JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

SUPEROXIDE DISMUTASES (SODs) AND SOD MIMETICS: AN UPDATED REVIEW ON THEIR STRUCTURE, MECHANISM AND ROLE IN HUMAN DISEASES AND INDUSTRIAL APPLICATIONS

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Abstract: Superoxide dismutases (SODs) are metalloenzymes that play a major role in antioxidant defense against oxidative stress in the body. Oxidative stress has become widely viewed as an underlying condition in several diseases such as central nervous system disorders, cancer, cardiovascular conditions, ischemia-reperfusion disorders, and diabetes. Thus, natural, and synthetic antioxidants have been actively sought. SOD is a first line of defense against oxidative stress under physiological and pathological conditions. SOD supplementation may trigger the endogenous antioxidant machinery for the neutralization of free-radical excess. However, the applications of natural SODs have been severely limited by their structural instability and high cost. To overcome these limitations of natural SODs, several low molecular weight metal ion complexes are developed as SOD mimetics. This paper is aimed at a brief review of natural SODs and their applications. This paper also reviews briefly about different SOD mimetics and their applications.

Index Terms: **Superoxide dismutase (SOD), SOD mimetics, reactive oxygen species, human diseases, metal cation, mechanism**

I. INTRODUCTION

Reactive oxygen species (ROS) are consistently recognized as threat to living organisms, especially for human beings. Many short-lived and highly reactive ROS such as superoxide anion radical (O_2^-) and hydrogen peroxide (H_2O_2), are toxic and can create oxidative stress in cells, a response involved in the pathogenesis of numerous diseases depending on their concentration, location, and cellular condition. Superoxide dismutase activates an endogenous and exogenous cell defense mechanism resulting in the potential use of SODs in treating various diseases and improving food stuffs preparation and dietary supplements for human nutrition. Through their activity, SODs control the levels of a variety of ROS and reactive nitrogen species, thus both limiting the toxicity of these species and controlling broad aspects of cellular life that are regulated by their signaling.

Superoxide radical is a potent oxidizing agent. Excessive amounts lead to a cascade of reactions causing damage to important biological molecules such as DNA, lipids, and proteins. Excess superoxide plays a role in the pathogenesis of many disease states including cancers, cardiovascular disorders, and neurodegenerative diseases[1-3]. Along with superoxide radicles other reactive species such as nitric oxide (NO), hydrogen peroxide (H_2O_2), peroxy- nitrite ($ONOO^-$) and others have been widely recognized as signaling species that, by affecting redox-based cellular transcriptional activity, control inflammatory and immune responses and enhance secondary oxidative stress[4,5.] Mitochondria, the major producers of reactive species, are consistently found to play critical role in oxidative stress[6,7].

Clinical use of the native SOD enzyme has its limitations, which include, high-manufacturing costs, low cell permeability, antigenicity as well as a short circulating half-life. Similarly, to other enzymes that are used as drugs, SODs possess a charge density

and undergo a rapid renal clearance affecting the pharmacodynamics and pharmacokinetics of the enzyme in a negative way. The development of SOD mimetics demonstrates that it is viable to synthesize small molecules, which carry out the function of much larger enzyme systems, thus, having a number of metal complexes with therapeutic applications[8]. SOD mimetics, as opposed to SODs, have low molecular weights as well as longer half-lives in the blood. Such characteristics would provide better access to cells, be cheaper than SOD, and would also prevent immunogenic responses[9].

II GENERAL MECHANISM OF SOD ACTION

Oxidative damage is a double-edged sword. On one hand, it damages normal tissues and cells, while on the other hand, oxidative damage can induce cell senescence and apoptosis, which may be used for anti-tumor and antibacterial applications. Among various reactive oxygen species (ROS), superoxide radical is the principle one[10]. Superoxide radical ($O_2^{-\cdot}$) is produced in the mitochondria of the cells. SOD has the ability to scavenge $O_2^{-\cdot}$ which catalyzes the dismutation of $O_2^{-\cdot}$ to produce O_2 and H_2O_2 as shown in equations[11] (1) – (3):

$$M^{(n+1)+} + O_2^{-\cdot} \longrightarrow M^{n+} + O_2 \qquad \text{Equ. (1)}$$

$$M^{n+} + O_2^{-\cdot} \longrightarrow M^{(n+1)+} + H_2O_2 \qquad \text{Equ. (2)}$$

$$\text{Net reaction: } 2O_2^{-\cdot} \xrightarrow{\text{SOD}} O_2 + H_2O_2 \qquad \text{Equ. (3)}$$

Here, $M^{(n+1)+}$ represents oxidized form of the metal and M^{n+} represents the reduced form of the metal attached to the active site of SOD. This mechanism is also known as ping-pong effect because it involves the sequential reduction and oxidation of the metal centers[12]. H_2O_2 produced in the above reaction may also generate another reactive oxygen species, the hydroxide ion (OH^-) via the Fenton reaction in the presence of Fe^{2+} ion. Subsequently H_2O_2 is reduced to water and by the catalase (CAT) enzyme, glutathione peroxidase (GPx), and or/thioredoxin (Trx) dependent peroxiredoxin (Prx) enzymes (Fig.1).

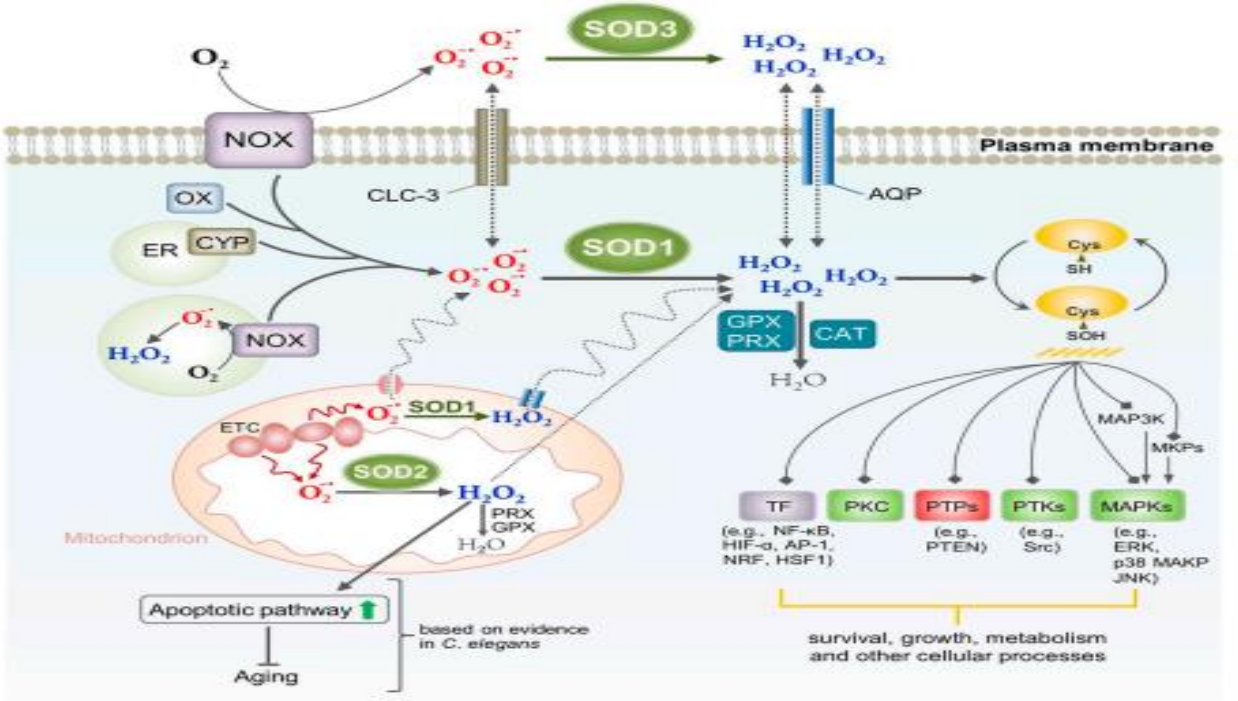

Figure 1: Cellular antioxidant machinery and oxidative stress

III NATURAL SUPEROXIDE DISMUTASES (NATURAL SODs)

SODs are metalloenzymes found naturally in eukaryotes and some prokaryotes. Since their discovery in 1969 by Joe McCord and Irvin Fridovich[13], their role as a major antioxidant defense has been firmly recognized. In the last half century, since the first discovery of SOD, it is strongly established that SODs are the first line of defense against oxygen free radicals, and the vast majority of organisms that live in the presence of oxygen express at least one SOD.

3.1 Structure, Types and Mechanism of Natural SOD Enzymes

Natural SODs, which are generally composed of proteins and metal cofactors are considered to be necessary for aerobic cells. Natural SODs exist in most prokaryotic cells and some eukaryotic cells and are widely present in various organelles. Bases on

different cofactors, natural SODs are divided into four types: copper-zinc SOD (CuZn-SOD), manganese SOD (Mn-SOD), iron SOD (Fe-SOD), and nickel SOD (Ni-SOD). Fe-SODs and Ni-SODs are prokaryotic whereas Fe-SOD is also present in chloroplast. CuZn-SODs are eukaryotic and Mn-SODs dwells within bacteria and all eukaryotes.

CuZn-SODs: CuZn-SODs are present in two different locations of eukaryotic cells – CuZn-SOD expressed in the cytosol and the mitochondrial intermembrane is classified as SOD1 and CuZn-SOD expressed in the extracellular compartment is classified as SOD3[14]. The structure of natural CuZn-SOD is composed of two subunits linked by histidine, each of which has an active center composed of metal atoms, Cu and Zn (Fig.2). In the active center, Cu acts as the catalytic site, while Zn maintains the stability of the enzyme[15]. The mechanism of catalytic action of CuZn-SOD is shown in equations (4) and (5).

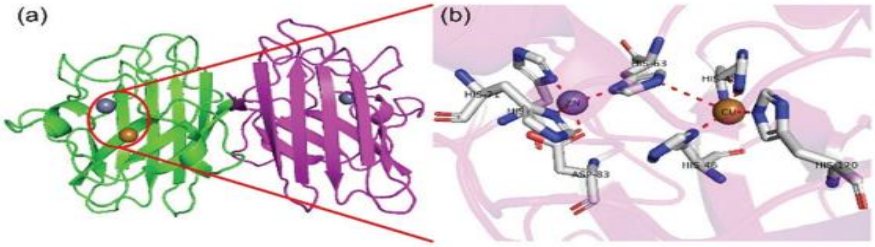

Figure 2: Human CuZn-SOD (a) structure and (b) active site

$$Cu^{2+}Zn^{2+}SOD + O_2^{-\cdot} \rightarrow Cu^{+}Zn^{2+}SOD + O_2 \quad \text{Equ. (4)}$$

$$Cu^{+}Zn^{2+}SOD + O_2^{-\cdot} + 2H^{+} \rightarrow Cu^{2+}Zn^{2+}SOD + H_2O_2 \quad \text{Equ. (5)}$$

Mn-SODs: Despite carrying out similar reaction, the active center structure of Mn-SOD is different from that of CuZn-SOD. Mn-SOD has both dimer and tetramer structures. For prokaryotes, (such as bacteria), Mn-SOD is in dimer form, while the Mn-SOD of eukaryotic cells is usually in the tetrameric form. The human Mn-SOD structure as an example is shown in Fig.3. There is one manganese site in each subunit, and each manganese is coordinated with four protein ligands. The Mn-SOD of eukaryotic cells is expressed in mitochondrial matrix and inner membrane and is classified as SOD2[16]. Like other SODs, the catalytic mechanism of Mn-SOD involves a cycle between Mn^{3+} and Mn^{2+}. However, due to the complexity of the structure, the catalytic mechanism of Mn-SOD involves more steps than other SODs[12,17] which are shown as equations (6) to (9).

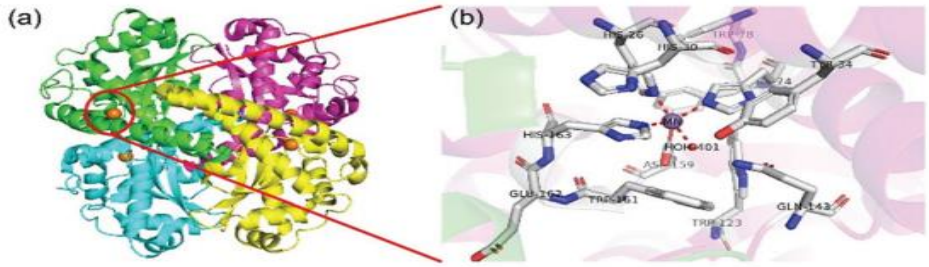

Figure 3: Human Mn-SOD (a) structure and (b) active site

$$Mn^{3+}SOD(OH^{-}) + O_2^{-\cdot} + H^{+} \rightarrow Mn^{2+}SOD(H_2O) + O_2 \quad \text{Equ. (6)}$$

$$Mn^{2+}SOD(H_2O) + O_2^{-\cdot} + H^{+} \rightarrow Mn^{3+}SOD(OH^{-}) + H_2O_2 \quad \text{Equ. (7)}$$

$$Mn^{2+}SOD(H_2O) + O_2^{-\cdot} \rightarrow Mn^{3+}SOD(H_2O) + O_2^{2-} \quad \text{Equ. (8)}$$

$$Mn^{3+}SOD(H_2O) + O_2^{2-} + H^{+} \rightarrow Mn^{3+}SOD(OH^{-}) + H_2O_2 \quad \text{Equ. (9)}$$

Fe-SODs and Ni-SODs: Fe-SOD was first discovered in prokaryotes and its structure is similar to that of Mn-SOD. As shown in Fig. 4, Fe-SOD is a dimer and each monomer has an iron-centered active site. The overall catalytic activity of Fe-SOD also relies on the conversion between Fe^{2+} and Fe^{3+} in a ping-pong manner[18]. Ni-SOD was discovered in recent years in prokaryotes. The structure of Ni-SOD is a homo-hexamer with a Ni and some amino acid residue bonded to the center of each monomer (Fig.5). The mechanism of catalytic activity of Ni-SOD is related to transformation between Ni^{2+} and Ni^{3+}.

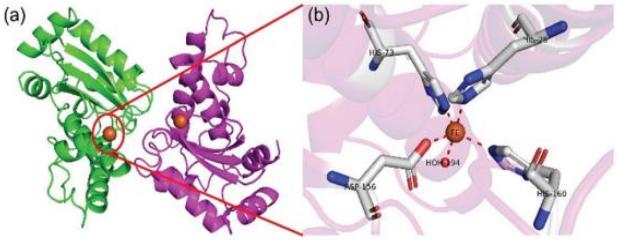

Figure 4: Fe-SOD (a) structure and (b) active site

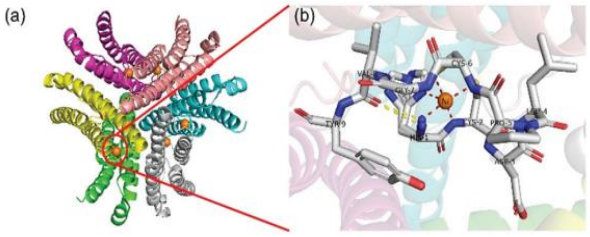

Figure 5: Ni-SOD (a) Structure and (b) Active site

3.2 Role of Natural SODs in Human Diseases and Industrial Applications

Role of Natural SODs in Human Diseases: SOD is the first detoxification enzyme and most powerful antioxidant in the cell. It is an important endogenous antioxidant enzyme that acts as a component of first line defense system against reactive oxygen species. Association between SOD deficiency and a number of pathologies has been observed in both animals and humans. It was observed that mitochondrial SOD deficiency in mice leads to neurodegeneration, myocardial injury, and perinatal death[19]. Mutant human SOD1 has been implicated in the disease commonly known as Lou Gehrig's disease which affects the nerve cells in the spinal cord and the brain[20]. Recently, superoxide deficiency promoted cerebral vascular hypertrophy and vascular dysfunction in hyperhomocysteinemia, has been reported[21]. Numerous studies have established safety of SODs drugs in animals and humans. Many studies reported using SODs as anti-inflammatory agents[22]. Reports also available for using SODs for treatment of skin ulcer lesions, especially due to burn and wounds. SOD is also used in reducing the severity of arthritic inflammation. Researchers discovered that SODs stimulate hair growth and decrease hair loss[23]. During irradiation treatment malignant diseases like breast cancer, radiation induced side effects such as radiation-induced sclerosis and radiation-induced fibrosis (radio-fibrosis), are treated using CuZn-SODs where no other effective therapy exists[24].

SOD enzyme deficiency is common. Hence, the enzyme is indispensable to cellular health, protecting body cells from excessive oxygen radicals, free radicals and other harmful agents that promote aging or cell death. The levels of SODs decline with age, whereas free radical formation increases. It has been suggested that proper daily SOD supplementation will protect the immune system and significantly reduce one's chances of diseases and ultimately slowdown aging process. It is encouraged to consume cabbage, Brussels sprouts, wheat grass, barley grass and broccoli as natural source of SOD[25].

Industrial Applications of Natural SODs: Commercial use of SOD in therapeutics has been investigated[26]. SODs are also used in cosmetics as an ingredient in anti-aging agents, anti-inflammatories, antioxidants, anti-pollution agents, anti-puff agents, anti-wrinkle agents, conditioning agents, firming agents/botox-like, moisturizing agents, nourishing agents, protective agents, smoothing agents, and soothing agents has been reported[27]. Liposomal SODs have been shown to be effective in cancer prevention of animal models. They have also passed safety tests during early phase clinical trials. Dietary supplement-based SOD cancer prevention provides another opportunity for industries to develop antioxidant-based cancer prevention agents[28].

IV SOD MIMETICS

As already mentioned above, using of natural SODs in therapy have many drawbacks like their high production cost, their large molecular size, which in turn limits their cell permeability, their limited circulating half-life in the body and their antigenicity. These aspects explain why natural SODs usage is restricted to drug applications in animals and to non-drug application in humans. To overcome these drawbacks associated with using natural SODs, in the last fifty years many efforts are being made by researchers to test different SOD-based compounds: from plant and animal extracts and SOD recombinant forms to SOD mimetics and SOD gene therapy. Among various available substituents for natural SODs, synthetic antioxidant enzymes, known as SOD mimetics, were found to be best alternative. SOD mimetics are characterized by low-molecular weight and better intestinal permeability when administered orally, but this also grants a higher circulating half-life and lower antigenicity. As Mn-SOD is crucial antioxidant enzyme in mitochondria, Mn-SOD mimetics are widely investigated by researchers for their pharmacokinetic properties for different applications.

4.1 Structure and Types of SOD Mimetics

According to literature sources available, SOD mimetics can be divided into different classes according to their structure: cyclic polyamines, MnPLEDs, MnP, salen-Mn complexes, metal-based compounds, and nitroxides. The structures of main types of manganese-based SOD mimetics are shown in Fig. 5 along with a nitroxide SOD mimetic[29].

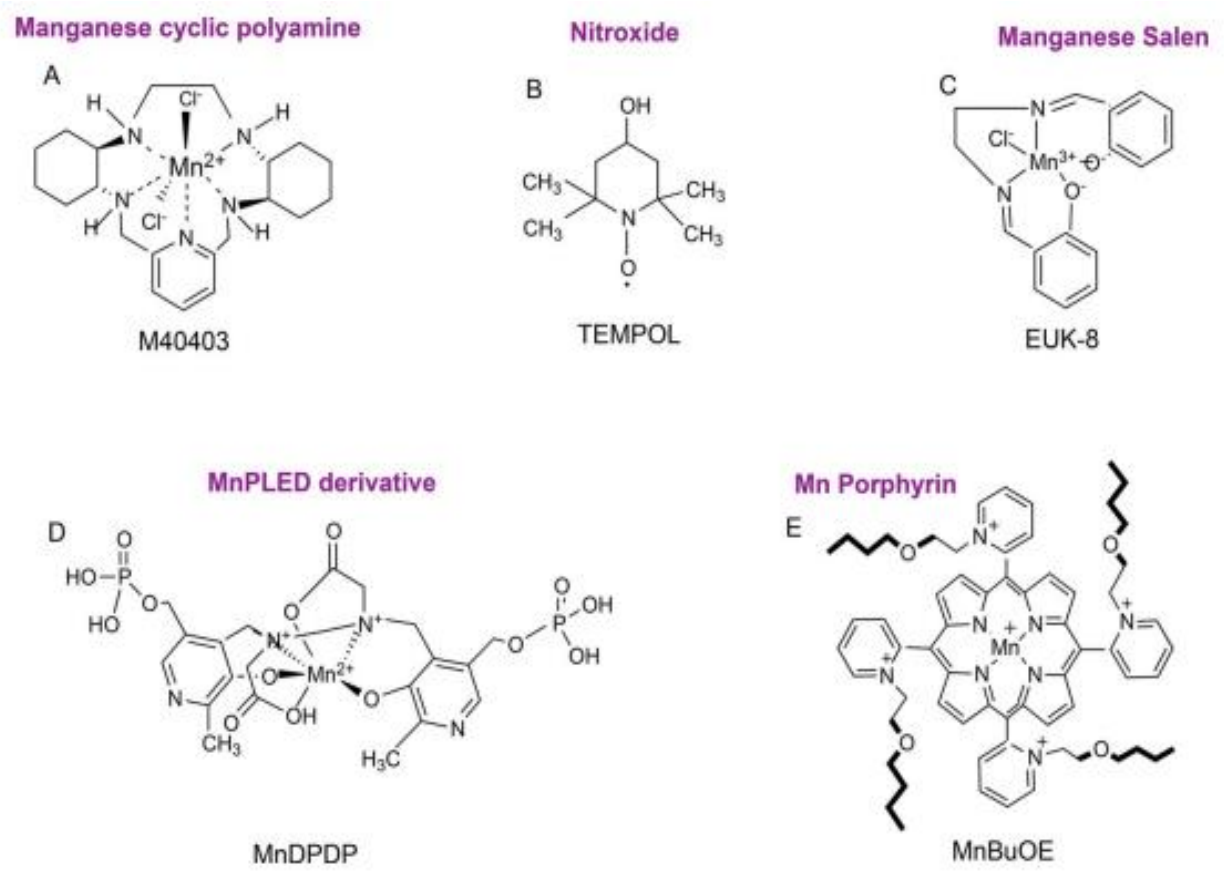

Figure 6: Different classes of SOD mimetics

The structural differences in different SOD mimetics can result in differing pharmacokinetic properties, including the route of administration and subsequent bioavailability. While the pharmacokinetics of MnPs have been widely investigated, a similar in-depth pharmacokinetic analysis is not available for other SOD mimetics[30].

4.2 Role of SOD Mimetics in Human Diseases

In vivo models have clearly shown the beneficial effects of SOD mimetics in protection against radiation injuries and improvement of the therapeutic index in anti-cancer drugs, ischemia reperfusion injury, protection against chemical stress, endotoxic shock, neuronal oxidative stress, diabetes, or inflammation[31]. Manganese cyclic polyamine SOD mimetics are reported to have therapeutic potential against models of ischemia and intestinal reperfusion, besides models of acute inflammation[32]. Nitroxide SOD mimetics like TEMPOL restricts CNS autoimmune disease and damage in established models of multiple sclerosis[33]. Manganese salen SOD mimetics are shown to have high therapeutic potential in numerous diseases related to oxidative and nitrosative stress. MnPLED SOD mimetics (mainly MnDPDP) have been tested in cancer patients and patients suffering from acute myocardial infarction, with promising results[34]. Manganese porphyrin SOD mimetics have therapeutic potential in numerous types of cancer that include head and neck, breast, brain, skin and lymphoma. These types of SOD mimetics have also shown promising characteristics, which could be used in the treatment of radiation injury, asthma, inflammation, bleomycin-induced pulmonary fibrosis and amyotrophic lateral sclerosis (ALS)[35].

V CONCLUSIONS

The brief and updated review reported herein, covering data published on the use of SODs for neurological, cardiovascular, respiratory, gastrointestinal, renal, skin, metabolic and ocular diseases, are indicative of the high efficacy of all SOD types tested, both natural SOD and SOD mimetics. Although SOD has been as attractive potential approach for 50 years, most of the published papers, and even more so in the case of recent works, deal with experimental preclinical studies, and only comparatively few clinical studies are ongoing. Notably the spread of the pandemic COVID-19 infection, causing the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), further renewed the interest in pharmacological strategies to counteract the oxidative stress response triggered by NOS. Since the spread of pandemic, in the last two and half years, numerous reports were published proposing many SOD mimic compounds to lower the inflammatory burden in critical SARS-CoV-2 infections[36]. However, none of the tested compounds have been approved to date. Several issues with the testing conditions and the type of compound evaluated have hampered translation of the evidence for SOD use from the bench to the bedside. Future research in Mn-SOD gene therapy in conjunction with radiotherapy will be able to avail from novel vectors that are safe for therapeutic purposes. SOD mimetics have expanding therapeutic potential in

oncology. These compounds can be used in combination with chemotherapy and radiotherapy, thus, enhancing the effectiveness of such treatments in cancer cells. While attenuating drug side-effects and toxicity issues related to radiation.

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