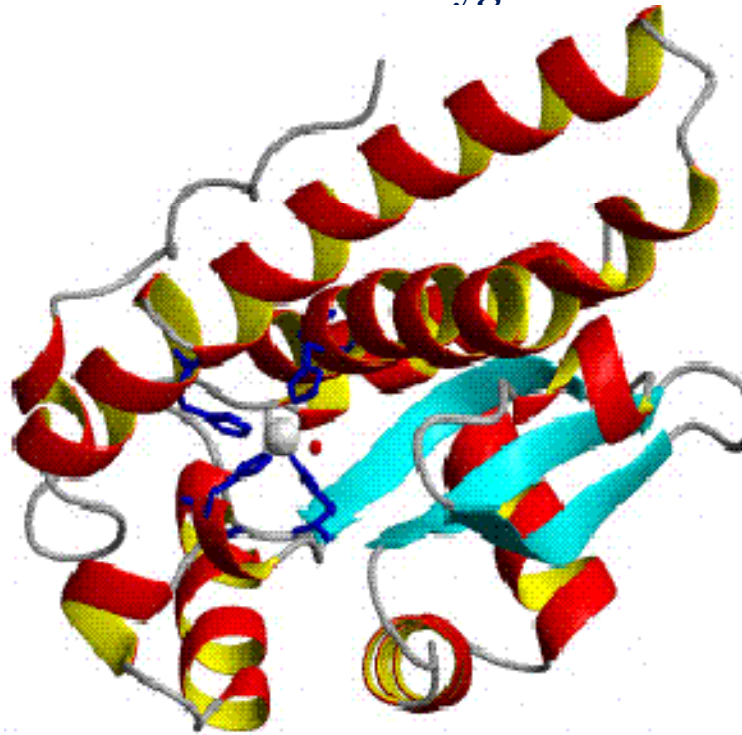
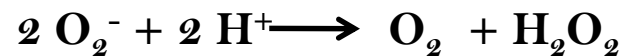


Mn(II)-base Superoxidase Dismutase Mimics...

SOD is an oxidoreductase that **catalyses the dismutation of superoxide to hydrogen peroxide and molecular oxygen**

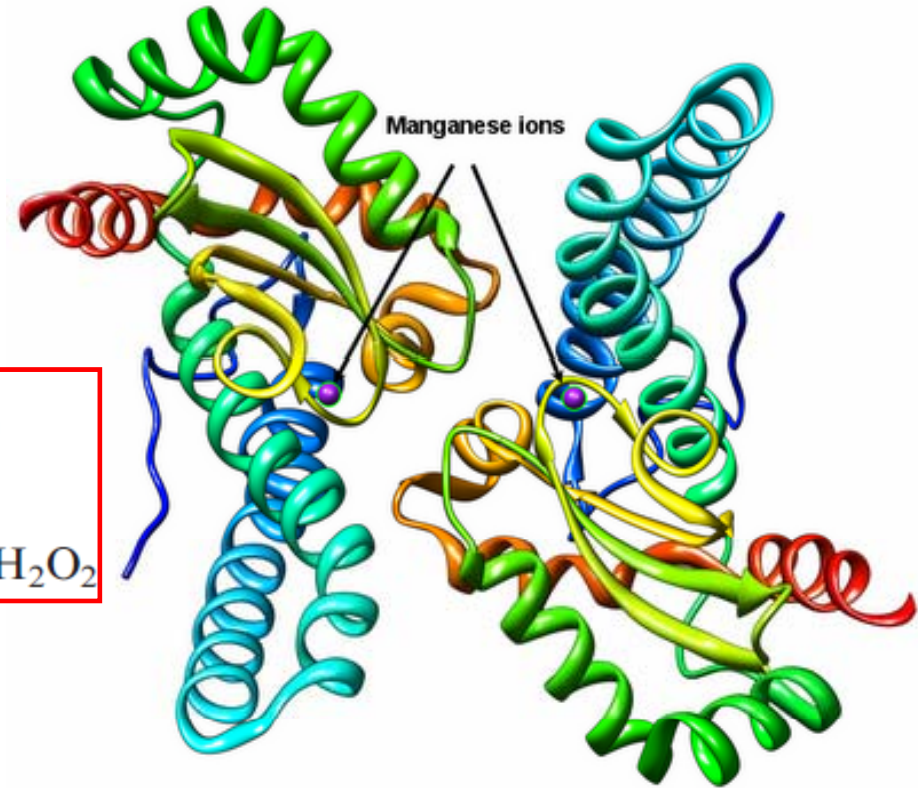
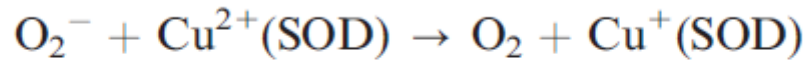


During normal respiration O_2 is consumed in living mammalian tissues but the biochemical processes involved may also produce **superoxide, O_2^-** or, in more acidic media, **HO_2** . Both of these are very reactive species capable of damaging DNA and initiating the auto-oxidation of membrane lipids and so are potentially damaging to tissues...

Manganese superoxide dismutase mimics

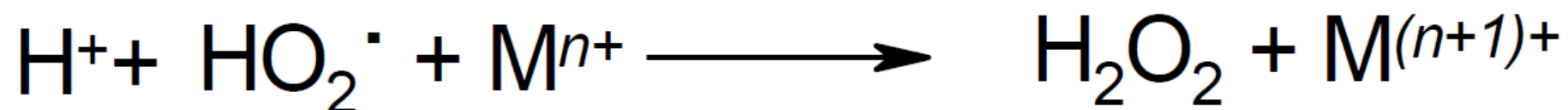
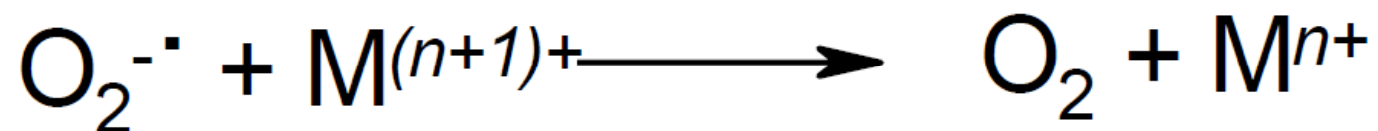
The functional activity of the enzyme superoxide dismutase (SOD) was discovered about 30 years ago (Fridovich and McCord).

A lot of effort has been made to develop the enzyme as a **therapeutic agent**. However there is only one clinically approved use of the recombinant SOD enzyme.

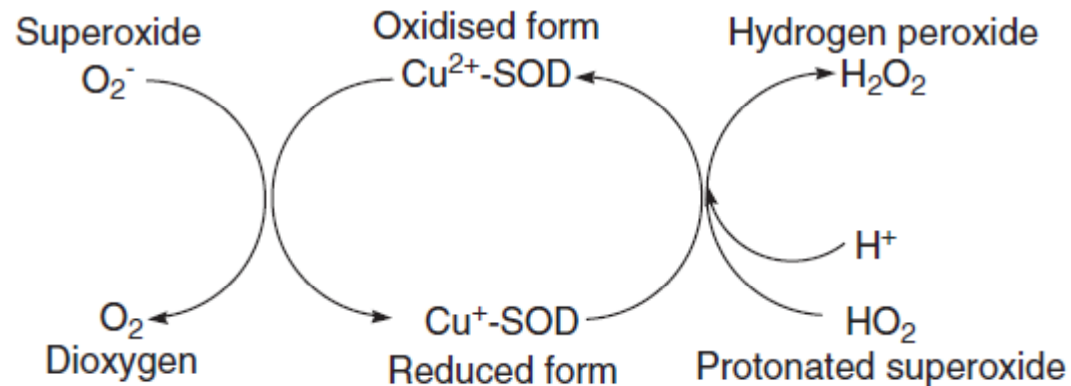
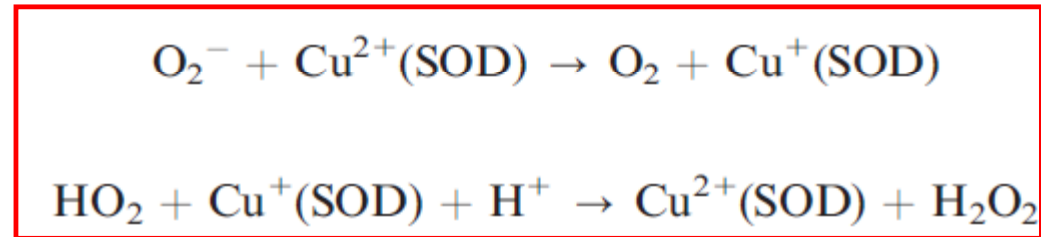


Catalysed by the SOD group of enzymes which exploit the **electron transfer properties** of certain metal ions. Examples are known with **Mn^{2+/3+}**, **Fe^{2+/3+}**, **Ni** or **both Cu and Zn** at the active site. The dioxygen produced in the reaction is, in effect, recycled while the **peroxide** is rapidly consumed *in vivo* by peroxidase enzymes carrying out biochemically controlled oxidations. Many forms of SOD enzymes are found in animal and plant species. **In humans there are two main types: in extracellular spaces contains both Cu and Zn at the active site, while in mitochondria contains Mn.**

SOD enzymes containing Fe or Ni are also known to occur but in other organisms.



In humans there are two main types: in extracellular spaces contains both Cu(II) and Zn(II) at the active site, while in mitochondria contains Mn(II).



Why Zn(II) ??? It's $3d^{10}$ and can not undergo electron transfer reactions... possibly, it has an electronic and structural effect on Cu(II)...

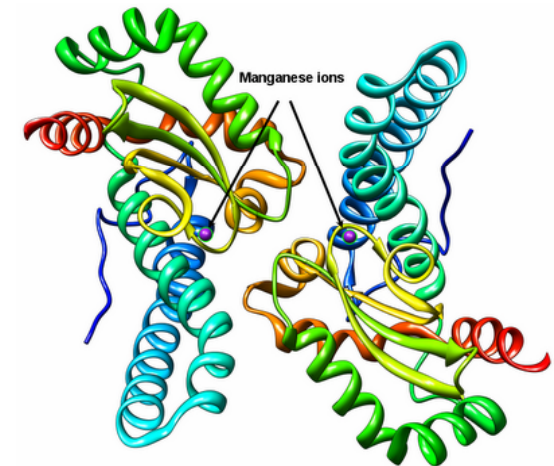
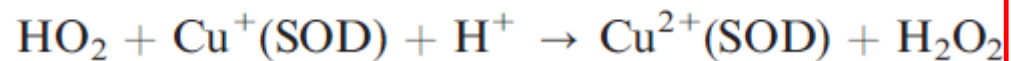
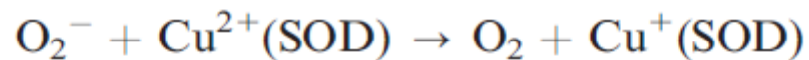
Very fast reactions in vivo ...

The rate of removal of superoxide is essentially determined by the rate at which O_2^- can **diffuse** to the enzyme.

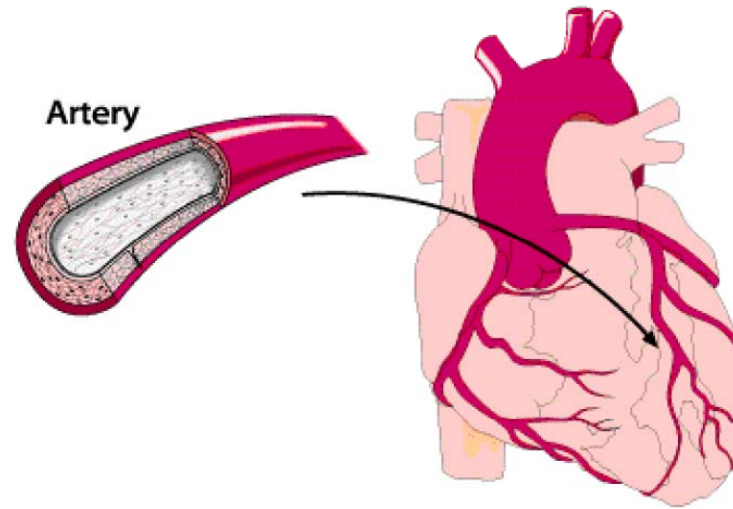
Mitochondrial SOD employs a similar catalytic cycle involving Mn^{3+} and Mn^{2+} while iron containing SOD similarly utilises a cycle involving Fe^{3+} and Fe^{2+} .

SOD enzymes containing Fe or Mn as the reactive metal react with O_2^- a bit slower than the Cu/Zn enzyme

Problems start in the case of disease or trauma...the amount of O_2^- produced can not be “destroyed” by the available SOD present...



The aim of treating injury from myocardial ischemia-reperfusion injuries has not been reached.



In addition, over the last 15-20 years an increase in the amount of biochemical data suggests that $\text{O}_2^{\cdot-}$ and HO_2^{\cdot} are involved in a number of biological pathways relevant to disease states.

Biological processes involving the perhydroxyl radical include:

- autoxidation of lipid membranes
- inflammation mediation
- site selective DNA nick

Normally your healthy tissue can control the delicate balance of these reactive species. A number of disease states result in a situation where the body can no longer cope.

Reperfusion injuries can be caused by myocardial infarct or stroke. They are a prime target for a drug.

In addition a drug candidate may have action against diseases such as arthritis and neurological disorders such as Parkinson's disease.

What then?...

- 1) Provide additional SOD as a therapeutic agent...

DISADVANTAGES

- (1) a narrow efficacious dose by a bell-shaped dose-response curve
- (2) lack of an accurate and routine method to monitor and quantitate

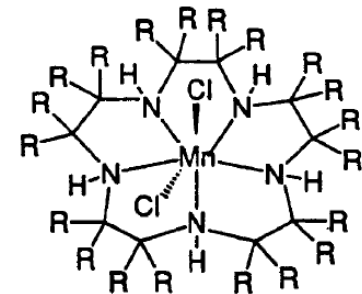
SOD activity

- (3) inability of the enzyme to gain access to the intracellular space
- (4) instability of the enzyme in blood (only for short period)

(5) *lack of oral bioavailability*

(6) Immunogenicity

(7) Cost



- 2) Development of synthetic, low-molecular weight mimetics of SOD...

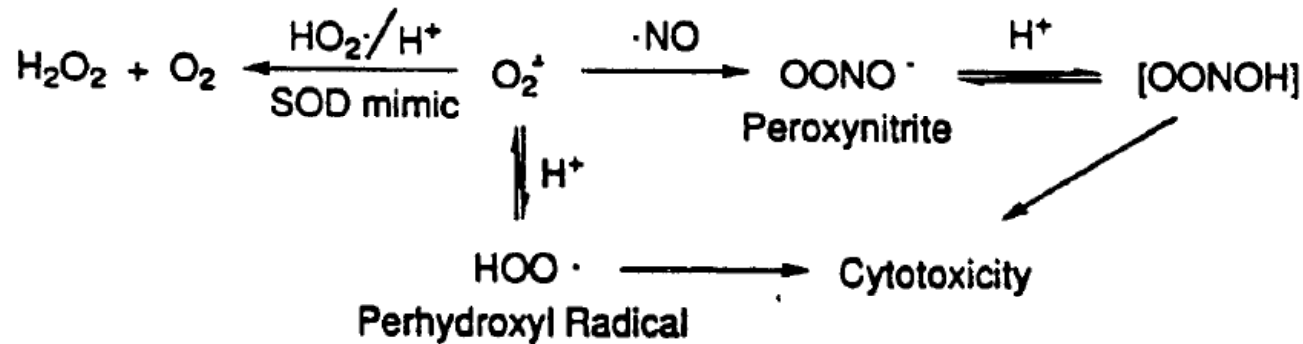


Figure 2 *Therapeutic strategy for the SOD mimics. The SOD mimic catalyzes the dismutation of superoxide, thereby preventing the formation of the cytotoxic oxidants, peroxynitrite and the perhydroxyl radical*

The basis of the therapeutic strategy utilizing SOD mimics is to more efficiently catalyze the dismutation of superoxide to generate the less innocuous non-radical compounds, **hydrogen peroxide** and O_2 , before cytotoxic superoxide-derived oxidants, such as peroxynitrite and the perhydroxyl radical can be produced. Superoxide reacts in a near diffusion controlled manner with nitric oxide to produce the potent oxidant peroxynitrite. **Under ischemic conditions where the pH of tissue can decrease**, the formation of the perhydroxyl radical ($\text{HO}_2\cdot$), which can abstract hydrogen atoms from unsaturated fatty acids to initiate lipid peroxidation will occur to a greater extent.

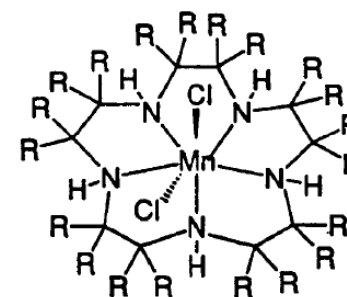
We need a catalytic reaction, not a “consuming” one (like the classic one where, *i.e.* an antioxidant reacts stoichiometrically and gets consumed)...

Design of Mn-based SOD mimics...

Why Mn and not Cu- and Fe – based?

Manganese-based complexes are particularly attractive as potential SOD mimics because they have a reduced capacity to react with **the dismutation product hydrogen peroxide** to generate cytotoxic hydroxyl radicals, whereas Cu- and Fe-based complexes and their aquated ions are known to carry out such undesirable chemistry...

Remember that we need a catalytic reaction, not a “consuming” one (like the classic one where, *i.e.* an antioxidant reacts stoichiometrically and gets consumed)...



In order to develop a SOD mimic for clinical use it is necessary to incorporate the metal in a suitable complex which has the **required catalytic effect** while showing **low toxicity** combined with **acceptable biodistribution** and **pharmokinetics**.

Mn^{3+/2+} : less prone to form hydroxyl radicals and lower toxicity in the free aquated form...

Requirements

- (1) Relatively easy to prepare,
- (2) Very stable as not to release the metal and produce unwanted toxicity,
- (3) The ligand system chosen needs to be resistant to attack from O_2^- , O_2 or HO_2^-
- (4) Acceptable biodistribution
- (5) Acceptable pharmacokinetics

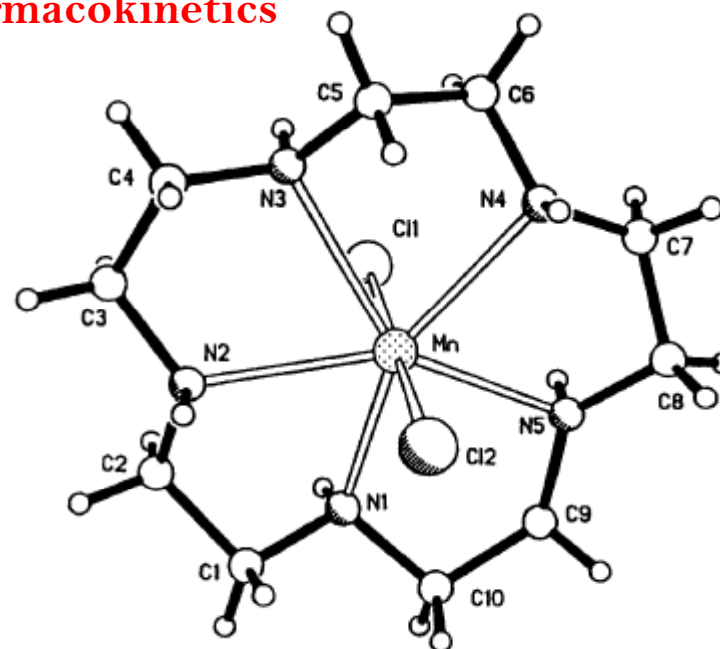
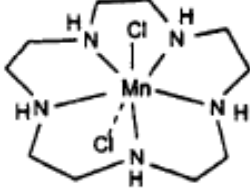


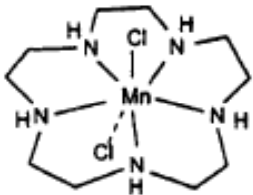
Table 1 *Advantages of Mn(II)-based SOD mimics over SOD enzymes*

Characteristic				Advantage of SOD mimic
		Bovine erythrocyte Cu, Zn SOD enzyme		
Molecular weight	341	32 500		Intracellular
Reactivity	O ₂ ^{-•} only	O ₂ ^{•-} , OONO ⁻ , H ₂ O ₂		Low toxicity
Structure	Non-peptidic	Peptide		Oral bioavailability; non-immunogenicity
Kinetic stability with EDTA	Stable	Unstable		Stability
Source	Synthesis	Isolation		Cost

Molecular Weight. The low molecular weight of the SOD mimics, lower than SOD enzyme by a factor of almost 100, permits the complexes to gain access to the intracellular space of certain cell types, whereas the cells are generally impermeable to the enzyme.

Reactivity. The SOD mimics catalyze the decomposition of superoxide only and do not react with hydrogen peroxide or with peroxynitrite. The Cu,Zn SOD enzyme reacts with both hydrogen peroxide and with peroxynitrite, inactivating the enzyme. The selective reactivity of the SOD mimics for superoxide makes these complexes useful probes to establish the role of superoxide in biological mechanisms

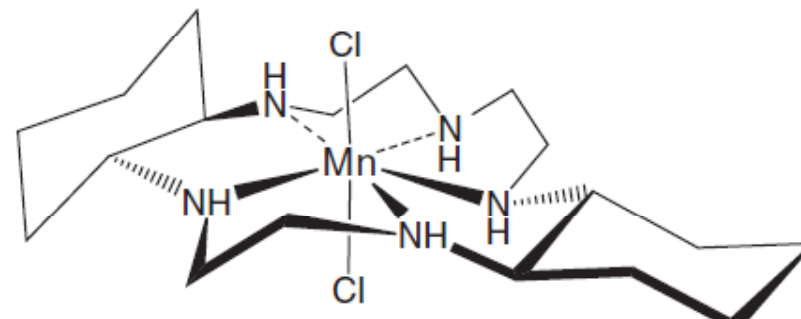
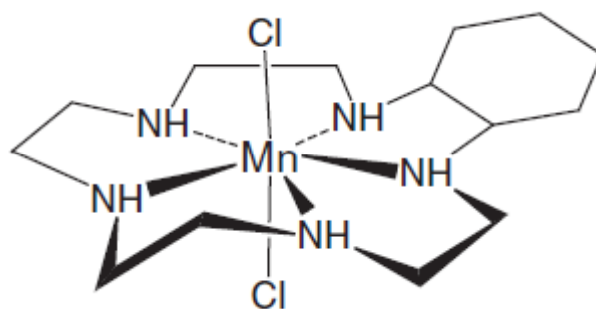
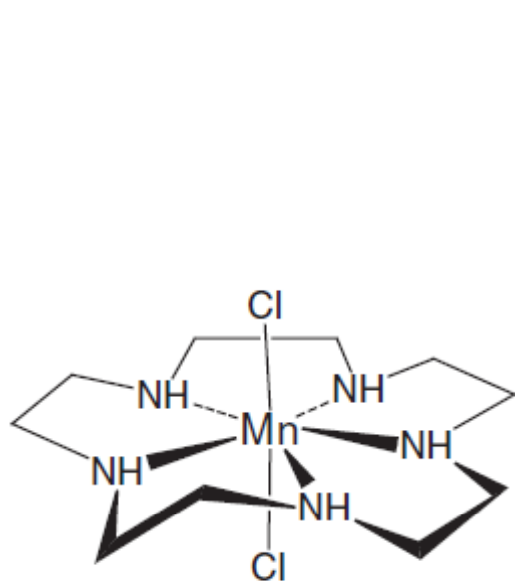
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Kinetic stability with EDTA	Stable	Unstable	Stability
Source	Synthesis	Isolation	Cost

Structure. The SOD enzyme structure is peptide-based and is thus readily degraded by **proteases**; therefore, it is not orally **bioavailable**. Immunogenicity problems are also a concern with the non-human-derived SOD enzymes, whereas the SOD mimics, which are not peptide-based, do not have that problem. It should also be possible to develop an orally effective Mn(II)-based SOD mimic.

Kinetic Stability. Due to the stabilizing effect of the macrocycle, the SOD mimics have high kinetic stability and, for example, are stable in the presence of the transition metal ion chelators, such as EDTA. However, the apoenzyme form of the Cu,Zn SOD enzyme can readily be prepared by dialyzing the enzyme with EDTA.

Source. The Mn(II)-based SOD mimics are prepared by total synthesis, whereas the enzymes are isolated from natural sources or prepared by recombinant DNA techniques. Consequently, the cost of manufacturing the SOD mimics would be potentially much less than that for manufacturing the enzymes.



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SC-54417

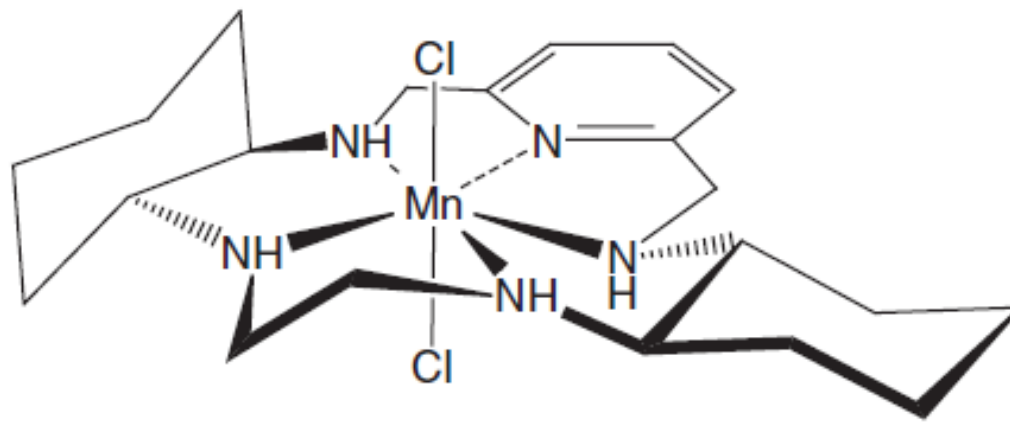
SC-55858

k_{cat} pH 7.4 $4.13 \times 10^7 \text{ M}^{-1} \text{ sec}^{-1}$ $9.09 \times 10^7 \text{ M}^{-1} \text{ sec}^{-1}$ $1.20 \times 10^9 \text{ M}^{-1} \text{ sec}^{-1}$

k_{diss} $2814 \text{ M}^{-1} \text{ sec}^{-1}$ $1375 \text{ M}^{-1} \text{ sec}^{-1}$ $33 \text{ M}^{-1} \text{ sec}^{-1}$

Increasing SOD Activity and Stability





Developed by Metaphore Pharmaceuticals Inc.

Clinical trials undertaken: Favourable results were obtained in a **Phase II clinical trial** of the enhanced analgesic effects of combining with morphine for **post-operative pain relief from dental operations**.

Similar trial of this combination approach was conducted on a bunionectomy post-operative pain model and a further trial is in progress to study the relief of moderate to severe pain in cancer patients.

Biological data

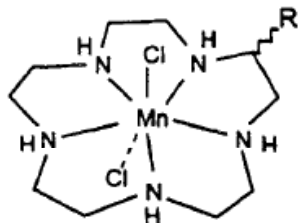
A rat animal model was used to simulate reperfusion injury. (In vivo rapid superoxide build up to about 2 micromolar)

After 30 minutes of the blood flow cut off, it was released and the drug was introduced.

About 40% of heart dead was reduced to 10% with the drug administered.

Lipophilicity...

Table 2 *Lipophilicities of Mn(II)-based SOD mimics*



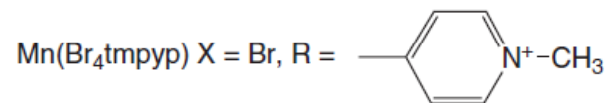
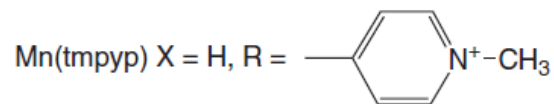
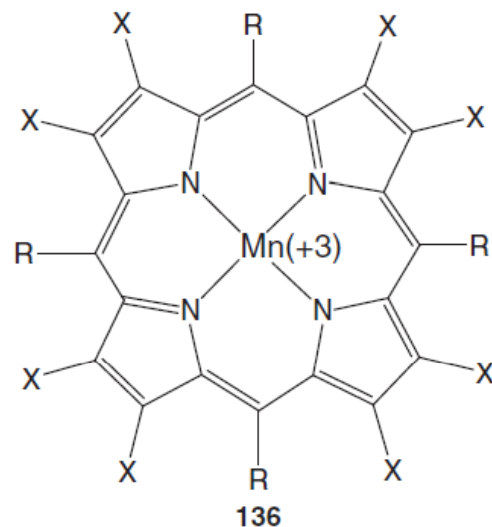
<i>R</i>	<i>log P</i>
1-Aminobutyl	− 4.1
Hydroxymethyl	− 3.4
H	− 2.9
Methyl	− 2.6
2-Propynyl	− 2.5
Isobutyl	− 1.5
Benzyl	− 1.3
Alkenyl	− 1.3
Phenyl	− 1.2
Cyclohexyl	− 0.87
Cyclohexylmethyl	− 0.30
1-Naphthyl	+ 0.02
<i>n</i> -Octadecyl	+ 0.18

P is the ratio of the partitioning of the complex between *n*-octanol and aqueous Hepes buffer, pH 7.4.

Lipophilicity increases



A potential advantage of the Mn(II)-based SOD mimics over the SOD enzymes is the ability of the SOD mimics to permeate the cell membrane. The **lipophilicity** of the complexes **can be controlled** by placing the appropriate substituents onto the carbon backbone of the macrocycle



Complexes containing macrocyclic porphyrin ligands have been found to show catalytic properties. The complex $\text{Mn}(\text{tmpyp})$ catalysed O_2^- dismutation with an apparent rate constant of $10^7 \text{ M}^{-1} \text{ s}^{-1}$ at pH 7.6 and 21°C while $\text{Mn}(\text{Br}_4\text{tmpyp})$ is said to have a SOD activity of about 12% that of the Mn enzyme itself.

PROBLEM: Under oxidising conditions porphyrin complexes of this type are prone to the formation of dimers through the formation of O^{2-} or OH^- bridges. These dimers are not active SOD catalysts. However, dimer formation could be suppressed by incorporating bulky substituents which prevent the close approach of two molecules necessary for dimer formation

Conclusions...

The radical **superoxide** (O_2^-) is over-produced in the body after i.e. ischemia followed by reperfusion, exposure to radiation, or activation of white blood cells in autoimmune conditions such as arthritis. Protonated superoxide HO_2^\cdot (the perhydroxyl radical) can initiate the autoxidation of lipid membranes and cause selective damage on DNA by abstracting the 5'-hydrogen from the deoxyribose ring of nucleotide units.

O_2^- is removed naturally by superoxide dismutase enzymes which are $\text{Cu}^{\text{I/II}} / \text{Zn}^{\text{II}}$ or $\text{Mn}^{\text{II/III}}$ enzymes in eukaryotic cells.

- $\text{O}_2^- + \text{M}^{\text{n+1}} \rightarrow \text{O}_2 + \text{M}^{\text{n}}$
- $\text{HO}_2^\cdot + \text{H}^+ + \text{M}^{\text{n}} \rightarrow \text{H}_2\text{O}_2 + \text{M}^{\text{n+1}}$
- When these enzymes cannot cope with O_2^- overproduction, the use of a mimic (a **synzyme**) may be useful in therapy.