCord and Fridovich. They showed that superoxide dismutase catalyzes the superoxide ion dismutation to hydrogen peroxide and molecular oxygen [4]. Thus just 30 years after the isolation of SOD protein by Mann and Keilin and the discovery of superoxide ion by Pauling, McCord and Fridovich showed that this protein in vivo catalyzes the two-stage reaction of superoxide ion dismutation to hydrogen peroxide [1, 5].

Although SOD’s reaction mechanism was understood, the physiological role of O$_2^-$ was still unknown, and it was not known why it is neutralized by SOD. A discovery by Babior et al. linked together all the known observations concerning SOD [6]. They found that phagocytes producing neutrophils produce large amounts of superoxide ion, which might indicate its bactericidal function. Soon it was shown in work by McCord that superoxide ion produced by neutrophils is responsible for the oxidative damage to tissue associated with the inflammatory process, and SOD could protect cells from this damage [7].

In the 1970s, the growing interest in SOD allowed for the discovery of other superoxide dismutase isoenzymes in addition to the copper-zinc form of superoxide dismutase (CuZnSOD,
SOD1). In 1970, Keele et al. isolated a superoxide dismutase that contained a manganese ion in its active center from *E. coli* cells and they called it MnSOD (SOD2) [8]. Soon thereafter, MnSOD was found in the mitochondria of mammalian cells [9]. In 1973, ferric dismutase (FeSOD), a homologue of MnSOD, was isolated from *E. coli* cells. It exists mainly in prokaryotic and in several lower eukaryotic organisms, e.g. yeasts [10]. The last SOD isoenzyme was discovered relatively late, in 1982: extracellular superoxide dismutase (EC-SOD, SOD3). Its presence was revealed in extracellular fluids such as serum, lymph, synovial fluid, and cerebrospinal fluid. This enzyme also exists on extracellular matrix and the outer surface of cells because of the strong affinity to proteoglycans commonly found on the outer cell surface. EC-SOD contains copper and zinc ions in its active center; however, it does not have the immunospecificity typical for CuZnSOD and it has a larger molecular mass. It is the only enzyme that removes superoxide ion from extracellular spaces [11]. Following years have been a time of intensive research on SOD isoenzymes, characterizing their structures, properties, and functions.

**Functions of SOD**

Superoxide dismutase (SOD, EC 1.15.1.1.) is found in all aerobic organisms, both pro- and eukaryotic. It is a metalloprotein, containing metal ions (Fe, Ni, Cu, Zn, Mn) in its active center [12]. This enzyme catalyzes the two-stage reaction of superoxide ion (O₂⁻) dismutation to hydrogen peroxide (H₂O₂).

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2O_2^- + 2H^+ \rightarrow H_2O_2 + O_2 \\
\text{k = 2 } \times 10^9 \text{ M}^{-1}\text{s}^{-1}
\]

In the human organism, SOD functions in different forms which altogether form the system of superoxide ion neutralization in the mitochondria, nucleus, cytosol, and extracellular space. There are three dismutase isoenzymes: copper-zinc (CuZnSOD, SOD1), found in the cytosol and nucleus; manganese (MnSOD, SOD2), found almost exclusively in mitochondria; and the extracellular superoxide dismutase (EC-SOD, SOD3) in the extracellular space and fluids [12, 13]. Available scientific data indicates that superoxide dismutase is a key enzyme which scavenges the ROS precursor superoxide ion both *in vitro* and *in vivo*. Under conditions of oxidative stress, which often accompany many pathologies (atherosclerosis, hypertension, tumor, liver cirrhosis, and autoimmune and neurodegenerative diseases), SOD has particular significance.

Among the SOD isoenzymes, the mitochondrial superoxide dismutase (i.e. MnSOD) is the first and most important line of defense against toxic superoxide ion [14, 12]. The second line of defense is CuZnSOD in the cytosol. This isoenzyme supports the protective function of MnSOD by neutralizing the O₂⁻ generated in the cytosol and leaking from the mitochondria. Such cooperation provides efficient elimination of superoxide ion excess [15]. EC-SOD scavenges O₂⁻ on outer surfaces of cells and in the extracellular matrix and fluids. Besides protecting cell surface, it removes O₂⁻ from the vasculature. It has great importance in preserving the bioactivity of NO in the cardiovascular circulation and endothelial tissue [16]. Scientific data indicate that superoxide dismutase, by maintaining a balance between O₂⁻ removal and H₂O₂ generation in cells, participates in the regulation of the most important life processes, such as proliferation and apoptosis [17].

The functions of the SOD isoenzymes in cells indicate that they play a significant role in the pathologies of many diseases, e.g. atherosclerosis, diabetes, neurodegenerative diseases, acute rheumatoid arthritis, and tumors [18–21].

**Capabilities of SOD’s Therapeutic Application**

Growing scientific data point to the great importance of both ROS and SOD in numerous physiological processes. ROS participate in an unusually wide range of processes, which suggests that free radicals might contribute to many diseases of unknown origin or mechanism. There are also many suggestions for the direct application of SOD in the therapy of free-radical-based diseases.

As a precursor of all ROS, superoxide ion in large measure contributes to the damage of biologically important molecules (DNA, proteins, lipids) in cells and, consequently to tissue injury. The accumulation of structural damage could be the cause of many diseases (coronary artery disease, tumors, arthritis, Crohn’s disease, autoimmune and neurodegenerative diseases, diabetes, and others) [14, 22, 23]. Many scientific publications indicate the possibility of applying antioxidants in the treatment of free-radical-based diseases. By neutralizing excess ROS, antioxidants might efficiently prevent or palliate disease symptoms. Therefore it is reasonable that superoxide dismutase, a superoxide ion scavenger, should demonstrate exceptionally beneficial therapeutic effects.

However, the application of SOD as a therapeutic agent faced some problems. Initially, CuZnSOD isolated from bovine erythrocytes was
mainly used, and introducing the alien protein to body fluids (blood or synovial fluid) induced a strong immunological response and allergic reaction in the organism. Applying human recombinant CuZnSOD was also inconvenient. Due to its low molecular weight, CuZnSOD undergoes rapid renal clearance and the duration halftime of this protein in the vasculature (6–10 minutes) is too short for effective therapy. In addition, this protein penetrates cells from body fluids weakly.

Still, the possibilities of the therapeutic use of SOD hold promise and attempts are underway to solve problems which appear. It was shown that covalent binding of CuZnSOD to a large polymeric molecule, e.g. polyethylene glycol, inhibits CuZnSOD renal clearance and extends its halftime in the vasculature to up to 30 hours and decreases its immunogenicity. Covalent binding of CuZnSOD with hydrophobic anions or fatty acids could be another solution which leads this modified protein to bind with albumins or anchoring in cell membranes. Linking CuZnSOD with hialuronnic acid also allows its adherence to cell surfaces and increases its halftime in the vasculature [24, 25]. CuZnSOD protein could also be packed in liposomes and introduced in this form to the blood circulation. This method extends CuZnSOD’s halftime in the vasculature and facilitates its penetration to cells because the liposomes are absorbed by cells as entire particles. Introducing SOD protein absorbed by special carriers, i.e. hydrogels, to the organism yielded interesting results. Recent research showed that this network of polymers containing great amounts of water can very efficiently absorb SOD molecules and release them after 72 hours from introduction to the organism [26]. Increased growth of fibroblast cultures was observed after the addition of hydrogels containing SOD, demonstrating its significance in the therapy and regeneration of injured tissues [24]. Due to the greater stability of MnSOD compared with CuZnSOD in the circulation (5–6 hours), its application might bring much better therapeutic effects.

The particular mechanisms of the activation and function of SOD isoenzymes and their role in the pathogenesis of many diseases are the subjects of numerous studies in molecular biology. These studies, besides explaining the details of the development of free-radical-based diseases, also contribute to introducing new therapeutic strategies. At the current stage of research, SOD isoenzymes are applied as potential therapeutic agents using the following strategies: 1) enhancing cells’ abilities to remove superoxide ion [27], 2) application of SOD mimetics [28, 29], and 3) supplementary enzyme therapy [30].

The first strategy is aimed at increasing native SOD activity and/or gene expression. This may be with drugs that are already in common use, such as the anti-hypertensive (candesartan, an angiotensin receptor antagonist) and inhibitors of angiotensin I to angiotensin II converting enzymes (ACE), such as ramipril. Upregulation of CuZnSOD activity in heart tissue was shown as well as prevention against the downregulation of EC-SOD expression in the vascular endothelium, and the functioning of the vascular endothelial smooth muscles was also improved [31–33]. These results found confirmation in large-scale studies conducted recently involving the application of selected drugs (ramipril and vitamin E) to decrease cardiovascular disease risk and outcome (HOPE, The Heart Outcomes Prevention Evaluation). The HOPE studies showed that vitamin E did not decreased cardiovascular disease risk. However, ramipril decreased blood pressure, significantly reduced the risks of stroke, myocardial infraction, atherosclerosis, and diabetic nephropathy [34–36]. Since ramipril is an inhibitor of angiotensin I-converting enzymes, it protects against a decrease in EC-SOD expression in the endothelium, providing better antioxidant protection to blood vessels and increasing the bioavailability of nitric oxide.

In the case of SOD mimetic drugs, compounds such as a porphyrin-based drug containing Mn(III) (MnTBAP), salen-manganese complexes (EUK-8), nitro oxide derivatives (tempol), and low-weight manganese or iron chelators, that can mimic either SOD or catalase activity (or both), are administered over long periods at protective doses [37].

Supplementary enzyme therapy relies on complementation of SOD activity by other antioxidant enzymes. An interesting approach was the application of a SOD-catalase conjugate. Both enzymes in this conjugate are linked by a glutaryl dialdehyde molecule. The halftime of this conjugate in the blood of rats was about 4 hours. The SOD part present in the conjugate was protected by the catalase part against inactivation by H₂O₂ [38, 39].

Recently, a so-called “super-SOD” was constructed. This chimeric protein contains an MnSOD (SOD2) primary structure plus the 26-amino-acid C-terminus of EC-SOD (SOD3). This tail allows SOD2/3 adherence to endothelial surfaces. As the chimera is less positively charged than SOD3, it does not bind as strongly to cell surfaces, allowing it successful intravascular administration [40, 41].

Clinical Trials of SOD Application

Research into the clinical application of SOD has been underway for many years. Positive effects of SOD were shown in the treatment of
numerous free-radical-based diseases in many experiments. Initially, before clinical trials of SOD application, studies of its use in animal models were conducted. The administration of CuZnSOD, catalase, and allopurinol showed protective effects in experimental rat acute pancreatitis [42]. CuZnSOD or catalase also suppressed experimental autoimmune neuritis symptoms in rats [43]. The administration of polyethylene glycol-conjugated CuZnSOD and desferroxamine reduced oxidative injuries in rat brains in experimental brain ischemia and experimental meningitis [44, 45]. Similarly, CuZnSOD showed strong anti-inflammatory effects in experimental ischemia in island skin flaps. Infusion of CuZnSOD also prevented against skin injuries during ischemia-reperfusion [46]. An increase in rat resistance to daily exposure to 100% oxygen was achieved by injecting polyethylene glycol-conjugated CuZnSOD to their lungs [47]. CuZnSOD conjugated with polyethylene glycol showed its effectiveness in the therapy of heart muscle ischemia and reperfusion arrhythmia, protected lungs against damage caused by E. coli, and decreased ischemia-reperfusion injuries in liver and kidneys [48].

Besides conjugation with polyethylene glycol, SOD may also be linked to a polyanionic compound such as divinyl ether and maleic anhydride, known as DIVEMA. Administration of the SOD-DIVEMA conjugate to rats with liver fibrosis led to decreased ROS generation in liver cells and inhibition of liver inflammation [49]. SOD-DIVEMA protected rat lungs also against lung edema and inflammation changes [50].

Protective and palliative activities of SOD were shown also in the case of skin burns. The administration of CuZnSOD packed to liposomes to rabbits with skin burns or to blood in the form of a gel reduced edema, decreased the area of tissue injury and necrosis, and accelerated wound healing [51].

Because EC-SOD scavenges superoxide ion from cell surfaces and vasculature lumen, this enzyme functions as a protector of NO bioactivity in the cardiovascular circulation and endothelial tissue. Therefore its application is possible in the therapy of coronary artery disease, hypertension, diabetes, and in lung degenerative diseases. This found confirmation in experiments in which EC-SOD administration protected rats against brain vessel injuries induced by acute hypertension [52].

During organ transplantation, ischemia-reperfusion is a frequent phenomenon. It is a source of large amounts of ROS generated after the fresh influx of oxygenated blood to tissues. These ROS contribute to transplant tissue injuries. Therefore, the application of SOD during transplantation should demonstrate positive effects. Indeed, the addition of CuZnSOD and catalase to the perfusion fluid during rat liver transplantation increased the survival rate of the transplanted organs [53]. Recently it was shown that the application of SOD linked with lecithin protects endothelial cells against ischemia-reperfusion injury [54].

Superoxide dismutase is also utilized in protecting cell cultures in vitro against toxic ROS. Addition of SOD to the medium in cultured cell lines increased their survival rates [25]. It was observed that ROS in in vitro cultured mouse embryos blocked the first zygote division, and the presence of SOD in the culture medium allowed their further normal development [55]. Positive effects were shown in studies on the overexpression of MnSOD in cell cultures. The human melanoma cell line A375 and the human histiocytic lymphoma cell line U937, with MnSOD overexpression achieved by gene transduction, showed greater resistance to doxorubicin (an antitumor drug that induces the generation of large amounts of ROS). It could ensure greater viability of normal cells during chemotherapy and, on the other hand, increase the sensitivity of tumor cells to doxorubicin [56].

The positive results of SOD application in cell cultures and animal models opened the way to applying SOD isoenzymes in clinical trials.

Palliative effects of SOD application were shown in patients with diseases such as rheumatoid arthritis, bladder inflammation resulting from irradiation, Crohn’s disease, and lupus erythematosus [57, 58]. Application of a topical gel containing liposomally encapsulated recombinant SOD in patients with painful Peyronie’s disease allowed a reduction in pain in as much as 89% of cases [59]. Lecithin-bound SOD decreased the sizes of defect in the cornea caused by keratinization, thermal or chemical burns, and corneal surgery. This has particular significance for patients who do not respond to conventional therapy [60]. In premature infants, intratracehal treatment with recombinant human CuZnSOD partially prevented pulmonary injuries, which eventually lead to bronchopulmonary dysplasia. Additional benefits of this therapy were decreased incidence and symptoms of lung disease over the first year of life of premature infants [61, 62].

There are also preliminary studies showing strategies of treating amyotrophic lateral sclerosis (ALS) by means of gene therapy with application of CuZnSOD. The strategy would be to inhibit all CuZnSOD mutants leading to ALS and also wild-type CuZnSOD by special RNAi. Then the wild-type CuZnSOD form is replaced by a form of CuZnSOD designed to be resistant to RNAi [63].
At the present moment, CuZnSOD is most widely applied in transplantology, where it is employed as one of the components in some perfusion fluids together with other antioxidant compounds, e.g. reduced glutathione (GSH), allopurinol, and desferroxamine mezylan (Carolin’s fluid) [25].

**EC-SOD as a Therapeutic Agent**

Due to its function in the human organism, the most promising therapeutic agent seems to be EC-SOD. The decreased activity of this enzyme observed in many diseases indicates it as a potential target in antioxidant therapy. There are a lot of experimental data concerning the application of EC-SOD. EC-SOD’s duration halftime in blood vessels (20 h) and strong affinity to heparan sulfate in vessel walls both endorse the application of EC-SOD in therapy. Due to its properties, the administration of a synthetic EC-SOD (recombinant rEC-SOD) might efficiently prevent disturbances related to exaggerated production of superoxide ion [64, 65].

Numerous studies (most of all on animal models) have shown the usefulness of EC-SOD in the therapy and prevention of such diseases as arthritis, liver intoxication, coronary disease, pathology of brain blood vessels, and others [66–68]. The usefulness of EC-SOD in genomic therapy was also examined, as it is a factor protecting cells against oxidative damage [67]. The ability of EC-SOD to bind to the cell surface allows better protection of heart and blood vessels walls than other SOD isoenzymes administered intravascularly because they cannot penetrate to the intracellular space. Whereas EC-SOD, administered in the form of a viral vector, is the only SOD isoenzyme which is naturally secreted from cells, it is exceptionally well adapted to production in the liver and further transport in the organism.

Genomic therapy of rabbits using EC-SOD enhances the possibilities of vasculature protection against atherosclerosis and particularly against myocardial infarction. It has great importance in the development of new cardioprotection strategies [69]. It was also shown that rat intravascular injections of adenoviruses containing EC-SOD decreased impaired relaxation of vascular endothelium smooth muscles caused by LPS (lipopolysaccharides) [70]. In addition, EC-SOD gene transfer to mice liver cells distinctly contributed to a decrease in liver cell injury and necrosis resulting from paracetamol [71]. Application of EC-SOD genomic therapy in the treatment of murine collagen-induced arthritis reduced numerous symptoms of the disease [67].

The possibility of modifying EC-SOD activity and protein level in the organism could be useful in the treatment of many pathologies. Therefore, a better understanding of EC-SOD’s role in the pathogenesis of disease should provide knowledge about its potential therapeutic capabilities.

The authors conclude that since the discovery of superoxide dismutase 68 years ago, research into the function, structure, and physiological role of SOD has been conducted persistently. This research yielded many unexpected discoveries and provided massive knowledge about superoxide dismutase. An exact understanding of the functions and activity mechanisms of SOD isoenzymes in significant living cell processes allows us to understand the molecular and biochemical bases of the initiation and development of free-radical-based diseases. Understanding these processes allows for better prophylaxis, diagnostics, and therapy of these diseases. Research conducted in this field has already proved the efficiency of SOD, particularly EC-SOD, as a therapeutic factor and has overcome the initial problems related to its application in therapy. Although most of the studies were conducted on animal models, the possibilities of applying SOD are unusually wide and give hope for more effective treatment of diseases in which reactive oxygen species participate.

**References**


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