Design and Development of Superoxide Dismutase Mimetics as Therapeutics
Traditional Approach
Block negative effects of enzymes

MetaPhore’s Approach
Amplify positive effects of enzymes
SYNZYMES: A CASE STUDY

Mimics of Superoxide Dismutase: mSOD
Superoxide Shunts

\[ \text{O}_2 + \text{H}_2\text{O}_2 \]

\[ \text{HOO}^- \xrightarrow{\text{H}^+} \text{O}_2^- \]

SOD Catalyst

Neutrophil-Driven Immune Responses

Normal Hydrogen Peroxide Detoxification

Catalase or Glutathione Peroxidase

\[ \text{O}_2 + \text{H}_2\text{O} \]

\[ \text{OCl}^- - \text{bacteriocide} \]

(Myeloperoxidase) + \text{Cl}^-
$O_2^+$ in Health and Disease
Reactive Oxygen Metabolites

\[ \text{Arginine} \rightarrow \text{NOS} \rightarrow \text{NO}^- \rightarrow \text{O}_2^- \rightarrow \text{HOO}^- \rightarrow \text{O}_2 + \text{H}_2\text{O}_2 \rightarrow \text{Cellular Damage} \]

- Peroxynitrite (OONO^-)
- Hydroxyl Radical (\text{\cdot}OH)
- Peroxynitrite Cytotoxic
- Lipids, DNA, Catecholamines, Steroids, etc.
- SOD Catalyst
- Fe^{III} Storage Proteins
- Fe^{II}
- [OONO\text{OH}]
- \text{Chain Autoxidation Products}
- \text{Enzyme Mimetic}

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Biochemical Impact of Excess Superoxide Anions

- Degrades Synovial Fluid & Collagen
- Depolymerizes Hyaluronic Acid
- Cytokine Release
- Stimulates Release of Inflammatory Mediators
- DNA Damage
- ONOO⁻ Generation
- Lipid Peroxidation
Superoxide is eliminated under physiological conditions by a set of superoxide dismutase enzymes that convert it to hydrogen peroxide which is then converted to water and oxygen by catalase.

Manganese superoxide dismutase is localized in mitochondria while Cu/Zn superoxide dismutases are in the cytoplasm and extracellular.

Bovine Cu/Zn SOD (Orgatein®) was used as a pharmaceutical in Europe for many years for the treatment of osteoarthritis.

In animal models, SOD enzymes have been shown efficacious in models of ischemia-reperfusion injury, inflammation, radiation damage, etc.
DISADVANTAGES OF PROTEIN SOD THERAPY

- Lack of Oral Activity
- Lack of Access to Intracellular Space where Superoxide is Produced
- Immunogenicity
- Short Half-Lives in Serum
Project Goal: SOD mimics

To develop a revolutionary advance in the small molecule approach to pharmacological intervention.

- Synzymes: Small molecules that achieve the destruction of superoxide at a rate of tens of millions of times per second per molecule, mechanistically similar to SOD, for acute and chronic therapy.
OBJECTIVE “SYNZYMME” – A SYNTHETIC ENZYME THAT CATALYZED CONVERSION OF SUPEROXIDE TO HYDROGEN PEROXIDE

Ribbon diagram of crystal structure of human superoxide dismutase dimer; active sites are within blue ribbons
TECHNOLOGY: mSODs

10 years of research
>$50 Million

Monsanto/ Searle

SOD
- 88kDA
- $k_{cat} = 10^8-10^9$ M$^{-1}$s$^{-1}$
- superoxide $\Rightarrow$ hydrogen peroxide

mSOD
- ca. 500Da
- $k_{cat} = 10^7-10^9$ M$^{-1}$s$^{-1}$
- superoxide $\Rightarrow$ hydrogen peroxide
Monsanto/Searle Synzyme Project

I did an evaluation of a synthetic enzyme project at Monsanto as a consultant.

I was extremely excited about the scientific achievements and therapeutic potential.

Due to external events (Monsanto purchased $6 billion worth of seed companies and COX-2 inhibitors were still in clinical trials), the decision was made to shelf the synzyme project.

I offered to out-license the project and finance it (over $90 million so far).
M40403 IS NOT A COPY OF ACTIVE SITE OF Mn SOD, BUT A MIMIC OF CATALYTIC MECHANISM

Active Site of human SOD – 3 His and 1 Asp coordinate with Mn

M40403 has 5 nitrogen ligands to Mn from its pentaazacrown crown scaffold
HOW WAS TECHNOLOGY DEVELOPED THAT GENERATED CLINICAL CANDIDATE?

M40403

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Enzyme Mimetic
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SEVERAL CHOICES WERE MADE:

Choice of metal – Mn, Cu or Fe?
- All found in SOD enzymes; only Mn not involved in Fenton chemistry that produces hydroxyl radicals

Choice of ligand – stability of complex a crucial feature; cyclization was known to enhance complex stability

Redox potential had to match that of both superoxide and anion and not that of other potential substrates; nitrogen coordination was appropriate
Screen Mn complexes of azacrownethers

Initial SAR
1. MnCl₂ complex prepared for each macrocycle
2. The MnCl₂ complex of one ligand, SC-52608, showed substantial SOD activity

Figure 2. Stopped-flow decays of superoxide measured at 245 nm at 21 °C, where [SO]₀ ~ 100 μM, in the presence and absence of complex 1. All reactions were carried out using 80 mmol of HEPES buffer at pH = 7.75. A: 8.80 × 10⁻⁶ M [Mn([15]aneN₅)Cl]Cl. B: 2.20 × 10⁻⁶ M [Mn([15]aneN₅)Cl]Cl. C: uncatalyzed.
Traditional Approach to Synthesis of Pentaazacrowns Gave Poor Yields

Scheme 1. Richman–Atkins type synthesis of the monomethyl-substituted ligand 16 accomplished in an overall yield of ~4%.
Cyclic Peptide Approach to Cyclopentaazaacrowns

SOD Mimetics Used to Develop Models of Mechanisms

Changes in Chirality of Substituents and Introduction of Cyclic Constraints Modified Complex Geometry


Figure 1. Structures of Mn(II) complexes (axial ligands omitted for clarity) with the macrocyclic ligands utilized for generating a modeling database. The stereochemistry of substituents and NH's is included along with the nitrogen labels: N° (outer-sphere NH fold) and N' (inner-sphere NH fold).
Stereochemical Effects

$k_{cat}$ (pH=7.4) = $1.2 \times 10^8$ M$^{-1}$ s$^{-1}$

$R,R,S,S$-isomer is rigidly planar; i.e., will not fold, thus no catalytic activity

$k_{cat}$ (pH=7.4): Inactive
Ligands Used to Test Predictive Ability of Model

*Figure 5.* Ligands utilized for testing the predictive ability of the folding paradigm for MM calculations.
Crystal Structure of M40403
The folded six-coordinate Mn(II) structure is very close to that of the six-coordinate Mn(III) indicating a favorable geometry for facile electron transfer & hence fast SOD catalysis. (Science, 286, 304 (1999)).
RATIONALIZATION FOR CATALYSIS

Marcus – lower reorganizational energy correlates with lower activation energy and higher rates of catalysis

Pauling – enzyme catalysis is based on stabilization of the transition state
MECHANISMS FOR CONVERSION OF SUPER-OXIDE TO HYDROGEN PEROXIDE VIA INNER SPHERE OR OUTER SPHERE OXIDATION OF Mn(II)


Figure 5. Mechanistic scheme depiction for the formation of a unique six-coordinate intermediate complex which gives rise to both the inner-sphere and outer-sphere pathways for oxidation of Mn(II).
Development of the SOD Mimic: Advances in Chemistry (from SC-68328 to M40403)

6000-fold stability increase, while retaining catalytic activity
M40403 – Lead Candidate Properties

Manganese-containing biscyclohexylpyridine mSOD

- Catalytic activity equivalent to that of the native enzyme
- Very stable in vivo: extracted intact (97%) from plasma, urine, bile after iv dosing
- Protective in various models of acute and chronic inflammation
Synthesis of M40403

1. TrtCl, CH₂Cl₂
2. Glyoxal, MeOH 96%

NH₂

\[
\text{OHC} - \text{CHO} + \text{NH₂H₂N} + \text{MnCl₂} \rightarrow \text{M40400} \]

\[
\text{M40400} \rightarrow \text{Pd/C, 150 psig H₂ ropanol} \]

\[
\text{M40402} \rightarrow \text{1 hr, >98% yield} \]

\[
\text{Enzyme Mimetic M40403} \]

99.3% purity in 1 pot reaction-- >93% Chem Yield with No Chromatography or Crystallization needed
Molecular Modeling has made possible the design of more stable compounds

M40403  M40401  M40484

SAR studies utilizing the \([\text{Mn}([15]\text{aneN}_5)\text{Cl}_2]\) lead reveals that extremely high chemical stability is achieved with added substituents to carbon centers of the macrocyclic ligand (Inorg. Chem., 35, 5213, 1996).

SAR studies reveal that catalytic activity can be dramatically affected with the stereochemistry, number, and position of such substituents (JACS, 119, 995 (1997)).

Mechanistic understanding developed that led to a molecular modeling paradigm (MM) allowing prediction of catalytic activity (Inorg. Chem., 38, 1908 (1999); and ibid., 40(8), 1779 (2000).)

\textit{Bis}-cyclohexylpyridine class of SOD mimic affords high activity and high stability with excellent \textit{in vivo} efficacy (Science, 286, 304 (1999)).
Metabolism of M40403

\[ k_{\text{cat}} (\text{pH}=7.4) = 1.6 \times 10^7 \]
\[ \log P = -0.3 \]

Liver Oxidases

\[ k_{\text{cat}} (\text{pH}=7.4) = 1.35 \times 10^7 \]
\[ \log P = -0.9 \]

M40403

M40414

Enzyme Mimetic

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One of two subunits.

Native Enzyme

M40403

O\textsubscript{2}^-

Inflammation
- Pain
- IBD/Crohn’s
- Osteoarthritis
- Rheumatoid arthritis
- Psoriasis

Oncology
- Pain
- Inhibition of tumor growth
- Reduction of side effects associated with chemotherapy and radiation therapy

Cardiovascular/CNS
- Ischemia-reperfusion injury
- Septic shock
- Organ transplantation
- Stroke
Superoxide – central role

SOD mimetics effective in multiple models
- Rheumatoid arthritis
  - Blocks TNFα release
- Asthma
- Many diseases

Collagen-induced Arthritis
Traditional NSAIDs provide unsatisfactory relief in models of severe chronic pain (neuropathic pain, visceral pain, cancer pain).

Opportunities exist beyond opioids and NSAIDs for the treatment of acute and severe pain.
Roles of Superoxide Anions ($O_2^{-}$) in Pain

- Sensitize primary afferent neurons
- Increase spinal release of excitatory amino acids (NMDA)
- Deactivate NE, a potent endogenous analgesic
- Damage nerve endings
  - Direct algesic action

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Enzyme Mimetic
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Intraplantar Injection of Superoxide Anion Evokes Pain in Rats

M40404 which lacks SODm activity does not block this response. Drugs given i.v. 15min before challenge.
Reversal of Acute Hyperalgesia by M40403 in Rat Footpad Carrageenan Model

Rapid onset

Long duration

Time (min) post drug

Time (hr) post drug

% inhibition of hyperalgesia

drug administered iv 3 hr after carrageenan

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Enzyme Mimetic

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M40403 Restores Analgesia in Morphine-tolerant Mice

Naive

Tol 0.01

100

20

Compound given 5 min prior to morphine

mg/kg, i.v

% Analgesia

120

100

80

60

40

20

0

Naive

Tol

0.01

0.03

1
Mechanism: SOD-mimics in Ischemia – Reperfusion-Mediated Injury

Ischemia

Reperfusion

$O_2^-$

Lipid peroxidation & cellular damage

TNF$\alpha$

Neutrophil activation/sequestration

Primary & Secondary Organ Damage

SOD-mimics
SC-67066 Inhibits the Burst of $\text{O}_2$ Release and Reduces Myocardial Damage upon Reperfusion of the Ischemic Rabbit Heart

- Control
- I (30min)
- I+R (60s)
- +SC-67066 (0.65 µmol/kg)
- +SC-67066 (1.25 µmol/kg)
- +SC-67066 (2.5 µmol/kg)
- +SC-65512 (2.5 µmol/kg)

$\text{O}_2$ concentration

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Catecholamine Autoxidation

A Single Reaction with HO$_2^-$ Initiates a
"Chain Autoxidation" Affording Multiple
Conversions to "Adrenochromes"!

Salvemini, MacArthur, Riley, *PNAS*, 2000, 97(17), 9753

MetaPhore Pharmaceuticals
Targeting SOD mimetics to Receptors

Use of Chiral Metal-Azacrowns as Peptidomimetics

Marshall et al., CCB, WUMS, St. Louis


PAC Conformational Template vs. Type I $\beta$-Turn

Pentaazacrown complexed with Mn(II, red ball) showing overlap of i, i+1, and i+2 side-chain orientations (yellow)
Targeting SOD mimetics to Receptors

Use of Chiral Metal-Azacrowns as Peptidomimetics

Marshall et al., CCB, WUMS, St. Louis

RGD Bioactive Conformation Mimicked by Chiral MAC

Peptide Backbone (yellow): cyclo(Arg-Gly-Asp-D-Val-Phe)
Overlay: PAC - cyclo(Arg-Gly-Asp-cyclohex-Ala)
Targeting Metal Complexes to Receptors

Use of Chiral Metal-Azacrowns as Peptidomimetics

Marshall et al., CCB, WUMS, St. Louis

MIMICRY OF CHIRAL MAC WITH WRY SEQUENCE OF TENDAMISTAT THAT INHIBITS α-AMYLASE
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Entropy and Solvation


Structured Waters

Fig. 1. World energies and excess entropies of water molecules in the principal hydration sites of the binding cavities. The world energy is the energy of interaction of the water molecules with the entire system. Shown are data for principle hydration sites that are proximal to hydrophobic protein groups. The points labeled 1, 2, and 3 represent data for hydration sites with unusually high ordering.

Fig. 2. World energies and excess entropies of water molecules in the principal hydration sites of the binding cavities. The world energy is the energy of interaction of the water molecules with the entire system. Shown are data for principle hydration sites that are proximal to hydrophobic protein groups. The point labeled 4 represents data for a hydration site with unusually high ordering.
Waters in Strepavidin

Fig. 3. The binding cavity of strepavidin and a typical solvating water configuration. Also shown is the protein structure that stabilizes the ring. The green lines represent hydrogen bonds. The hydrogen bonds between the ring water molecules and the protein are the correlated hydrogen bonds referred to in the text. The gray scaffolding is the protein that encloses the ring from above.

Fig. 5. A typical configuration for a water molecule in principle hydration site 1 from Fig. 1 in the 1DBJ binding cavity. The molecule is orientationally constrained such that its oxygen atom maintains a hydrogen bond with Asn-35. It is also flanked on three sides (to the left, right, and below) by hydrophobic groups. The two hydrogen bond vectors point toward additional solvent with which the water molecule can hydrogen bond. The purple shading is to the scale of a Van der Waals radius for a water molecule.
“Mutating Trp-79 to Phe was found to enthalpically stabilize biotin binding by 1.5 kcal/mol but entropically destabilize it by 2.4 kcal/mol (10). This mutation effectively enlarges the cavity and partially removes the hydrophobic enclosure, resulting in more entropically favorable binding-cavity solvation. This result explains why Poisson–Boltzmann-based methods, which cannot capture molecular-length scale solvation physics, underestimate the binding affinity, as measured by the disassociation constant, of the streptavidin–biotin complex by three to six orders of magnitude, whereas explicit solvent simulations predict the binding affinity within chemical accuracy (11–13).”