Microbial superoxide dismutase enzyme as therapeutic agent and future gene therapy

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All aerobically growing organisms come into contact with reactive oxygen species or free radicals, generated as a byproduct of normal respiratory processes or from encounter with exogenous oxidants. To counter the damaging effect of reactive oxygen species, cells have evolved anti-oxidant defense systems, whose expression is usually induced by reactive oxygen species and/or oxidants. One of the most important antioxidant defense systems in nearly all cells exposed to oxygen is the production of superoxide dismutase enzyme. Superoxide dismutases discovered by Irwin Fridovich and Joe McCord, are class of metal cofactored enzymes that detoxify these free radicals by catalyzing the dismutation of superoxide into oxygen and hydrogen peroxide. Seen to their continuous exposure to high oxidative stress during growth and metabolism, aerobic microorganisms represent an excellent source for production of superoxide dismutases. Many studies clarified the efficacy of superoxide dismutases as a therapy in the treatment of many diseases, in addition to their inclusion in cosmetic products to reduce free radical damage to skin. Recently, superoxide dismutases became target for gene therapeutic approaches. In the present chapter, the history of the discovery of superoxide dismutase enzymes, their types, structure, therapeutic and cosmetic uses, in addition to their therapeutic potential in future gene therapy, have been reviewed and discussed.

Keywords superoxide dismutase; microorganisms; gene therapy

1. Introduction

An inevitable consequence of life in an oxygen-rich environment is the formation of reactive oxygen species (ROS), which are unavoidable by-products of aerobic metabolism. ROS produced from metabolic events, such as respiration (as a consequence of the incomplete reduction of oxygen) or fatty acid oxidation or due to environmental processes. Oxidative stress occurs when the rate of generation of reactive compounds exceeds the detoxification capacity of the cell [1]. If not disposed off efficiently, ROS can cause cellular and genetic damage leading to carcinogenesis, senescence, and neurodegenerative disorders [2, 3]. Because cells under oxidative stress are at risk for lethal or mutagenic damage, all aerobic organisms have evolved defense mechanisms to cope with ROS. Part of this response involves the reprogramming of gene expression to increase levels of antioxidant enzymes such as superoxide dismutase and catalase, which limit the levels of superoxide ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2), respectively.

2. Reactive oxygen species (ROS)

Molecular oxygen (O_2) is not reactive with most compounds due to its two unpaired electrons [4]. The activity of oxygen increases when it accepts one, two, or three electrons to form superoxide anions ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), and the hydroxyl radicals (OH^-), respectively. These mediators of oxidative stress ($O2^{\cdot-}$, H_2O_2, and OH^-) are known as reactive oxygen species (ROS). Reactive oxygen species (ROS) is a term which encompasses all highly reactive, oxygen-containing molecules, including free radicals. Types of ROS include the hydroxyl radical, the superoxide anion radical, hydrogen peroxide, singlet oxygen, nitric oxide radical, hypochlorite radical, and various lipid peroxides. All are capable of reacting with membrane lipids, nucleic acids, proteins and enzymes, and other small molecules, resulting in cellular damage [5]. ROS are generated by exposure to radiation, heavy metals, and redox active compounds, and also occur under normal metabolism [6]. ROS affect normal cell functions by activating a number of enzymatic cascades and pathological processes in many diseases by inducing oxidative stress [7, 8]. Oxygen toxicity causes widespread damage to DNA, RNA, protein, and lipids when the degree of oxidative stress exceeds the capacity of the cell defense systems. There is growing evidence that ROS are important for the induction of apoptosis (the process of programmed cell death: PCD) [9]. There is now universal agreement that free radicals are involved in the physical, biochemical, and pathological changes associated with aging [10]. And there is growing evidence that free

radicals are involved in the initiation of cellular injury observed in neurodegenerative diseases such as: Alzheimers's disease (AD), Huntington's disease (HD), Parkinson's disease (PD) [11]. In addition, ROS can act in all stages of carcinogenesis leading to cancer development [12]. There is also increasing evidence that ROS plays a significant role in the development of diabetes. To counter the damaging effect of reactive oxygen species, cells have evolved anti-oxidant defense systems, whose expression is usually induced by reactive oxygen species and/or oxidants [13].

3. Antioxidants

The term "antioxidant" refers to any molecule capable of stabilizing or deactivating free radicals before they attack cells. Humans have evolved highly complex antioxidant systems (enzymic and nonenzymic), which work synergistically, and in combination with each other to protect the cells and organ systems of the body against free radical damage. The antioxidants exist endogenously or obtained exogenously e.g., as a part of a diet or as dietary supplements. Some dietary compounds that do not neutralize free radicals, but enhance endogenous activity may also be classified as antioxidants [10]. Non-enzymatic antioxidants include uric acid, glutathione, bilirubin, thiols (glutathione, thioredoxin and lipoic acid), albumin, melatonin, carotenoids, natural flavonoids and other compounds and nutritional factors including vitamins such as E and C and phenols [14]. Enzymatic antioxidants include the superoxide dismutases, the glutathione peroxidases [GSHPx] and catalase [15]. The most important source of antioxidants is provided by nutrition (Exogenous antioxidant); many belonging to the phenol family. Nutritional antioxidants act through different mechanisms and in different compartments, but are mainly free radical scavengers: 1) they directly neutralize free radicals, 2) they reduce the peroxide concentrations and repair oxidized membranes, 3) they quench iron to decrease reactive oxygen species production, 4) via lipid metabolism, short-chain free fatty acids and cholesteryl esters neutralize reactive oxygen species [16].

4. The history of discovery of superoxide dismutase

Superoxide dismutases (SOD, EC 1.15.1.1) discovered by American biochemist Irwin Fridovich and his graduate student Joe McCord in 1969 [17]. Interestingly, the original paper was cited 39 times in just the year 2009 - forty years after original publication. PubMed lists over 49,000 papers published on superoxide dismutase since its discovery in 1965. Few other biochemists have had such an impact on the field of biochemistry. SOD has been found in most organisms, aerobic and anaerobic, and plays a key role in cellular protection against oxidative stress conditions [18].

5. Function of superoxide dismutase

SODs enzymes were previously thought to be several metalloproteins with unknown function (for example, CuZnSOD was known as erythrocuprein) [19]. But now it is proven that SOD catalyzes the conversion of superoxide anions to dioxygen and hydrogen peroxide; the latter being broken in turn to water by catalase or peroxidase. SOD neutralizes superoxide ions by going through successive oxidative and reductive cycles of transition metal ions at its active site [20].

6. Sources of SODs

Most organisms, microorganisms, plants and animals have at least one superoxide dismutase enzyme [21]. While one of the exceedingly rare exceptions is *Lactobacillus plantarum* and related lactobacilli, which use a different mechanism [22 ,23]. It is widely accepted that a plant-based diet with high intake of fruits, vegetables, and other nutrient-rich plant foods may reduce the risk of oxidative stress-related diseases [24]. SODs were also produced efficiently by many microbial species [25, 26]. Seen to their continuous exposure to high oxidative stress during growth and metabolism, aerobic microorganisms represent an excellent source for production of superoxide dismutases.

Of many aerobic microorganisms considered as a potent source of superoxide dismutase, *Corynebacterium glutamicum*, an industrial relevant producer of amino acids and vitamins, is considered as an excellent candidate for this purpose seen to its high need of oxygen during amino acid production, nominating it to have a hyper antioxidant defense system including production of abundance superoxide dismutase enzyme [27, 28]. Cloning techniques reported to be used successfully with many corynebacterial genes [29, 30, 31, 32]. Thus it would be interesting to enhance superoxide dismutase production using cloning strategies. In addition other microbial species should also be considered for extraction of different superoxide dismutase types.

7. Types of SODs

SODs are distinguished into several types depending on the type of metal cofactors.

(1) SOD containing either manganese (Mn-SOD) or iron (Fe-SOD) has been found in the human mitochondria in addition to the cytoplasm of prokaryotic cells and have very similar sequences and structures [33, 34, 35, 27, 28].

(2) Copper and zinc-containing SOD (Cu,Zn-SOD) has been described in humans as cytosolic and extracellular SOD. Until relatively recently, Cu,Zn-SOD was considered to be an almost exclusively eukaryotic enzyme, and its presence in bacteria, originally identified in a very small number of microorganisms, was thought to be an exception rather than a rule [36, 26]. Cu,Zn-SOD has been found in the periplasm of several Gram-negative pathogenic and endosymbiotic bacteria and are evolutionarily unrelated to Mn,Fe-SODs [37, 38, 39, 40, 36, 41, 35].

(3) In addition, two novel SODs containing nickel (Ni-SOD) or both iron and zinc (FeZn-SOD) have been characterized as cytoplasmic enzymes of *Streptomyces griseus* and *Streptomyces coelicolor* [42, 43]. Another type of small metalloprotein with superoxide dismutase activity, unrelated to classical SODs, has also been recently identified in the sulphate-reducing bacterium *Desulfovibrio* [44].

8. Structure of SODs

In humans (as in all other mammals and most chordates), three forms of superoxide dismutase are present. SOD1, SOD2 and SOD3.

SOD1: It is a dimer (consists of two units) SOD located in the cytoplasm, containing Cu, Zn (copper and zinc) and has two identical subunits with a molecular weight of 32 kDa and each of the subunit contains as the active site, a dinulcear metal cluster constituted by copper and zinc ions, and it specifically catalyzes the dismutation of the superoxide anion to oxygen and water.

SOD2: It is the mitochondrial SOD having the Mn (manganese) in its reactive centre. Mn-SOD is a homotetramer with a molecular weight of 96 kDa and contains one manganese atom per subunit [15], and it cycles from Mn(III) to Mn(II), and back to Mn(III) during the two-step dismutation of superoxide.

SOD3: Extracellular superoxide dismutase contains Cu, Zn (copper and zinc), and is a tetramer (consists of four subunits) [15].

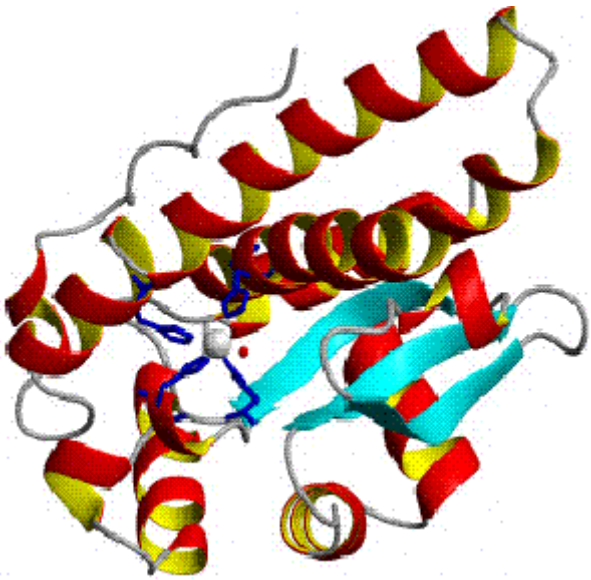

Fig. 1. Structure of the monomeric unit of Mn-human mitochondrial superoxide dismutase (SOD2) [45].

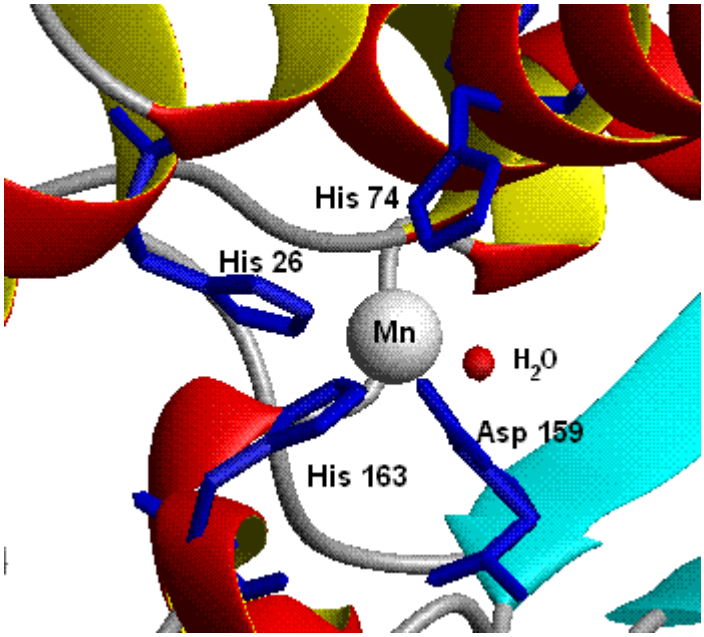

Fig. 2. Structure of the active site of Mn-human mitochondrial superoxide dismutase (SOD2) [45].

9. SOD as a pharmaceutical product

Superoxide dismutase, by scavenging of free oxygen radicals, might interrupt inflammatory cascades and thereby limit further disease progression [46]. Numerous studies have established safety of superoxide dismutases drugs in animals and man. Many studies reported using superoxide dismutases as an anti-inflammatory agent [47]. Superoxide dismutases were reported for the treatment of not only systemic inflammatory diseases but also skin ulcer lesions, especially due to burn and wounds, where liposomal-encapsulated SOD injection was effective [48]. Besides direct inhibition of joint tissue destruction, the mechanism of action of SOD in reducing the severity of arthritic inflammation includes limitation of existing positive feedback between secretion of ROS and inflammatory cytokine production [49]. SOD in its pharmaceutical form "Orgotein" is a potent anti-inflammatory agent approved in many countries, and because of their effectiveness in the general treatment of inflammatory and degenerative diseases, researchers are constantly looking at SODs and related agents in experimental animal models of disease. In the course of such studies, researchers discovered that SODs stimulate hair growth and decrease hair loss [50], ameliorate radiation-induced side effects [51] such as radiation-induced sclerosis [52] and radiation-induced fibrosis (radiofibrosis) following irradiation for breast cancer [53, 54, 55] using Cu/Zn superoxide dismutase where no other effective therapy exists [56]. SOD might be proposed as a potent antagonist of this major fibrogenic growth factor [53], and as a potential antifibrotic drug for hepatitis C related fibrosis [57]. In addition SODs are used for preparation of many pharmaceutical compositions for treatment of many diseases including myocardial ischemia [57, 58], Peyronie's Disease [46], multiple sclerosis [59], colitis [60]; and in improving a clinical irradiation treatment of malignant diseases such as breast cancer [61]. There is also increasing evidence that radical scavengers like superoxide dismutase may influence the outcome and progression of diabetic retinopathy [62]. Dramatic improvement of clinical active Behçet's syndrome symptoms treated by CuZn SOD was also reported [63].

Superoxide dismutase mimetics [64] such as tempol has also a beneficial effects in several experimental models of hypertension and acute kidney injury [65]; while sodium thiopental and propofol are used to treat reperfusion injury and traumatic brain injury [66]. Superoxide dismutase mimetics have also been reported to extent the mean life-span of the wild-type worm *Caenorhabditis elegans* by a mean of 67 percent increase [67].

The topical application of SOD cream was concluded to be effective for rapidly improving of many cases such as progressive systemic sclerosis (PSS), systemic lupus erythematosus (SLE), Behçet's disease, herpes simplex and burns, even when the symptoms were stabilized for several weeks before the treatment [68]. Wound excision in conjunction with SOD-treatment might be therapeutic in the management of severe burns suggesting that superoxide radicals may play a critical role in the pathogenesis following thermal injury [69, 70].

10. SOD as a future gene therapy

Gene therapy is the insertion of genes into an individual's cells and tissues to treat disease, such as cancer [71, 72], where deleterious mutant alleles are replaced with functional ones. Although the technology of gene therapy is still in its infancy, it has been used with some success. Nowadays, in vivo gene therapy is considered as an attractive therapy for the treatment of many diseases [73, 74].

Gene therapy may be classified into the two following types: the first is the germ line gene therapy: in this case, germ cells, i.e., sperms or eggs, are modified by the introduction of functional genes, which are ordinarily integrated into their genomes. Therefore, the change due to therapy would be heritable and would be passed on to later generations. This new approach, theoretically, should be highly effective in counteracting genetic disorders and hereditary diseases. However, many jurisdictions prohibit this for application in human beings, at least for the present, for a variety of technical and ethical reasons. The second is the somatic gene therapy: in this case, the therapeutic genes are transferred into the somatic cells of a patient. Any modifications and effects will be restricted to the individual patient only, and will not be inherited by the patient's offspring or later generations [75].

Viral-mediated gene-delivery systems are used as tools in gene therapy [76]. Some types of viruses physically insert their genes into the host's genome, thus they were employed in gene therapeutic uses, such as retroviruses, adenoviruses, adeno-associated viruses (from the parvovirus family), envelope protein pseudo-typing of viral vectors, replicating vectors, cis and trans-acting elements, and herpes simplex viruses.

Besides virus-mediated gene-delivery systems, there are several non-viral options for gene delivery [77]. The simplest method is the direct introduction of therapeutic DNA into target cells. This approach is limited in its application because it can be used only with certain tissues and requires large amounts of DNA. Another non-viral approach involves the creation of an artificial lipid sphere with an aqueous core (the liposome). This liposome, which carries the therapeutic DNA, is capable of passing the DNA through the target cell's membrane. Researchers also are experimenting with introducing a 47th (artificial human chromosome) into target cells. A problem with this potential method is the difficulty in delivering such a large molecule to the nucleus of a target cell. Due to presence of shortcomings for each gene transfer method, some hybrid methods developed combining two or more techniques; virosomes are an example of combining liposomes with an inactivated HIV or influenza virus.

Gene therapeutic strategies were reported to be used successfully in reduction of symptoms generated by radiotherapy. The total allowable radiotherapeutic dose is always limited by the potential for developing irradiation-induced cystitis [78]. Successful reduction and protection against irradiation therapy side effects such as cystitis was reported using manganese superoxide dismutase gene therapy. Induction of manganese superoxide dismutase by gene therapy using plasmid/liposome complex (MnSOD-PL) contained the complete human manganese SOD (MnSOD) transgene did not prevent disruption of barrier function by irradiation but led to rapid regeneration of the urothelium and recovery of barrier function [79]. It is also reported that overexpression of a transgene for human (MnSOD-PL) delivered by plasmid-liposome, or adenovirus to the mice lungs, before irradiation exposure, demonstrated to decrease the late effects of whole lung irradiation [80]. It is also found that inhalation radioprotective gene therapy using (MnSOD-PL) provided a practical and effective method for delivery of lung-specific radioprotection during fractionated radiotherapy protocols [81]. Radioprotective gene therapy through retroviral expression of manganese superoxide dismutase may be applicable to the haematopoietic compartment, enabling radiation dose escalation in cancer therapy [82]. Ionizing irradiation-induced murine mucosal cell cycling and apoptosis was found to be decreased using intraoral (MnSOD-PL) radioprotective gene therapy [83]. Previous data provided a rational basis for the design of gene therapy approaches to organ protection from irradiation damage.

Many reports showed that SOD gene therapy has been shown to attenuate tissue damages and improve the recovery of the tissue injuries [84]; to provide the heart with substantial protection against myocardial stunning without the need for concomitant administration of catalase [85]; to ameliorate delayed diabetic wound healing [86]; to stent-induced vascular injury [87]; to attenuates ischemia-reperfusion injury of testes in rats [88]; to attenuates ischemia-reperfusion injury in the rat kidney **i**f delivered to the kidney by intravenous injection [89]; to reduce portal pressure in CCl_4 cirrhotic rats with portal hypertension [90]; to reduce restenosis and may be useful for the prevention of intimal hyperplasia after vascular manipulations [91]; and to reduce oxidative stress in erectile dysfunction [92]. Results also suggested that if MnSOD gene could be effectively delivered to a tumor in vivo using the adenovirus paradigm, effective tumor growth suppression could be observed in hamster [93].

Reports also demonstrated that MnSOD gene therapy could be applied in combination with other therapies, for example with chemotherapy as a dual therapy achieving synergetic action in suppressing the tumor growth of colorectal cancer [94]; another example is in the rabbit model of vein graft disease, combination extracellular SOD gene therapy, antiinflammatory, and antiproliferative genes was found to be effective in decreasing neointimal formation [95].

11. Conclusions

Studies clarified the efficacy of superoxide dismutase as a therapy in the treatment of many diseases such as myocardial ischemia, Peyronie's disease, multiple sclerosis, Behçet's syndrome, colitis; and in improving the clinical irradiation treatment of malignant diseases such as breast cancer. There is also increasing evidence that radical scavengers like superoxide dismutase may influence the outcome and progression of diabetic retinopathy. Superoxide dismutase was also used successfully as an antifibrotic drug and anti-inflammatory agent. In addition, superoxide dismutases are included in cosmetic products to reduce free radical damage to skin.

Microorganisms represent an economic source for production of different superoxide dismutases. Aerobic microorganisms with high oxygen demand such as *Corynebacterium glutamicum* are suggested to have a hyper antioxidant defense system including production of abundance superoxide dismutase enzyme, so special attention should be paid to ameliorate the superoxide dismutase production capacity of those microorganisms. Recently, superoxide dismutase became a promising genetically therapeutic target for reduction of oxidative stress involved in pathophysiology aging and many disorders. For this purpose it is important to develop more efficient gene transfer techniques to deliver the therapeutic gene superoxide dismutase precisely and efficiently into the target cells and tissues.

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