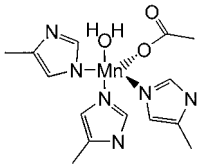
The Pharmaceutical Potential of Manganese-Based Superoxide Dismutase Mimics

Roland Krämer*

A considerable part of O_2 metabolized by the human organism is converted by one-electron reduction to the highly reactive superoxide radical anion, $O_2^{\bullet-}$. Sources of superoxide include "leaks" in the cytochrome c oxidase mediated O_2-to-H_2O transformation pathway in mitochondria, various autooxidation reactions (e.g. of glutathione in red blood cells) but also the controlled production of $O_2^{\bullet-}$ by membrane-bound NAD(P)H oxidase in phagocytes to support immune defense against bacterial and fungal infections.

Endogenous overproduction of $O_2^{\bullet-}$ (and a number of highly reactive oxidizing agents thus formed) combined with reduced ability to eliminate radicals (= oxidative stress) clearly has the potential to inflict considerable damage on biological systems.[1] In particular, the protonated form HO_2 ($pK_a = 4.8$) is a very potent initiator for the selective autooxidation of lipid membrane components at the allylic position of unsaturated fatty acids and is also an active agent for the abstraction of ribose hydrogen atoms in DNA leading to extremely mutagenic DNA damage.



Scheme 1. Mn^{II} coordination sphere in manganese superoxide dismutase from human mitochondria.[7]

The presence of superoxide in biological systems was first revealed by the discovery in 1969 of superoxide dismutase (SOD) enzymes by McCord and Fridovich.[2] In healthy organisms radical accumulation is suppressed by these enzymes which contain, in the active site, either a manganese ion (Mn-SOD, Scheme 1, present in mitochondria) or a dinuclear Cu/Zn unit (Cu/Zn-SOD, present in the cytosol and extracellular space). The second-order rate constant for the spontaneous disproportionation of $O_2^{\bullet-}$ to O_2 and H_2O_2 is $5 \times 10^5\,M^{-1}s^{-1}$ at 21 °C and pH 7.4. This corresponds to a half-life of about 0.2 seconds at an initial $O_2^{\bullet-}$ concentration of $10^{-5}\,M$ ($t_{1/2}$ is proportional to $1/[O_2^{\bullet-}]$), sufficient to effect serious damage to cell components. The SOD enzymes dramatically accelerate $O_2^{\bullet-}$ decay with second-order catalytic rate constants of up to $2 \times 10^9\,M^{-1}s^{-1}$ for the Cu/Zn-SOD system (diffusion controlled reaction).

In the reduced state the metal (Mn^{II} or Cu^{I}) converts superoxide into H_2O_2 by a one electron reduction [Eq. (1)], followed by oxidation of a second equivalent $O_2^{\bullet-}$ to O_2 by the Mn^{III} or Cu^{II} center, respectively [Eq. (2)]. The less aggressive H_2O_2 is destroyed or metabolized by other enzymes such as catalase.

$$O_2^{\bullet-} + M^{n+} + 2H^+ \longrightarrow H_2O_2 + M^{(n+1)+} \quad (1)$$

$$O_2^{\bullet-} + M^{(n+1)+} \longrightarrow O_2 + M^{n+} \quad (2)$$

Net reaction:

$$2\,O_2^{\bullet-} + 2H^+ \longrightarrow H_2O_2 + O_2 \quad (3)$$

Oxidations mediated by superoxide are currently believed to participate in the pathogenesis of a number of important degenerative diseases.

The typical course of myocardial or cerebral infarct includes transient ischemia (stopping of the blood flow) of tissue areas followed by reperfusion with oxygenated blood. Since during the hypoxic events the cell is continuing to build reducing equivalents, reperfusion results in high levels of superoxide which are responsible to a great extent for cell death and tissue damage. In autoimmune conditions oxidants produced by activated neutrophiles at sites of inflammation can have serious consequences for the organism, for example, joint damage in arthritis. Severe neurological disorders, such as amyotrophic lateral sclerosis (ALS), Parkinson's and Alzheimer's disease may be related to nerve cell damage caused by superoxide. Some types of cancer are thought to arise from oncogene (tumor forming) mutations caused by oxidative damage to DNA.

Application of SOD enzymes to animals has clearly shown beneficial effects in some of the above mentioned diseases. Cu/Zn-SOD preparations (trade names: palosein, orotein) are available for the treatment of inflammatory diseases in dogs and horses. In spite of encouraging results in animal models, SOD is not yet used for the treatment of human disease, a result of complications in clinical trials, in particular immunogenic (allergic) responses.

[*] R. Krämer
Anorganisch-Chemisches Institut
Im Neuenheimer Feld 270
69120 Heidelberg (Germany)
Fax: (+49) 6221-548599
E-mail: roland.kraemer@urz.uni-heidelberg.de

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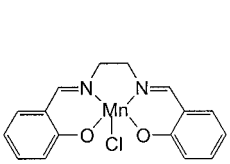
Low molecular weight mimics could have significant advantages over SOD enzymes such as lack of immunogenic response, longer half-life in the blood, improved access to cells and intercellular space, potential for oral delivery, and low costs. The effects of metal-based SOD analogues on biological systems have been investigated for two decades,[3–5] but the requirements for a mimic that could be used as a human pharmaceutical agent are high: high chemical and metabolic stability, SOD enzyme like activity and specificity under physiological conditions, low toxicity, and favorable biodistribution. In this respect, the manganese complexes **1** and, in particular, **2** (discovered by the research group of D. Salvemini and P. Riley at MetaPhore Pharmaceuticals[6]) are among the most promising candidates. Complex **2** was designed on the basis of molecular modeling considerations. These studies predicted that, of a class of complexes derived from the 1,4,7,10,13-pentaazacyclopentadecane framework, the properties for **2** were most favorable.



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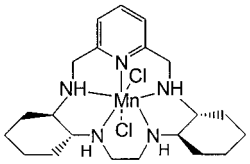

2

Complex **2** is thermodynamically stable, the constant for its formation from the macrocycle and Mn^{II} ions in water is >17 ($\lg K$). After intravenous injection in rats it is taken up into the heart, lungs, brain, liver, and kidneys and is excreted intact. The second-order rate constant for catalytic breakdown of superoxide is $2 \times 10^8\,M^{-1}s^{-1}$ at pH 6 and 21 °C and is comparable to that of manganese SOD enzymes. This is a rare example for a low molecular weight enzyme mimic that displays structural similarities to the enzyme active site (Mn^{II}/Mn^{III} redox cycle, nitrogen donors) and at the same time approaches the catalytic rate of the enzyme under physiological conditions. Complex **2** is selective for superoxide and does not react with NO and H_2O_2.

The anti-inflammatory effect of **2** was tested in rats in which paw edema (swelling) was provoked by a local injection of carrageenan.[6] The increase of paw volume, a consequence of inflammatory response, as well as the infiltration by neutrophile blood cells was almost completely blocked by administering complex **2** (10 mg kg^{-1}) 30 min before the carrageenan injection.

In another rat experiment certain arteries were occluded to generate prolonged ischemia of intestinal tissue. Subsequent reperfusion results in neutrophile infiltration of the intestine, increased plasma levels of lipid peroxidation products and cytokines, severe hypotension , and finally in circulatory shock. The consequence is a high mortality rate within the first two hours. All these effects are significantly reduced when 15 min before reperfusion infusions of **2** (1 mg kg^{-1} rat) are given, with survivals rates of 90% after 4 hours. Thus superoxide radical anions appear to be one of the mediators for the above mentioned events.

In summary the potent SOD mimic **2** which is about 60 times smaller than the natural enzyme has strong anti-inflammatory and cytoprotective effects in rats. It is a powerful pharmacological tool to explore the selective role of superoxide in physiological processes. The potential of SOD mimics such as **2** for the treatment of a broad range of inflammatory and cardiovascular diseases and their efficiency compared with other antioxidants has to be explored by clinical studies.

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