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Graphical Review

An educational overview of the chemistry, biochemistry and therapeutic aspects of Mn porphyrins – From superoxide dismutation to H₂O₂-driven pathways

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ABSTRACT

Most of the SOD mimics thus far developed belong to the classes of Mn-(MnPs) and Fe porphyrins(FePs), Mn(III) salens, Mn(II) cyclic polyamines and metal salts. Due to their remarkable stability we have predominantly explored Mn porphyrins, aiming initially at mimicking kinetics and thermodynamics of the catalysis of O₂^{•−} dismutation by SOD enzymes. Several MnPs are of potency similar to SOD enzymes. The in vivo bioavailability and toxicity of MnPs have been addressed also. Numerous in vitro and in vivo studies indicate their impressive therapeutic efficacy. Increasing insight into complex cellular redox biology has been accompanied by increasing awareness of complex redox chemistry of MnPs. During O₂^{•−} dismutation process, the most powerful Mn porphyrin-based SOD mimics reduce and oxidize O₂^{•−} with close to identical rate constants. MnPs reduce and oxidize other reactive species also (none of them specific to MnPs), acting as reductants (antioxidant) and pro-oxidants. Distinction must be made between the type of reactions of MnPs and the favorable therapeutic effects we observe; the latter may be of either anti- or pro-oxidative nature. H₂O₂/MnP mediated oxidation of protein thiols and its impact on cellular transcription seems to dominate redox biology of MnPs. It has been thus far demonstrated that the ability of MnPs to catalyze O₂^{•−} dismutation parallels all other reactivities (such as ONOO[−] reduction) and in turn their therapeutic efficacies. Assuming that all diseases have in common the perturbation of cellular redox environment, developing SOD mimics still seems to be the appropriate strategy for the design of potent redox-active therapeutics.

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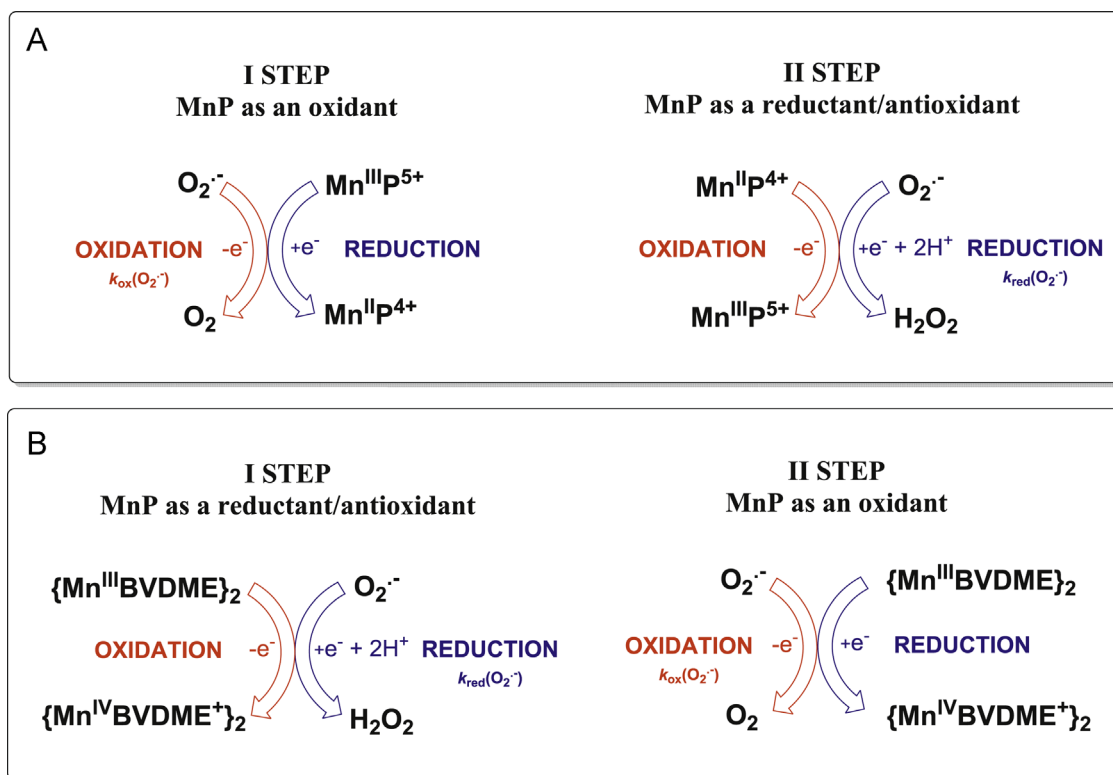
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Introduction

The necessity of SOD enzymes for all aerobic life [1,2] led to the development of SOD mimics as therapeutics [3]. The macrocyclic

structure of a porphyrin ring, by the analogy to the structure of the critical porphyrin-containing proteins, such as hemoglobin, myoglobin, nitric oxide synthases and cyt P450 family of enzymes, provides limitless stability to a metal complex and preserves



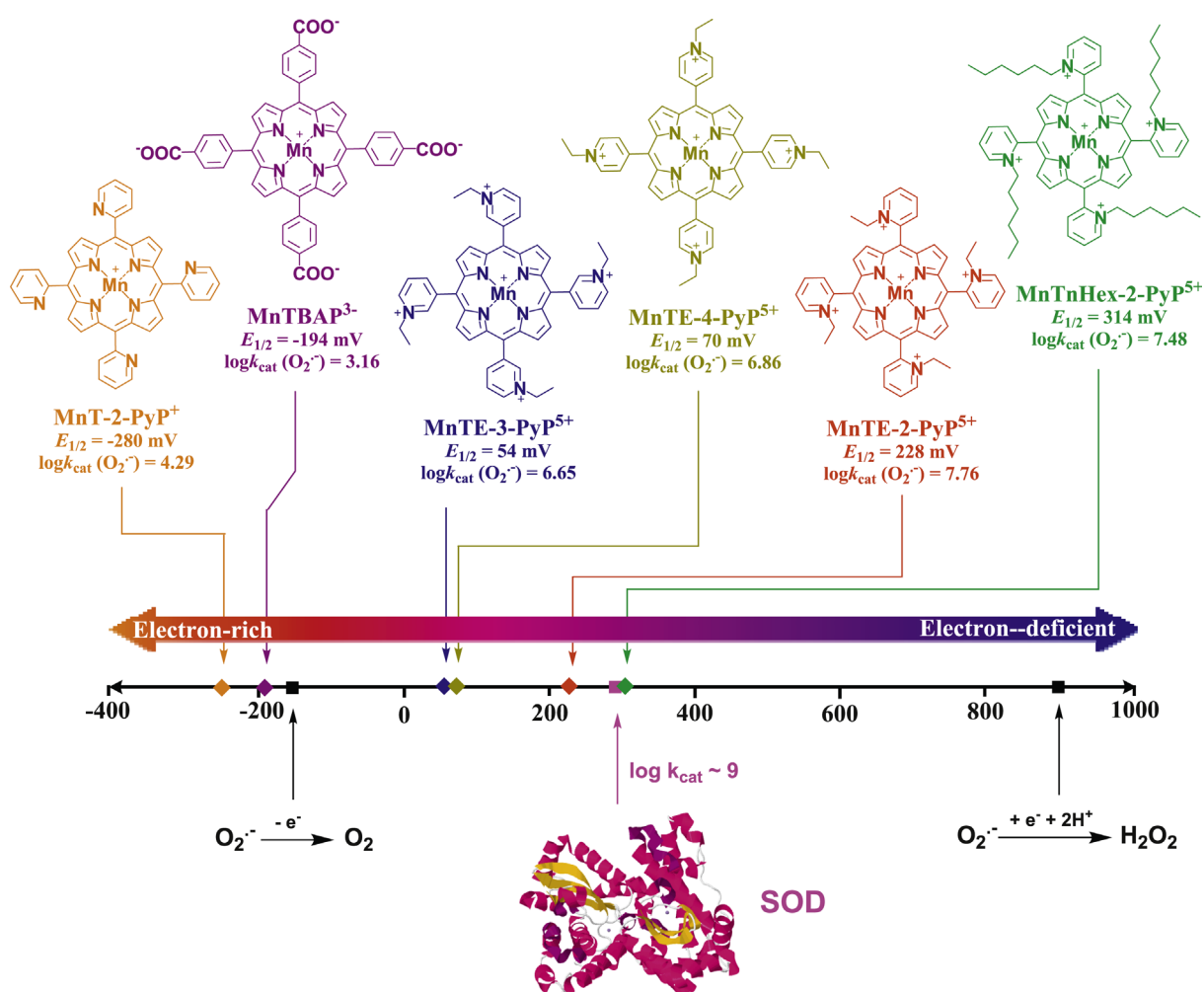
Scheme I. The $O_2^{\bullet -}$ dismutation process catalyzed by pentacationic Mn porphyrins (A) and Mn biliverdin (and its derivatives) (B). A. Dismutation of $O_2^{\bullet -}$ by pentacationic Mn complexes involves Mn^{III}/Mn^{II} redox couple. The dismutation process shown here communicates important information that SOD mimics exhibit PRO- and ANTI-oxidative actions during $O_2^{\bullet -}$ dismutation, i.e. they can accept (1st step) electron from $O_2^{\bullet -}$ producing O_2 , and give away electron to another molecule of $O_2^{\bullet -}$ (2nd step) producing H_2O_2 if their metal-centered reduction potential, $E_{1/2}$ for $Mn^{III}P/Mn^{II}P$ redox couple is within the range of +100 to +500 mV vs NHE. Such $E_{1/2}$ values indicate that SOD mimics are mild oxidants and reductants/antioxidants and that their reduction potentials are biologically compatible and allow them to couple with numerous other biological targets. Yet, there is another side of dismutation process. The 2nd step (while antioxidative in its nature with regards to $O_2^{\bullet -}$) produces an oxidant, H_2O_2 . The SOD enzymes and their mimics may in turn be only viewed as anti-oxidative defense when (under physiological conditions) are coupled with multiple H_2O_2 removing systems. It explains why in numerous in vivo reactions (oxidation of thiols, ascorbate, lipids, NADPH, NADH, see below) MnP acts as pro-oxidant, while still GENERATING therapeutic EFFECTS. Thus the additional distinction must be made here between the actions of redox-active compounds and therapeutic effects observed. Please note that MnPs with very negative $E_{1/2} < 0$ mV vs NHE (very electron rich such as MnTBAP³⁻ or MnTSP³⁻) are fairly redox-inert and cannot be reduced in a 1st step with $O_2^{\bullet -}$, but can act as anti-oxidants (reductants) during oxidation with strong oxidants (such as ONOO⁻, ClO⁻, lipid reactive species). Since they are electron-rich, and oxidation with those species involves their binding to Mn site, such reactions are less favored than those with electron-deficient Mn porphyrins (such as MnTE-2-PyP⁵⁺) with $E_{1/2}$ in between +100 and +500 mV vs NHE [7]. Note, also that MnPs with $E_{1/2} > +450$ (very electron-deficient, $Mn^{II}Br_8TM-3$ (or 4)-PyP⁴⁺ and $Cl_3Mn^{II}TE-2$ -PyP⁴⁺), are stabilized in +2 oxidation state and lose Mn readily at pH 7.8. For comparison those Mn(III) N-alkylpyridylporphyrins with Mn in +3 oxidation state (indicated with III) resist even 98% sulfuric and 36% hydrochloric acids. Such compounds therefore start dismutation by reducing $O_2^{\bullet -}$ while oxidizing Mn in a 1st step, and get reduced to Mn(II) while oxidizing $O_2^{\bullet -}$ in a 2nd step, reversal of Scheme A. B. Dismutation of $O_2^{\bullet -}$ by Mn(III) biliverdin and its analogs employ Mn^{IV}/Mn^{III} redox couple, shown in Scheme for Mn(III) biliverdin dimethylester (BVDME). Mn(III)- and Fe(III) corroles employ metal +3/+4 redox couple also [8]. In turn, in a 1st step those metal sites reduce $O_2^{\bullet -}$ while undergoing oxidation to M(IV) complex, whereas in backward reaction they get reduced with $O_2^{\bullet -}$ (and restored as catalysts) (see [3] for details). Regardless of which metal redox couple is used (+3/+2 or +3/+4), $O_2^{\bullet -}$ only cares if the metal easily accepts electron from and donates the electron to it, thus operating at $E_{1/2}$ value that is around the midway potential between the oxidation and reduction of $O_2^{\bullet -}$; consequently both steps of dismutation are facilitated to a similar extent. That indeed is true, the $E_{1/2}$ ranges between +440 and +470 mV vs NHE for Mn(III) biliverdin analogs, while $E_{1/2} = +468$ and +480 mV vs NHE for $Br_8Mn^{II}TM-3$ -PyP⁴⁺ for $Br_8Mn^{II}TM-4$ -PyP⁴⁺ [9].

integrity of the metal site where all actions occur. Consequently, porphyrin appears to be the most appropriate ligand for an SOD mimic. Further, the porphyrin structure allows for limitless modifications whereby the redox property, bioavailability and toxicity could be optimized. Over years in search for the best therapeutics, metalloporphyrins with high $k_{\text{cat}}(\text{O}_2^{\cdot-})$ reaching that of SOD enzyme and with high stability towards the loss of Mn, have been synthesized and characterized. Such properties give them the advantage over other classes of SOD mimics. Most recent research indicate that SOD-like activity parallels ability of MnPs to undergo other reactions (such as ONOO^- reduction) and their therapeutic efficacy. None of the reactions appear specific to MnPs. For additional insight see also other articles in Antioxidant & Redox Signaling 2014 Forum Issues on “Superoxide dismutases” (vol. 20/10)

and “SOD therapeutics” (vol.20/15).

$\text{O}_2^{\cdot-}$ dismutation process

The dismutation process consists of two steps indicated below for Mn porphyrin-based SOD mimics. It is critical to note that: (i) SOD enzyme or MnP-based mimic acts as a (pro)oxidant in a 1st step, and as an anti-oxidant in a 2nd step, whereby closing the catalytic cycle, and (ii) an oxidant, H_2O_2 , is produced in a 2nd step. Under physiological conditions it is taken care of. Thus, H_2O_2 is either dismuted to O_2 and H_2O , or H_2O_2 is reduced to H_2O , with enzymatic systems such as catalases, glutathione peroxidases (GPx), peroxiredoxins, etc. Therefore both SOD and its mimic could



Scheme II. SOD mimics were designed in a process where the kinetics and thermodynamics of $\text{O}_2^{\cdot-}$ dismutation catalyzed by superoxide dismutases were mimicked. The kinetics of the catalysis $\text{O}_2^{\cdot-}$ dismutation relates to the electrostatic facilitation of the approach of anionic $\text{O}_2^{\cdot-}$ towards the metal site. In the case of enzyme the anionic superoxide is pulled towards the metal site through the tunnel of amino acids with cationic residues. Those MnPs which have cationic charges on periphery are ~ 2 or 2.5 orders of magnitude faster catalysts than those which are either neutral or anionic at the periphery, respectively. The thermodynamics relates to the redox properties of the metal site (described with metal-centered reduction potential, $E_{1/2}$) which would allow Mn to equally easily accept electron from one $\text{O}_2^{\cdot-}$ and give it to another $\text{O}_2^{\cdot-}$. Cationic compounds with $E_{1/2}$ for $\text{Mn}^{\text{III}}\text{P}/\text{Mn}^{\text{II}}\text{P}$ redox couple around the $E_{1/2}$ of SOD enzymes ($\sim +300 \text{ mV}$ vs NHE) were synthesized and demonstrated to efficiently catalyze $\text{O}_2^{\cdot-}$ dismutation in aqueous solution with high rate constants. In addition, they are extremely stable complexes towards the loss of Mn. Such $E_{1/2}$ of SOD enzyme and its mimics sits around the midway potential between the $E_{1/2}$ for $\text{O}_2^{\cdot-}$ reduction at $+890 \text{ mV}$ vs NHE and its oxidation at -180 mV vs NHE. In turn, it allows equal facilitation for both steps of dismutation process [3,9]. Indeed both steps occur with the same rate constants with SOD enzyme as was demonstrated for its mimic, MnTE-2-PyP⁵⁺ [10]. The most powerful SOD mimics belong to the class of Mn(III) *ortho* (*N*-substituted pyridinium-2-yl)porphyrins. They possess both favorable electrostatics, as they bear 4 positive charges at the periphery to attract $\text{O}_2^{\cdot-}$, and favorable thermodynamics, $E_{1/2} \sim +200$ to $+450 \text{ mV}$ vs NHE. Among them the *ortho*, *meta* and *para* pyridyl MnPs which are β -halogenated (with chlorines at β positions of pyrrole rings, see Scheme IV) possess the highest $k_{\text{cat}}(\text{O}_2^{\cdot-})$, close to that of SOD enzymes; yet they are the least stable. Adapted from [3].

be considered an antioxidative defense only when coupled to H_2O_2 removing systems. Growing data indicate that in cancer such systems fail. Reports indicate downregulation or inactivation of enzymes such as GPx and peroxiredoxin, while up-regulation of SOD during cancer progression [4–6]. In turn, H_2O_2 gets accumulated and used by cancer cell for its proliferation; under such conditions both SOD and its mimic will increase oxidative stress and cannot be anymore considered an antioxidative defense [3,5,6].

Design of SOD mimics

MnP-based SOD mimics have been developed with the goal to mimic the kinetics and thermodynamics of the catalysis of $\text{O}_2^{\cdot-}$ dismutation by SOD enzymes (Scheme II and III). MnPs, which $k_{\text{cat}}(\text{O}_2^{\cdot-})$ is close to that of enzyme, were synthesized: $\text{Br}_8\text{MnTM-3-PyP}^{4+}$ ($\log k_{\text{cat}}(\text{O}_2^{\cdot-}) \geq 8.85$), $\text{Br}_8\text{MnTM-4-PyP}^{4+}$ ($\log k_{\text{cat}}(\text{O}_2^{\cdot-}) \geq 8.67$) and $\text{Cl}_5\text{MnTE-2-PyP}^{4+}$ ($\log k_{\text{cat}}(\text{O}_2^{\cdot-}) = 8.41$) (Scheme VI). Such results teach us that even with low-molecular weight compounds the properties of an enzyme could be mimicked. The $\log k_{\text{cat}}(\text{O}_2^{\cdot-})$ of SOD enzymes in the range 8.84–9.30 were reported [1,9]. Yet the protein structure assures the specificity of SOD enzymes to $\text{O}_2^{\cdot-}$. Despite favorable thermodynamics, the SOD enzymes react with other species, such as ONOO^- , orders of magnitude slower than cationic MnTE-2-PyP^{5+} .

Over years diverse compounds were synthesized and/or obtained from commercial sources of either high, modest or no SOD-like activities. Mn porphyrins with high ($\log k_{\text{cat}}(\text{O}_2^{\cdot-}) > 7$),

modest $\log k_{\text{cat}}(\text{O}_2^{\cdot-}) < 7$ and no SOD-like activity ($\log k_{\text{cat}}(\text{O}_2^{\cdot-}) \leq 5.6$) are listed in Schemes IV, V and VII. Those of no SOD-like activities have been often incorrectly used to support $\text{O}_2^{\cdot-}$ -related pathways.

Structure–activity relationships, SAR, for mimicking SOD enzymes

See Scheme VI.

Mn porphyrin-based non-SOD mimics

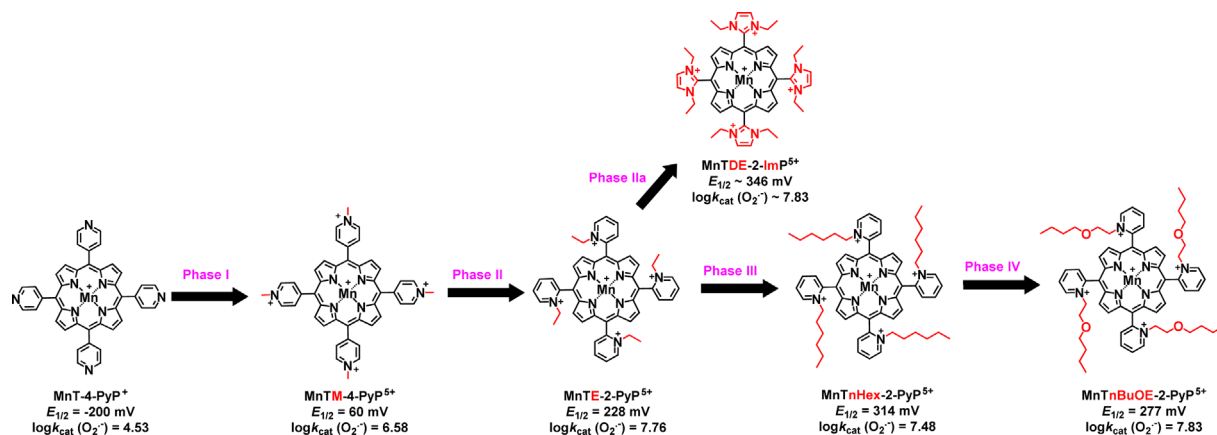
See Scheme VII.

Fe porphyrin-based SOD mimics

See Scheme VIII.

Reactivities of MnPs towards diverse low-molecular weight reactive species

The reactivity of *ortho* Mn(III) *N*-substituted pyridylporphyrin towards a whole spectrum of reactive species with corresponding rate constants (estimated based on reactions of MnTE-2-PyP^{5+}) is



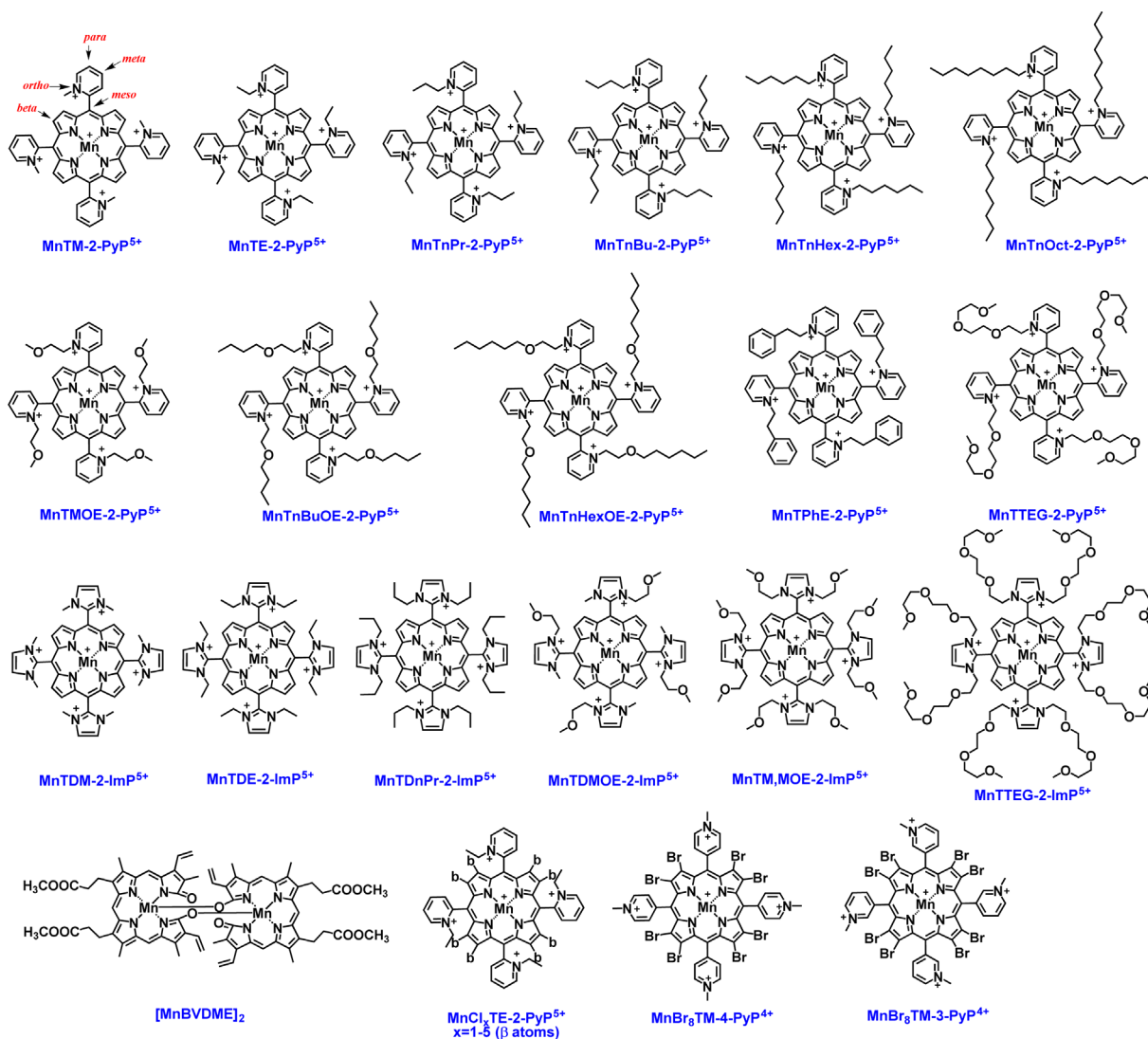
Scheme III. The design of SOD mimics occurred through phases I to IV. The unsubstituted electron-rich MnT-4-PyP^+ has $E_{1/2}$ value for $\text{Mn}^{\text{III}}/\text{Mn}^{\text{II}}$ redox couple of -200 mV vs NHE which is too negative (more negative than $E_{1/2} = -180$ mV vs NHE for $\text{O}_2^{\cdot-}$ oxidation) and precludes its reduction (along with $\text{O}_2^{\cdot-}$ oxidation) to start the 1st step of dismutation process. In addition, there is no electrostatic facilitation; subsequently it has no SOD like activity, $\log k_{\text{cat}}(\text{O}_2^{\cdot-}) = 4.53$. In order to decrease metal center electron density to enable its acceptance of electron and reduction with $\text{O}_2^{\cdot-}$, the alkyl groups were added to pyridyl nitrogens in *Phase I*; subsequently nitrogens became cationic and withdrew the electron density from Mn site. In turn, Mn becomes electron-deficient and prone to be reduced at biologically feasible $E_{1/2}$ of $+60$ mV vs NHE, which results in a modest $\log k_{\text{cat}}(\text{O}_2^{\cdot-}) = 6.58$. Such rate constant is still ~ 3.5 orders of magnitude lower than that of SOD enzyme. Shifting nitrogens from para (4) positions to *ortho* (2) positions in *Phase II* moves positive charges closer to Mn site, which enhances both the electron-withdrawing effects and the attraction of anionic superoxide to cationic Mn porphyrin. In turn, $E_{1/2}$ of MnTM-2-PyP^{5+} is increased for another order of magnitude, $\log k_{\text{cat}}(\text{O}_2^{\cdot-}) = 7.79$. Very importantly alkyl chains in *ortho* positions are stuck above or below the porphyrin plane and suppress the interaction of MnP with nucleic acid [11]. Such interaction demonstrated with planar para MnTM-4-PyP^{5+} prevent its SOD-like activity and may impose toxicity. *Ortho* isomers exist as 4 atropoisomers which all have the same $E_{1/2}$ and same $\log k_{\text{cat}}(\text{O}_2^{\cdot-})$, but likely have different biodistribution [12]. Once the knowledge on key *ortho* impact has been gained, *ortho N*-ethylpyridyl analog MnTE-2-PyP^{5+} and its di-*ortho N,N'*-diethylimidazolyl derivative, MnTDE-2-ImP^{5+} , were synthesized in *Phase II* and *Phase IIa* and are among the most frequently studied compounds both in vitro and in vivo. Both compounds are pentacoordinate and thus highly hydrophilic. Initial studies suggested that, by increasing lipophilicity, we should be able to enhance their accumulation within organs and cells and mostly so brain. While cationic charge is a driving force, lipophilicity is another major factor that affects their transport across the blood brain barrier and mitochondrial accumulation and in turn their therapeutic efficacy [80–82,89]. The lipophilicity was addressed in *Phase III*. Several lipophilic compounds have been synthesized. $\text{MnTnHex-2-PyP}^{5+}$ ($\sim 6,500$ -fold more lipophilic than MnTE-2-PyP^{5+}) has been the most frequently studied in vitro and in vivo. The longer the hydrophobic alkyl chains the higher their micellar properties and the higher ability to damage lipid membranes. This is however outbalanced with the much higher accumulation of $\text{MnTnHex-2-PyP}^{5+}$ within cell; in turn, up to 120-fold lower doses are needed for the efficacies identical to those produced by hydrophilic MnTE-2-PyP^{5+} . This further allowed for a wider therapeutic window for the hexyl than for ethyl analog [13]. The micellar property and in turn toxicity was suppressed by ~ 4 – 5 -fold relative to $\text{MnTnHex-2-PyP}^{5+}$ when polar oxygen atoms were introduced into alkyl chains: $\text{MnTnBuOE-2-PyP}^{5+}$ was synthesized in *Phase IV* [14] and has been aggressively developed towards clinic.

summarized in Scheme IX. The related reactions are described by equations in Scheme X. These reactions may dominate their in vivo actions and may not necessarily be related to SOD mimicking. Most of those reactions are not catalytic, and their rate constants refer to either reduction or oxidation of MnP, i.e. or oxidation or reduction of respective reactive species. The reactivities of MnPs could become catalytic in vivo, i.e. MnP as a catalyst could be recovered. This could happen when the reduction or oxidation of MnP is coupled with other reactive species or cellular reductant. In addition to direct reactions with small reactive species listed in Schemes IX and X, MnPs react directly with small thiols such as GSH and cysteine and with protein thiols of signaling proteins/transcription factors (Schemes XI and IV) as well as with ascorbate (Scheme XII) and tetrahydrobiopterin (Scheme XIII). MnPs interactions with thiols may or may not involve H₂O₂. If driven by H₂O₂, various MnP redox couple will be involved (Schemes XI, XII and XIV). MnP/H₂O₂-mediated oxidation of NADH and NADPH was demonstrated also (Scheme XIV).

Researchers need to pay attention to the type of rate constants they refer to. Immediate translation of data from aqueous solutions to cellular and animal models is not straightforward and often requires substantial insight into extremely complex chemistry of those redox active drugs, their biodistribution (affected by their lipophilicities, polarities, charge, size and bulkiness) at sufficient concentrations and at targeted location [3,5,9,15,22–24].

Reactivity of MnPs towards cellular reductants – thiols, ascorbate and tetrahydrobiopterin

The interactions of Mn(III) *ortho* *N*-substituted pyridylporphyrins with cellular reductants seems to play critical role in the biology of MnPs, in great part due to high levels of cellular reductants. Such interactions have been explored in aqueous solutions, in vitro and in vivo and are depicted in Schemes IX–XIV [3,18,30,40–44]. Such interactions also play major role in MnP/H₂O₂ biology depicted



Scheme IV. Powerful Mn porphyrin-based SOD mimics ($\log k_{\text{cat}}(\text{O}_2^{\cdot-}) > 7$). The most powerful compounds bear *ortho* cationic pyridyl- or di-*ortho* imidazolyl nitrogens which carry positive charges to guide anionic $\text{O}_2^{\cdot-}$ to the metal site and tune $E_{1/2}$ to be in between +200 and +500 mV vs NHE. For numerical values of $k_{\text{cat}}(\text{O}_2^{\cdot-})$ please see [3,9,15].

in Schemes XIV, XVII and XIX.

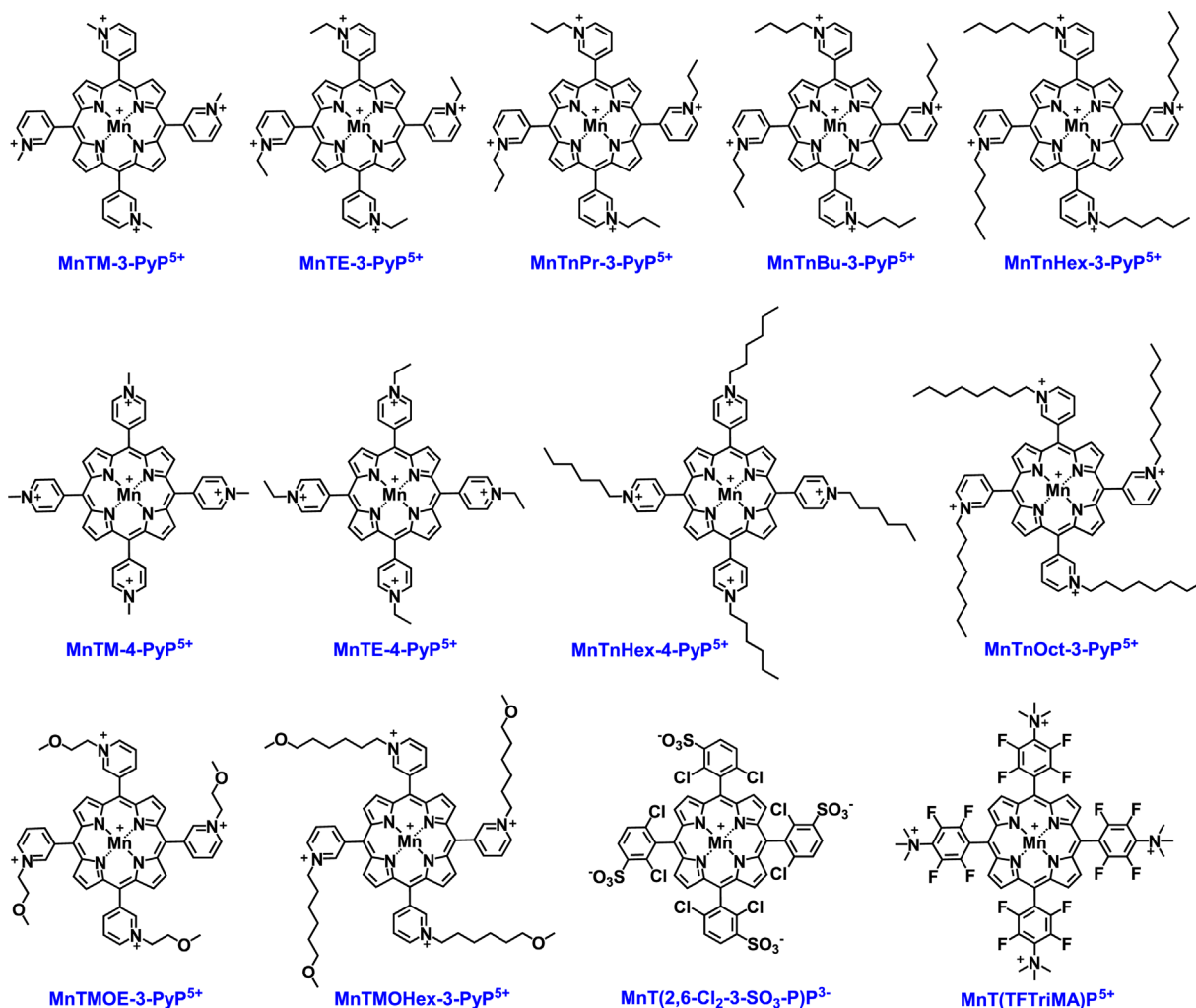
Role of H₂O₂ in the actions of MnPs

While MnPs are not functional mimics of catalase [30], H₂O₂ seems to be critical in the redox biology of MnPs. MnP produces H₂O₂ in a reaction of reduced Mn^{II}P with oxygen, O₂ (Eq. (7) in Scheme X) and superoxide, O₂^{•-} during cycling with either thiol or ascorbate or tetrahydrobiopterin (Schemes XI, XII, XIII). Subsequently it can use it to oxidize biological targets listed in Scheme XIV. Such scenario may allow for maintenance of the (unchanged) levels of H₂O₂; this was indeed demonstrated by Tome's group in lymphoma studies when lymphoma cells were treated with MnP/dexamethasone [43]. Many aspects of H₂O₂ in the biology of MnPs are listed in Scheme XIV; there may still be reactions which we have not yet explored. Present data point to a complex MnP redox-biology.

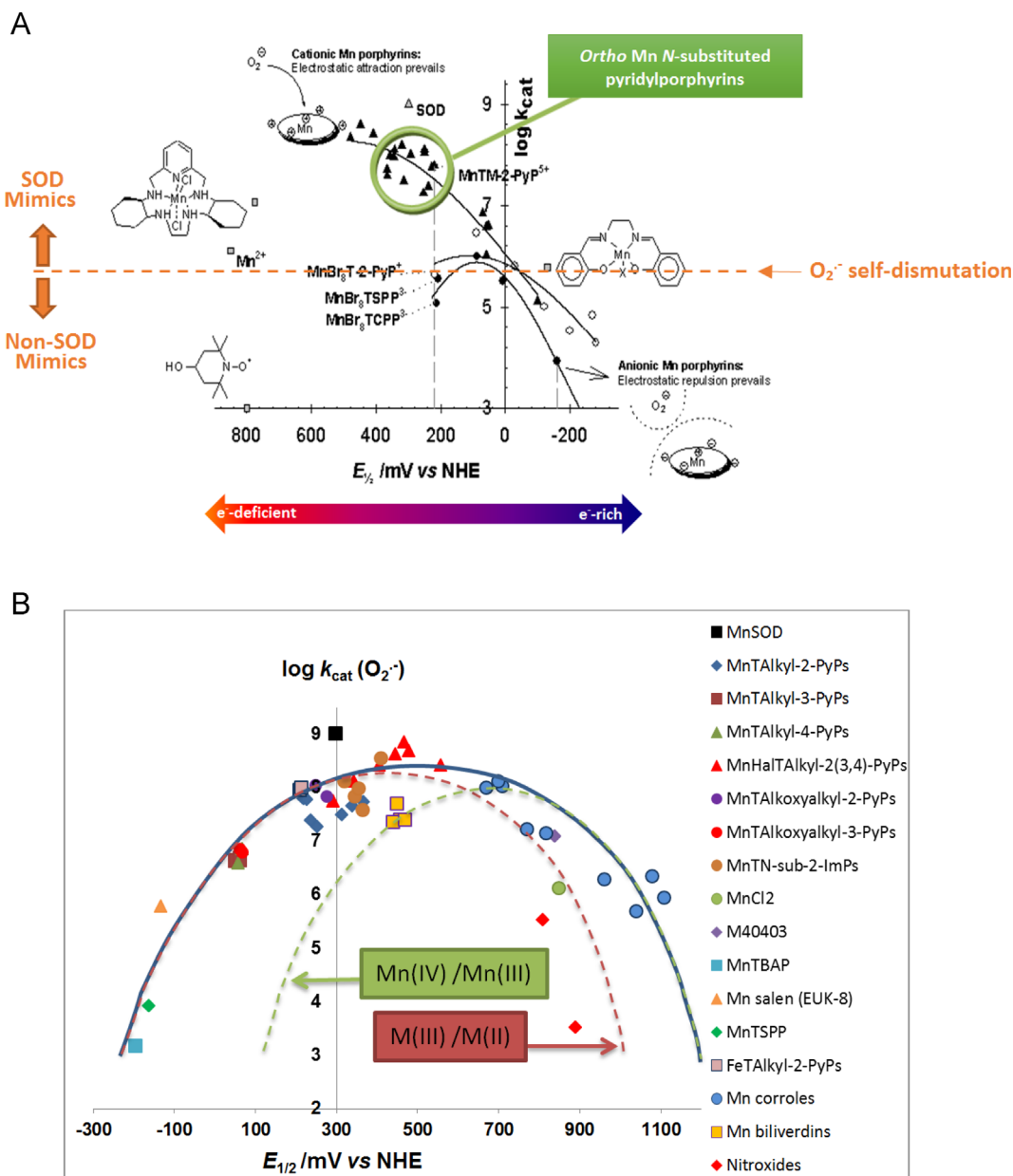
SOD-like activity parallels all other reactivities and in turn controls therapeutic effects of MnPs

We came a long way-from SOD mimicking to H₂O₂-related pathways

We initially believed that MnPs are specific SOD mimics. In 2006, Dr. Radi approached us at the 13th Annual Meeting of the SFRBM suggesting that MnPs may react with ONOO⁻ also. Peroxynitrite was an emerging species at that time. Intuitive reasoning would say that nature would not make a more dangerous species from two less dangerous and physiologically important radicals, O₂^{•-} and [•]NO. Moreover, it seems that nature has not developed an enzymatic system which could take care of ONOO⁻ specifically, unless peroxiredoxins serve that purpose [56,57]. The studies on ONOO⁻ in collaboration with Radi's group reveal that reactivities towards ONOO⁻ and O₂^{•-} are similar, only few fold away from each other [7,58]. That has opened the door to considerations that MnPs may play crucial role in in vivo destiny of reactive species other than O₂^{•-}. Along with the growing awareness of H₂O₂ as a central modulator in cellular redox processes, the



Scheme V. Modest mimics of SOD enzymes ($\log k_{\text{cat}}(\text{O}_2^{\bullet-}) = 5.7\text{--}7$). Some of those compounds have increased lipophilicity which allows higher intracellular accumulation and compensates largely for their inferior SOD-like activity. That has been most strikingly demonstrated with *ortho* MnTE-2-PyP⁵⁺ and its *meta* isomer, MnTE-3-PyP⁵⁺. With 10-fold lower $k_{\text{cat}}(\text{O}_2^{\bullet-})$, but with 10 fold higher cellular accumulation, MnTE-3-PyP⁵⁺ protected SOD-deficient *E. coli* to the same extent as did MnTE-2-PyP⁵⁺. For values of $k_{\text{cat}}(\text{O}_2^{\bullet-})$ please see [3,9,15].



Scheme VI. (A) Structure–activity relationships, SARs, relate $E_{1/2}$ to $\log k_{\text{cat}}(\text{O}_2^{\cdot-})$ for cationic, anionic and compounds which are neutral on periphery. All Mn^{III} Ps carry one + charge on Mn center. SARs have been initially established for Mn porphyrins and later proven to have wider applicability to any redox active metal complex (Scheme VI B). As compared to cationic MnPs, the ~ 2 – 2.5 orders of magnitude lower $k_{\text{cat}}(\text{O}_2^{\cdot-})$ was determined with neutral or anionic compounds than with cationic MnPs, where either no attraction exists or anionic $\text{O}_2^{\cdot-}$ is repulsed from anionic porphyrin. The additional importance of such SARs lies in the fact that the magnitude of the interactions of SOD mimics with other reactive species (thus far explored, Schemes IX–XIV), and their therapeutic effects are directly proportional to their SOD-like activity; in turn SAR established for SOD-like activity, can offer best guidance for the development of powerful therapeutics. Compounds which dismute $\text{O}_2^{\cdot-}$ with rate constants lower than that for self-dismutation, $\log k_{\text{cat}}(\text{O}_2^{\cdot-}) < 5.7$, are not mimics of SOD. A number of Mn porphyrins with no SOD-like activities reportedly demonstrate beneficial therapeutic effects in vitro and in vivo models of diseases (Scheme XXV). Such data suggest that mechanism of action, other than SOD-like, may be operative [4]. Adapted from [16]. (B) Structure activity relationships, SARs, relates $E_{1/2}$ to $\log k_{\text{cat}}(\text{O}_2^{\cdot-})$ for all compounds irrespectively of their electrostatics, their size and shape and type of redox couple involved. The blue curve relates to all compounds irrespectively of the type of redox couple involved: either Mn(III)/Mn(II) or Mn(IV)/Mn(III). The $\text{O}_2^{\cdot-}$ does not care which type of redox couple is involved, as long as the magnitude of $E_{1/2}$ is adequate. The red dashed curve relates to the compounds which operate via Mn(III)/Mn(II) redox couple, while the green one relates to compounds that utilize Mn(IV)/Mn(III) redox couple for $\text{O}_2^{\cdot-}$ dismutation. While we have not explored it further, the maximum seems to be shifted right for compounds which use Mn(IV)/Mn(III) redox couple; therefore the dashed curves are suggested. The larger deviation from the plot is demonstrated with compounds which have unfavorable electrostatics (anionic, bulky compounds). The largest deviation is seen with non-metal-based SOD mimics such as nitroxides. When the $E_{1/2}$ is very negative, left arm of bell-shape curve, the reduction of Mn^{III} P to Mn^{II} P is a rate limiting step in $\text{O}_2^{\cdot-}$ dismutation. As the $E_{1/2}$ increases, reaching maximal $\log k_{\text{cat}}$ values, both steps of dismutation become similar and offer similar thermodynamics for the dismutation process, such conditions favor high $k_{\text{cat}}(\text{O}_2^{\cdot-})$. Once the electron-density of Mn center gets overly reduced, $E_{1/2}$ of Mn(III)/Mn(II) redox couple $> +450$ mV vs NHE, the compound becomes stabilized in Mn +2 oxidation state (the right arm of the bell-shape curve). The consequence of this is that Mn(II) porphyrins are unstable (Mn gets easily lost) and are therefore not prospective therapeutics. Under such conditions the 1st and 2nd steps of dismutation process are reversed. With Mn(II) porphyrins, as the $E_{1/2}$ becomes too positive, the 1st step of dismutation (oxidation of Mn^{II} P to Mn^{III} P and reduction of $\text{O}_2^{\cdot-}$ to H_2O_2) becomes unfavorable and rate limiting; in turn the $k_{\text{cat}}(\text{O}_2^{\cdot-})$ decreases again (the right arm of bell-shape curve). Adapted from [3].

role of ONOO⁻ in the chemistry and biology of MnPs has been increasingly appreciated (Scheme XV).

Differential (therapeutic) effects of MnPs on cancer vs normal cell

Differential effects of MnPs in cancer vs normal cell have been shown and reported in a number of cellular models (Schemes XVI and XVII).

Differential (therapeutic) effects of MnPs on cancer vs normal tissue in animal models

Differential effects of MnPs in cancer vs normal cell have also been shown and reported in a number of animal models. Radio-protection of salivary glands and mouth mucositis in non-tumor bearing mice receiving daily MnTnBuOE-2-PyP⁵⁺ treatment was recently demonstrated. At the same time, radiosensitization of head and neck tumor in a sc mouse tumor xenograft model was demonstrated [Ashcraft et al., unpublished]. The radioprotective effects of MnPs have been demonstrated in numerous other models as well. Most striking data were obtained with mouse prostate radiation with complete reversal of radiation-induced erectile dysfunction (ED) ([65], Koontz, Batinic-Haberle, Tovmasyan et al., unpublished). Such treatment has a chance to reduce prostate radiation-induced ED which would encourage the men

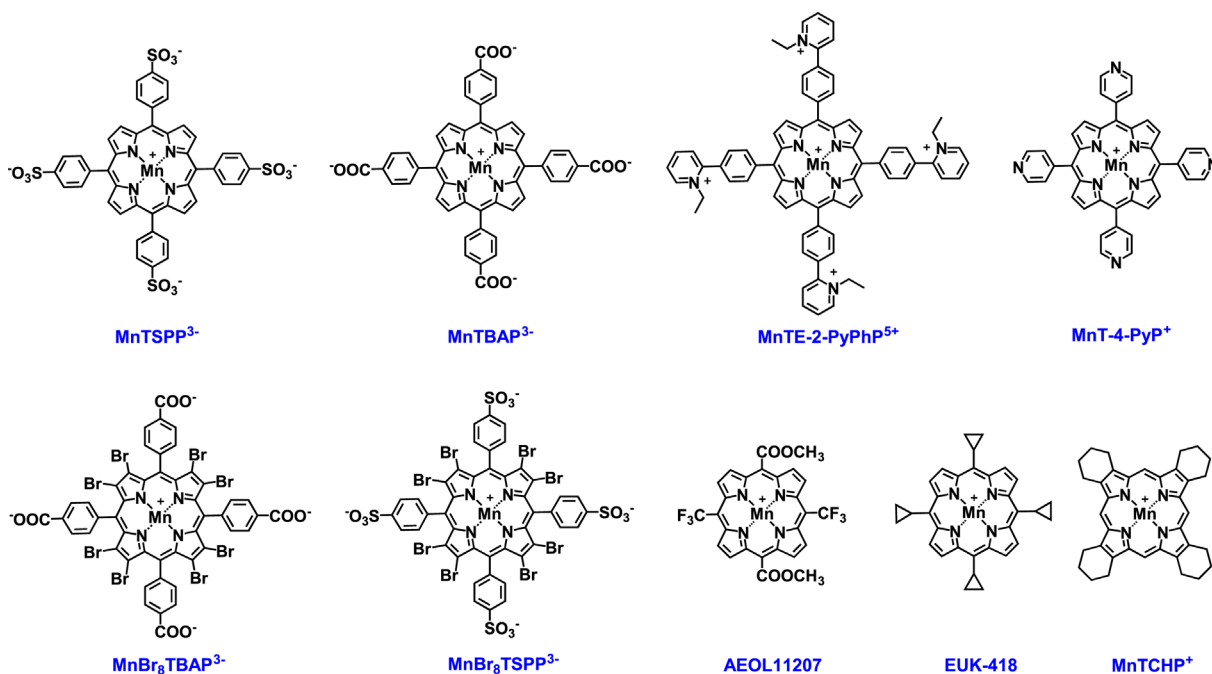
with prostate cancer, who have thus far been reluctant, to undergo radiation therapy. Scheme XVII describes the data on the radio-protection of a normal brain in non-tumor bearing mice and radiosensitization of brain tumor in a mouse sc glioma xenograft model [53] (Scheme XVIII).

Impact of MnPs on cellular transcription

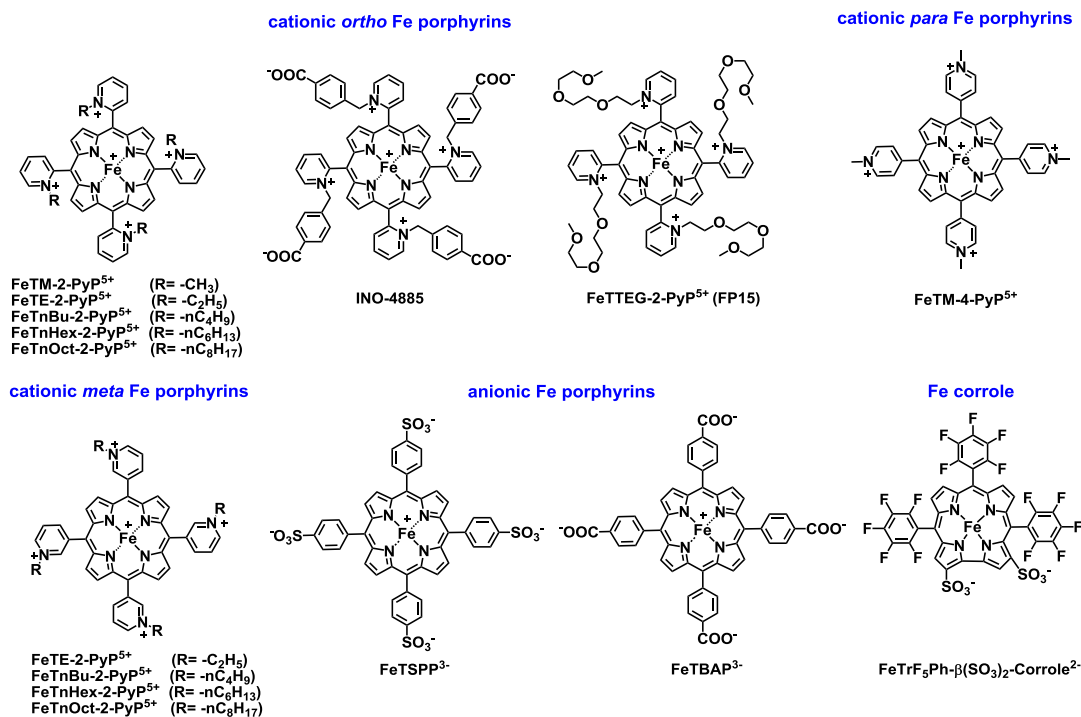
At present stage of our knowledge, the *direct* reactions of MnPs with thiols of transcription factors, such as NF-κB or Keap1/Nrf2, and/or coupled to other species, may present major contributions to the therapeutic efficacy of MnPs. In addition to direct reactions, the *indirect* impact of MnP on NF-κB via scavenging reactive species, which would have otherwise signaled its activation, cannot be ruled out [53] (Scheme XIX).

Suppression of cellular energetics of a cancer cell with MnPs

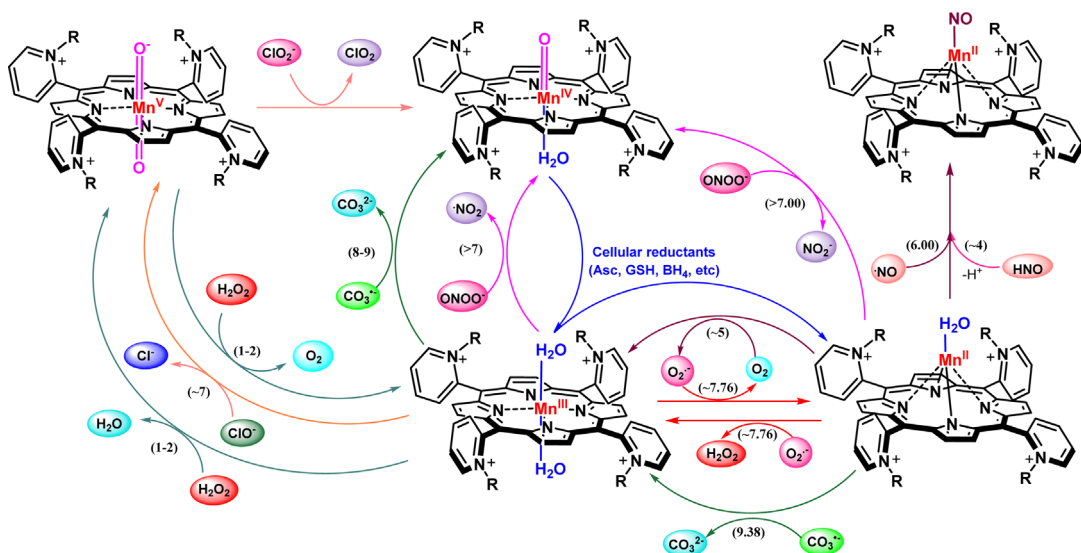
S-glutathionylation of complexes I, III and IV of mitochondrial electron transport chain with subsequent inactivation of complexes I and III and suppression of ATP production of lymphoma cells when those were treated with MnP/dexamethasone was reported by Tome's group [45]. In addition to the radiosensitizing actions of MnPs, such chemosensitization may further contribute to their anticancer effects [3] (Scheme XX).



Scheme VII. Compounds with $k_{\text{cat}}(\text{O}_2^{\cdot-})$ lower than the value for $\text{O}_2^{\cdot-}$ self-dismutation, i.e. below 5.7 are not SOD mimics. Can non-SOD mimic be a good therapeutic? A simple answer is: yes. In addition to other groups, our group has also reported therapeutic effects of MnTBAP³⁻ which has a very low $\log k_{\text{cat}} = 3.16$ and is thus not an SOD mimic. The nature of the actions of this compound resulting in therapeutic effects is not yet understood. Some of the reactive species, such as ONOO⁻ (see Scheme X), are highly oxidizing and reportedly oxidize even fairly inert compounds such as MnTBAP³⁻, AEOL11207 or MnTCHP⁺. The resulting O=Mn^{IV}P species may be reduced back to Mn^{III}P with $\text{O}_2^{\cdot-}$ affecting in turn the in vivo levels of $\text{O}_2^{\cdot-}$ and restoring physiological redox environment. Reduction of O=Mn^{IV}P species to Mn^{III}P may occur by cellular reductants also (Schemes XI and XII). This being said, the accurate assignment of the effects observed in vivo is needed as that would ultimately help us in understanding the relations between the redox reactions of those compounds and redox-based pathways of a cell. This will in turn positively affect drug development helping us understand what kind of drugs may be the best therapeutics.



Scheme VIII. Fe porphyrin-based SOD mimics. Fe porphyrins and Fe complex with shrunk porphyrin, corrole, which is missing a methine group at one *meso* position, are listed here. Cationic Fe(III) *N*-substituted pyridylporphyrins (FePs) have different chemistry from Mn analogs. Both have same conformation with two axially ligated aqua ligands, but under different conditions. Such conformation of FeP exists at pH=2, but not at pH=7.8. The $E_{1/2}$ of (H₂O)₂FeTE-2-PyP⁵⁺ is ~160 mV more positive than $E_{1/2}$ of (H₂O)₂MnTE-2-PyP⁵⁺. Thus, at pH=7.8, one aqua ligand of FeP gets deprotonated: (OH)(H₂O)FeTE-2-PyP⁴⁺ is formed. MnP however has two aqua ligands at pH=7.8. Aqua ligands are not indicated in formulas in this review (unless involved in reactions) though all Mn(III) porphyrins bear 2 aqua ligands at pH=7.8, while all FePs discussed here have one hydroxo and one aqua ligands. The presence of electron-donating hydroxo ligand stabilizes Fe in +3 oxidation state. This results in a shift of $E_{1/2}$ from +380 to +215 mV vs NHE. Consequently, the $E_{1/2}$ becomes identical to the one of MnTE-2-PyP⁵⁺ (+228 mV vs NHE); thus MnPs and FePs have similar SOD-like activities [17,18]. Another difference, FePs undergo faster degradation *in vivo* than MnPs. At $\leq 1 \mu\text{M}$ concentration, FeTE-2-PyP⁵⁺ is as efficacious as $\leq 1 \mu\text{M}$ Fe(II) citrate in protecting aerobic growth of SOD-deficient *E. coli*. That is due to the release of Fe from Fe porphyrin which subsequently restores Fe-bearing proteins which have lost Fe due to the superoxide-mediated oxidative damage. At 20 μM , though, where MnP is efficacious, FeP is toxic. FePs have been successfully used in many animal models even at fairly high concentrations similar to those of MnPs [3,18–20]. One of them, INO-4885 is in clinical trials. Another porphyrin-like class of compounds with therapeutic potential – Fe corroles – has been explored by Gross's group [8,21]. Some members of corrole class, including FeTrF₅Ph- β (SO₃)₂-corrole²⁻, are advancing towards clinic.



Scheme IX. Reactivity of Mn(III) *ortho N*-substituted pyridylporphyrins towards different low-molecular weight reactive species. In late 1990s, along with growing awareness of highly oxidizing species, ONOO⁻, it became obvious that all potent SOD mimics can effectively reduce ONOO⁻ either one- (if starting from Mn^{III}P) or two-electronically (if starting from Mn^{IV}P), producing either highly oxidizing [•]NO₂ or benign NO₂⁻, respectively. The ONOO⁻ reduction by MnP is only few-fold slower than the catalysis of O₂^{•-} dismutation, demonstrating that all potent SOD mimics are potent peroxynitrite scavengers. Compounds which cannot dismute O₂^{•-}, such as MnTBAP³⁻, could be oxidized with strong oxidant, such as ONOO⁻, in a 1st step and reduced with either ascorbate or thiol or O₂^{•-} in a 2nd step. If O₂^{•-} is involved in a 2nd step, such compounds can affect *in vivo* levels of O₂^{•-}. Yet, the lower the SOD-like activity, the lower is the ability to reduce ONOO⁻. Indeed, MnTBAP³⁻ is ~2 orders of magnitude less able reductant of ONOO⁻ than is MnTE-2-PyP⁵⁺. Coupling of ONOO⁻ reduction to O₂^{•-} oxidation, has been explored with MnTM-4-PyP⁵⁺ [25], and may be operative with other compounds. Coupling of ONOO⁻ reduction to cellular reductants is likely operative with potent SOD mimics also (Scheme X). Due to high levels of carbonate *in vivo*, ONOO⁻ forms an adduct with CO₂. The ONOOCO₂⁻ adduct degrades to CO₃^{•-} and [•]NO₂ radicals. Due to its radical nature, all potent SOD mimics react rapidly with CO₃^{•-} in a pH-dependent manner, log $k_{\text{red}}(\text{CO}_3^{\bullet-})$ ranging between 8 and 9 (Scheme X, Eqs. 5 and 6). Most of the rate constants provided here were based on those determined for MnTE-2-PyP⁵⁺. The linear relationship between log $k_{\text{cat}}(\text{O}_2^{\bullet-})$ and log $k_{\text{red}}(\text{ONOO}^-)$ allows for calculations of other $k_{\text{red}}(\text{ONOO}^-)$ (see Scheme XIV).

MnPs as inducers of adaptive responses via (pro)oxidative mechanism(s)

Oxidative modification of Keap1 thiols with subsequent activation of Nrf2 and upregulation of endogenous antioxidative defenses was shown with another class of SOD mimics, M40403 [72]. Such data along with results shown in Scheme XXI strongly suggest that MnP could act in a similar manner as inducer of endogenous antioxidative defenses. The upregulation of different SOD enzymes in rat kidney ischemia/reperfusion injury strongly suggests that MnP does not act as SOD mimic. A lipophilic analog, MnTnHex-2-PyP⁵⁺ was tested.

MnTBAP³⁻ – the actions behind its therapeutic effects are still largely unknown

See Scheme XXII.

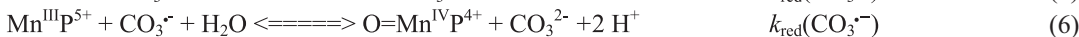
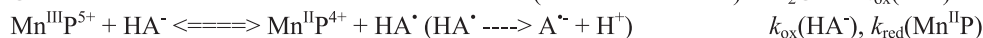
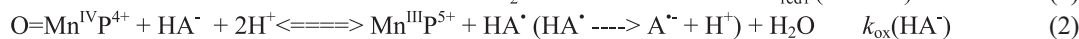
Bioavailability – pharmacokinetic studies

Scheme XXIII.

Targeting mitochondria

Due to the critical role mitochondria has in all organisms, the compounds that are able to cross two mitochondrial membranes and preferentially target its matrix have been sought. Excellent work has been done on the basis of mitochondrial transport by Skulachev's and Murphy's groups [87,88]. Based on their research, the essential properties of mitochondrially-targeted drug are identified to be cationic charge and lipophilicity. Most efficacious MnPs bear penta-cationic charge. Moreover the lipophilic analogs bear long hydrophobic substituents. Such facts justified extensive studies of mitochondrial accumulation [80–82,89]. We have demonstrated that MnPs accumulate in mouse heart mitochondria, and those that are more lipophilic do so to a higher extent than the hydrophilic compounds, such as MnTE-2-PyP⁵⁺. Lipophilic MnTnHex-2-PyP⁵⁺ and MnTnBuOE-2-PyP⁵⁺ were found in mouse

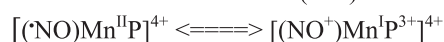
Reduction of ONOO⁻ and CO₃⁻ with MnP



Oxidation of MnP with oxygen



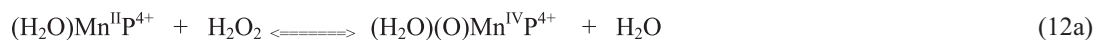
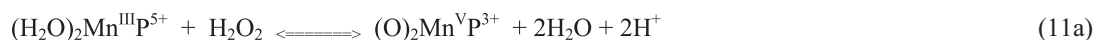
Nitrosylation of MnP with $\cdot\text{NO}$



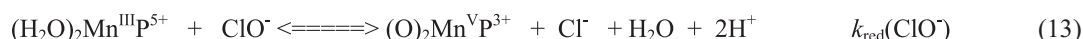
Nitroxylation of MnP with nitroxyl, HNO



Catalase-like activity of MnP



Reduction of hypochlorite, ClO⁻, with MnP



brain mitochondria also. We were not able to measure the hydrophilic MnTE-2-PyP⁵⁺ in brain mitochondria as its levels were below LCMS/MS detection limit [90]. The mitochondrial/cytosolic ratios of MnTnHex-2-PyP⁵⁺ and MnTnBuOE-2-PyP⁵⁺ in heart and brain mitochondria are similar [68]. The remarkable impact of lipophilicity on the accumulation of cationic MnPs in mitochondria relative to cytosol of *Saccharomyces cerevisiae* is shown in Scheme XXIV.

Diminishing toxicity

While most of the requirements for a good MnP-based redox-active drug are met, lowering MnP toxicity remains a challenge. Since, most but not all MnPs cause acute toxicity due to the blood pressure drop (BPD), we are far away from understanding that phenomenon. MnPs and FePs of similar redox properties but different lipophilicities and polarities, and with differently hindered cationic charges, that may affect their interactions with biomolecules, induce BPD of different magnitude. Some, such as (OH)FeTnHex-2-PyP⁴⁺ and (OH)FeTnBuOE-2-PyP⁴⁺, for reasons not yet understood, do not cause BPD. However, their Mn analogs, with similar $k_{\text{cat}}(\text{O}_2^{\cdot-})$, cause BPD [18]. MnPs that are of relatively high lipophilicity, such as MnTnHex-2-PyP⁵⁺ and MnTnBuOE-2-PyP⁵⁺, cross blood brain barrier to significant extent and tend to damage membranes and become toxic at higher levels. Therapeutic window needs to be identified for each MnP. The most frequently studied

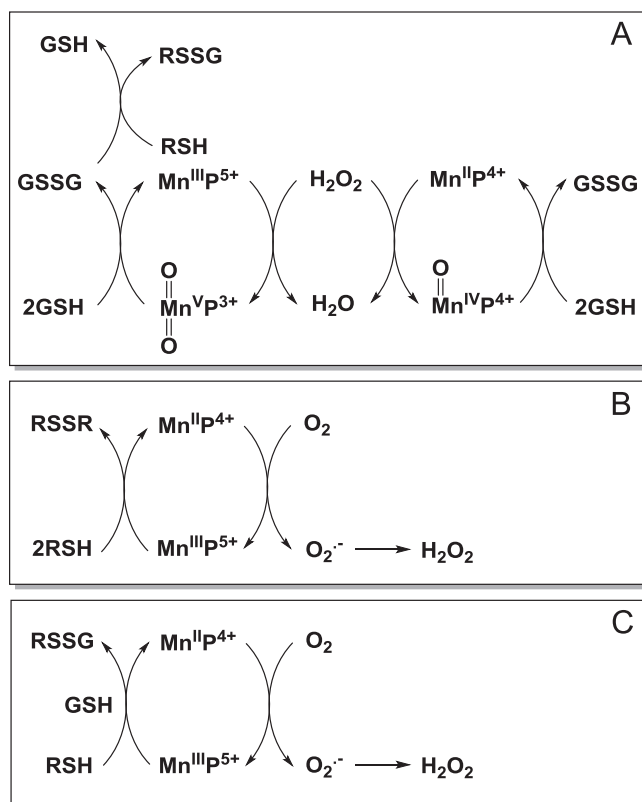
compound with excellent safety/toxicity profile, MnTE-2-PyP⁵⁺, [92] has two “weaknesses”: (i) low transport across the blood brain barrier which reduces its chances to be used for treatment of CNS injuries, and (ii) short patent life of only 4 years which precludes Pharma’s investment into its development.

Therapeutic effects

What controls the magnitude of MnPs therapeutic effects?

Bioavailability and redox properties control therapeutic effects. Redox properties are best described with $E_{1/2}$ for Mn^{III}P/Mn^{II}P redox couple which controls $\log k_{\text{cat}}(\text{O}_2^{\cdot-})$, i.e. SOD-like activities of MnPs (Scheme XV, plot A). $E_{1/2}$ describes best the electron-density of metal site. Electron-density of MnP in turn controls (i) predominantly outer-sphere electron transfer in $\text{O}_2^{\cdot-}$ dismutation and (ii) propensity of Mn center to bind other stronger oxidants (ONOO^- , ClO^- , H_2O_2 , $\text{CO}_3^{\cdot-}$) in a 1st step which is followed by electron transfer in a 2nd step. Thermodynamics of electron transfer, which involves either Mn +3/+4 or Mn +3/+5 oxidation states, seems to be similar for all MnPs. In turn, $E_{1/2}$ of Mn^{III}P/Mn^{II}P redox couple (which involves Mn +3/+2 oxidation states) parallels SOD-like and all other activities of MnPs thus far explored (see Schemes X and XV). *Bioavailability* describes levels (concentrations) of MnPs at targeted sites (organs and cellular

Scheme X. Reactions of Mn(III) *ortho* *N*-substituted pyridylporphyrins with different reactive species presented by equations. A number of reactions whose equations are listed have been studied and rate constants determined [3]. Most of those have been determined with frequently explored MnTE-2-PyP⁵⁺. It is very likely that many other reactions are possible in vivo and await exploration. *Peroxyxynitrite reduction with MnP: Are Mn(III) and Fe(III) porphyrins specific peroxyxynitrite decomposition catalysts?* Simple answer – they are not. Firstly, to the best of our knowledge none of them are specific to ONOO⁻ [20]. Most compounds reported as peroxyxynitrite decomposition catalysts are potent SOD mimics also (see Scheme XV also) [20]. Secondly, the reported rate constants for Mn porphyrins relate to the reduction of ONOO⁻ only (Eqs. (1) and (4)), and are not of catalytic nature and cannot be described as $\log k_{\text{cat}}(\text{ONOO}^-)$. What happens in vivo is another story and type of process involved (catalytic with FeTM-4-PyP⁵⁺ or non-catalytic with MnTE-2-PyP⁵⁺) may be irrelevant in elimination of ONOO⁻; very likely MnP will be reduced with abundant cellular reductants (Eq. (3)), most so with ascorbate, and will then reduce ONOO⁻ in a 2nd step (Eq. (4)), closing the catalytic cycle. This would happen *only* if MnP encounters ONOO⁻ in its immediate neighborhood and at high enough concentration. The second re-oxidation step of Mn^{II}P⁴⁺, given the fairly high $k_{\text{red}}(\text{O}_2)$ and the higher in vivo levels of oxygen than ONOO⁻, could occur with oxygen, O_2 and will give rise to $\text{O}_2^{\cdot-}$ and subsequently H_2O_2 . For FeTM-4-PyP⁵⁺, however, the catalytic rate constant was reported; same may be valid for other similar FePs [26]. *Oxidation of MnP with oxygen.* Reactivity towards oxygen, O_2 is much lower than towards $\text{O}_2^{\cdot-}$ ONOO⁻ or ClO^- . However, given the orders of magnitude higher in vivo levels of O_2 , such reactivity may play a role and be a critical step in the interactions of MnPs with cellular reductants, most so with ascorbate (see Scheme XII). *Oxidation of *NO with MnP – nitrosylation of MnP.* The report by Meyer et al. tempted us to study the reactions of Mn^{III}P⁵⁺ and Mn^{II}P⁴⁺ with *NO (Eqs 8 and 9) [27]. Mn^{III}P may be reduced in a slow reaction with *NO (Eq. (8)). Yet, in vivo Mn^{III}P⁵⁺ would rather be reduced rapidly with ascorbate (Eq. (3)) in a 1st step followed by nitrosylation (Eq. (9)) of Mn^{II}P⁴⁺ to give rise to (NO)Mn^{II}P⁴⁺ in a 2nd step. Given the electron deficiency of Mn center, the distribution of electron in a complex of *NO with Mn^{II}P may be better described as [(NO⁺)Mn^{II}P³⁺]⁴⁺. The (NO)Mn^{II}P⁴⁺ species is not very stable and under aerobic conditions releases *NO slowly which subsequently gets oxidized to nitrite and/or nitrate [28]. Possibly (NO)Mn^{II}P⁴⁺, formed rapidly, may serve as a depot for slow release of *NO. *HNO oxidation with MnP – nitroxylation of MnP:* reported by Doctorovich’s group, the reaction of MnP with HNO gives rise to the same product as reaction of MnP with *NO [29]. *Catalase-like activity of MnP:* A poor ability of 13 Mn porphyrins to act in a catalase-like fashion (2-electron reduction and oxidation of H_2O_2 , Eq. 11) has been demonstrated. MnTE-2-PyP⁵⁺ has the highest $k_{\text{cat}}(\text{H}_2\text{O}_2) = 63 \text{ M}^{-1} \text{ s}^{-1}$. The lowest $k_{\text{cat}}(\text{H}_2\text{O}_2)$ is $5.6 \text{ M}^{-1} \text{ s}^{-1}$ for MnTBAP³⁻. The $k_{\text{cat}}(\text{H}_2\text{O}_2)$ for FeTE-2-PyP⁵⁺ is $803 \text{ M}^{-1} \text{ s}^{-1}$. The most potent MnPs and FePs have only ~0.006% and 0.06% of catalase enzyme activity [30–32]. Both MnPs and FePs could undergo similar turnovers: slower oxidative degradation of MnPs compensates for their lower $k_{\text{cat}}(\text{H}_2\text{O}_2)$. The catalysis of dismutation by MnPs, is unlikely to happen in vivo due to high levels of cellular reductants; the catalytic cycle may be closed by the reduction of oxo species in a 2nd step with cellular reductants, [35] Eq. (2); in such scenario H_2O_2 would still be eliminated/reduced to water in a 1st step. The dismutation may also involve $\text{O}=\text{Mn}^{\text{IV}}\text{P}/\text{Mn}^{\text{III}}\text{P}$ via Eq. 12. Fe corrole is a complex with shrunken porphyrin ligand [8,33]. It has ~10-fold higher $k_{\text{cat}}(\text{H}_2\text{O}_2)$ than FeTE-2-PyP⁵⁺ [33,34]. It also has higher turn-over number, due to higher stability resulting from tri-anionic nature of corrole ligand vs di-anionic nature of porphyrin ligand. In turn, Fe(III) corrole can dismute larger quantities of peroxide relative to either MnPs or FePs. The overall behavior of Fe corrole in vivo may be different than that of either FeP or MnP. For example, Fe(III) corrole, due to the stabilization of higher oxidation state, cannot be reduced in vivo to Fe(II) corrole with cellular reductants. The coupling with cellular reductants could happen only in a 2nd reduction step once Fe(III) corrole is in a first step oxidized to higher (+4 or +5) oxidation state with either $\text{O}_2^{\cdot-}$ or ONOO⁻. The relevance of such coupling and the biological relevance of catalysis of H_2O_2 by metallocorroles, and the possible advantage over MnPs (claimed by Gross’s group, [35]) needs to be carefully explored. Most so in the light of recent data which indicate that MnPs, rather than removing H_2O_2 , take advantage of it. Thus MnPs couple with H_2O_2 in a pro-oxidative and catalytic manner (see below), presumably in glutathione peroxidase or thiol oxidase fashion while imparting therapeutic effects (see Schemes XI and XIV). Note that in a first step H_2O_2 is reduced with MnP which in essence represents elimination of H_2O_2 (Scheme XIA). Such interactions vs H_2O_2 dismutation may be major or at least one of the major actions of metalloporphyrins or metallocorroles in vivo. Further, none of such compounds would experience H_2O_2 concentrations high enough to degrade them to any significant extent. Further studies are needed to address the issue of the advantage of higher $k_{\text{cat}}(\text{H}_2\text{O}_2)$ of Fe corrole (Scheme VIII) over MnPs, which has been used by Gross’s group to discuss the therapeutic advantage of Fe corroles over Mn porphyrins [35]. *Reduction of lipid reactive species with MnP:* while those reactions have not been quantified, the indirect evidence suggests that Mn^{III}P scavenges lipid reactive species whereby preventing lipid peroxidation. However, that happens *only* in the presence of cellular reductants. When the low-density lipoproteins were exposed to low flux of ONOO⁻, the 5 μM MnTE-2-PyP⁵⁺, MnTnOct-2-PyP⁵⁺ and MnTBAP³⁻ prevented lipid peroxidation, i.e. the formation of cholesteryl ester hydroperoxide in the presence of uric acid. The most lipophilic MnTnOct-2-PyP⁵⁺ was the most efficacious and ~2-fold better than MnTE-2-PyP⁵⁺. The MnTBAP³⁻ was ~2-fold less efficacious than MnTE-2-PyP⁵⁺. Also α and γ tocopherol were spared. Yet in the absence of uric acid MnPs acted as a pro-oxidant; the consumption of tocopherols was potentiated and more so than more redox-active cationic MnPs than with MnTBAP³⁻ [36]. Similar impact of MnTE-2-PyP⁵⁺ on lipid reactive species has been suggested in another study also [37]. *Hypochlorite reduction with MnP.* While only preliminary data are collected on MnTalkyl-2-PyP⁵⁺, the reactivity towards hypochlorite seems to be of the same order of magnitude as towards ONOO⁻ [38,39]. The reaction described by Eq. (13) is based on the Umile et al. data on imidazolyl analog, MnTDM-2-PyP⁵⁺ [38].



Scheme XI. Interaction of MnPs with thiols/ H_2O_2 in thiol oxidase or Gpx fashion. While catalase-like action may be of no in vivo relevance due to low $k_{\text{cat}}(\text{H}_2\text{O}_2)$, the key role of H_2O_2 in the actions of MnPs, other than catalase-like, has already been established and occur in reactions of MnPs with cellular reductants (see also Schemes XIII and XIX). Note that H_2O_2 is reduced to water (H_2O is eliminated) in Scheme A, while it is generated in Schemes B and C. The GSSG species can exchange thiol spontaneously with RSH whereby glutathionylated protein is formed. Reactivity towards thiols may predominate in therapeutic effects of MnPs. Studies on CNS injuries, diabetes and cancer show that oxidation of cysteines of p50 and p65 subunits by MnP (enhanced with dexamethasone as a producer of H_2O_2) suppresses/precludes the NF- κ B activation and in turn suppresses inflammation rescuing the diseased cell and/or precluding the survival of cancer cell [45–49]. In addition S-glutathionylation of cysteines of complexes I, III and IV and subsequent inactivation of complexes I and III of mitochondrial respiration may play key role in reducing ATP levels in tumor cell whereby suppressing tumor growth (see Scheme XX) [46]. The impact of MnP on ATP levels via glycolysis has been demonstrated also [50] (Scheme XVII) $\text{Mn}^{\text{III}}\text{P}^{5+}$ can either oxidize thiols directly (B and C), undergoing one-electron reduction to $\text{Mn}^{\text{II}}\text{P}^{4+}$ whereby thiol radical is formed in a thiol oxidase fashion; or it can act in Gpx fashion while coupling with H_2O_2 to S-glutathionylate thiols (A and C). Indeed, studies on lymphoma cells showed clearly that S-glutathionylation happens only in the presence of H_2O_2 and GSH [45,47]. The H_2O_2 may originate either from: (i) already increased oxidative stress of a cancer cell, or (ii) additional generation via radio- or chemotherapy, or (iii) catalytic cycling of MnP with ascorbate or thiols.

organelles) and is in turn controlled by lipophilicity, polarity, charge, size, shape and bulkiness of a molecule. The reactivities and in turn therapeutic effects are further controlled by the levels or reactive species in immediate neighborhood of MnPs. Based on all said, the best strategy in the design of an excellent therapeutic able to restore normal physiological redox environment may be to: (i) assure high metal/ligand stability, and in turn provide integrity of metal site; (ii) target as high as possible SOD-like activity; (iii) maintain positive charges to enhance electrostatically the reactions of MnPs with predominantly anionic reactive species, while enhancing lipophilicity. Enhanced lipophilicity would result in (iv)

enhanced accumulation of MnPs at all sites abundant with anionic phosphates such as mitochondria and brain. Importantly, the efforts need to be continuously invested in (v) minimizing toxicity of MnPs (Scheme XXV).

Distinction must be made between anti- and pro-oxidative reactions and anti- and pro-oxidative therapeutic effects

See Scheme XXVI.

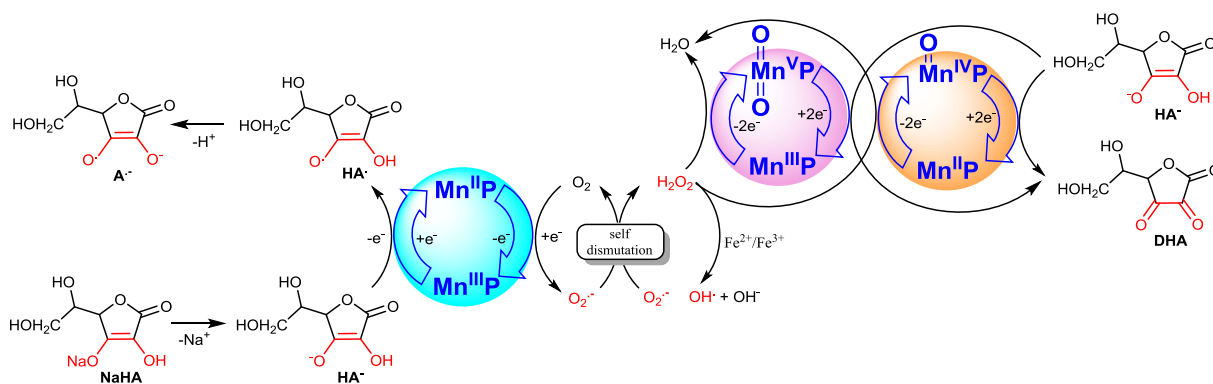
SOD mimics – the story continues

The perfect therapeutic should promote adaptive responses and help our body with upregulation of our own endogenous antioxidative defenses. The most obvious case of such adaptive response is demonstrated during regular physical exercising. Increasing number of reports support pro-oxidative actions of MnPs, nitroxides and flavonoids which result in adaptive responses. During 2 decades of research, we have gone a long way from initially viewing MnPs as antioxidants exclusively (SOD mimics) to considering them (pro)oxidants. Interestingly enough, with data right in front of our noses, it took us a long time to consider all aspects of $\text{O}_2^{\cdot-}$ dismutation process. The incorrect viewing of SOD enzyme and its mimic as antioxidants lies in 2 facts. Firstly, the two reactions involved in a dismutation process require a compound to be equally good anti- and pro-oxidant (Scheme I). Secondly, the dismutation process produces an oxidant, H_2O_2 . In turn, the SODs and their mimics can only be considered antioxidative defenses if H_2O_2 is removed rapidly in conjunction with catalases or peroxidases. Such considerations help us understand that, based on identical thermodynamics of metal centers and depending upon the cellular redox environment (levels of oxidative stress) plethora of reactions are possible with SOD enzymes and MnPs. Yet, due to steric hindrance imposed by large protein structure such reactions of SODs occur, if at all, with orders of magnitude lower rate constants as compared to their small molecular-weight mimics.

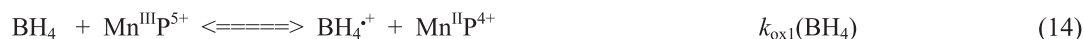
A number of other issues need to be addressed as the MnPs advance towards Clinic. *How long the treatment with MnP, following injury, should last?* Will we be able to ever restore the physiological redox status, or as soon as the therapy is discontinued some level of inflammation will be re-established. Studies on CNS indicate that the longer the treatment lasts, the longer the reduction in stroke volume post-injury was demonstrated [93]. *What are the therapeutic concentrations of MnPs at the sites of injury?* We have only marginal knowledge how much of MnP we need in target tissues. It seems that ~ 30 nM brain levels afford protection with MnTnHex-2-PyP $^{5+}$ in a stroke model [94]. Yet, we do not know if lower levels would produce such effects also. Clarification of such issues is costly but eventually will be needed.

Several metalloporphyrins are progressing towards or are in Clinical Trials. MnTDE-2-ImP $^{5+}$ passed Safety/toxicity Phase I Clinical Trials on ALS patients [95]. MnTE-2-PyP $^{5+}$ is in ongoing Clinical Trials, whereas STTR/NIH support has been granted for the Phase I/II Clinical trials on Head and Neck cancer patients on radioprotection of salivary glands and mouth mucosis. Another STTR/NIH funding for Phase I/II Clinical Trials on radioprotection of normal brain (with brain tumor patients) is pending. Another type of SOD mimics, M40403, is in Clinical Trials for the radioprotection of mouth mucosis as well. Fe porphyrin INO-4885 is in Clinical Trials for the use in contrast-induced nephropathy [20].

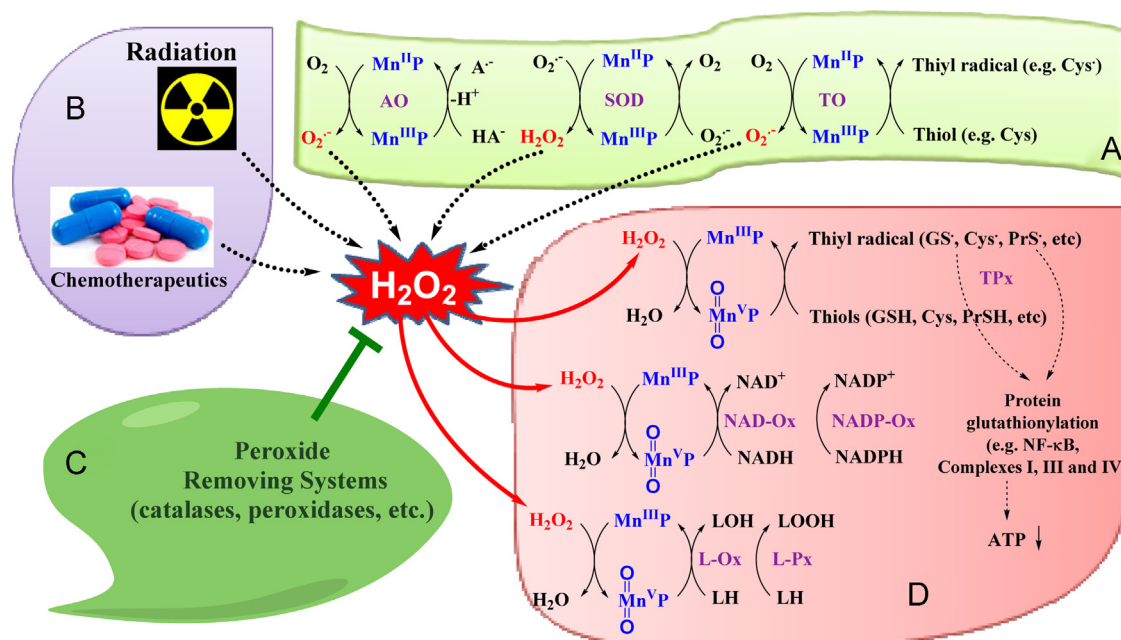
Summary. We may thus conclude our story with our most recent understanding that cationic Mn(III) substituted pyridyl- or



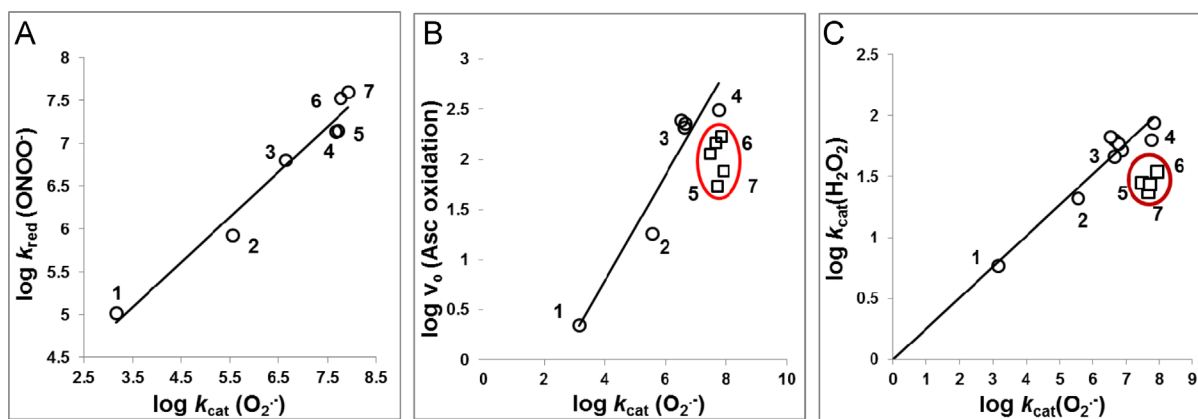
Scheme XII. Interaction of MnPs with ascorbate (NaHA—sodium ascorbate, HA⁻ - monodeprotonated ascorbate, major species at pH 7.8). Due to high intracellular levels of ascorbate (up to 0.1 mM), its interaction with MnP has been of our continuous interest since 1998 [3,6,17,41,43,44,51,52]. Due to its catalytic nature, such cycling generates high levels of H₂O₂, which are limited by levels of either ascorbate or oxygen. H₂O₂ formed can alternatively oxidize Mn^{II}P and Mn^{III}P to (O)Mn^VP and (O)₂Mn^VP, respectively (Eqs. 11a and 12a), with concomitant reduction to H₂O. The oxidized MnPs can be reduced back to two-electronically with HA⁻ producing dehydroascorbate (DHA). MnP/ascorbate system carries the therapeutic potential as anticancer therapeutic strategy [3,43]. MnP will in vivo likely couple with endogenous ascorbate due to its high levels. Yet therapeutic effects may be enhanced with exogenous ascorbate [42,44]. The system MnP + ascorbate, with regards to its mechanism of action, resembles to other major anticancer therapies which generate H₂O₂ (radiation and chemotherapy) where MnPs act as radio- and chemosensitizers [3,47,53] See also Schemes XVI–XVIII.



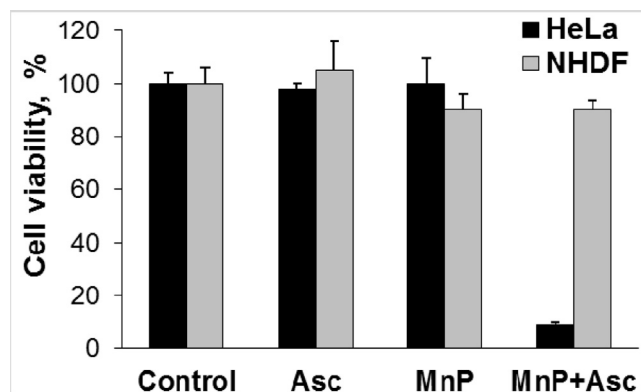
Scheme XIII. Interaction of MnP with tetrahydrobiopterin. Tetrahydrobiopterin is a cofactor of nitric oxide synthase, NOS. The reaction with tetrahydrobiopterin, BH₄, would result in a loss of reducing equivalents for NOS and could in turn result in NOS inhibition. Under such conditions NOS would make O₂^{•-} instead of *NO. The $k_{\text{ox1}}(\text{BH}_4)$ was found to be $1.0 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$ for one-electron oxidation of BH₄ to BH₄^{•+} and $k_{\text{ox2}}(\text{BH}_4^{+})$ $1.5 \times 10^3 \text{ M}^{-1} \text{ s}^{-1}$ for one-electron oxidation of BH₄^{•+} to 6,7-(8H)-BH₂ [54]. Cycling with BH₄ could result in tumor growth suppression via inhibition of its angiogenesis [54]. Recovery of MnP catalyst may lead to H₂O₂ production via Eq. (7). Alternatively, BH₄ may be oxidized with high-valent, highly oxidizing O=Mn^VP⁺ species formed in reactions with ONOO⁻ or H₂O₂ (Eqs. (1) and (12a)).



Scheme XIV. Role of H₂O₂ in the actions of MnPs. Over years, our data have contributed to our understanding that H₂O₂ plays a key role in the redox biology of MnPs. The Scheme summarizes all aspects of MnP/H₂O₂ interactions addressed thus far ([3,30,31,45,47]. The direct evidence was provided that H₂O₂ is essential for the impact of MnP/dexamethasone on cellular transcription and cell viability in lymphoma cellular model [45]. In such model MnP/dexamethasone system produces H₂O₂ which MnP employs to S-glutathionylate NF-κB which in turn causes cellular apoptosis and reduces number of viable cells. When catalase over-expressor CAT2 and CAT38 cells were treated with MnP/dexamethasone the identical number of viable cells was obtained as with MnP alone. Further, depletion of GSH with buthionine sulfoximine inhibitor of GSH synthesis suppressed S-glutathionylation of NF-κB. The same effect on NF-κB inhibition, demonstrated with MnP/dexamethasone, was replicated with NF-κB inhibitor, SN50 [3,31,45,47]. The oxidation of NADH and NADPH in the presence (but not absence) of H₂O₂ was demonstrated in aqueous system [31]. Hydroxylation of cyclophosphamide with MnP/asc - H₂O₂ generator system - was reported (see Scheme XXI) [52,55]. Adapted from [55] TPx—thiol peroxidation; TO—thiol oxidation; L-Ox—lipid oxidation; L-Px—lipid peroxidation.



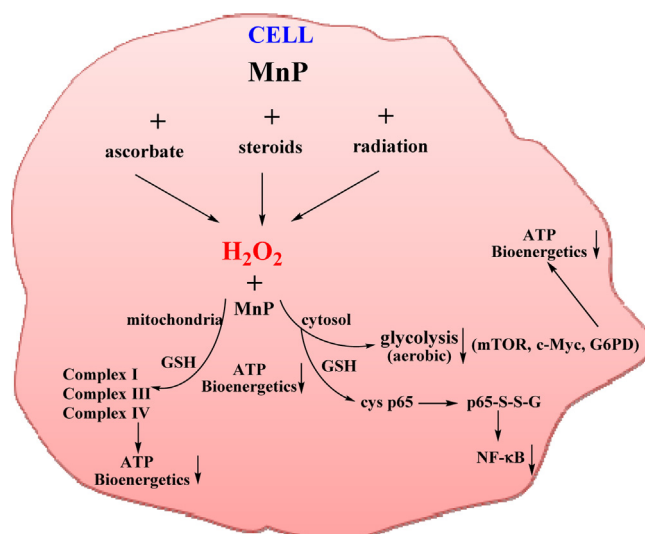
Scheme XV. SOD-like activity parallels other reactions of MnPs thus far studied as exemplified here with reduction of ONOO^- , oxidation of ascorbate and catalysis of H_2O_2 dismutation by MnP. Why potent SOD mimics are effective scavengers of other species and why in turn they may still be the most prospective therapeutic agents (assuming they are optimized for bioavailability and toxicity)? (1) MnTBAP $^{3-}$, (2) MnTE-2-PyPh $^{5+}$, (3) MnTE-3-PyP $^{5+}$, (4) MnTE-2-PyP $^{5+}$, (5) MnTPhE-2-PyP $^{5+}$, (6) MnTnHexOE-2-PyP $^{5+}$, and (7) MnTnOct-2-PyP $^{5+}$. In addition to those MnPs numerated, encircled in B are MnTnHex-2-PyP $^{5+}$ and MnTnBuOE-2-PyP $^{5+}$ and in C is MnTnHex-2-PyP $^{5+}$. The maximal $k_{\text{cat}}(\text{O}_2^{\cdot-})$ has been obtained at $E_{1/2}$ for $\text{Mn}^{\text{III}}\text{P}/\text{Mn}^{\text{II}}\text{P}$ of $\sim +200$ to $+450$ mV. The values of $E_{1/2} \geq +400$ mV stabilize Mn in $+2$ oxidation state and either $\text{Mn}^{\text{II}}\text{P}$ or a very weak $\text{Mn}^{\text{III}}\text{P}$ complex is formed. At values of $E_{1/2} \leq +100$ mV Mn is stabilized in $+3$ oxidation state to such extent that it does not favor reduction to $\text{Mn}^{\text{II}}\text{P}$ complex in a 1st step in order to start the $\text{O}_2^{\cdot-}$ dismutation. The $E_{1/2}$ of around $+200$ to $+400$ mV vs NHE is around the $E_{1/2}$ of SOD enzymes where both steps of dismutation process occur with similar rate constants and are thus similarly thermodynamically favored. The $E_{1/2}$ describes the magnitude of metal electron density which in turn reflects electron density of porphyrin ligand. $E_{1/2}$ of metal center thus relates to the protonation equilibria at all porphyrin sites (at pyrrolic nitrogens of porphyrin ligand and at waters axially ligated to metal site). Two such equilibria have been quantified at: (i) 3rd inner pyrrolic nitrogen of metal-free porphyrin, $\text{p}K_{\text{a}3} [\text{H}_3\text{P}^+ \leftrightarrow \text{H}_2\text{P} + \text{H}^+]$, and (ii) at 1st axially bound waters, $\text{p}K_{\text{a}(\text{ax})} [(\text{H}_2\text{O})_2\text{Mn}^{\text{II}}\text{P}^{5+} \leftrightarrow (\text{OH})(\text{H}_2\text{O})\text{Mn}^{\text{II}}\text{P}^{5+} + \text{H}^+]$. The inverted linear relationship exists between $E_{1/2}$ and $\text{p}K_{\text{a}(\text{ax})}$ and $\text{p}K_{\text{a}3}$ each: the higher the propensity of MnP to be reduced (the higher the $E_{1/2}$), the more-electron-deficient MnP is and less strongly it keeps its axial waters, thus easier (at lower pH) the proton is released and in turn the lower is the $\text{p}K_{\text{a}(\text{ax})}$. The same is true for $\text{p}K_{\text{a}3}$. The more pyrrolic nitrogen favors the proton association (the higher the $\text{p}K_{\text{a}3}$), the more electron-rich nitrogen is. In turn, such electron rich porphyrin ligand favors the formation of a stable complex with Mn. That means the higher the $\text{p}K_{\text{a}3}$ the less positive is the $E_{1/2}$ for $\text{Mn}^{\text{III}}\text{P}/\text{Mn}^{\text{II}}\text{P}$ redox couple. The consequence of such relationships is that the more electron-deficient the Mn complex is (lower the $\text{p}K_{\text{a}3}$), in addition to aqua ligand, the more it would favor binding different ligands, such as ONOO^- , ClO^- , CO_3^- and H_2O_2 . The binding of these species comprises the 1st step in their reductions. This further explains why the rate constants for the reduction of ONOO^- or ClO^- and H_2O_2 dismutation are related to the $E_{1/2}$ of the $\text{Mn}^{\text{III}}\text{P}/\text{Mn}^{\text{II}}\text{P}$ redox couple though it is not involved in such reactions (Scheme XII). As $E_{1/2}$ is proportional to $\log k_{\text{cat}}(\text{O}_2^{\cdot-})$, the $\log k_{\text{cat}}(\text{O}_2^{\cdot-})$ is proportional to both $\log k_{\text{red}}(\text{ONOO}^-)$ and $\log k_{\text{cat}}(\text{H}_2\text{O}_2)$ as shown in plots A and C. Importantly, the $E_{1/2}$ of electron transfer which follows the binding of those species, and involves Mn $+3/+4$ oxidation states (Eq. (1)), appears to be nearly identical for all MnPs [6,59]. In vivo, the 2nd step may preferentially involve two-electron electron transfer between Mn $+2$ and Mn $+4$ oxidation states (Eq. (4)). Under such conditions, the differences in $E_{1/2}$ for the $\text{Mn}^{\text{III}}\text{P}/\text{Mn}^{\text{II}}\text{P}$ redox couple will affect the $E_{1/2}$ of $\text{O}=\text{Mn}^{\text{IV}}\text{P}/\text{Mn}^{\text{III}}\text{P}$ redox couple. The ability of MnP to couple with ascorbate (plot B), catalyzing its oxidation follows the same trend as described for ONOO^- and H_2O_2 , as binding of ascorbate is involved in a 1st step of ascorbate oxidation. The deviation from the linearity has been demonstrated with compounds which have bulky pyridyl substituents (encircled in plots B and C). While not showed in plot A, the deviation from linearity has been seen also [7,10].



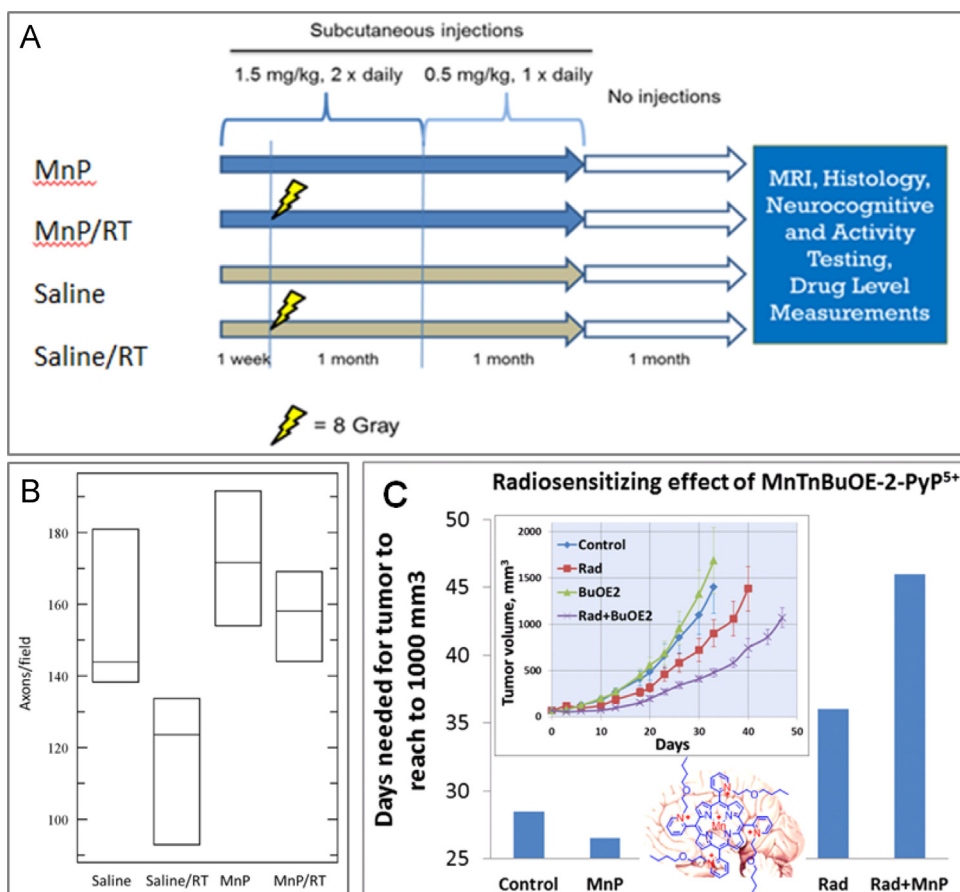
Scheme XVI. Differential therapeutic effects of MnP/ascorbate system on cancer (cervix cancer HeLa) vs normal cell line (human dermal fibroblasts, NHDF). MnP stands here for MnTE-2-PyP $^{5+}$. The differential therapeutic effect is based on differential redox environment of cancer vs normal cell [5,60]. Growing insight into the biology of cell and redox chemistry of MnPs taught us that we can take advantage of the rapid coupling of MnP with abundant cellular reductants [3,6,17,41,44,51,52]. Thus far we have shown that the catalytic oxidation of ascorbate with MnP (coupled to oxygen, Scheme XII) would enhance peroxide levels and could kill cancer without affecting much normal tissue [3,46]. The reason lies in fact that cancer is frequently low in H_2O_2 -removing systems (catalases, GPx, peroxiredoxins) and cannot cope with additional increase in H_2O_2 levels [4–6,60]. This reasoning is the same as the one behind the use of other H_2O_2 -producing strategies: radio- and chemotherapy. Oxidation of ascorbate at the expense of endogenous metalloproteins has already been employed in clinical trials in patients with pancreatic cancer [61,62]. The use of Mn porphyrin, MnTE-2-PyP $^{5+}$ and analogs of similar redox properties (MnTnHex-2-PyP $^{5+}$, MnTnBuOE-2-PyP $^{5+}$), optimized for ascorbate oxidation, has been reported by us [3,6,41,43,44] and subsequently by others to bear therapeutic potential [40,42,63]. The cytotoxicity of MnP/ascorbate has been shown with several other cancer cell lines, such as CaCo-2 (colorectal cancer), HCT116 (human colon cancer) MCF-7 (breast cancer), SUM149 (inflammatory breast cancer), 4T1 (mammary breast cancer), and 3 different gliomas (deltaGli36, U87MG, D-245MG) ([41,43] Batinic-Haberle I, Lam PYP, Roberts ERH et al., unpublished). Such system has been further shown by us and others not to be deleterious to normal cells [3,40,43,46]. Adapted from [3].

imidazolyl porphyrins suppress inflammatory processes in normal cell and cancer cell to a different extent but through identical actions – modifications of thiol-based signaling pathways. As SOD

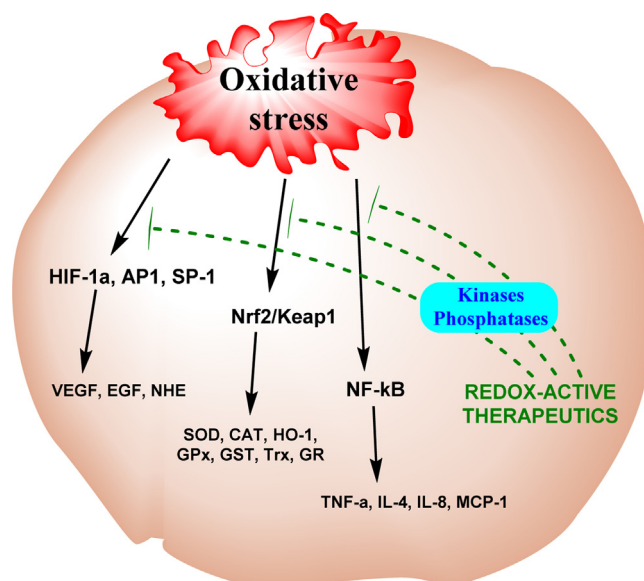
potency relates to the therapeutic effects of Mn porphyrins we alternate between describing them as SOD mimics or redox active therapeutics.



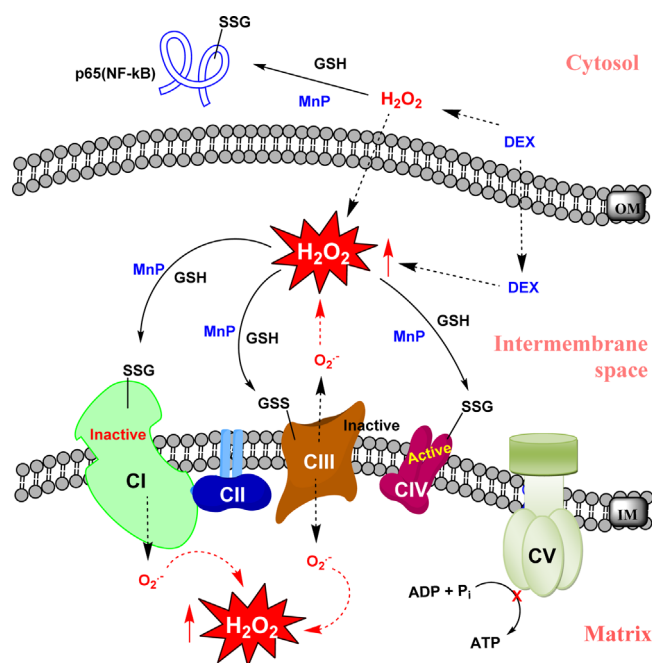
Scheme XVII. Differential effects of MnPs on normal vs cancer cell are controlled by their differential redox environments. Differential redox environment of normal and cancer cells promotes differential actions of MnPs. The rising data indicate that many antioxidant systems are differently regulated in cancer cell. Thus catalases, GPx, peroxiredoxins may be down regulated and often inactivated, while MnSOD may be upregulated which results in increased levels of H₂O₂ [4–6,60]. Reactions involved in the actions of MnPs are indicated in this Scheme and Scheme XIV and occur with different yield/magnitude in normal vs cancer due to their different levels of H₂O₂. Thus much larger levels of H₂O₂ in cancer vs normal cell would promote MnP/H₂O₂-driven oxidations to such extent that cancer cell would be forced to undergo apoptosis. Oxidations and/or S-glutathionylation (in GPx fashion) of thiols of NF-κB and complexes I and III of electron transport chain by MnPs/H₂O₂ may predominate in vivo [45,47–49]. MnP/H₂O₂-driven oxidations in normal cell would be of lesser extent that in cancer cell and thus would only suppress excessive inflammation which would result in healing of a normal cell [2,34,3,24]. *Note:* the beneficial therapeutic effects we observe may be different in nature. With normal cells, healing is demonstrated and thus the effects observed are of antioxidative nature. With cancer cells the actions of MnP/H₂O₂ are of magnitude high enough to cause cancer cell death: the therapeutic effects observed are pro-oxidative. Note that the actions behind such effects are pro-oxidative with both normal and cancer cell (oxidative modification of thiols), but due to differences in their magnitudes the therapeutic effects observed are of different type: *transient suppression of NF-κB in a normal cell similar to what is seen with aspirin and ibuprofen, but excessive NF-κB inhibition in a cancer cell similar to effects induced with steroid therapy leading to cell death* [64].



Scheme XVIII. Radioprotection of normal brain and radiosensitization of brain tumor by MnTnBuOE-2-PyP⁵⁺. (A) The treatment strategy for brain radioprotection with MnTnBuOE-2-PyP⁵⁺ (MnP) [53]. (B) Histological evaluation of myelinated axons in the corpus callosum. Representative images of toluidine blue-stained mid-sagittal corpus callosum tissue were imaged by brightfield microscopy ($n=3$ mice/group). Groups studied were (1) Saline, (2) MnP (3) Saline / radiation (RT), and (4) MnP/RT. Toluidine blue-stained mid-sagittal corpus callosum tissue were imaged by brightfield microscopy ($n=3$ mice/group). Three images from each mouse underwent automated axon counting that is quantitated in the box graph with 95% confidence intervals. ANOVA analysis of the group resulted in significant differences among the groups ($p=0.040$). (C) The impact of MnTnBuOE-2-PyP⁵⁺-based radiosensitization on tumor growth delay in a sc mouse xenograft D-245MG glioblastoma multiforme study. Patients-derived glioblastoma cells (kindly provided by The Preston Robert Tisch Brain Tumor Center at Duke University) were inoculated subcutaneously in the right flank of BALB/c nu/nu mice. Once tumors reached approximately 65 mm³ (Day 0), mice were randomly assigned to 4 groups with 8 mice per group: saline, saline/RT, MnP, and MnP/RT. Mice were injected subcutaneously with 1.6 mg kg⁻¹ MnP twice daily (beginning at 24 h before RT and continuing for the duration of the study). Tumors were irradiated for 3 days with 1 Gy day⁻¹ on day 2–4 (XRAD-320, Precision X-ray, North Branford, CT) [53]. The impact of similar magnitude was achieved with MnP/temozolomide treatment, where MnP acted as a chemosensitizer; the action is similar to MnP-driven sensitization of lymphoma cells to dexamethasone [45–47]. Both MnTnHex-2-PyP⁵⁺ [2] and MnTnBuOE-2-PyP⁵⁺ suppressed tumor growth of D-245MG to similar magnitude under identical experimental conditions. Adapted from [53].

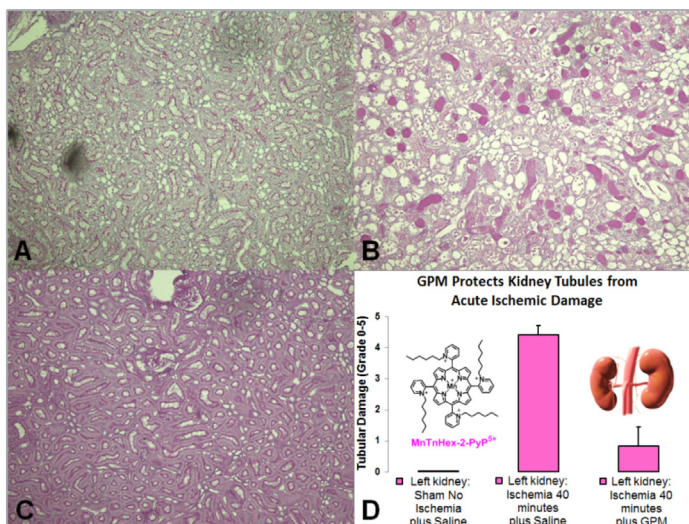


Scheme XIX. Impact of MnPs on cellular transcription. The reactions of MnPs were thus far explored with respect to several transcription factors, *NF-κB*, *AP-1*, *SP-1*, *HIF-1α* (see also [Scheme XIV](#)) [48]. MAPK/ERK pathway has been implicated also [41]. Oxidation of cysteine residues of p50 and p65 subunits of *NF-κB* by MnPs/ H_2O_2 (the pro-oxidative actions of MnPs) prevents the activation of *NF-κB*, and in turn suppresses excessive inflammation and thus produces therapeutic effects which we identify as anti-oxidative [3]. Data obtained by us on MnPs ([Scheme XXI](#)) [66,67] and others on a member of other class of SOD mimics, Mn(II) cyclic polyamines, M40403 [68], suggest the impact of MnPs on the activation of Nrf2. Upon oxidation of redox sensitive cysteines of Keap1, Nrf2 transcription factor is activated, which results in upregulation of endogenous antioxidative defenses [67,68]. Nitroxides were shown to activate that pathway also [69–71]. It may be mechanism of actions of flavonoids also [70,71] (see also [Scheme XX](#)).

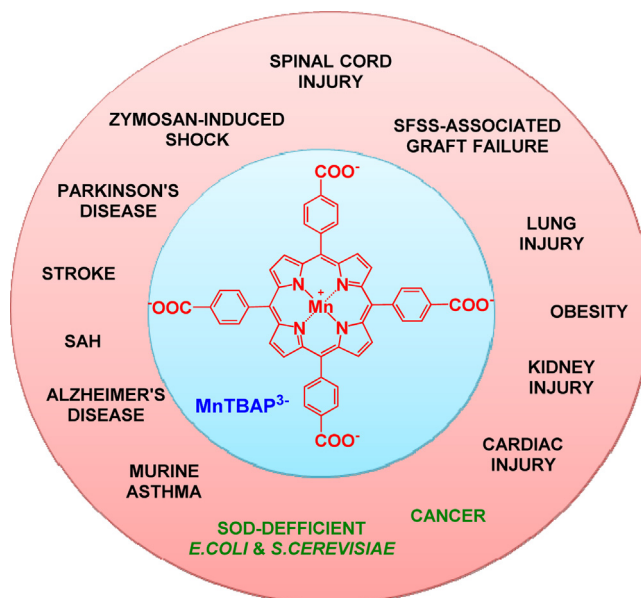


Scheme XX. MnP inactivates complexes I and III via their S-glutathionylation when combined with dexamethasone in lymphoma cellular model. MnTE-2-PyP⁵⁺ was tested in lymphoma cellular model where it enhanced cell killing in the presence of dexamethasone via enhancing H_2O_2 production [45,46]. H_2O_2 in turn was employed by MnP to catalyze S-glutathionylation of protein thiols. Such action, depicted in [Scheme XI](#), is in essence either GPx- or thiol oxidase-like activity of MnP. Such oxidative modification has been demonstrated on both cytosolic and mitochondrial levels. In cytosol cysteine units of p50 and most so p65 of *NF-κB* seem to be modified, while complexes I, III and IV were S-glutathionylated in mitochondria. Also oxidation of p50 by MnP in nucleus has been suggested by Piganelli's group [48]. All proteins except complex IV (though S-glutathionylated) were inactivated by such oxidative modifications. That indicates that MnP under conditions of high oxidative stress, i.e. high H_2O_2 levels, which may be produced by different treatment modalities such as radiation and chemotherapy (dexamethasone or ascorbate or temozolamide, [3,41,42,44,45,47,53]), would suppress anti-apoptotic, survival pathways and cellular energetics, killing cancer cell on two metabolic fronts. Adapted from [45,46].

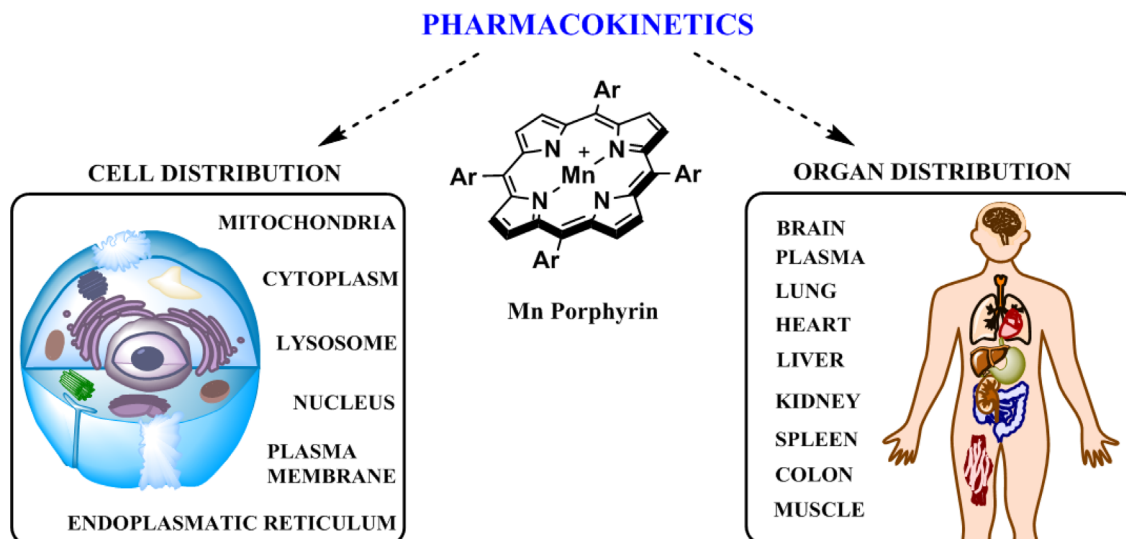
Genes	Fold-increase
Glutathione peroxidase 1	9.8
Glutathione peroxidase 3	23.9
Lactoperoxidase	16.7
NADPH oxidase 4	13.9
Peroxiredoxin	
2	9.6
5	9.8
3	6.6
Superoxide dismutase	
mitochondrial	10.2
extracellular	12.3
Inducible nitric oxide synthase	15.2
Thioredoxin	
Thioredoxin reductase	6.9
Interacting protein	7.3
Flavin monooxygenase	10.5
Catepsin B	12.2
Apolipoprotein E	16.2
Prostaglandin peroxidase synthetase 1	11.1



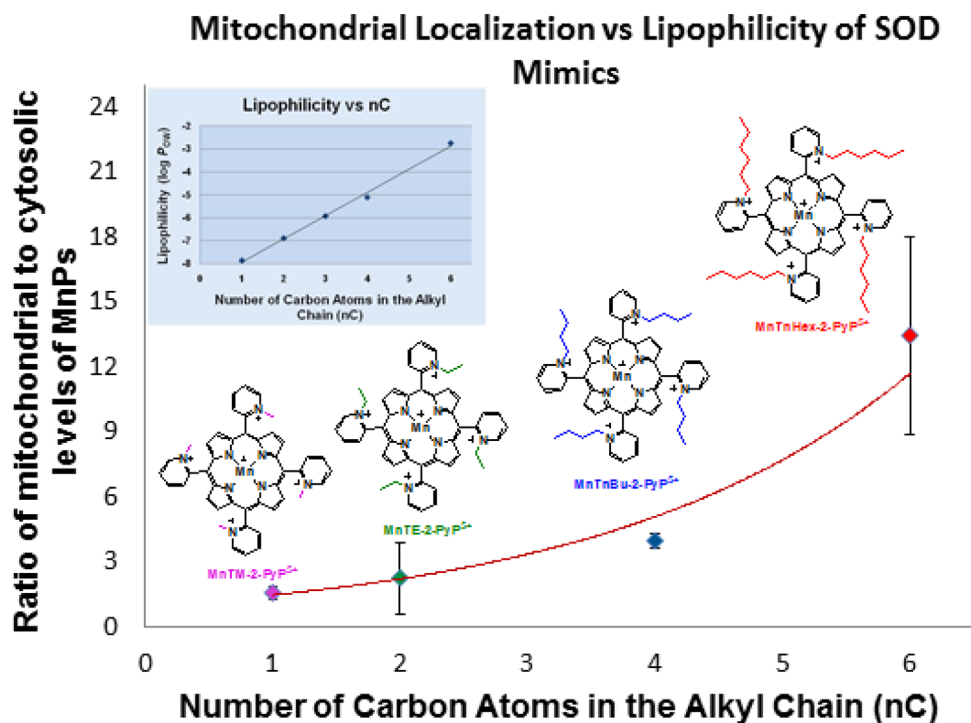
Scheme XXI. Increase in antioxidative genes due to MnP-based treatment of rats which underwent kidney ischemia–reperfusion I/R. The GPM mixture treatment contained: bFGF, recombinant EPO, SDF-1 and BMP-7, and a mixture of mitochondria protective amino acids, including α -ketoglutarate and L-aspartic acid, and MnTnHex-2-PyP⁵⁺ [67]. Note that major endogenous antioxidative defenses are up-regulated indicating that MnP under given conditions could not have acted as SOD mimic. During I/R the H₂O₂ is produced which MnP supposedly employs for oxidative modifications of protein thiols as indicated in other studies (Schemes XI, XIV and XX). We hypothesized that in this model MnP oxidizes Keap1 cysteine and in turn activates Nrf2 which then upregulates endogenous antioxidative defenses. Indeed the Keap1 oxidation and Nrf2 activation were implicated with another class of SOD mimic, M40403 [68], nitroxides, curcumin [73] and flavonoids [70,71]. The following groups, with 10 male Sprague–Dawley rats each, were studied: (a) sham-operated; (b) I/R with saline only; (c) I/R with growth factors only (G); (d) I/R with MnP only (P); (e) I/R a mixture of α -keto-glutarate and L-aspartic acid (M), and (f) GPM mixture. GPM was given 24 h before 40-min ischemia and 24 h post-reperfusion. The study was terminated at 48 h post-reperfusion. Periodic acid–Schiff (PAS) stains and tubular damage grading was assessed at 48 h post-I/R. All individual components of GPM mixture produced beneficial effects which were lower than those produced with whole GPM mixture. (A) Normal PAS staining of non-ischemic sham operated left kidney; (B) dramatically increased PAS positive necrosis in ischemic left kidney; (C) reversal of necrosis in ischemic+GPM treated left kidney; (D) significant decrease in the acute tubular necrosis grading in ischemic left kidney treated with the GPM mixture ($p < 0.0003$). In next kidney I/R study, the authors introduce N-acetyl cysteine into the GPM mixture; all effects were enhanced; note that MnP cycles with thiols producing H₂O₂ (Scheme XI) [66,67]. Adapted from [67].



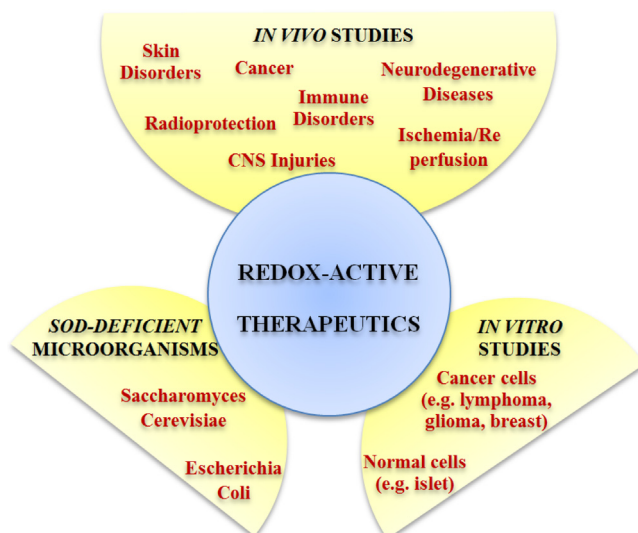
Scheme XXII. MnTBAP³⁻, while not an SOD mimic, produces therapeutic effects. The therapeutic effects have been reported in all cases listed in the Scheme, other than in cancer and in O₂^{•-}-specific aerobic growth of *E. coli* and *S. cerevisiae*. The impure MnTBAP³⁻ obtained from Alexis or Calbiochem has often been used in past [74]. It contained SOD-active Mn oxo/hydroxo/acetato complexes; in turn the origin of effects has been dubious [75]. Still even when pure MnTBAP³⁻ was used the therapeutic effects have been observed, though of lesser magnitude relative to cationic Mn(III) *N*-substituted pyridylporphyrins [4,76,77]. 50 nM MnTE-2-PyP⁵⁺, 1 μ M FeTM-4-PyP⁵⁺ while 100 μ M MnTBAP³⁻ were able to attenuate ATN-224-induced peroxynitrite-dependent death of lymphoma cells which are oxidative stress-resistant and are over-expressing Bcl-2. ATN-224 is a Cu chelator, choline tetrathiomolybdate, that reduces the activity of Cu,ZnSOD enzyme. While not an SOD mimic, MnTBAP³⁻ reduces ONOO⁻, thus exhibiting some specificity towards elimination of ONOO⁻ over O₂^{•-} [77]. This suggests that much about its in vivo actions awaits further clarification. In a rat spinal cord ischemia/reperfusion model, under same dosing regime, MnTBAP³⁻ suppressed NF- κ B expression as does a powerful SOD mimic, MnTnHex-2-PyP⁵⁺. Such action prevented excessive NF- κ B-driven inflammation [4]. The dose-dependence and the mechanism behind NF- κ B inactivation by those MnPs are under further investigation. Adapted from [4].



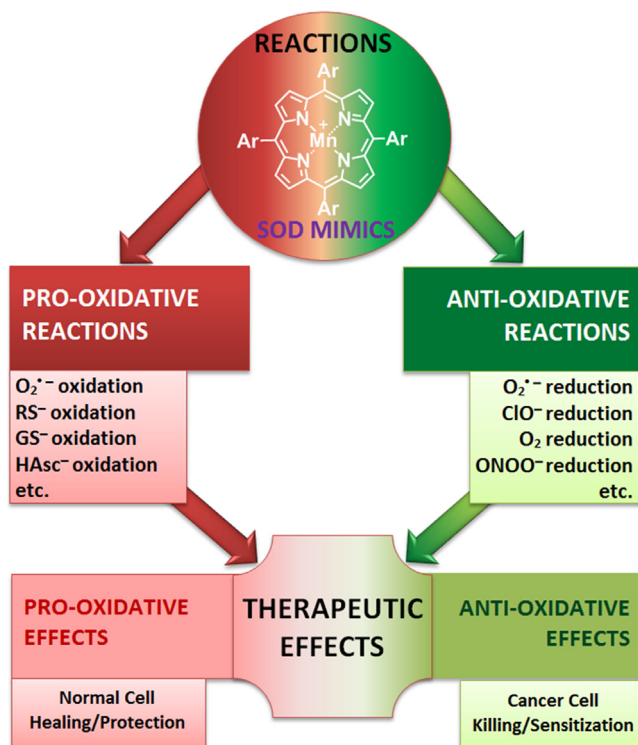
Scheme XXIII. Pharmacokinetic and bioavailability studies with MnPs. Bioavailabilities of MnPs depend upon their lipophilicities, polarities, charge, size, shape, and bulkiness. Comprehensive studies have been reported on MnPs that bear similar redox properties (MnTE-2-PyP⁵⁺, MnTM-2-PyP⁵⁺, MnTnBu-2-PyP⁵⁺, MnTnHex-2-PyP⁵⁺ and MnTnBuOE-2-PyP⁵⁺ in different organisms via various administration routes. The PK studies involved plasma and different organs such as liver, kidney, lungs, spinal cord, brain, spleen, heart, colon, salivary glands and tongue. Also intracellular accumulation has been addressed on ZnPs and MnPs and data published on membrane, cytosolic, nuclear, mitochondrial, endoplasmic reticular and lysosomal accumulation [78–83]. Largest body exposure of MnPs, expressed as AUC (area under pharmacokinetic curve) has been obtained via subcutaneous route; this route also has another advantage as it imposes the lowest acute side effects in MnP administration – blood pressure drop. Highest levels of MnPs are found in liver and kidneys (at μM levels), while the lowest were detected in brain (at nM levels). Lipophilic and cationic, MnTnHex-2-PyP⁵⁺ and MnTnBuOE-2-PyP⁵⁺ accumulate several fold more in brain (driven there by the anionic phospholipids) than does hydrophilic MnTE-2-PyP⁵⁺ [Spasojevic, Tovmasyan, Weitner et al., unpublished]. Given the highly positive charge, MnTE-2-PyP⁵⁺ accumulates ~ 3 -fold more in nucleus than in cytosol of macrophages and LPS stimulated macrophages [48]. Such accumulation seems to be important given the suggestion by Tse and Piganelli that MnP oxidizes p50 in nucleus [48,49]. Cationic MnPs were also found to favor accumulation in cell wall of *E. coli* due to abundance of negatively charged phospholipids [84]. Extensive distribution studies of fluorescent Zn analogs using confocal fluorescent microscopy indicate their accumulation in membranes, lysosomes and endoplasmic reticulum also [78,85]. The study was carried out on adenocarcinoma cells where lipophilic ZnTnHex-2-PyP⁵⁺ was also found in mitochondria in the vicinity of cytochrome c oxidase [79]. The only difference between Zn and Mn analogs is the lack of single charge on Zn center. Yet, with longer alkyl analogs, such as MnTnHex-2-PyP⁵⁺ no difference in their lipophilicities exists as single cationic charge on Mn is shielded from the solvent by long hexyl chains. Moreover, in vivo MnPs get readily reduced with cellular reductants, lose single charge on metal site and become similarly hydrophilic as ZnPs [86]. Thus, accumulation data on ZnPs (in particular those with long alkylpyridyl substituents) can be readily applied to MnPs.



Scheme XXIV. Impact of lipophilicity on mitochondrial vs cytosolic accumulation of cationic MnPs in *S. cerevisiae*. Impressive levels of otherwise hydrophilic compounds were found in mitochondria relative to cytosol and are presumably due to their high penta-cationic charge. The striking impact of lipophilicity on their mitochondrial accumulation was demonstrated [91]. Even the most hydrophilic MnTM-2-PyP⁵⁺ and MnTE-2-PyP⁵⁺ prefer mitochondria to cytosol. Such data suggest that pentacationic charge, rather than lipophilicity of pyridyl alkyl chains, controls mitochondrial accumulation of MnPs.



Scheme XXV. Therapeutic effects of MnPs. The $O_2^{\cdot-}$ specific models of SOD-deficient single-cell pro- and eukaryotic organisms, *E. coli* and *S. cerevisiae*, are the first and major steps in evaluating therapeutic potential of MnPs. Once they are successfully tested in unicellular organisms, MnPs are forwarded to various cellular and animal models. During the last two decades numerous cellular and animal studies (on mice, rats, rabbits and monkeys), conducted on models of diverse diseases, support remarkable therapeutic potential of metalloporphyrins; thus we hope that some will successfully Clinical Trials. Therapeutic effects demonstrated in models of CNS injuries, immune disorders, cancer and radioprotections were summarized in several manuscripts of *Forum Issue "SOD therapeutics"* of *Antioxidants & Redox Signaling* 2014.



Scheme XXVI. MnPs undergo anti- and pro-oxidative reactions which may be demonstrated either as anti- or pro-oxidative therapeutic effects. While such reasoning and experimental data behind it have been a main theme across this review, it is important to stress it here again: researchers need to differentiate between the type of reactions of MnPs and nature of therapeutic effects they observe. For example, oxidation of cysteines of NF- κ B by MnP suppresses its activation which in turn suppresses secondary oxidative stress. This is demonstrated as reduced inflammation – commonly accepted as anti-oxidative therapeutic effects. Further, the oxidation of cysteine of Keap1 by MnP would activate Nrf2 which would upregulate endogenous antioxidative defenses (catalases, SODs, peroxidases, etc) in an adaptive response. This would result in suppression of inflammation – clear case of antioxidative effect. Again pro-oxidative reaction of MnP results in anti-oxidative therapeutic effects. Please note that not all reactions thus far studied are listed in this Scheme, such as reactions with tetrahydrobiopterin, H_2O_2 and $CO_3^{\cdot-}$ (for further information see other chapters). Many more in vivo reactions of MnPs are likely yet to be identified.

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