Rational Design of Superoxide Dismutase (SOD) Mimics: The Evaluation of the Therapeutic Potential of New Cationic Mn Porphyrins with Linear and Cyclic Substituents

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Supporting Information

ABSTRACT: Our goal herein has been to gain further insight into the parameters which control porphyrin therapeutic potential. Mn porphyrins (MnTnOct-2-PyP5+, MnTnHex-OE-2-PyP5+, MnTE-2-PyPhP5+, and MnTPhE-2-PyP5+) that bear the same positive charge and same number of carbon atoms at meso positions of porphyrin core were explored. The carbon atoms of their meso substituents are organized to form either linear or cyclic structures of vastly different redox properties, bulkiness, and lipophilicities. These Mn porphyrins were compared to frequently studied compounds, MnTE-2-PyP5+, MnTE-3-PyP5+, and MnTBPPh. All Mn(III) porphyrins (MnPs) have metal-centered reduction potential, $E_{1/2}$ for MnIII/MnII redox couple, ranging from $-194$ to $+340$ mV versus NHE, log $k_{cat}(O_2^{**})$ from $3.16$ to $7.92$, and log $k_{cat}(ONOO^-)$ from $5.02$ to $7.53$. The lipophilicity, expressed as partition between n-octanol and water, log $P_{O/W}$ was in the range $-1.67$ to $-7.67$. The therapeutic potential of MnPs was assessed via: (i) in vitro ability to prevent spontaneous lipid peroxidation in rat brain homogenate as assessed by malondialdehyde levels; (ii) in vivo $O_2^{**}$ specific assay to measure the efficacy in protecting the aerobic growth of SOD-deficient Saccharomyces cerevisiae; and (iii) aqueous solution chemistry to measure the reactivity toward major in vivo endogenous antioxidant, ascorbate. Under the conditions of lipid peroxidation assay, the transport across the cellular membranes, and in turn shape and size of molecule, played no significant role. Those MnPs of $E_{1/2}$ ~ $+300$ mV were the most efficacious, significantly inhibiting lipid peroxidation in 0.5–10 μM range. At up to 200 μM, MnTBPPh (E1/2 = −194 mV vs NHE) failed to inhibit lipid peroxidation, while MnTE-2-PyPhP5+ with 129 mV more positive E1/2 (~65 mV vs NHE) was fully efficacious at 50 μM. The E1/2 of MnIII/MnII redox couple is proportional to the log $k_{cat}(O_2^{**})$, i.e., the SOD-like activity of MnPs. It is further proportional to $k_{cat}(ONOO^-)$ and the ability of MnPs to prevent lipid peroxidation. In turn, the inhibition of lipid peroxidation by MnPs is also proportional to their SOD-like activity. In an in vivo S. cerevisiae assay, however, while E1/2 predominates, lipophilicity significantly affects the efficacy of MnPs. MnPs of similar log $P_{O/W}$ and E1/2 that have linear alkyl or alkoxalkyl pyridyl substituents, distribute more easily within a cell and in turn provide higher protection to S. cerevisiae in comparison to MnP with bulky cyclic substituents. The bell-shape curve, with MnTE-2-PyP5+ exhibiting the highest ability to catalyze ascorbate oxidation, has been established and discussed. Our data support the notion that the SOD-like activity of MnPs parallels their therapeutic potential, though species other than $O_2^{**}$, such as peroxynitrite, $H_2O_2$, lipid reactive species, and cellular reductants, may be involved in their mode(s) of action(s).

INTRODUCTION

Our continuous goal has been to learn how to improve the therapeutic potential of porphyrin-based SOD mimics for the treatment of disorders with perturbed cellular redox environment, commonly described as oxidative stress. While maintaining the most appropriate thermodynamics and kinetics for SOD-like activity, the efforts have recently been directed toward the increase in the biodistribution of SOD mimics and decrease in their toxicity. The structure–activity relationship
(SAR), which has guided us in our efforts to improve the drug quality,1,2 correlates the thermodynamic (metal-centered reduction potential, $E_{1/2}$ for Mn$^{3+}$/Mn$^{2+}$ redox couple) and kinetic properties of Mn(III) porphyrins (MnPs), log $k_{\text{cat}}(O_2^−)$ (O$_2^−$/superoxide). The $k_{\text{cat}}(O_2^−)$ describes the ability of MnP to catalyze O$_2^−$ dismutation to O$_2$ and H$_2$O$_2$. SAR is universally valid, not only for metalloporphyrins but for other redox-active drugs also.3,4 Further, $k_{\text{cat}}(O_2^−)$ parallels the ability of MnPs to reduce peroxynitrite, described by the rate constant for NOON$^-$ (peroxynitrite) reduction, $k_{\text{cat}}$(ONOON$^-$).4 Both properties are controlled by the electron-deficiency of a metal site which favors exchanging electrons with O$_2^−$ (reducing and oxidizing it during dismutation process) and binding of electron-rich ONOO$^-$ with its subsequent reduction to either NO$_2^-$ (one-electronically) or NO$_2^-$ (two-electronically).6 We have further shown that such property of the metal site also favors reactions with other electron-rich nucleophiles such as ClO$^-$ (deprotonated hypochlorite), 7 HO$_2^-$ (a deprotonated reactive species of H$_2$O$_2$), lipid radicals,8,9 CO$_2^-$,4 ascorbate, HA$^-$ (monodeprotonated ascorbic acid), and deprotonated thiols, RS$^-$,10–12. The reaction of MnPs with simple and protein thiols as well as with ascorbate coupled to peroxide production seems to be heavily involved in their mechanism(s) of action(s).2,10

With the goal to enhance the biodistribution of MnPs, we modified the original structure of MnTE-2-PyP$^{5+}$ (AEOL10113, Mn(III) meso-tetrakis(N-ethylpyridinium-2-yl)porphyrin) and synthesized a first generation of lipophilic analogs, via lengthening the alkyl chains of MnTE-2-PyP$^{5+}$ to MnTnOct-2-PyP$^{5+}$ (Mn(III) meso-tetrakis(N-octylypyridinium-2-yl)porphyrin).13 MnTnHex-2-PyP$^{5+}$ (Mn(III) meso-tetrakis(N-hexylpyridinium-2-yl)porphyrin) has been a well-explored lipophilic analog with much higher brain and mitochondrial distribution than MnTE-2-PyP$^{5+}$.14,15 Yet, its toxicity at higher concentration and prolonged administration may limit its use. We thus designed and characterized MnTnBuOE-2-PyP$^{5+}$ (BMX-001; Mn(III) meso-tetrakis(N-(2′-n-butoxyethyl)-pyridium-2-yl)porphyrin), which has 4–5-fold reduced toxicity relative to MnTnHex-2-PyP$^{5+}$, while lipophilicity and redox-activity have not been compromised.16

Herein we continued with the rational design of MnPs. New Mn porphyrins were synthesized and compared to MnTnOct-2-PyP$^{5+}$ and several other compounds (Figures 1 and 2) mostly studied by us and others, MnTE-2-PyP$^{5+}$, MnTE-3-PyP$^{5+}$ (Mn(III) meso-tetrakis(N-ethylpyridinium-3-yl)porphyrin), and MnTBAP$^{5−}$ (Mn(III) meso-tetrakis(4-carboxylatophenyl)porphyrin).17 While of entirely different redox properties, both an SOD mimic, MnTE-2-PyP$^{5+}$, and a non-SOD mimic, MnTBAP$^{5−}$, reportedly exhibit beneficial effects in in vitro and in vivo models of numerous oxidative stress-related disorders, such as stroke, cancer, lung diseases, radiation injuries, spinal cord injury, Alzheimer disease, cardiac injuries, pain, and morphine tolerance and autoimmune diseases, some of which are shown in Figure 1.18–68

We have been puzzled with the therapeutic efficacy of the MnTBAP$^{5−}$ [see also Results and Discussion] on the basis of our present knowledge, this compound has inferior redox properties ($E_{1/2} = −194$ mV vs NHE), relative to MnTE-2-PyP$^{5+}$ ($E_{1/2} = +228$ mV vs NHE) and thus does not favor interactions with biological targets. Most of the reactive species are anionic and would disfavor interacting with anionic MnTBAP$^{5−}$ on electrostatic grounds.69 Still strongly oxidizing species such as ONOO$^-$ and CO$_3^{2-}$ are able to oxidize it.4,5,9 Despite claims,70 MnTBAP$^{5−}$ is neither reactive toward O$_2^−$ nor to H$_2$O$_2$. Recent data indicate that its RNS-related chemistry may account for its biological effects;71 the neutrality of NO or HNO would work in favor of such reactions.72 Finally, its negative charge would not facilitate its transport across anionic phospholipid membranes. The fact that impure preparations of MnTBAP$^{5−}$, provided by several commercial sources, were often used without prior characterization and purification has complicated things further.69,73 Still the abundance of published data, including a few of our studies, indicates that under certain conditions MnTBAP$^{5−}$ is efficacious.5,9,18–42,72 Recently, a manuscript was published where PEG-ylated amid of MnTBAP$^{5−}$ was synthesized and characterized.74 Such derivatization removed the unfavorable electron-donating effect of COO$^-$ groups upon Mn site. In turn, the $E_{1/2}$ is nearly 200 mV more positive relative to MnTBAP$^{5−}$. Along with the improved electrostatics, this modification increased log $k_{\text{cat}}$(O$_2^−$) from 3.16 to 5.6.

Figure 1. Structures of MnTBAP$^{5−}$,18–43 and ortho (2) and meta (3) isomers, MnTE-2(3)-PyP$^{5+}$.43–68 Also listed are their in vivo efficacy studies.
METAL-CENTERED REDUCTION POTENTIAL, $E_{1/2} (\text{Mn}^{III}/\text{Mn}^{II})$

\[ k_{\text{cat}} (\text{O}_2^- \text{ dismutation}) \quad v_0 (\text{Asc oxidation}) \quad k_{\text{red}} (\text{ONOO}^- \text{ reduction}) \]

LIPOPHILICITY

MOLECULAR STRUCTURE

(shape, size, bulkiness)

Inhibition of Lipid Peroxidation

Growth of SOD-deficient \(S.\) cerevisiae

Figure 2. Impact of structural features of MnTnOct-2-PyP\(^{5+}\) and three new Mn porphyrins (MnP) on their \(in\) \(vitro\) and \(in\) \(vivo\) therapeutic potential. The figure illustrates which properties of MnPs were studied herein with a goal to (i) further our knowledge on their impact on the therapeutic potential of redox-active drugs, and in turn (ii) facilitate drug development. Metal-centered reduction potential, $E_{1/2}$ of Mn\(^{III}/\text{Mn}^{II}\), controls the rate constant for the catalysis of $\text{O}_2^-$, $k_{\text{cat}}(\text{O}_2^-)$, rate constant for the peroxynitrite reduction, $k_{\text{red}}(\text{ONOO}^-)$, as well as the ability of MnP to catalyze ascorbate oxidation to ascorbyl radical $A^\bullet$. The \(in\) \(vitro\) consequences of appropriate thermodynamics were also witnessed in the lipid peroxidation of rat brain homogenate. This is so because the reduction of highly reactive species, such as ONOO$^-$ and lipid reactive species, involves their binding to Mn site in the first step. Binding is controlled by electron-deficiency of porphyrin and its Mn site and could be best described by the protonation equilibria of porphyrin inner pyrrolic nitrogens\(^7\&\) and axial waters,\(^7\) which in turn control the $E_{1/2}$ of Mn\(^{III}/\text{Mn}^{II}\). With $E_{1/2}$ value beyond 0 mV vs NHE, the fair deficiency in electron density of the metal site is indicated which in turn suggests the high affinity of Mn toward binding of an electron-rich ligand, such as ONOO$^-$ or lipid reactive species. Ligand binding is followed by Mn\(^{III}\) oxidation to O\(=\text{Mn}^{III}\). Therefore, the $E_{1/2}$ of Mn\(^{III}/\text{Mn}^{II}\) redox couple correlates well with rates of reactions involving the O\(=\text{Mn}^{III}/\text{Mn}^{III}\) redox couple. The $E_{1/2}$ of O\(=\text{Mn}^{III}/\text{Mn}^{II}\) redox couple is similar for a variety of different Mn and Fe porphyrins, implying that the ligand (such as ONOO$^-$) binding is a rate-limiting step in metal oxidation and ligand reduction (see also Results and Discussion). The other major property that controls the therapeutic potential of MnP is its lipophilicity, and it was herein explored in aerobic growth of SOD-deficient yeast \(S.\) cerevisiae.

impact of such derivatization agrees well with our data on the contribution of electrostatics in $\text{O}_2^-\text{•}^{-}$ dismutation.\(^7\) In order to gain further insight into the possible therapeutic effects of MnTBAP\(^7\), we have used it in all studies performed herein.

From a therapeutic point of view, if the drug is efficacious it may not quite matter what exactly it is doing in \(in\) \(vivo\). This fact may not preclude its clinical development. Yet, understanding what exactly it is doing in \(in\) \(vitro\) may not preclude its clinical development. Metal-centered reduction potential, $E_{1/2}$ of Mn\(^{III}/\text{Mn}^{II}\), controls the rate constant for the catalysis of $\text{O}_2^-$, $k_{\text{cat}}(\text{O}_2^-)$, rate constant for the peroxynitrite reduction, $k_{\text{red}}(\text{ONOO}^-)$, as well as the ability of MnP to catalyze ascorbate oxidation to ascorbyl radical $A^\bullet$. The \(in\) \(vitro\) consequences of appropriate thermodynamics were also witnessed in the lipid peroxidation of rat brain homogenate. This is so because the reduction of highly reactive species, such as ONOO$^-$ and lipid reactive species, involves their binding to Mn site in the first step. Binding is controlled by electron-deficiency of porphyrin and its Mn site and could be best described by the protonation equilibria of porphyrin inner pyrrolic nitrogens\(^7\&\) and axial waters,\(^7\) which in turn control the $E_{1/2}$ of Mn\(^{III}/\text{Mn}^{II}\). With $E_{1/2}$ value beyond 0 mV vs NHE, the fair deficiency in electron density of the metal site is indicated which in turn suggests the high affinity of Mn toward binding of an electron-rich ligand, such as ONOO$^-$ or lipid reactive species. Ligand binding is followed by Mn\(^{III}\) oxidation to O\(=\text{Mn}^{III}\). Therefore, the $E_{1/2}$ of Mn\(^{III}/\text{Mn}^{II}\) redox couple correlates well with rates of reactions involving the O\(=\text{Mn}^{III}/\text{Mn}^{III}\) redox couple. The $E_{1/2}$ of O\(=\text{Mn}^{III}/\text{Mn}^{II}\) redox couple is similar for a variety of different Mn and Fe porphyrins, implying that the ligand (such as ONOO$^-$) binding is a rate-limiting step in metal oxidation and ligand reduction (see also Results and Discussion). The other major property that controls the therapeutic potential of MnP is its lipophilicity, and it was herein explored in aerobic growth of SOD-deficient yeast \(S.\) cerevisiae.
Aldrich. Xanthine, equine ferricytochrome c (lot 7752), and (+)-sodium l-ascorbate (>98%) were from Sigma, whereas xanthine oxidase was prepared by R. Wiley.1 Triethylamine (Et3N) of >99.5% purity was obtained from Thermo Scientific Pierce. All chemicals were used as received without further purification. The 1H NMR spectra were recorded on a spectrometer “Mercury Varian 300” with deuterated chloroform as solvent.

**Synthesis of meso-Tetrakis(N-substituted pyridinium-2-yl)porphyrins.** The general synthetic procedure for meso-tetrakis(N-substituted pyridinium-2-yl)porphyrins and their Mn complexes is shown in Figure 3. The synthesis, isolation, purification, and characterization of Mn porphyrins, MnTPhE-2-PyPCl5, MnTnHexOE-2-PyPCl5, were performed as described earlier. The appropriate tosylates, phenylpyridinium-2-yl)tosylates, and 2-n-hexoxyethyl p-toluenesulfonate, were obtained, purified, and characterized according to the methods earlier reported for analogous compounds.40 The synthesis of new porphyrinic ligands H2TPhE-2-PyPCl4 ((meso-tetrakis(N-(2′-phenylethyl)pyridinium-2-yl)porphyrin tetrachloride)) and H2TnHexOE-2-PyPCl4 ((meso-tetrakis(N-(2′-n-hexoxyethyl)-phenyl-4-(2′-pyridyl))porphyrin tetrachloride)) and their Mn complexes is illustrated in Figure 3.

**H2TPhE-2-PyPCl4** meso-Tetrakis(N-(2′-phenylethyl)pyridinium-2-yl)porphyrin Tetrachloride. H2T-2-PyP (100 mg; 0.162 mmol) was dissolved in 4.6 mL of DMF preheated for 10 min at 115 °C. To the resulting solution was added the 9 g (0.032 mol) of phenylpyridinium p-toluenesulfonate. The course of N-quaternization was followed by thin-layer chromatography (TLC) on silica gel plates using acetonitrile/KNO3(sat)/water = 8/1/1 as a mobile phase. The pH of the solution dropped to 7.6. The stirring was continued at 100 °C for 3.5 h until metalation was completed. The precipitate was dried in vacuum oven in the form of Cl− salt. Yield (calculated based on elemental analysis): 180 mg (94.3%).

**Sodium L-ascorbate** (+)-sodium L-ascorbate (>98%) were from Sigma, whereas xanthine oxidase was prepared by R. Wiley.1 Triethylamine (Et3N) of >99.5% purity was obtained from Thermo Scientific Pierce. All chemicals were used as received without further purification. The 1H NMR spectra were recorded on a spectrometer “Mercury Varian 300” with deuterated chloroform as solvent.
H_{2}T-2-PyPhP, meso-Tetraakis(phenyl-4-(2′-pyridyl))porphyrin. 4-(2-Pyridyl)benzaldehyde (8 g, 0.027 mol) was added to a boiling propionic acid (100 mL). Pyrrole (1.85 g, 0.027 mol) was added to the reaction mixture and was stirred for 45 min. The solution was stirred for another 2 h at room temperature and was left overnight in dark. The precipitate formed was filtered, washed with dilute aqueous solution of NaHCO₃, cold water, hot water, cold water and finally with small portions of methanol, and was left overnight to dry. The obtained violet crystals were dissolved in chloroform and were purified by column chromatography (absorptive, alumina; eluent, chloroform). The solvent was evaporated under reduced pressure, and the porphyrin was air-dried. Yield: 1.1 g (17.47%).

H_{2}T-2-PyPhP porphyrin (C_{6}H_{4}N_{2}) M = 923.1. 1H NMR (300 MHz; CDCl₃; δ ppm; J) = 2.72 (2H, s, pyrrole-NH); 7.38 (4H, d, J = 7.5, 7 = 4.8, pyridine-4); 7.94 (4H, d, J = 8.0, J = 7.5, 7 = 1.8, pyridine-5); 8.16 (4H, d, J = 8.0, pyridine-6); 8.31–8.36 (8H, m, phenyl-H); 8.49–8.54 (8H, m, phenyl-H); 8.79 (4H, ddd, J = 4.8, J = 1.8, J = 0.9, pyridine-3, H); 8.91 (8H, s, β-pyrrrole-H). Elemental Analysis H₂T-2-PyPhP: H, 5.00; C, 79.36; N, 11.37%. UV-Vis Spectroscopy. UV−vis spectra were recorded in water at room temperature on a UV-2501PC Shimadzu spectrophotometer with 0.5 nm resolution in 1 cm quartz cuvette (Table 1). The UV−vis spectra for new compounds are provided in Supporting Information (Figures S1−S3).

Table 1. Spectral Properties of Porphyrins and their Mn Complexes

<table>
<thead>
<tr>
<th>(metal)</th>
<th>porphyrin</th>
<th>λ_{max} (nm) (log ε) *</th>
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<tr>
<td>MnTBAP*</td>
<td>230.0 (4.93), 290.0 (4.49), 318.0 (4.84), 410.0 (4.84), 420.0 (4.70), 468.0 (5.04), 515.0 (3.92), 566.0 (4.16), 599.0 (4.07), 684.0 (4.32), 712.0 (4.32), 780.0 (3.24), 811.0 (4.37), 819.0 (4.37)</td>
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<tr>
<td>MnTE-2</td>
<td>363.5 (4.48), 409.0 (4.32), 454.0 (5.14), 499.0 (3.75), 558.0 (4.08), 782.0 (3.26)</td>
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<tr>
<td>MnTE-3</td>
<td>240.0 (4.77), 260.0 (4.60), 370.0 (4.74), 395.0 (4.78), 460.0 (5.19), 502.0 (3.83), 557.0 (4.16), 674.0 (3.25), 766.0 (3.37), 837.0 (2.40)</td>
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<tr>
<td>MnTnHexOE-2-PyPhP</td>
<td>264.4 (4.38), 419.4 (5.33), 515.5 (4.27), 545.5 (3.64), 586.4 (3.86), 640.5 (3.83)</td>
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<tr>
<td>MnTnHexOE-2-PyPhP</td>
<td>212.5 (4.72), 261.7 (4.56), 365.4 (4.74), 411.4 (4.39), 455.5 (5.26), 561.1 (4.16), 786.5 (3.38)</td>
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<tr>
<td>MnTPHE-2-PyPhP</td>
<td>263.2 (4.41), 419.4 (5.34), 514.6 (4.24), 585.6 (3.85), 638.6 (3.26)</td>
<td></td>
</tr>
<tr>
<td>MnTPHE-2-PyPhP</td>
<td>260.7 (4.60), 364.9 (4.72), 455.5 (5.27), 560.4 (4.17), 783.1 (3.41)</td>
<td></td>
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<tr>
<td>MnTE-2</td>
<td>274.2 (4.62), 414.2 (5.77), 515.5 (4.29), 521.2 (3.95), 579.8 (3.88), 634.1 (3.64)</td>
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</tr>
<tr>
<td>MnTE-2</td>
<td>273.1 (4.71), 378.7 (4.82), 400.4 (4.83), 466.3 (5.06), 514 (3.90), 562.5 (4.15), 597.1 (4.01), 773.8 (3.27)</td>
<td></td>
</tr>
<tr>
<td>MnTeOct-2-PyPhP</td>
<td>364.0 (4.72), 414.0 (4.44), 454.5 (5.24), 500.5 (3.84), 559.5 (4.14), 781.0 (3.25)</td>
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*Spectra were recorded in water at room temperature unless otherwise noted. Molar absorption coefficients (M⁻¹ cm⁻¹) were determined within 5% errors. λ_{max} (nm) were determined with errors inside ±0.5 nm. *Data are taken from ref 79.

Electrospray-Ionization Mass Spectrometry. Electrospray ionization mass spectrometric (ESI-MS) analyses were performed on Applied Biosystems MDS Sciea 3200 Q Trap LC/MS/MS spectrometer at Duke Comprehensive Cancer Center, Shared Resource PK Laboratories, as described elsewhere.²⁷,³⁸ Samples of ~1 µM concentrations were prepared in acetonitrile/H₂O mixture (1/1, v/v) containing 0.1% v/v heptfluorobutyric acid, and infused for 1 min at 10 µL/min into the spectrometer (curtain gas 20 V, ion spray voltage 3500 V, ion source 30 V, T = 300 °C, declustering potential 20 V, entrance potential 1 V, collision energy 5 V, gas N₂). Under given conditions, in the presence of ion-pairing heptfluorobutyrate anion (HFBA⁻), no fragmentation was observed; the data relate to species originally present in solutions. The absence of peaks associated with partially alkylated and nonmetalated species unambiguously indicates...
the purity of the sample. Data are summarized in Table 2. All MS spectra are provided in Supporting Information (Figure S4).

Lipophilicity. Both TLC retention factor, Rf (compound path/solvent path), and the partition coefficient between n-octanol and water, log P OW, are equal valid parameters in assessing lipophilicity of the free ligands and their Mn complexes.79,81 Rf was obtained on silica gel plates using acetonitrile/KNO3(sat)/water = 8/1/1 as previously described.83 As it is difficult to impossible to fully reproduce the Rf values from one experiment to another, we are routinely comparing all the compounds of interest in a single experiment. The log P OW values of the newly synthesized compounds were determined as reported by Kos et al.81 The log P OW values (the partition between water-saturated n-butanol and n-butanol-saturated water) were determined experimentally using the following equation: log P OW = log(C_{n-octanol}/C_{water}). The log P OW values were converted to log P OW using the equation: log P OW = 1.55 × log P OW - 0.54,32,83 The log P OW values for the most hydrophilic porphyrins, MnTE-2-PyP5+ and MnTE-3-PyP5+, were determined using the following equations: log P OW = 12.207 × Rf - 8.521 for ortho Mn(III) N-alkylpyridyl porphyrins (i.e., MnTE-2-PyP5+), and log P OW = 8.764 × Rf - 8.198 for meta Mn(III) N-alkylpyridyl porphyrins (i.e., MnTE-3-PyP5+).81,83 The Rf and log P OW values are given in Table 3.

Table 3. Lipophilicity of MnPs Determined in Terms of TLC Retention Factor, Rf, and Partition Coefficient between n-Octanol and Water, log P OW

<table>
<thead>
<tr>
<th>Mn porphyrin</th>
<th>( R_f )</th>
<th>( \log P_{OW} )</th>
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<tr>
<td>MnTE-2-PyP5+</td>
<td>0.07</td>
<td>-6.67</td>
</tr>
<tr>
<td>MnTE-3-PyP5+</td>
<td>0.12</td>
<td>-7.15</td>
</tr>
<tr>
<td>MnTnHexOE-2-PyP5+</td>
<td>0.50(0.53)</td>
<td>-1.67</td>
</tr>
<tr>
<td>MnTPHE-2-PyP5+</td>
<td>0.40(0.47)</td>
<td>-5.90</td>
</tr>
<tr>
<td>MnTE-2-PyPhP4+</td>
<td>0.32(0.45)</td>
<td>-5.51</td>
</tr>
<tr>
<td>MnTnOct-2-PyPhP4+</td>
<td>0.48</td>
<td>-2.27</td>
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</table>

Lipophilicities of porphyrin ligands of the related Mn complexes are given in parentheses. The TLC was done on silica gel plates using acetonitrile/KNO3(sat)/water = 8/1/1 as a mobile phase. Determined experimentally using n-butanol and water biphasic system and converted to log P OW according to the equation \( \log P_{OW} = 1.55 \times \log C_{n-octanol}/C_{water} \). The supporting electrolyte in all calibration with the standard buffer (pH 7.8). The pH values were determined on a Denver Instrument Model 250 pH-meter using a glass electrode calibrated with the standard buffers (pH 4.00, 7.00, and 10.00). The concentrations of MnPs were determined spectrophotometrically. All potentials are reported versus the normal hydrogen electrode (NHE). MnTE-2-PyP5+ with E1/2 = +228 mV versus NHE was used as a reference.16,80,81 Its voltammetry was performed before and after each series of measurements. The data are presented in Table 4.

Catalysis of O2•− Dismutation (Cytochrome c Assay). The ability of newly synthesized Mn metalloporphyrins to dismute O2•− was evaluated via cytochrome c assay. The validity of assay was proven with pulse radiolysis and stopped-flow methodology.84–86 The cyt c assay is based on O2•− production via xanthine/xanthine oxidase reaction and metalloporphyrin ability to compete with ferricytochrome c in scavenging O2•−. The experiments were conducted at room temperature (25 ± 1 °C) in 0.05 M potassium phosphate buffer, pH 7.8, and 0.1 mM EDTA as previously described in detail.84 The reduction of cytochrome c was followed at 550 nm. MnTE-2-PyP5+ was used as a standard. Data are summarized in Table 4. Kinetic traces, plots (\( t_n/s \) vs [MnP]), and the information on the calculation of \( k_{cat}(O_2^{•−}) \) from such plots are provided in Figures S12 and S13 of Supporting Information.

Peroxynitrite Reduction with MnIIIP. Oxidation of MnIIIPs with peroxynitrite was carried out under pseudo-first-order conditions with peroxynitrite in excess over MnP. In all cases, peroxynitrite (dissolved in a NaOH) was mixed with MnPs dissolved in sodium phosphate buffer. The final concentrations upon mixing were the following: MnPs 0.5 μM (MnTnHexOE-2-PyP5+), 10-fold excess of peroxynitrite, and 0.05 M sodium phosphate buffer, pH 7.4, 0.1 mM DTPA. The temperature was maintained at (37.0 ± 0.1) °C, and the pH of the reaction mixtures was measured at the outlet of the stopped flow. The reaction was monitored as a change in the absorbance of the Soret band at the following: 456 nm for MnTPHE-2-PyP5+, 455 nm for MnTnHexOE-2-PyP5+, 467 nm for MnTE-2-PyP5+, 454 nm for MnTE-3-PyP5+, and 460 nm for MnTE-3-PyP5+. The pseudo-first-order rate constants, \( k_{obs} \) (s−1), were determined by fitting the stopped-flow data to a single exponential function. The second-order rate constant was determined from the slope of \( k_{obs} \) versus [O2•−] plot. All kinetic runs were performed on a stopped-flow spectrophotometer (Applied Photophysics, SX20). Data are summarized in Table 4. The raw data (kinetic traces, \( k_{obs} \) versus [O2•−] plots, and time-resolved equilibrium spectra for all new compounds are provided in Supporting Information (Figures S5–S11).

Lipid Peroxidation Assay. The lipid peroxidation was triggered spontaneously. Rat brains were homogenized on ice in 5 volumes (w/v) of cold 50 mM potassium phosphate buffer, pH 7.00. The 200 μL aliquots were diluted to a final volume of 1.0 mL with 50 mM potassium phosphate buffer and incubated 30 min at 37 °C on a shaking water bath. Under such standardized conditions the 2.2 ± 0.18 μmol/L malondialdehyde (MDA) was produced. If MDA production was not within specified limits, the homogenate was discarded. The level of MDA produced under standardized conditions was taken as 100% lipid peroxidation. In order to measure preformed MDA, as well

Table 2. Electrospray Ionization Mass Spectrometry (ESI-MS) Data for New Porphyrins, H2P, and their Mn(III) Complexes

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<tbody>
<tr>
<td>([P^n+ + HBFa]^{−+} / (n - 1))</td>
<td>449.4 (449.2)</td>
<td>350.2 (350.2)</td>
<td>417.8 (417.2)</td>
<td>326.4 (326.1)</td>
<td>417.4 (417.2)</td>
<td>326.6 (326.1)</td>
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<td>([P^n+ + 2HBFa]^{−+} / (n - 2))</td>
<td>780.2 (780.4)</td>
<td>537.7 (537.9)</td>
<td>732.9 (732.2)</td>
<td>505.6 (505.8)</td>
<td>732.1 (732.2)</td>
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<tr>
<td>([H3P]^{−+} / 4)</td>
<td>283.6 (283.7)</td>
<td>260.2 (259.6)</td>
<td>260.2 (259.6)</td>
<td>259.9 (259.6)</td>
<td>260.2 (259.6)</td>
<td>259.9 (259.6)</td>
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<tr>
<td>([H3PnH− – H−] / 3)</td>
<td>378.1 (377.9)</td>
<td>346.5 (345.8)</td>
<td>346.5 (345.8)</td>
<td>346.5 (345.8)</td>
<td>346.5 (345.8)</td>
<td>346.5 (345.8)</td>
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<tr>
<td>([P^n+ + H+ + 2HBFa]^{−+} / (n - 1))</td>
<td>673.3 (673.4)</td>
<td>625.9 (625.2)</td>
<td>625.9 (625.2)</td>
<td>625.9 (625.2)</td>
<td>625.9 (625.2)</td>
<td>625.9 (625.2)</td>
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<tr>
<td>([P^n+ + H+ + 3HBFa]^{−+} / (n - 2))</td>
<td>870.8 (878.3)</td>
<td>840.3 (839.2)</td>
<td>840.3 (839.2)</td>
<td>840.3 (839.2)</td>
<td>840.3 (839.2)</td>
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*−1 μM solution of porphyrins and metalloporphyrins in 1:1 v/v acetonitrile/H2O [containing 0.01% v/v heptfluorobutyric acid (HFBA)] mixture, 20 V cone voltage; \( n = 4 \) or 5 corresponding to H2P or MnP accordingly.

Electrochemistry. Cyclic voltammetry measurements were performed under argon in a glass cell on CH Instruments model 600 voltammetric analyzer, as described previously.84,85 Stock solutions of MnPs were prepared by dissolving solids in deionized water. Working solutions of 2 mM MnPs were prepared in 0.05 M phosphate buffer (pH = 7.8). The supporting electrolyte in all measurements was 0.1 M NaCl. The pH values were determined on a Denver Instrument Model 250 pH-meter using a glass electrode calibrated with the standard buffers (pH 4.00, 7.00, and 10.00). The concentrations of MnPs were determined spectrophotometrically. All potentials are reported versus the normal hydrogen electrode (NHE). MnTE-2-PyP5+ with E1/2 = +228 mV versus NHE was used as a reference.16,80,81 Its voltammetry was performed before and after each series of measurements. The data are presented in Table 4.
Table 4. Metal-Centered Reduction Potential, $E_{1/2}$ vs NHE of MnIII/P/MnP Redox Couple, Proton Dissociation Constant of First Axial Water, $pK_{a1}$, log $k_{cat}(O_2^{•−})$ for the Catalysis of $O_2^{•−}$ Dismutation, log $k_{red}(ONOO−)$ for the ONOO− Reduction, and Initial Rates for the Catalysis of Ascorbate HA− Oxidation with MnPs, $v_0$(HA− Oxidation). Relative Molecular Masses, $M_r$ are Listed Also

<table>
<thead>
<tr>
<th>compd</th>
<th>$M_r$</th>
<th>$pK_{a1}$</th>
<th>$E_{1/2}$ mV vs NHE</th>
<th>log $k_{cat}(O_2^{•−})$</th>
<th>log $k_{red}(ONOO−)$</th>
<th>$v_0$ (HA− oxidation), nM s−1</th>
<th>$a$</th>
<th>$b$</th>
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<td>MnTBAP3−</td>
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<td>−194$^b$</td>
<td>3.16$^b$</td>
<td>5.02$^b$</td>
<td>2.26</td>
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<td>$e$</td>
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<tr>
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<td>12.0</td>
<td>−65</td>
<td>5.55</td>
<td>5.93</td>
<td>18.24</td>
<td>$f$</td>
<td>$g$</td>
</tr>
<tr>
<td>MnTE-3-pyP5+</td>
<td>965.1</td>
<td>11.5$^a$</td>
<td>54$^a$</td>
<td>6.65$^a$</td>
<td>6.81</td>
<td>229.96</td>
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<td></td>
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<tr>
<td>MnTE-2-pyP5+</td>
<td>965.1</td>
<td>11.0$^a$</td>
<td>228$^a$</td>
<td>7.76$^a$</td>
<td>7.54$^a$</td>
<td>312.84</td>
<td></td>
<td></td>
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<tr>
<td>MnTPHe-2-pyP5+</td>
<td>1269.5</td>
<td>10.8$^a$</td>
<td>259</td>
<td>7.66</td>
<td>7.14</td>
<td>147.21</td>
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<tr>
<td>MnTnHexOE-2-pyP5+</td>
<td>1365.8</td>
<td>10.7$^a$</td>
<td>313</td>
<td>7.92</td>
<td>7.61</td>
<td>76.33</td>
<td></td>
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<tr>
<td>MnTnOct-2-pyP5+</td>
<td>1301.8</td>
<td>10.5$^a$</td>
<td>340</td>
<td>7.71$^a$</td>
<td>7.15$^a$</td>
<td>54.29</td>
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</table>

$^a$E_{1/2} of MnIII/P/MnP redox couple is determined in 0.05 M phosphate buffer (pH 7.8, 0.1 M NaCl). $^b$k_{cat}(O_2^{•−}) is determined by cytochrome c assay in 0.05 M potassium phosphate buffer [pH 7.8, at (25 ± 1) °C]. $^c$k_{red}(ONOO−) is determined by stopped-flow technique in 0.05 M potassium phosphate buffer [pH 7.4, at (37 ± 0.1) °C]. $^d$v_0, initial rate for HA− oxidation, was determined spectrophotometrically under aerobic conditions: $^e$μM MnP, 0.15 mM sodium ascorbate, 5 mM EDTA, pH 7.4 maintained with 0.05 M Tris buffer and at (25 ± 1) °C. The mono-deprotonated HA− is the main ascorbate species at pH 7.8. $^f$pK_{a1} values were estimated on the basis of the relationship $pK_{a1}$ vs E_{1/2} of MnIII/P/MnP redox couple published in ref 4.

as the MDA generated during tissue handling and homogenization, butylated hydroxytoluene (BHT) was added before incubation to a final concentration of 60 mM. The MDA content of samples containing BHT did not exceed 0.0254 ± 0.0089 μmol MDA per L homogenate. After 60 min of incubation at 37 °C, BHT (60 mM) was added to all samples, and MDA was initially assessed by colorimetric thiobarbituric acid (TBA) assay.89 The TBA assay lacks specificity. Thus, all the results were re-evaluated by HPLC analysis as previously described.90

Catalysis of Ascorbate Oxidation with MnPs. Initial rates of MnP-catalyzed ascorbate, HA−, oxidation to ascorbonyl radical, HA− (which readily deprotonates to A•−), were determined with 5 μM metalloporphyrin, 5 mM EDTA, and 0.15 mM sodium ascorbate under aerobic conditions at (25 ± 1) °C and at pH 7.4 maintained with 0.05 M Tris buffer. The buffer was initially treated with Chelex-100 ion-exchange resin (200−400 mesh sodium form, Bio-Rad Life Science) to remove the adventitious metals present in the solution. Ascorbate oxidation was followed at 265 nm on UV−vis spectrophotometer (Shimadzu UV-2550). The molar absorptivity of ascorbate was re-evaluated to be ε_{265} = 14 000 M−1 cm−1. The initial rates, v_0’s (HA− oxidation) (nM s−1), which were calculated on the basis of the linear kinetic traces obtained for the first 100 s, are summarized in Table 4. The background rate for noncatalyzed ascorbate oxidation was subtracted from the catalyzed reaction rates.77

Superoxide-Specific Biological Models. Aerobic Growth of S. cerevisiae strains with mutations in the cytoplasmic CuZnSOD gene (sod1Δ) exhibit amino-acid auxotrophies for lysine and methionine. Only those compounds which are capable of catalyzing the dismutation of superoxide at a rate higher than O_2^{•−} self-dismutation substitute for the missing SOD enzyme, thus restoring the aerobic growth of a mutant in a medium lacking lysine or methionine.67 Such growth impairment makes the SOD-deficient yeast a good system for testing the therapeutic potential of an SOD mimic. The wild type S. cerevisiae strain used in this study was EG103, while its corresponding sod1Δ mutant was EG118.91,92 Stock and test cultures were grown as previously described.67 Tests were performed in 96-well plates in triplicates. Aqueous solutions of MnPs were filter-sterilized (0.22-μm filter, Whatman, Middlesex, U.K.) and added to wells containing 200 μL aliquots of yeast culture in SD medium supplemented with all amino acids except methionine. Cultures in 96-well plates were grown aerobically at 30 °C and 220 rpm on a thermostatic shaker. Since yeast cells tend to clump irrespective of the vigorous shaking, wells were mechanically stirred at regular time intervals using a specifically designed 96-pin sterilized stirrer. In control samples the volume of MnP solution was compensated with sterile distilled water. Growth was followed turbidimetrically at 600 nm using ELISA reader.

### RESULTS AND DISCUSSION

The series of MnPs, MnTnOct-2-PyP5+, MnTPHe-2-PyP5+, and MnTE-2-PyPhP5+, was synthesized and characterized (Figure 2). With the same porphyrin core, all meso substituents have 8 carbon atoms differently organized in either linear or cyclic conformations resulting in compounds of vastly different properties.

Two porphyrins, MnTnOct-2-PyP5+ and MnTnHexOE-2-PyP5+, bear linear 8 atom-long and 9 atom-long pyridyl substituents, respectively (Figure 2). The latter has one oxygen atom buried so deeply in each of 4 alkylpyridyl chains that the surrounding medium does not sense them. In turn, the lipophilicity of MnTnHexOE-2-PyP5+ is higher than of MnTnOct-2-PyP5+. On the basis of a large amount of lipophilicity measurements reported,16,68,81,93 we can safely assume that lipophilicity of MnTnHexOE-2-PyP5+ will be similar to that of MnTnNon-2-PyP5+ (Mn(III) meso-tetraakis(N-nonylpyridinium-2-yl)porphyrin). Both compounds have equal 9-atom long pyridyl substituents, but the latter does not contain oxygen atoms.

Two other porphyrins, MnTPHe-2-PyP5+ and MnTE-2-PyPhP5+, bear two cyclic aromatic rings, one phenyl and one pyridyl. The rings are differently organized: in MnTPHe-2-PyP5+, the pyridyl ring precedes phenyl, and in MnTE-2-PyPhP5+ the pyridyl ring follows phenyl ring (Figure 2). In the first case the positively charged quaternary nitrogen atoms are close to the metal site and affect favorably the $E_{1/2}$. In the second case they are far away, separated from the porphyrin core by the phenyl ring and in turn have minimal impact on $E_{1/2}$. Besides the effect on $E_{1/2}$, such distribution of charges affects differentially the shape of the molecule, and in turn the solvation/lipophilicity of these MnPs and their interactions with biotargets.

Compounds were characterized in terms of the following: (i) elemental analysis; (ii) NMR spectroscopy; (iii) UV−vis spectral properties (Table 1); (iv) electrospray mass spectrometry, ESI-MS (Table 2); (v) lipophilicity in terms of $P_{ow}$ and $R_f$ (Table 3); (vi) electrochemistry (metal-centered reduction potential $E_{1/2}$ of MnIII/P/MnP redox couple) (Table 4); (vii) ability to catalyze $O_2^{•−}$ dismutation, $k_{cat}(O_2^{•−})$ (Table 4); (viii) ability to reduce ONOO−, $k_{red}(ONOO−)$ (Table 4); and (ix) ability to catalyze ascorbate, HA− oxidation, described by initial rate, $v_0$(HA−) (Table 4, Figure 7).
Figure 5. Lipophilicities of Mn(III) porphyrins expressed in terms of chromatographic retention factor, $R_f$ (A), and partition coefficient between n-octanol and water, log $P_{OW}$ (B). The $R_f$ values are linearly related to log $P_{OW}$ values. The small differences in $R_f$ values translate into large differences in log $P_{OW}$ values.

Figure 6. Structure−activity relationships between the kinetic parameters, log $k_{cat}$ ($O_2^{•−}$) and log $k_{red}$ (ONOO$^{−}$), and thermodynamic parameters, $E_{1/2}$ for Mn$^{III}$/Mn$^{II}$ redox couple; (A) log $k_{cat}$($O_2^{•−}$) vs $E_{1/2}$ for Mn$^{III}$/Mn$^{II}$ redox couple; (B) $pK_{a1}$ vs $E_{1/2}$ for Mn$^{III}$/Mn$^{II}$ redox couple; (C) log $k_{red}$ (ONOO$^{−}$) vs $pK_{a1}$; (D) log $k_{red}$ (ONOO$^{−}$) vs $E_{1/2}$ for Mn$^{III}$/Mn$^{II}$ redox couple; (E) log $k_{red}$ (ONOO$^{−}$) vs $pK_{a1}$ and log $k_{red}$($O_2^{•−}$) vs $E_{1/2}$ for Mn$^{III}$/Mn$^{II}$ redox couple; (F) log $k_{cat}$($O_2^{•−}$) vs log $k_{red}$ (ONOO$^{−}$). Numerical values and experimental conditions for $k_{cat}$ ($O_2^{•−}$), $k_{red}$ (ONOO$^{−}$), $pK_{a1}$, and $E_{1/2}$ (mV vs NHE) are given in Table 4; empty squares in parts B, C, and E are estimated values: (1) MnTBAP$^{3−}$, (2) MnTE-2-PyPhP$^{5+}$, (3) MnTE-3-PyP$^{5+}$, (4) MnTE-2-PyP$^{5+}$, (5) MnTPhE-2-PyPhP$^{5+}$, (6) MnTnHexOE-2-PyPhP$^{5+}$, and (7) MnTnOct-2-PyPhP$^{5+}$. The kinetics of Mn$^{III}$ oxidation to O═Mn$^{IV}$P, involved in reduction of ONOO$^{−}$ as well as reduction of lipid reactive species (see Figure 8), relates to the thermodynamics of Mn$^{III}$/Mn$^{II}$ redox couple. For explanation, see text; in brief, the electron transfer from Mn to ONOO$^{−}$ is preceded with ONOO$^{−}$ ligand binding which is dependent upon the electron-deficiency of Mn site. The latter is described by proton dissociation equilibrium of first axial water, $pK_{a1}$, which parallels $E_{1/2}$ of Mn$^{III}$/Mn$^{II}$ redox couple and is shown in part B. There appears to be no difference between the $E_{1/2}$ values for O═Mn$^{IV}$P/Mn$^{III}$P for various structurally diverse metalloporphyrins (Supporting Information Table S1).
Lipophilicity of MnPs. The linear relationship between the chromatographic retention factor, \( R_g \) and log \( P_{OW} \) has been established for water-soluble cationic Mn N-alkylpyridylporphyrins.77,92 It guided us not only in the design and development of lead drug candidates but also in the safe prediction of the partition coefficients of those compounds which are highly hydrophilic and for which log \( P_{OW} \) could not be assessed, such as MnTE-2-PyP\( ^{5+} \) and MnTE-3-PyP\( ^{5+} \).81,94 The log \( P_{OW} \) and \( R_g \) values of the series of ligands and related MnPs are listed in Table 3 and Figure 5 and are related to other properties of MnPs in Figure 6. While water-soluble MnPs do not distribute readily into n-octanol (as illustrated by highly negative log \( P_{OW} \) value), multiple positive charge is a driving force for their distribution into brain and mitochondria.83 In mitochondria they mimic mitochondrial matrix MnSOD,83,95–97

The different reorganization of 8 carbon atoms in \( \text{meso} \) substituents resulted in the following observations depicted in Figure 5: (1) lipophilicity of the molecule dropped noticeably by >3.5 log units when 8-carbon atom alkyl chains rearrange into aromatic phenyl or pyridyl substituents; (2) significant increase in lipophilicity was observed when an oxygen atom was introduced into n-octyl chains to form n-octoxyethyl chains [log \( P_{OW} \) (MnTnOct-2-PyP\( ^{5+} \)) < log \( P_{OW} \) (MnTnHexOE-2-PyP\( ^{5+} \))]. We have previously reported that the introduction of one methoxy group at the periphery of each of four hexyl chains reduced significantly the lipophilicity of MnTMOHex-3-PyP\( ^{5+} \) relative to MnTnHex-3-PyP\( ^{5+} \).80 Such a drop was considerably minimized when the oxygen atoms were buried deeper into the alkyl chains of MnTnBuOE-2-PyP\( ^{5+} \).16 Consequently, this porphyrin has only slightly lower lipophilicity relative to MnTnHex-2-PyP\( ^{5+} \) [log \( P_{OW} \) (MnTnBuOE-2-PyP\( ^{5+} \)) = -4.10 versus log \( P_{OW} \) (MnTnHex-2-PyP\( ^{5+} \)) = -3.86]. The oxygen atoms in MnTnHexOE-2-PyP\( ^{5+} \) are buried even deeper within the lipophilic n-octyl chains. In turn, the solvation is largely suppressed. We can predict that the chains of a n-octoxyethyl analog would behave similarly to linear 9-carbon atom substituents in MnTnNon-2-PyP\( ^{5+} \); the latter is estimated to have partition coefficient log \( P_{OW} \) = -1.18 based on reported data for a series of Mn(III) N-alkylpyridylporphyrins.81,94

With phenyl rings at the periphery, such as in MnTPhE-2-PyP\( ^{5+} \), the compound is more lipophilic than MnTnBuOE-2-PyP\( ^{5+} \) where the pyridyl cationic charges are exposed at the periphery. As expected, both compounds are much more lipophilic than either MnTn-2-PyP\( ^{5+} \) or MnTn-3-PyP\( ^{5+} \).

Structure–Activity Relationships among \( E_{1/2} \), \( pK_{a1} \), \( k_{cat} \left( O_2^{•−} \right) \), and \( k_{red} \left( \text{ONO}^− \right) \). Cationic Mn(III) porphyrins are among the most potent SOD mimics. They have been tested in numerous oxidative stress related models and have shown remarkable therapeutic potential which is attributed to their ability to interact not only with \( O_2^{•−} \), but also with numerous other reactive species, such as \( \text{ONO}^− \), \( \text{CO}_3^{•−} \), \( \text{H}_2\text{O}_2 \), \( \text{ClO}^− \), ascorbate, lipid reactive species, and thiols, RS\( ^− \). Data, thus far obtained, provide evidence that the ability of MnP to efficiently eliminate \( O_2^{•−} \) closely parallels its therapeutic efficacy.\(^2\) As already noted in the Introduction, this is due to the appropriate electron-deficiency of Mn site which favors reactions with nucleophiles, not only \( O_2^{•−} \) but other species, some of which are listed above. SOD enzymes have the same thermodynamic property of metal site as MnPs, but steric hindrance imposed by large protein structure provides specificity toward \( O_2^{•−} \). Thus, their reactivity toward other species is a few orders of magnitude lower than that of SOD mimics.

In order to mimic the kinetics and thermodynamics of the enzymatic catalysis of \( O_2^{•−} \) dismutation (eqs 1 and 2), the metal-centered reduction potential should be around the midpoint (\( +300 \text{ mV vs NHE} \)) and \( k_{cat} \left( O_2^{•−} \right) \) should be assessed. Such MnTnOct-2-PyP\( ^{5+} \) and MnTnHexOE-2-PyP\( ^{5+} \), and MnTPhE-2-PyP\( ^{5+} \) also, as all of them have \( E_{1/2} \) values of \( +300 \text{ mV vs NHE} \) and exhibit a high ability to catalyze \( O_2^{•−} \) dismutation, \( k_{cat} \left( O_2^{•−} \right) \approx 7.8 \) (Table 4). In addition to eq 1, Mn\( ^{III} \)P could be reduced to Mn\( ^{II} \)P with ascorbate shown by eq 1b, and reoxidized with \( O_2 \) to Mn\( ^{III} \)P as superoxide reductase like rubredoxin oxidoreductase,\(^102\) a likely scenario in vivo due to the abundance of ascorbate.

\[
\text{Mn}^{III}P + O_2^{•−} \rightleftharpoons \text{Mn}^{II}P + O_2 \quad \text{(1)}
\]

\[
\text{Mn}^{III}P + HA \rightleftharpoons \text{Mn}^{II}P + HA^− (HA^− \rightarrow A^− + H^+ \quad \text{(1b)}
\]

\[
\text{Mn}^{II}P + 2H^+ + O_2^{•−} \rightleftharpoons \text{Mn}^{III}P + H_2O_2 \quad \text{(2)}
\]

Mn\( ^{III} \)P reduces \( \text{ONO}^− \) via one-electron reaction giving rise to toxic \( \text{NO}_2 \) (eq 3).\(^2\) In vivo, the reduction of \( \text{ONO}^− \) by Mn\( ^{III} \)P is likely coupled to cellular reductants such as ascorbate.\(^5\) In a first step Mn\( ^{III} \)P gets reduced with ascorbate to Mn\( ^{II} \)P (eq 1b). In a subsequent step Mn\( ^{II} \)P gets oxidized two-electronically to O\( =\text{Mn}^{III} \)P while benign nitrite, \( \text{NO}_2^− \), is formed. The rate constant for reaction 4 has been estimated for MnTn2-PyP\( ^{5+} \), and is equal or higher than for the reaction given by eq 3.\(^6\) The O\( =\text{Mn}^{III} \)P is a highly oxidizing species. Its damage to biological targets is largely suppressed at the expense of cellular reductants as they readily reduce it to Mn\( ^{II} \)P.

\[
\text{Mn}^{III}P + \text{ONO}^− \rightleftharpoons \text{O} = \text{Mn}^{IV}P + \text{NO}_2 \quad \text{k_{red}(\text{ONO}^−)} \quad \text{(3)}
\]

\[
\text{Mn}^{IV}P + \text{ONO}^− \rightleftharpoons \text{O} = \text{Mn}^{IV}P + \text{NO}_2^− \quad \text{k_{red}(\text{ONO}^−)} \quad \text{(4)}
\]

The \( E_{1/2} \) of Mn\( ^{III} \)P/Mn\( ^{II} \)P redox couple for the series of compounds studied varies from \( -194 \) to \( +340 \text{ mV vs NHE} \). Strong correlations have been found between \( E_{1/2} \) and \( k_{cat} \left( O_2^{•−} \right) \) (eqs 1 and 2; Figure 6A). We observed earlier with Mn(III) N-alkylpyridylporphyrins,\(^5\) and here with new series of porphyrins, that \( k_{red} \left( \text{ONO}^− \right) \) correlates with \( E_{1/2} \) for Mn\( ^{III} \)P/Mn\( ^{II} \)P even though the reaction of Mn\( ^{II} \)P with
peroxynitrite, studied in this work, involves the O==MnP/P vs MnIIIP redox couple. This can be accounted for by a two-step process: (i) binding of ONOO− to the Mn site, and (ii) subsequent reduction of ONOO− yielding O==MnP/P species. The first step is dependent upon the Mn site electron-deficiency, a property well-described by the E1/2 of MnIII/P/MnIIIP couple. This E1/2 has previously been reported to lineally correlate with metal-free porphyrin protonation equilibria of its inner pyrrolic nitrogens,7,6 and (ii) with the protonation equilibria of axial waters of MnPs, depicted herein with the proton dissociation constant of either pH 11 are all around +800 mV in excess. Those MnPs with negative potentials do not favor reduction, while those with too positive potential do not favor reoxidation of MnIIIP. (D) Redox cycling of MnP with ascorbate, which involves the reoxidation of MnIIIP with O2 (preferred over O2− due to its higher in vivo levels) to close the catalytic cycle. The conditions are 5 μM MnP, 0.15 mM sodium ascorbate at pH 7.4 maintained with 0.05 M Tris buffer with 5 mM EDTA, (25 ± 1) °C. The numerical assignments in part C are identical to those described in the Figure 6 caption.

\[(\text{H}_2\text{O})_2\text{Mn}^{\text{III}}\text{P}^{5+} \rightleftharpoons (\text{H}_2\text{O})(\text{OH})\text{Mn}^{\text{III}}\text{P}^{4+} + \text{H}^+ \quad \text{pK}_{\text{a1}} \quad (5)\]

Therefore, the MnPs of more positive E1/2 for MnIII/P/MnIIIP redox couple and lower pk\textsubscript{a} values (Figure 6B) are more electron-deficient and favor binding of an electron-rich ligand (ONOO− in this case) which in turn gives rise to higher k\textsubscript{red}(ONOO−) (Figure 6C).2 Such data explain why the log k\textsubscript{red}(ONOO−) correlates as well with E1/2 for MnIII/P/MnIIIP couple (Figure 6D) as does log k\textsubscript{red}(O2−*) (Figure 6A). It thus explains why there is a linear relationship between log k\textsubscript{red}(ONOO−) and k\textsubscript{red}(O2−*) (Figure 6F). The second step of the reaction of MnIIIP with ONOO− is related to the E1/2 values for the O==MnP/P redox couple. Of note, as already indicated, the oxidation of MnPs with ONOO− in vivo may involve the O==MnP/P redox couple (eq 4), as MnIIIPs would likely be readily reduced to MnPs by cellular reductants. The E1/2 of the O==MnP/P redox couple is controlled in part by the MnIII/P/MnIIIP redox couple and thus differs among MnPs, for MnTE-2-PyP\textsubscript{5+}, MnTE-3-PyP\textsubscript{5+}, and MnTnBuOE-2-PyP\textsubscript{5+}. The E1/2 (O==MnP/P) values were calculated to be +317, +253, and +343 mV versus SHE,12 resulting in a higher driving force and therefore thermodynamically favoring the two-electron reduction of ONOO−.

An interesting phenomenon has been observed when two plots [log k\textsubscript{red}(O2−*) vs E1/2 and log k\textsubscript{red}(ONOO−) vs pk\textsubscript{a}] are overlapped (Figure 6E). The differences observed between the highest and the lowest rate constants for O2−* dismutation and ONOO− reduction at identical E1/2 values are 4.55 [Δk\textsubscript{red}(O2−*)] and 2.13 [Δk\textsubscript{red}(ONOO−)]. This diagram supports the fact that the reported beneficial effects of MnTBAP were could be rather attributed to its peroxynitrite reducing ability, and not superoxide scavenging.

The therapeutic effects observed with cationic ortho Mn(III) N-substituted pyridylporphyrins cannot be safely assigned to a specific reactive species. Implementing multiple approaches, including pharmacological and genetic, along with direct measurements of MnP subcellular localization may allow us to safely identify the location of MnP within tissue and cell/ cellular fragments and the nature of reactive species involved in its mode of action.

MnP-Catalyzed Ascorbate Oxidation. Understanding the reactivity of MnPs toward ascorbate is biologically relevant due to: (i) high intracellular ascorbate concentrations; (ii) high ability of MnPs to oxidize ascorbate; (iii) coupling of ascorbate with O2−* and ONOO−; reduction of MnIIIP to MnIIIP with ascorbate in vivo is likely a first step in its redox cycling with O2−* and ONOO− in such scenario MnP acts as a rate limiting step dependent upon the electron deficiency/richness of Mn site and thus best characterized with proton dissociation constants of either porphyrin pyrrolic nitrogens, or axial waters,4,7,6 or the E1/2 value of the MnIII/P/MnIIIP redox couple. Therefore, the MnPs of more positive E1/2 for MnIII/P/MnIIIP redox couple and lower pk\textsubscript{a} values (Figure 6B) are more electron-deficient and favor binding of an electron-rich ligand (ONOO− in this case) which in turn gives rise to higher k\textsubscript{red}(ONOO−) (Figure 6C).2 Such data explain why the log k\textsubscript{red}(ONOO−) correlates as well with E1/2 for MnIII/P/MnIIIP couple (Figure 6D) as does log k\textsubscript{red}(O2−*) (Figure 6A). It thus explains why there is a linear relationship between log k\textsubscript{red}(ONOO−) and k\textsubscript{red}(O2−*) (Figure 6F).
PyP5+ with \( \text{vcatalyze ascorbate oxidation (eq 1b), described as initial rate, tumor therapy.} \)

The therapeutic potential of MnP/ascorbate as a ROS generator for tumor therapy would produce higher levels of \( \text{H}_2\text{O}_2 \) and be more efficient in a peroxide production, may distinguish which compound

The ratio of the stabilities of Mn\( +2 \) and \( +3 \) oxidation states has been discussed in Figure 6 and in text in the Structure–Activity Relationships section. The bulkiness of the molecule, i.e., the steric hindrance toward lipid reactive species plays a minimal role. The impact of \( E_{1/2} \) was better visualized in plot B where the percent of lipid peroxidation was plotted vs \( E_{1/2} \) at 5 \( \mu \text{M MnP}. \) At that concentration, no inhibition of lipid peroxidation was observed with MnTBAP\( ^{3+} \) (1) and MnTE-2-PyPhP\( ^{5+} \) (2). As \( E_{1/2} \) increases from MnTBAP\( ^{3+} \) and MnTE-2-PyPhP\( ^{5+} \) to MnTE-3-PyP\( ^{5+} \), the inhibition of lipid peroxidation increases (3) and reaches maximum at \( \sim +300 \) mV vs NHE with MnTE-2-PyP\( ^{5+} \) (4), MntPhE-2-PyPhP\( ^{5+} \) (5), and MntHexOE-2-PyP\( ^{5+} \) (6). The somewhat lower inhibition with MntOct-2-PyP\( ^{5+} \) (7) is likely due to the steric hindrance imposed by long \( \text{N}-pyridyl substituents toward the approach of lipid reactive species.}

The magnitude of the reoxidation of Mn\( II \), resulting eventually in the cycling of MnP with ascorbate.

In addition to ascorbate, glutathione and cysteine (and likely protein thiols based on their exposure) may reduce MnP also. Under such conditions the differential impact of MnP/peroxide on cancer versus normal cell is discussed in the next paragraph.

**Differential Impact of MnP/Peroxide on Cancer versus Normal Cell.** The interaction of MnP with peroxide produced in its cycling with ascorbate (or when combined with radiation or other chemotherapies such as dexamethasone) will eventually cause cancer cell death, while either sparing or protecting normal cell. While little is still known on the differential biology of cancer versus normal cells, the prevailing opinion is that this is largely based on the differential redox environments of those cells; in turn, the differential impact of MnP/peroxide is dependent upon such differences also. It has been established that cancer relative to normal cell is under increased oxidative stress. While cancer cell often up-regulates MnSOD in efforts to control oxidative stress, this seems frequently not to be accompanied by up-regulation of appropriate levels of peroxide removing enzymes. Thus, an increase in circulating MnSOD frequently results in increased peroxide levels and is positively correlated with tumor reoccurrence. Moreover, down-regulation of a number of peroxide removing enzymes, such as thioredoxin reductase, peroxiredoxin, catalase, and glutathione peroxide, was reported; in turn, the peroxide levels get increased as tumor progresses. Malignant properties were reportedly reversed by up-regulation of catalase. Under such conditions of high oxidative stress, any addition of a reducto-active compound such as MnP, that further enhances the levels of RS via cycling with cellular reductants such as ascorbate (added exogenously), will further increase the levels of superoxide/peroxide and will enhance cancer cell death, the observation we

\[ \text{O}_2^{+} \text{ reductase rather than superoxide dismutase; and (iv) therapeutic potential of MnP/ascorbate as a ROS generator for tumor therapy.} \]

We have herein demonstrated that the ability of MnPs to catalyze ascorbate oxidation (eq 1b), described as initial rate, \( v_0(\text{HA}^-) \), depends upon the electron deficiency of the metal center, \( E_{1/2} \). The bell-shape curve was established for MnPs in the range \( E_{1/2} \) \( \sim +194 \) to \( +340 \) mV versus NHE (Figure 7). The highest rate of ascorbate oxidation was reached for MnTE-2-PyP\( ^{5+} \) at \( E_{1/2} \) of \( \sim +228 \) mV versus NHE, and dropped afterward. The \( k_{\text{cat}}(\text{O}_2^-) \) and \( k_{\text{red}}(\text{ONOO}^-) \), though, reached a plateau at MnTE-2-PyP\( ^{5+} \) but did not drop afterward (Figure 6A,D).

The ratio of the stabilities of Mn \( +2 \) and \( +3 \) oxidation states has larger impact on the catalysis of ascorbate oxidation than it has on \( \text{O}_2^{+} \) dismutation, where the interplay of solvation and lipophilicity of alkyl chains results in similarly high \( k_{\text{cat}}(\text{O}_2^{+}) \) of MntOct-2-PyP\( ^{5+} \) and MnTE-2-PyP\( ^{5+} \) (Figure 6A). We have shown that \( k_{\text{red}}(\text{O}_2^{+}) = k_{\text{cat}}(\text{O}_2^{+}) \) for MnTE-2-PyP\( ^{5+} \) with \( E_{1/2} \) \( \sim +228 \) mV vs NHE. Thus, both \( +3 \) and \( +2 \) oxidation states are equally stabilized. With MnOct-2-PyP\( ^{5+} \), at \( +340 \) mV versus NHE, the Mn \( +2 \) oxidation state is more stabilized and disfavors reoxidation with either \( \text{O}_2 \) or \( \text{O}_2^{+} \) (whichever species predominates in vivo in MnP neighbor.

In addition to ascorbate, glutathione and cysteine (and likely protein thiols based on their exposure) may reduce MnP also. The magnitude of the reoxidation of Mn\( II \), resulting eventually in a peroxide production, may distinguish which compound would produce higher levels of \( \text{H}_2\text{O}_2 \) and be more efficient in employing it subsequently in oxidation of biological targets.

**Figure 8.** Attenuation of lipid peroxidation by various MnPs as a function of their metal-centered reduction potentials. (A) The ability of MnPs to prevent lipid peroxidation of rat brain homogenates in terms of malondialdehyde, MDA, expressed as % of control (taken as 100% of lipid peroxidation) measured by HPLC method. Butylated hydroxytoluene (BHT) was used as positive control which prevented \( \sim 90\% \) of lipid peroxidation. The \( E_{1/2} \) of Mn\( II \)/Mn\( II \) governs the ability of MnPs to attenuate lipid peroxidation. The possible reasons why the oxidation of Mn\( II \) with lipid reactive species relates to the \( E_{1/2} \) of Mn\( II \)/Mn\( II \) have been discussed in Figure 6 and in text in the Structure–Activity Relationships section. The bulkiness of the molecule, i.e., the steric hindrance toward lipid reactive species plays a minimal role. The impact of \( E_{1/2} \) was better visualized in plot B where the percent of lipid peroxidation was plotted vs \( E_{1/2} \) at 5 \( \mu \text{M MnP}. \) At that concentration, no inhibition of lipid peroxidation was observed with MnTBAP\( ^{3+} \) (1) and MnTE-2-PyPhP\( ^{5+} \) (2). As \( E_{1/2} \) increases from MnTBAP\( ^{3+} \) and MnTE-2-PyPhP\( ^{5+} \) to MnTE-3-PyP\( ^{5+} \), the inhibition of lipid peroxidation increases (3) and reaches maximum at \( \sim +300 \) mV vs NHE with MnTE-2-PyP\( ^{5+} \) (4), MntPhE-2-PyPhP\( ^{5+} \) (5), and MntHexOE-2-PyP\( ^{5+} \) (6). The somewhat lower inhibition with MntOct-2-PyP\( ^{5+} \) (7) is likely due to the steric hindrance imposed by long \( \text{N}-pyridyl substituents toward the approach of lipid reactive species.}
have frequently demonstrated. Such enhancement of oxidative stress via radiation or chemotherapy has been regularly used as therapeutic modality. Thus, the enhancement of the anticancer effect in a lymphoma cellular study by the joint action of MnP and dexamethasone has been reported. The cancer cell killing by MnP in a lymphoma model occurred via MnP/peroxide-driven oxidation of thiols of antiapoptotic transcription factor NF-κB with subsequent suppression of its transcription. The inactivation of mitochondrial complexes I and III, and the impact on the glycolysis by MnP/dexamethasone, has been implicated in cancer cell death also.

In a normal cell, though, the dismutation of $O_2^{•−}$ catalyzed by MnP and MnP cycling with ascorbate, both giving rise to peroxide, has no significant toxic impact as peroxide is readily removed by abundant peroxide-removing enzymes maintaining physiological redox balance. If anything, and in diseased cell, the MnP may suppress excessive inflammation which would have otherwise lead to death of a normal cell. This could occur via suppression of NF-κB transcription by oxidation of its thiols, yet to a limited extent as levels of peroxide are much lower than those in a cancer cell. We have indeed frequently reported on the differential effects of MnP on normal versus cancer cells. Please see for further discussion Miriyala et al. and Batinc-Haberle et al.

The type of cell, cancer or normal, will control the suitability of particular MnP as a therapeutic of choice: MnTE-2-PyP and MnTE-3-PyP would be preferred when applied along with therapeutic doses of ascorbate to destroy cancer cells due to the highest rate of catalysis of ascorbate oxidation with subsequent peroxide production. Equally active and more lipophilic MnPs, such as MnTnOct-2-PyP and MnTnHexOEt-2-PyP, may be selected for the application in normal tissue oxidative stress related models as they are less efficacious in catalyzing ascorbate oxidation. However, much is still needed to fully understand therapeutic effects of redox-active drugs as they depend not only on their redox properties but on cellular and subcellular accumulation and colocalization with targeted species, many of those likely not yet identified.

Inhibition of Lipid Peroxidation by MnPs. Lipid peroxidation, i.e., the oxidative damage to polyunsaturated fatty acids, is initiated by the attack of reactive oxygen species, such as hydrogen peroxide, singlet oxygen, and hydroxyl radical. This gives rise to lipid peroxyl, ROO$^{−}$, and alkoxyl RO$^{•}$ radicals and lipid hydroperoxides which propagate the lipid peroxidation. As most of the proteins are closely associated with membranes, the lipid peroxidation damages not only lipids but proteins also. Lipid peroxidation is involved in pathogenesis of a number of diseases such as cancer, atherosclerosis, diabetes, Alzheimer’s disease, and Parkinson’s disease, etc. The peroxidation of arachidonic, linolenic, and docosahexanoic acids gives rise to malondialdehyde, MDA. MDA is also formed enzymatically during eicosanoid metabolism. Due to the intrinsic aldehyde instability, the MDA is reactive toward DNA and amino acids, in particular lysine. HPLC-based thiobarbituric acid (TBA)-assay eliminates most of the interference that plagues the simple TBA assay and is therefore useful in screening the biological tissues on lipid peroxidation. Figure 8 shows the magnitude of spontaneous lipid peroxidation affected by MnPs and measured as MDA with HPLC method. The data are expressed as percentage of peroxidation in control samples, which was taken as 100%.

The data in Figure 8 demonstrate a direct link between the $E_{1/2}$ of Mn$^{2+}$/Mn$^{3+}$ and log $k_{cat}(O_2^{•−})$, i.e., SOD-like activity, and the ability of MnPs to suppress lipid peroxidation. As $E_{1/2}$ parallels the log $k_{cat}(O_2^{•−})$, it in turn correlates with the ability

Figure 9. Aerobic growth of the wild type SOD-proficient (EG 103) and SOD-deficient (EG118) S. cerevisiae in the presence and absence of MnPs. Yeast grew in a restricted medium where the impact of MnPs is enhanced. All samples were run in triplicate. Growth was followed turbidimetrically by measuring the absorbance at 600 nm using ELISA reader. Inset: The lipophilicity, $Y$, and the SOD-like activity, described by log $k_{cat}(O_2^{•−})$, are plotted to demonstrate their impact on the growth of SOD-deficient yeast. The plots show that compounds of high lipophilicity (bioavailability) and high log $k_{cat}(O_2^{•−})$ are the most efficacious in protecting SOD-deficient yeast and in turn bear the highest therapeutic potential.
of MnP to inhibit lipid peroxidation. Such a relationship [among the $E_{1/2}$ log $k_{cat}(O_2^{•−})$, and log $P_{ow}$] MnPs could be divided into 3 groups: (1) lipophilic and SOD-inactive [(of negative $E_{1/2}$ and log $k_{cat}(O_2^{•−})$), the latter being lower than 5.7, situated in the right part of the figure; (2) lipophilic and SOD-active, situated in the left part of the figure, and (3) hydrophilic and SOD-active situated in the middle, i.e., in the minimum of the lipophilicity plot (Figure 5). Those MnPs that are lipophilic, SOD-active, and of positive $E_{1/2}$ values, the most efficacious in in vivo $S. cerevisiae$ assay and therefore bear the highest therapeutic potential.

**CONCLUDING REMARKS**

A series of MnPs with a wide range of metal-centered reduction potentials ($E_{1/2}$ from $−190$ to $+340$ mV vs NHE) and lipophilicities (log $P_{ow}$ from $−7.67$ to $−1.67$) have been synthesized and evaluated for their redox activities [log $P_{ow}$, $k_{cat}(O_2^{•−})$, $k_{red}(ONOO^{•})$, and ν$_d$(HA$^+$ oxidation to A$^{•+}$, ascorbyl radical)] and in vitro (lipid peroxidation) and in vivo therapeutic effects.

**Effect of MnPs on the Aerobic Growth of SOD-Deficient $S. cerevisiae$.** The aerobic growth of SOD-deficient $S. cerevisiae$, which lacks CuZnSOD, is an excellent in vivo model for the evaluation of the therapeutic potential of relatively lipophilic compounds within a class of water-soluble MnPs. It is also O$_2^{•−}$ specific in vivo model of oxidative stress. The combined impact of $E_{1/2}$, lipophilicity, and bulkiness (size, shape) was demonstrated in yeast study. The most lipophilic compounds MnTnOct-2-PyP$_{5^+}$ and MnTnHexOE-2-PyP$_{5^+}$ are the most efficacious MnPs, presumably due to their higher accumulation in the cell and the higher $k_{cat}(O_2^{•−})$ values relative to other MnPs. At 1–5 μM both compounds allow SOD-deficient yeast to grow as well as wild type (Figure 9). At higher concentrations both MnPs become toxic. The lipophilic, but bulkier, MnTPhE-2-PyP$_{5^+}$ has lower accumulation and is thus less efficient, but less toxic also. As seen before in E. coli assay, the higher lipophilicity of MnTE-3-PyP$_{5^+}$ compensates for its lower $E_{1/2}$ and lower $k_{cat}(O_2^{•−})$ and is thus equally efficacious as MnTE-2-PyP$_{5^+}$. Both MnTE-3-PyP$_{5^+}$ and MnTE-2-PyP$_{5^+}$ become efficacious at concentrations above 5 μM. The compounds with very negative $E_{1/2}$ lacking SOD-like activity, MnTBAPO$_3^−$ and MnTE-2-PyPhP$_{5^+}$, are not protective to SOD-deficient $S. cerevisiae$.

![Figure 10. Schematic representations of the dominant properties of MnPs which control their therapeutic potential: $E_{1/2}$, log $k_{cat}(O_2^{•−})$, and log $P_{ow}$. MnPs could be divided into 3 groups: (1) lipophilic and SOD-inactive [(of negative $E_{1/2}$ and log $k_{cat}(O_2^{•−})$), the latter being lower than 5.7, situated in the right part of the figure; (2) lipophilic and SOD-active, situated in the left part of the figure, and (3) hydrophilic and SOD-active situated in the middle, i.e., in the minimum of the lipophilicity plot (Figure 5). Those MnPs that are lipophilic, SOD-active, and of positive $E_{1/2}$ are the most efficacious in in vivo $S. cerevisiae$ assay and therefore bear the highest therapeutic potential.](image-url)
potential (aerobic growth of SOD-deficient S. cerevisiae). Those porphyrins could be divided in 3 groups by their $E_{1/2}$ (which parallels $\log k_{cat}(O_2^{•+})$) and lipophilicity as shown in Figure 10.

Two of those groups contain MnPs which are similarly lipophilic, yet in one group are the MnPs with negative $E_{1/2}$ and in the other with positive $E_{1/2}$. MnPs with negative $E_{1/2}$, despite high bioavailability, were inferior or non-efficient in both in vitro and in vivo assays. MnPs with positive $E_{1/2}$ are the most efficacious ones. The third group comprises very hydrophilic ortho and meta MnTE-2-PyP(S) and MnTE-3-PyP(S) which are efficacious but several-fold less than lipophilic analogs of equal $E_{1/2}$. In summary our observations are the following: (1) The $E_{1/2}$ for the MnIIIP/MnIIP redox couple, dominated by the electron deficiency of porphyrin and its metal site, not only controls the ability of MnPs to eliminate $O_2^{•+}$ and ONOO$^-$ but also the ability to prevent lipid peroxidation. The SOD-like activity appears to be proportional to the efficacy of MnP in preventing lipid peroxidation. The MnPs with highly positive $E_{1/2}$ (≥+228) and high SOD-like activity demonstrated strong inhibition of lipid peroxidation. MnTBAP(S) cannot inhibit lipid peroxidation, and MnTE-2-PyPhP(S) shows activity only at high concentration (≥50 μM). (2) The catalysis of ascorbate oxidation which involves reduction of MnIIIP is controlled by the thermodynamics of Mn IIIP/MnIIP redox couple. A bell shaped curve was observed for the MnP-driven catalysis of ascorbate oxidation with the highest $v_0$(HA$^-$) observed at $E_{1/2} = +228$ mV versus NHE (MnTE-2-PyP(S)). The data on ascorbate oxidation by MnP leading to a cytoxic peroxide production (Figure 7) indicate the superior activity of MnTE-2-PyP(S), while the data on lipid peroxidation and S. cerevisiae suggest that lipophilic MnTnOct-2-PyP(S) and MnTnHexOE-2-PyP(S) may be superior as therapeutics. Hence, the latter compounds may be preferably applied in normal tissue injuries with oxidative stress background, whereas MnTE-2-PyP(S), in combination with exogenous ascorbate, would be a therapeutic choice for tumor treatment. (3) Only MnPs which disproportionate $O_2^{•+}$ with a rate constant higher than the one for noncatalyzed, $O_2^{•+}$ self-dismutation, i.e., $\log k_{self-dismutation}(O_2^{•+}) = 5.70$, mimic the SOD enzyme in protecting SOD-deficient yeast. MnTE-2-PyPhP(S) [log $k_{cat}(O_2^{•+}) = 5.55$] or MnTBAP(S) [log $k_{cat}(O_2^{•+}) = 3.16$] did not show any beneficial effect as they are not true SOD mimics. While $E_{1/2}$ controls the efficacy of MnPs in aqueous solution [i.e., $\log k_{cat}(O_2^{•+})$ and $\log k_{red}(ONOO^-)$], the lipophilicity plays critical role in vivo also, as it governs the cellular and intracellular distribution of MnPs. Therefore, the compounds of somewhat lower SOD-like potency, such as MnTE-3-PyP(S), still support the yeast growth as good as the MnTE-2-PyP(S) of higher log $k_{cat}(O_2^{•+})$, as their cellular uptake is enhanced (Figure 9). (4) Enhanced toxicity of MnPs to SOD-deficient yeast is observed (Figure 9) with very lipophilic MnPs as they accumulate within a cell to higher levels and tend to localize in membranes disrupting their integrity. While fully protective in the region 1–5 μM, the MnTnOct-2-PyP(S) and MnTnHexOE-2-PyP(S) were already toxic at 20 μM. While MnTnHexOE-2-PyP(S) is a better inhibitor of lipid peroxidation, it is somewhat inferior to MnTnOct-2-PyP(S) in protecting S. cerevisiae which is likely due to the polar interactions between oxygen atoms and membrane structures. Jointly, high $k_{cat}(O_2^{•+})$, high lipophilicity, and lower bulkiness contribute to the therapeutic potential of MnPs in S. cerevisiae. (5) We have originally developed MnPs as SOD mimics. Over the past decade we have shown that MnPs are involved in other actions, some of which may even predominate in vivo. Yet, thus far the experimental evidence (provided herein and elsewhere) indicates that the higher the SOD-like activity, the higher the therapeutic potential MnPs possess, even when reactions in question do not involve $O_2^{•+}$ elimination. We can therefore safely conclude that the modification of a porphyrin molecule to enhance its SOD-like activity may still comprise the best experimental strategy in the design of redox-active drugs. (6) None of the data obtained here on MnTBAP(S) explain therapeutic effects reported elsewhere. Yet its in vivo efficacy justifies its future exploration. Its ONOO$^-$-related chemistry is ∼100-fold slower than that of Mn(III) N-substituted pyridylporphyrins (Table 4). When compared to cationic pyridylporphyrins, MnTBAP(S) cannot be in vivo reduced to Mn$^0$ by cellular reductants, such as ascorbate, in order to produce peroxide in subsequent reoxidation step (Figure 7). No reactivity toward lipid reactive species was observed (Figure 8). The lack of its SOD-like activity was proven here with SOD-deficient S. cerevisiae (Figure 9). It has insignificant catalase-like activity (Maia et al., submitted). Reactivity toward H$_2$O$_2$ may thus not play a major role in its actions. The role of reactive nitrogen species, other than ONOO$^-$, awaits further explorations.

**ASSOCIATED CONTENT**

Supporting Information

Additional figures and tables. This material is available free of charge via the Internet at http://pubs.acs.org.

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**Notes**

The authors declare no competing financial interest.

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**REFERENCES**


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