COMPREHENSIVE INVITED REVIEW

Mn Porphyrin-Based Redox-Active Drugs: Differential Effects as Cancer Therapeutics and Protectors of Normal Tissue Against Oxidative Injury

Ines Batinic-Haberle,¹ Artak Tovmasyan,¹ and Ivan Spasojevic^{2,3}

Abstract

Significance: After approximately three decades of research, two Mn(III) porphyrins (MnPs), MnTE-2-PyP⁵⁺ (BMX-010, AEOL10113) and MnTnBuOE-2-PyP⁵⁺ (BMX-001), have progressed to five clinical trials. In parallel, another similarly potent metal-based superoxide dismutase (SOD) mimic—Mn(II)pentaaza macrocycle, GC4419—has been tested in clinical trial on application, identical to that of MnTnBuOE-2-PyP⁵⁺—radioprotection of normal tissue in head and neck cancer patients. This clearly indicates that Mn complexes that target cellular redox environment have reached sufficient maturity for clinical applications.

Recent Advances: While originally developed as SOD mimics, MnPs undergo intricate interactions with numerous redox-sensitive pathways, such as those involving nuclear factor κ B (NF- κ B) and nuclear factor E2-related factor 2 (Nrf2), thereby impacting cellular transcriptional activity. An increasing amount of data support the notion that MnP/H₂O₂/glutathione (GSH)-driven catalysis of S-glutathionylation of protein cysteine, associated with modification of protein function, is a major action of MnPs on molecular level.

Critical Issues: Differential effects of MnPs on normal *versus* tumor cells/tissues, which support their translation into clinic, arise from differences in their accumulation and redox environment of such tissues. This in turn results in different yields of MnP-driven modifications of proteins. Thus far, direct evidence for such modification of NF- κ B, mitogen-activated protein kinases (MAPK), phosphatases, Nrf2, and endogenous antioxidant defenses was provided in tumor, while indirect evidence shows the modification of NF- κ B and Nrf2 translational activities by MnPs in normal tissue.

Future Directions: Studies that simultaneously explore differential effects in same animal are lacking, while they are essential for understanding of extremely intricate interactions of metal-based drugs with complex cellular networks of normal and cancer cells/tissues. *Antioxid. Redox Signal.* 29, 1691–1724.

Keywords: SOD mimics, Mn porphyrins, BMX-001, M40403/GC4403, cancer, radioprotection, protein cysteine oxidation

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Departments of ¹Radiation Oncology and ²Medicine, Duke University School of Medicine, Durham, North Carolina.

³PK/PD Core Laboratory, Pharmaceutical Research Shared Resource, Duke Cancer Institute, Durham, North Carolina.

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I. Introduction

A. Progress of Mn porphyrins toward clinical trials

Cationic Mn(III) *N*-substituted pyridylporphyrins [Mn(III) porphyrins, MnPs] were initially developed as powerful superoxide dismutase (SOD) mimics—catalysts of superoxide ($O_2^{\bullet-}$) dismutation. Subsequent studies showed that they can rapidly react with number of other species and modify the activity of transcription factors, such as nuclear factor κ B (NF- κ B), hypoxia inducible factor (HIF)-1 α , nuclear factor E2-related factor 2 (Nrf2), activator protein 1 (AP-1), and specificity protein 1 (SP-1) (75–77, 83–85, 164, 169, 178, 195, 196). Our data demonstrate that the magnitude of the SOD-like activity of MnPs is proportional to the magnitude of their reactivities toward other reactive species (RS). Moreover it parallels their remarkable efficacy in treating those diseases where physiological redox status has been perturbed, such as cancer, diabetes, and central nervous system injuries.

The wealth of the efficacy data supported the progress of several MnPs toward clinical trials. The Mn porphyrin MnTE-2-PyP⁵⁺ (or AEOL10113, BMX-100, or MnE [Mn(III) *meso*-tetrakis(*N*-ethylpyridinium-2-yl)porphyrin]) was in a clinical trial (NCT02457858) for a noncancer application—protection of islet cells during transplants. It did not improve marginal mass engraftment in a murine donation after circulatory death islet transplantation model, but it did reduce oxidative stress in islets (34). The efficacy of MnTnBuOE-2-PyP⁵⁺ (or BMX-001 or MnBuOE [Mn(III) *meso*-tetrakis(*N*-(2'-*n*-butoxyethyl)pyridinium-2-yl)porphyrin]) in the same application has been also evaluated (35). MnTE-2-PyP⁵⁺ is now tested in clinical trial NCT03381625 on another noncancer condition—atopic dermatitis and plaque psoriasis. Furthermore, MnTnBuOE-2-PyP⁵⁺ is presently in three clinical trials as a radioprotector of normal brain (NCT02655601), salivary glands and mouth mucosa (NCT02990468), and rectal, gastrointestinal (GI), and genitourinary (GU) systems (NCT0338650) in glioma, head and neck, and anal cancer patients, respectively. In addition, Aeolus Pharmaceuticals is initiating Phase I trials on di-imidazolyl analog, MnTDE-2-ImP⁵⁺ (AEOL10150) (21), as a radioprotector of normal tissue.

B. Chemistry of Mn porphyrins controls their therapeutic effects

The very rich chemistry of redox-active drugs, coupled with complexity of cellular milieu, limits our knowledge on the interactions of MnPs with cellular targets. To further our insight into the redox biology of MnPs, we have synthesized numerous Mn and Fe porphyrins and explored other types of metal complexes of different redox properties, charges, lipophilicities, shapes, bulkiness, and bioavailabilities (16, 17, 24, 25, 27, 28, 115, 172). Aqueous chemistry, combined with *in vitro* and *in vivo* data, furthered our understanding of the actions of MnPs on cellular transcription and metabolic pathways. Our most recent data, discussed herein, provide

clues to what lies behind such effects. Rather than acting as direct scavengers of RS, therefore eliminating the prerequisite for the RS-mediated activation of transcription factors (as was originally thought to be the case), MnPs can, in the presence of H₂O₂ and glutathione (GSH), catalyze the oxidation/*S*-glutathionylation of signaling protein cysteines (83, 84). Several sets of data, including redox proteomics (169), point to the oxidation of NF- κ B to be at the core of MnP activity. Most recent data indicate that the MnP-dependent oxidation of Nrf2/Kelch-like ECH-associated protein 1 (Keap1) and different kinases, endogenous antioxidative defenses, and phosphatases, all of which have redox-active signaling cysteines, could play a role as well (42, 147, 169).

The *in vitro* and *in vivo* studies demonstrate that the therapeutic effects produced by MnPs are beneficial at the level of both normal and tumor tissues; in other words, MnPs suppress tumor growth, while either healing the normal cell/tissue injury or preventing such injury. Meanwhile, two different types of redox-active drugs, an Mn(III) porphyrin, MnTnBuOE-2-PyP⁵⁺, and an Mn(II) pentaazamacrocycle, GC4419 (the enantiomer of M40403, which effects are discussed herein), have entered clinical trials on the same application—radioprotection of normal tissues during cancer radiotherapy. This clinical development of Mn complexes represents a major step toward understanding the therapeutic potential of metal-based redox-active drugs on a molecular level.

II. Chemistry of Mn Porphyrins

A. Design of powerful Mn porphyrin-based SOD mimics

Over the course of nearly two decades, four powerful MnP-based SOD mimics were identified and explored *in vitro* and *in vivo* (16, 17, 24, 25, 27, 28, 115, 172) (Fig. 1). First, studies aimed at mimicking the thermodynamics and kinetics of enzyme-driven catalysis of $O_2^{\bullet-}$ dismutation (Eqs. [1] and [2]) and triumphed in the design of the signature drug. The subsequent studies improved bioavailability and reduced the toxicity.

1. Design of a signature drug. MnTE-2-PyP⁵⁺ is based on the structure/activity relationship (SAR) between the kinetic and thermodynamic parameters: catalytic potency in dismuting $O_2^{\bullet-}$ $k_{cat}(O_2^{\bullet-})$, and redox property of Mn site described as metal-centered reduction potential, half-wave reduction potential ($E_{1/2}$). Its design represents a breakthrough discovery of the major structural features that make Mn porphyrins powerful SOD mimics and redox-active drugs and enabled the design of a series of analogs with modified bioavailability and toxicity profiles.

Unsubstituted analogs, such as MnT-2-PyP⁺, are too electron rich (thus stabilized in Mn +3 oxidation state) to

FIG. 1. Design of powerful SOD mimics. Starting from nonsubstituted and not SOD-active analog MnT-4-PyP⁺ (150), the methyl (M) chains were attached to pyridyl nitrogens, which produced a modest SOD mimic, MnTM-4-PyP⁵⁺. The SOD-like properties of this compound and its iron analog have been studied by several groups (65, 127, 184). Yet, due to the planarity of this molecule and its five positive charges, the MnTM-4-PyP⁵⁺ associates with nucleic acids to such an extent that it loses its SOD-like activity (12). Next, we moved the methylated pyridyl nitrogens from *para* [4] to *ortho* [2] positions; the closer vicinity of positive charges to the Mn site in MnTM-2-PyP⁵⁺ than in MnTM-4-PyP⁵⁺ produced a larger electron-withdrawing effect on the Mn center. Moreover, the limited rotation of methypyridyls around the *meso* positions in MnTM-2-PyP⁵⁺ confined them to the vertical positions relative to the porphyrin plane. This feature makes a porphyrin bulky and less prone to interactions with nucleic acids (12). To enhance the lipophilicity and suppress the interactions with nucleic acids, methyl groups were replaced with ethyls (E), giving birth to MnTE-2-PyP⁵⁺ (AEOL10113, BMX-010, MnE), (19), which remains the most frequently studied Mn porphyrin (16, 17, 24, 25, 27, 28, 115, 172) with an excellent safety/toxicity profile (72). The SOD-like activity of some analogs [MnBr₈TM-3(or 4)-PyP⁴⁺] (54) is very close to that of SOD enzymes (17, 172); yet the +2 oxidation state of Mn in these compounds makes them unstable at pH 7.8, due to the dissociation of the redox-active Mn from the porphyrin ligand. Lengthening of the alkyl groups resulted in MnTnHex-2-PyP⁵⁺, which has markedly increased ability to cross the blood/brain barrier and accumulate into mitochondria relative to most of the other analogs (24, 100, 185). The introduction of oxygen atoms into alkyl chains of MnTnHex-2-PyP⁵⁺ gave rise to a less toxic butoxyethyl analog, MnTnBuOE-2-PyP⁵⁺ (BMX-001, MnBuOE) (73, 100, 139). Charges on MnPs are omitted in some Figures for simplicity; MnBr₈TM-3(or 4)-PyP⁴⁺, Mn(II) *beta*-octabromo-*meso*-terakis(*N*-methylpyridinium-3(or 4)-yl)porphyrin; MnPs, Mn(III) porphyrins; MnT-4-PyP⁺, Mn(III) *meso*-tetrakis(pyridinium-4-yl)porphyrin; *meso* relates to 5, 10, 15, and 20 positions on porphyrin ring; MnTE-2-PyP⁵⁺ (AEOL10113, BMX-010, MnE), Mn(III) *meso*-tetrakis(*N*-ethylpyridinium-2-yl)porphyrin; MnTM-2-PyP⁵⁺, Mn(III) *meso*-tetrakis(*N*-methylpyridinium-2-yl)porphyrin; MnTM-4-PyP⁵⁺, Mn(III) *meso*-tetrakis(*N*-methylpyridinium-4-yl)porphyrin; MnTnBuOE-2-PyP⁵⁺ (BMX-001, MnBuOE), Mn(III) *meso*-tetrakis(*N*-(2'-n-butoxyethyl)pyridinium-2-yl)porphyrin; MnTnHex-2-PyP⁵⁺, Mn(III) *meso*-tetrakis(*N*-n-hexylpyridinium-2-yl)porphyrin; SOD, superoxide dismutase. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

undergo the first step of dismutation process (Eq. [1])—acceptance of an electron from O₂^{•−}. The substitution in the *ortho* [2] positions of four *meso* pyridyl rings bearing positive charges exert strong electron-withdrawing power on the Mn center making it electron deficient enough to accept electron from O₂^{•−}, while positive charges afford electrostatic attraction toward anionic O₂^{•−}. Along with pyridylporphyrins, and based on the same *ortho* concept, the analogous di-*ortho* Mn(III) alkylsubstituted imidazolylporphyrin-based potent SOD mimics were developed (16, 17, 21, 24, 25, 27, 28, 88, 115, 172). One of these analogs, Mn(III) *meso*-tetrakis(*N,N'*-diethylimidazolium-2-yl)porphyrin, MnTDE-2-ImP⁵⁺

(21) has been pursued toward clinical trials as a lung radioprotector (Fig. 1) (109). MnTDE-2-ImP⁵⁺ was also found to function as a protectant against middle cerebral artery occlusion (MCAO)-induced stroke in a rat model (146), sulfur mustard-induced skin injuries (129, 163), status epilepticus—a disorder induced by nerve toxin, pilocarpine (112), and as a rescue treatment after toxic gas lung injury (113). Over years we synthesized, and obtained from commercial sources, numerous Mn and Fe porphyrins (19) and other redox-active drugs (153). We demonstrated that all of them follow the SAR originally developed for Mn porphyrins (24).

2. Improvement in the bioavailability of Mn porphyrins. Improvement in the bioavailability of Mn porphyrins was achieved by lengthening the pyridyl substituents from ethyl in MnTE-2-PyP⁵⁺ to *n*-octyl in MnTnOct-2-PyP⁵⁺ [Mn(III) *meso*-tetrakis(*N*-*n*-octylpyridinium-2-yl)porphyrin]. Such modification increased lipophilicity by ~10-fold per CH₂ group. Several alkylpyridyl analogs were synthesized, of which hexyl porphyrin, MnTnHex-2-PyP⁵⁺ (or MnHex [Mn(III) *meso*-tetrakis(*N*-*n*-hexylpyridinium-2-yl)porphyrin]), has the optimally balanced bioavailability and toxicity (20).

3. Decrease in the toxicity of Mn porphyrins. Decrease in the toxicity of Mn porphyrins was accomplished, while favorable redox properties and bioavailability of the earlier hexyl analog were maintained. It is well known that Na lauryl ether sulfate is a less toxic version of dodecyl sulfate, frequently used in cosmetic preparations. By the analogy, we decided to incorporate oxygen atoms into hexyl chains. The first attempt to insert oxygen atoms at the periphery of the hexyl chains failed. The methoxyhexyl analog of insufficient purity was made (175). Moreover, the peripherally located polar oxygen atoms established hydrogen bonding with water molecules. In turn, the compound became less lipophilic than hexyl.

To increase the purity and to maintain the high lipophilicity comparable with that of the hexyl porphyrin, we buried the oxygen atoms deep within the porphyrin cavity formed by four pyridyl substituents. Oxygen atoms were no more exposed to water molecules. In a major breakthrough, a butoxyethyl derivative, MnTnBuOE-2-PyP⁵⁺, of high lipophilicity (identical to the one of hexyl analog) and high SOD-like activity was created (138, 139). The challenges encountered along the synthesis are discussed in detail (138). BMX-001 is presently in three clinical trials as a radio-protector of normal tissue (16, 24, 25, 27, 28, 115, 172). Recent efforts in the development of Mn porphyrins have resulted in the synthesis and characterization of the fluorinated Mn(III) *N*-alkylsubstituted pyridylporphyrins with excellent redox properties and therapeutic efficacy (167, 173).

Mn porphyrins of clinical potential have Mn in +3 oxidation state, which allows them to be stable and not lose redox-active Mn center. Yet, some of the most potent porphyrin-based SOD mimics have Mn in the +2 oxidation state: MnBr₈TM-3-PyP⁴⁺ and MnBr₈TM-4-PyP⁴⁺ [Mn(II) *beta*-octabromo-*meso*-terakis(*N*-methylpyridinium-3(or 4)-yl)porphyrin] (14, 54). Although these molecules contributed to the design of stable potent redox-active drugs and establishment of SAR, they are unstable due to the low cationic charge and therefore lose Mn and thus have limited biological and clinical applications.

Along the way, studies of porphyrin derivatives and other redox-active compounds allowed us to expand our conclusions on the reactivity of redox-active drugs and their impact on cellular metabolism (24, 25, 27). Those are as follows: Mn(III) complexes with biliverdin and its analogs, Mn(III) corroles (Mn complexes with shranked porphyrin ligands bearing three instead of four *meso* positions present in porphyrins), other nonporphyrin macrocycles [Mn(III) salen compounds (Mn complexes with semicyclic ligand salen and its derivatives) and Mn(II) pentaazamacrocycles], and metal-free compounds (such as nitroxides and flavonoids) (17, 23–25, 27). While redox active, the differences in their structures, which impose differences in thermodynamics of the Mn site, result in different chemistry and biology of those Mn com-

plexes [some of which are discussed in Section VI, and in detail in Batinic-Haberle *et al.* (17)].

It must be noted that the O₂^{•−} dismutation is not limited to the Mn^{III}/Mn^{II} redox couple (as shown in Eqs. [1] and [2]), but can also occur *via* Mn^{IV}/Mn^{III} couple with Mn corroles (61, 124) and Mn biliverdins (153). As long as the redox ability, that is, thermodynamics of Mn center described by its reduction potential, *E*_{1/2}, is appropriate for the exchange of electrons with O₂^{•−}, the efficient catalysis of O₂^{•−} dismutation by such complexes will occur. The catalysis of O₂^{•−} dismutation can also be initiated with Mn^{II}, which first gets oxidized to Mn^{III} and is then reduced back to Mn^{II}. Such scenario happens with Mn(II) pentaaza macrocycles—M40403 and GC4419. For details on the consequences of such chemistry on the redox biology of M40403, see elsewhere (16, 24, 25, 27, 28, 115, 166, 172) and in Section VI.A.

Since our first design steps, we have showed clearly that in addition to redox ability, the bioavailability of Mn porphyrins is a second major factor that controls their therapeutic effects (91). Development of the liquid chromatography/tandem mass spectrometry, LC-MS/MS, technique was another breakthrough that enabled direct quantification of Mn porphyrins in tissues, cells, and organelles (100, 154, 155, 158, 185), and furthered our understanding of the differential effects of these compounds in normal and tumor cells/tissues.

4. MnSOD enzyme *versus* its Mn porphyrin-based mimics. MnSOD enzyme and MnP-based mimics of MnSOD have nearly identical thermodynamics of the metal site, that is, they can undergo similar types of reactions. Yet they differ with regard to their kinetic properties. The kinetics of MnSOD is dominated by the large protein structure, which endows it with a high specificity toward O₂^{•−}. The reaction with peroxynitrite (ONOO[−]), a larger molecule than O₂^{•−}, is about 1000-fold slower with MnSOD than with MnP (67, 68). MnP, in essence, can react indiscriminately with numerous RS due to its fully exposed Mn center.

The production of H₂O₂ by MnSOD is limited by the amount of O₂^{•−}. MnPs, however, can produce H₂O₂ by first undergoing rapid one-electron reduction with endogenous or exogenous ascorbate or thiols, and then being reoxidized by oxygen or superoxide (see below Eqs. [4] and [5]). As a result, the H₂O₂ production by MnP is only limited by the supply of oxygen, which is more abundant than O₂^{•−} (25).

Depending on the magnitude of oxidative stress in the tissue of interest, which is, in large part, controlled by the peroxide-removing systems, both MnPs and MnSOD enzyme can act as tumor promoters or suppressors [(115) and references therein]. See also Iskandar *et al.* (82), Kumar *et al.* (94), and Loo *et al.* (107) on the impact of cellular redox on the apoptotic *versus* surviving processes.

B. From SOD mimicking to reacting with other low-molecular-weight RS

1. SOD-like activity is proportional to all other activities of Mn porphyrins. Starting in the late 1990s, it became obvious that those MnPs, which are potent SOD mimics, react with numerous other RS (24, 25, 27). Diverse reactivities are due to several properties of MnPs. (i) Lack of a large quaternary enzymatic structure and attendant lack of specificity toward O₂^{•−}. (ii) Biologically compatible *E*_{1/2} of the Mn^{III}P/

Mn^{II}P redox couple (ranging from approximately +100 to +400 mV vs. normal hydrogen electrode [NHE]) makes such compounds electron deficient and allows them to bind and react with diverse biologically relevant species (174). (iii) Pentacationic charge of MnPs affords electrostatic facilitation for the reactions of MnPs with RS, most of which are negatively charged (*i.e.*, deprotonated at physiological pH): ONOO[−] (68, 168), carbonate radical, CO₃^{•−} (68), nitric oxide, •NO (151), deprotonated hydrogen peroxide, HO₂^{•−} (37, 166, 169, 170), thiols, HS[−] and RS[−] (deprotonated GSH and protein thiol) (22, 37, 83, 84), ascorbate, HA[−] (monodeprotonated form of ascorbic acid, Asc, is a major species in aqueous solution at pH 7.8, pK_a=4.45) (18, 63, 166, 171, 191), and hypochlorite, HClO[−] (Fig. 2) (39). (iv) Bioavailability of four oxidation states of manganese (Mn⁺², Mn⁺³, Mn⁺⁴, and Mn⁺⁵) allows for ample reactions with biological targets.

Table 1 summarizes those kinetic and thermodynamic properties of MnPs that are relevant for discussion of the mechanism of action of MnPs: redox property of the Mn site (described by metal-centered reduction potential, *E*_{1/2}), and abilities of MnP to react with O₂^{•−}, ONOO[−], H₂O₂, ascorbate, GSH, and protein thiols. Interaction with ascorbate resulting

FIG. 2. Rich reactivity of cationic Mn(III) substituted pyridylporphyrins toward low- and high-molecular-weight RS. These Mn porphyrins, described here with MnTR-2-PyP⁵⁺, have the biologically compatible reduction potential of the Mn(III) site (*E*_{1/2} for Mn^{III}/Mn^{II} approximately +100 to +400 mV vs. NHE). Such value of *E*_{1/2} allows them to easily accept and donate electrons, acting as both antioxidants and pro-oxidants. Such actions are most obvious in the catalysis of a two-step O₂^{•−} dismutation process, depicted in Equations [1] and [2]. It is very likely that many other reactions are possible *in vivo*. The recently discovered reaction has been the oxidation of sulfite into sulfite radical (180). The implication of this discovery to sulfur biology needs further investigation. Number 2 in MnTR-2-PyP⁵⁺ indicates *ortho* positions of substituted pyridyls, which are critical for the facile redox chemistry of Mn center. BH₄, tetrahydrobiopterin; ClO[−], deprotonated hypochlorite; CO₃^{•−}, carbonate anion radical; *E*_{1/2}, half-wave reduction potential; GS[−], deprotonated glutathione; NHE, normal hydrogen electrode; O₂^{•−}, superoxide; R, alkyl or alkoxyalkyl *N*-pyridylsubstituents; RS, reactive species; RS[−], deprotonated protein thiol; SO₃^{2−}, sulfite anion.

in H₂O₂ formation and use of H₂O₂ with GSH and thiols in oxidative modification of proteins affect protein activities and in turn major cellular metabolic pathways. For details see Sections II.C and II.D. Table 1 also lists the lipophilicity of those compounds expressed as log value of their partition between water and n-octanol, log *P*_{OW}. This property controls the bioavailability and pharmacokinetics (PK) and consequently the magnitude of the therapeutic effects of MnPs.

Indeed, diverse SARs were subsequently developed and demonstrated that the ability of MnPs to react with all of those species, O₂^{•−} included, is proportional to *E*_{1/2} (Fig. 3). The bell shape of the SARs originates from the tight dependence of the kinetic parameters (the rate at which each of those reactions will occur) on *E*_{1/2} (thermodynamic parameter that tell us if the reaction may occur); it has been discussed in detail elsewhere (17, 19, 20, 24, 28). Briefly, When *E*_{1/2} is too negative, MnP is too electron rich and cannot be easily reduced. When *E*_{1/2} is too high (too positive), MnPs are already too stable in reduced Mn +2 oxidation state and disfavor oxidation. Collectively, SARs recognized how favorable *E*_{1/2} of Mn site dominates the reactivities of MnPs (Fig. 3). The newly discovered ability of MnPs to oxidize sulfite into sulfite radical points to their possible involvement in sulfur biology. It is highly likely, given the biologically compatible reduction potential of Mn center, that more reactions of MnPs will be revealed in future. Thus far, we know that the type and the amount of the species that MnP encounters *in vivo*, the levels of MnP, and the rate constants of the reactions that ensue are what control the types and the yields of reaction(s) that MnP undergoes *in vivo*.

2. SOD-like activity of Mn porphyrins is proportional to their therapeutic efficacies. The SOD-like potency also correlates well with the magnitude of the therapeutic effects inflicted by MnPs. Consequently, developing potent SOD mimics continues to control our synthetic efforts as a logical strategy for their clinical development.

Based on all said, most recently we tend to use a more correct term when describing MnPs: “redox-active drugs” rather than “SOD mimics.” We are pleased to see that such terminology has gained wide recognition.

3. Pro- versus antioxidative actions of Mn porphyrins. Although initially overlooked, the ability of MnPs to act as a pro-oxidant is not at all surprising. In the catalysis of O₂^{•−} dismutation (Eq. [4]), Mn^{III}P oxidizes O₂^{•−} in a first step (Eq. [1]) and reduces it in a second step, while reoxidizing itself from Mn^{II}P into Mn^{III}P (Eq. [2]). It thus acts as both reductant, giving away an electron (antioxidant, nucleophile), and oxidant (electrophile), accepting the electron from biological target. MnPs are therefore prone to react with both electron-rich nucleophiles and electron-poor electrophiles as long as both reactants have compatible thermodynamics. For example, MnP can oxidize O₂^{•−}, ascorbate, thiols, and tetrahydrobiopterin, while reducing O₂^{•−}, ONOO[−], hypochlorite (ClO[−]), and H₂O₂. Of note, and as detailed below and based on the data available, MnP appears to act *in vivo* primarily as pro-oxidant. The readers may benefit from visiting the Forman and Ursini article on how organic versus inorganic chemists versus biologists view concepts of electrophile, nucleophile, and antioxidants (26, 71).

TABLE 1. CHEMICAL AND PHYSICAL PROPERTIES OF Mn(III) PORPHYRINS AND OTHER Mn COMPLEXES

Mn complex	log P_{OW}	$E_{1/2}$, Mn ^{III} /Mn ^{II} mV vs. NHE	log $k_{cat}(O_2^{\bullet-})$ Cyt c assay	log $k_{red}(ONOO^-)$ Stopped-flow	$v_o(Asc)_{ox}$ nMs ⁻¹	$k_{cat}(H_2O_2)$ M ⁻¹ s ⁻¹	GPx-like activity Enzyme activity %
MnTE-2-PyP ⁵⁺	-7.67	+228	7.76	7.53	286	63	0.350
MnTnBuOE-2-PyP ⁵⁺	-4.10	+277	7.83	7.54	160	88	0.307
MnTnHex-2-PyP ⁵⁺	-3.84	+314	7.48	7.11	103	28	0.166
MnTBAP ³⁻		-194	3.16	5.02	4	6	0.008
EUK-8	Less than -0.90	-130	6.20		12	13	0.079
M40403	-0.38	+525 (ACN) +840 (CH ₃ OH)	7.08 (6.55)		3	8	0.0

Lipophilicities expressed here as log value of the partition of MnP between *n*-octanol, O, and water, W (log P_{OW}); redox properties expressed as a metal-centered reduction potential for Mn^{III}/Mn^{II} redox couple ($E_{1/2}$); ability to mimic SOD enzyme in the catalysis of $O_2^{\bullet-}$ dismutation, log $k_{cat}(O_2^{\bullet-})$; ability to reduce ONOO⁻ by one electron to [•]NO₂ radical using O=Mn^{IV}/Mn^{III} redox couple, log $k_{red}(ONOO^-)$; ability to oxidize ascorbate *via* using Mn^{III}/Mn^{II} redox couple expressed as initial rate, $v_o(Asc)_{ox}$; ability to catalyze H₂O₂ dismutation into H₂O and O₂ using O=Mn^V=O/Mn^{III} redox couple *via* two-electron process, log $k_{cat}(H_2O_2)$, and the ability to mimic GPx expressed in terms of percent of enzyme activity. All rate constants and initial rates were obtained at 25°C ± 1°C, except at 37°C ± 1°C for log $k_{red}(ONOO^-)$. Data are taken from Batinic-Haberle *et al.* (17, 19), Batinic-Haberle and Tovmasyan (23), Batinic-Haberle *et al.* (27), Bueno-Janice *et al.* (37), and Tovmasyan *et al.* (166, 170–172) and for log $k_{red}(ONOO^-)$ for MnTnBuOE-2-PyP⁵⁺ from Tovmasyan *et al.* (166).

$E_{1/2}$, half-wave reduction potential; EUK-8, Mn(III) salen complex; GPx, glutathione peroxidase; M40403, Mn(II) pentaazamacrocyclic; MnP, Mn(III) porphyrin; MnTBAP³⁻, Mn(III) *meso*-tetrakis(4-carboxylatopenyl)porphyrin; MnTE-2-PyP⁵⁺, Mn(III) *meso*-tetrakis(*N*-ethylpyridinium-2-yl)porphyrin; MnTnBuOE-2-PyP⁵⁺, Mn(III) *meso*-tetrakis(*N*-(2'-*n*-butoxyethyl)pyridinium-2-yl)porphyrin; MnTnHex-2-PyP⁵⁺, Mn(III) *meso*-tetrakis(*N*-*n*-hexylpyridinium-2-yl)porphyrin; NHE, normal hydrogen electrode; $O_2^{\bullet-}$, superoxide; ONOO⁻, peroxynitrite; SOD, superoxide dismutase.

C. From mimicking SOD enzymes to interacting with transcription factors

1. Normal cells/tissues. Early 2000s were marked by the shift in how we view MnPs. Rather than reacting with small RS, the emerging evidence has pointed to their interactions with transcription factors, such as Nrf2 and NF-κB. Such interactions seem to be involved in the healing of the normal tissue and suppression of tumor growth (16, 24, 25, 27, 28, 115, 172) (Fig. 4). Figure 5 summarizes therapeutic effects of cationic Mn porphyrins that bear substituents in *ortho* [2] pyridyl positions. The roles of NF-κB and Nrf2 in cellular metabolism are complex; studies have showed that they both may be oncogenes and tumor suppressors. The story with NF-κB is similar to the one with MnSOD. While protecting normal cell during precarcinogenic stage, both may become oncogenic once cell redox environment gets perturbed and carcinogenesis kicks in, Figure 6.

The very first studies related to the effects of MnPs on transcription factors were done on normal tissue injuries. In 2011, Dorai's group reported the MnTnHex-2-PyP⁵⁺-driven upregulation of endogenous enzymes (mitochondrial and extracellular SODs, glutathione peroxidase [GPx] 1 and 3, peroxiredoxins 2, 3, and 5, thioredoxin reductase, *etc.*), in turn reducing levels of RS (52, 56). Those were the very first data that demonstrated that MnP did not act as SOD enzyme in its own right. In other words, the data suggested that the major action of MnPs *in vivo* may not be the direct removal of $O_2^{\bullet-}$, but rather the upregulation of SOD enzymes *via* Nrf2 activation.

In 2007, Saba *et al.*'s data on rat renal ischemia/reperfusion (I/R) injury, which demonstrated that MnTnHex-2-PyP⁵⁺ increased MnSOD activity (in renal extracts by cytochrome c assay), may be understood also in the light of increased MnSOD expression *via* Nrf2 pathway (141). Finally, the increase in MnSOD (but not Cu,Zn-SOD) activity by MnTE-2-PyP⁵⁺ and MnTnHex-2-PyP⁵⁺, in a mouse model of morphine antinociceptive tolerance reported by Doyle *et al.* in 2009,

could be understood also in the light of Nrf2 activation (58). At that time, the involvement of Nrf2 did not occur to us as a possible mechanism of action. It is important to stress that the therapeutic outcome of MnP treatment by itself cannot readily distinguish among the types of reactions of MnPs at the molecular level. Comprehensive approach, encompassing pharmacological and genetic studies, is essential to correctly address mechanistic concerns. Nonetheless, the direct removal of $O_2^{\bullet-}$ in an SOD-like manner cannot be entirely excluded from consideration and will depend on the $O_2^{\bullet-}$ levels in the immediate environment of MnP.

The first *in vivo* studies on MnPs (MnTE-2-PyP⁵⁺, MnTDE-2-ImP⁵⁺, and MnTnHex-2-PyP⁵⁺) were conducted in the late 1990s on the MCAO I/R rat and mouse stroke models (143–146). MnPs were initially administered at the site of injury—intracerebroventricularly—to provide the proof-of-principle. The delivery of MnP *via* subcutaneous (sc) injections followed. MnTnHex-2-PyP⁵⁺ was the most successful compound in reducing the infarct volume; even if given *via* sc route it accumulates across the brain parts at higher levels than any other MnP thus far explored (16, 24, 25, 27, 28, 100, 115, 145, 172, 185, 186). One would expect that MnP, to be efficacious, that is, to remove the RS, needs to be in the brain at the time of reperfusion when the highest amount of these species is produced. However, even when given 6 h after MCAO, the effect was similar to the one when MnP was given 5 or 90 min postreperfusion (108). When MnTDE-2-ImP⁵⁺ was given for a week, starting at 90 min postreperfusion, the reduction in the stroke volume was still seen at 8 weeks poststroke (146). The data implicated the inhibition of NF-κB activity as a mechanism of action and strengthen our beliefs that MnP does not act predominantly at the level of RS, but rather at the level of cellular transcription. Soon afterward, the involvement of NF-κB was also demonstrated by Piganelli's group in diabetes (131, 178) and in a cellular lymphoma study by Tome's group (see Section II.D) (83–85).

In parallel, the pulmonary radioprotection studies by Vujaskovic and Batinic-Haberle's groups (51, 75–77, 135, 136, 181) demonstrated that MnTE-2-PyP⁵⁺ and MnTnHex-2-

PyP⁵⁺ downregulated HIF-1 α , its target gene vascular endothelial growth factor (VEGF), and transforming growth factor- β (TGF- β) (75–77). MnPs reduced expression of activated macrophages, DNA damage, and overall lung damage. The effects on HIF-1 α may be the consequence of the impact of MnP on NF- κ B. NF- κ B is known to control HIF-1 α (11, 31, 87, 125, 161). Recently, Oberley-Deegan's team demonstrated that MnTE-2-PyP⁵⁺-driven radioprotection of primary prostate fibroblasts occurs *via* inhibition of TGF- β /SMAD-mediated fibroblast activation pathway (46). Thus, the involvement of MnP in a cross talk between HIF-1 α , TGF- β , SMAD [homologs of both the *Drosophila* MAD, mothers against decapentaplegic protein and the *Caenorhabditis elegans* SMA protein (from gene *sm*a for small body size)] NADPH oxidase 4 (NOX4), and NF- κ B has emerged as a possibility (194).

Most recently, direct evidence has emerged in a study on hematopoietic stem/progenitor cells (HSPCs) by St. Clair's group, which supports Dorai *et al.*'s (56) data that MnPs affect

Nrf2 pathway and thus do not act predominantly as SOD mimics in their own right (24, 41, 42, 63, 147, 195). The study showed undoubtedly that MnTnBuOE-2-PyP⁵⁺ activates Nrf2 pathway upregulating genes controlled by it, catalase and MnSOD included (see Sections IV.A.3 and V) (195).

In addition to MnPs, other redox-active drugs also acted at the level of NF- κ B and Nrf2, including flavonoids (curcumin), nitroxides, and Mn(III) salen compounds [see reviews in this Forum (40, 48, 50, 89, 159)]. Yet, the molecular basis for the actions of redox-active drugs at the levels of transcription factors has only started to emerge (24, 63, 83, 84, 144, 146, 178).

2. Cancer. Cancer studies followed in the early 2000s. St. Clair and colleagues work on mouse skin carcinogenesis model showed that apoptosis of the cells in mouse epidermis, when treated with tumor promoter TPA (12-O-tetradecanoylphorbol-13-acetate), occurred before cell proliferation—6 *versus* 24 h (197). The data also demonstrated that MnSOD enzyme

FIG. 3. The reactivities of Mn porphyrins toward different RS are controlled by thermodynamics of the metal site.

To better make our point, that is, avoid crowding of the names of MnPs on the plots, we have chosen to list the compounds and their respective $E_{1/2}$ values in mV *versus* NHE in *parenthesis* at the end of the legend so that readers can easily locate those on each of the plots. This property of Mn porphyrins is best described with metal-centered reduction potential, $E_{1/2}$ of Mn^{III}P/Mn^{II}P redox couple. The value of $E_{1/2}$ in the range of –100 to +400 mV is biologically compatible and thus allows Mn porphyrins to rapidly exchange electrons with diverse RS. Four peripheral-positive charges of the pyridyl nitrogens and a single charge on the Mn site afford electrostatic facilitation toward mostly anionic biological species. We initially showed that the SOD-like activity (ability of MnP to catalyze O₂^{•–} dismutation) is proportional to the metal-centered reduction potential of Mn^{III}P/Mn^{II}P, $E_{1/2}$. Further studies discovered the ability of MnPs to react with other RS, some of which have been explored in detail and listed here: ascorbate, H₂O₂ (catalase-like activity), peroxynitrite, H₂O₂/RSH/GSH (in a GPx manner), and lipid radicals. The reactivity of MnPs toward all species thus far studied appears to be proportional to $E_{1/2}$ in a similar bell-shape manner. Data are taken from Batinic-Haberle *et al.* (17, 24, 25, 27) and Tovmasyan *et al.* (166, 172, 173, 175) and relate to the following compounds with the $E_{1/2}$ values indicated in the *parentheses*: MnTBAP^{3–} (–194), MnTSPP^{3–} (–160), MnTE-2-PyPhP⁵⁺ (–65), MnTM-3-PyP⁵⁺ (+52), MnTE-3-PyP⁵⁺ (+54), MnTM-4-PyP⁵⁺ (+60), MnTnPr-3-PyP⁵⁺ (+62), MnTnBu-3-PyP⁵⁺ (+64), MnTnHex-3-PyP⁵⁺ (+64), MnTMOE-3-PyP⁵⁺ (+64), MnTnHex-4-PyP⁵⁺ (+68), MnTMOHex-3-PyP⁵⁺ (+68), MnTE-4-PyP⁵⁺ (+70), MnTnOct-3-PyP⁵⁺ (+74), MnTM-2-PyP⁵⁺ (+220), MnTE-2-PyP⁵⁺ (+228), MnTnPr-2-PyP⁵⁺ (+238), MnTMOE-2-PyP⁵⁺ (+251), MnTnBu-2-PyP⁵⁺ (+254), MnTPhE-2-PyP⁵⁺ (+259), MnTnBuOE-2-PyP⁵⁺ (+277), MnCl₁TE-2-PyP⁵⁺ (+293), MnTnHexOE-2-PyP⁵⁺ (+313), MnTnHex-2-PyP⁵⁺ (+314), MnTDnPr-2-ImP⁵⁺ (+320), MnTnHep-2-PyP⁵⁺ (+342), MnCl₂TE-2-PyP⁵⁺ (+343), MnTDE-2-ImP⁵⁺ (+346), MnTM,MOE-2-ImP⁵⁺ (+356), MnTDMOE-2-ImP⁵⁺ (+365), MnTnOct-2-PyP⁵⁺ (+367), MnCl₃TE-2-PyP⁵⁺ (+408), MnTTEG-2-ImP⁵⁺ (+412), MnCl₄TE-2-PyP⁵⁺ (+448), MnBr₈TM-3-PyP⁴⁺ (+468), MnBr₈TM-4-PyP⁴⁺ (+480), MnCl₅TE-2-PyP⁵⁺ (+560), Mn corroles (+670 to +1110). GPx, glutathione peroxidase; GSH, glutathione; MnCl₁TE-2-PyP⁵⁺, Mn(III) β -chloro-*meso*-tetrakis(*N*-ethylpyridinium-2-yl)porphyrin; MnCl₂TE-2-PyP⁵⁺, Mn(III) β -dichloro-*meso*-tetrakis(*N*-ethylpyridinium-2-yl)porphyrin; MnCl₃TE-2-PyP⁵⁺, Mn(III) β -trichloro-*meso*-tetrakis(*N*-ethylpyridinium-2-yl)porphyrin; MnCl₄TE-2-PyP⁵⁺, Mn(III) β -tetrachloro-*meso*-tetrakis(*N*-ethylpyridinium-2-yl)porphyrin; MnCl₅TE-2-PyP⁵⁺, Mn(III) β -pentachloro-*meso*-tetrakis(*N*-ethylpyridinium-2-yl)porphyrin; MnP, Mn(III) porphyrin; MnTBAP^{3–}, Mn(III) *meso*-tetrakis(4-carboxylatophenyl)porphyrin; MnTDE-2-ImP⁵⁺, Mn(III) *meso*-tetrakis(*N,N'*-diethylimidazolium-2-yl)porphyrin; MnTDMOE-2-ImP⁵⁺, Mn(III) *meso*-tetrakis(*N,N'*-di(2-methoxyethyl)imidazolium-2-yl)porphyrin; MnTDnPr-2-ImP⁵⁺, Mn(III) *meso*-tetrakis(*N,N'*-di(*n*-propyl)imidazolium-2-yl)porphyrin; MnTE-2-PyPhP⁵⁺, Mn(III) *meso*-tetrakis(phenyl-4'-(*N*-ethylpyridinium-2-yl))porphyrin; MnTE-3-PyP⁵⁺, Mn(III) *meso*-tetrakis(*N*-ethylpyridinium-3-yl)porphyrin; MnTE-4-PyP⁵⁺, Mn(III) *meso*-tetrakis(*N*-ethylpyridinium-4-yl)porphyrin; MnTM-3-PyP⁵⁺, Mn(III) *meso*-tetrakis(*N*-methylpyridinium-3-yl)porphyrin; MnTM,MOE-2-ImP⁵⁺, Mn(III) *meso*-tetrakis(*N*-methyl-*N'*-methoxyethylimidazolium-2-yl)porphyrin; MnTMOE-2-PyP⁵⁺, Mn(III) *meso*-tetrakis(*N*-(2'-methoxyethyl)pyridinium-2-yl)porphyrin; MnTMOE-3-PyP⁵⁺, Mn(III) *meso*-tetrakis(*N*-(2'-methoxyethyl)pyridinium-3-yl)porphyrin; MnTMOHex-3-PyP⁵⁺, Mn(III) *meso*-tetrakis(*N*-(6'-methoxyhexyl)pyridinium-3-yl)porphyrin; MnTnBu-2-PyP⁵⁺, Mn(III) *meso*-tetrakis(*N*-*n*-butylpyridinium-2-yl)porphyrin; MnTnBu-3-PyP⁵⁺, Mn(III) *meso*-tetrakis(*N*-*n*-butylpyridinium-3-yl)porphyrin; MnTnHep-2-PyP⁵⁺, Mn(III) *meso*-tetrakis(*N*-*n*-heptylpyridinium-2-yl)porphyrin; MnTnHex-3-PyP⁵⁺, Mn(III) *meso*-tetrakis(*N*-*n*-hexylpyridinium-3-yl)porphyrin; MnTnHex-4-PyP⁵⁺, Mn(III) *meso*-tetrakis(*N*-*n*-hexylpyridinium-4-yl)porphyrin; MnTnHexOE-2-PyP⁵⁺, Mn(III) *meso*-tetrakis(*N*-(2'-*n*-hexoxyethyl)pyridinium-2-yl)porphyrin; MnTnOct-2-PyP⁵⁺, Mn(III) *meso*-tetrakis(*N*-*n*-octylpyridinium-2-yl)porphyrin; MnTnOct-3-PyP⁵⁺, Mn(III) *meso*-tetrakis(*N*-*n*-octylpyridinium-3-yl)porphyrin; MnTnPr-2-PyP⁵⁺, Mn(III) *meso*-tetrakis(*N*-*n*-propylpyridinium-2-yl)porphyrin; MnTnPr-3-PyP⁵⁺, Mn(III) *meso*-tetrakis(*N*-*n*-propylpyridinium-3-yl)porphyrin; MnTPhE-2-PyP⁵⁺, Mn(III) *meso*-tetrakis(*N*-(2'-phenylethyl)pyridinium-2-yl)porphyrin; MnTSPP^{3–}, Mn(III) *meso*-tetrakis(4-sulfonatophenyl)porphyrin; MnTTEG-2-ImP⁵⁺, Mn(III) *meso*-tetrakis(*N*-(1-(2-(2-(2-methoxyethoxy)ethoxy)ethyl)pyridinium-2-yl)porphyrin; ONOO[•], peroxynitrite. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

suppressed both apoptosis and proliferation of the cells. Contrary to the enzyme, the timely administration of its mimic, MnTE-2-PyP⁵⁺, is possible. When given at 12 h, after apoptosis but before proliferation of the cells, a reduced number of papillomas from 31 in control to 5 in treated group were measured. Authors also showed the suppression of oxidative stress, AP-1 activation, and cell proliferation (196). Based on such data, the only plausible explanation offered (at that time) was as follows: MnP reduced levels of RS to such an extent that precluded the activation of redox-sensitive transcription factors.

The connection between transcription factor, HIF-1 α , and RS was first established in a 4T1 breast cancer cellular model. H₂O₂ and *NO activated HIF-1 α in 4T1 cells, while MnTE-2-PyP⁵⁺ prevented such activation (118). In a mouse model, MnP suppressed tumor growth in the presence and absence of radiation therapy (RT). In the absence of RT, MnTE-2-PyP⁵⁺ suppressed tumor growth only when injected throughout the study at a high dose of 15 mg/kg/day. The reduction in oxidative stress, as measured *via* levels of HIF-1 α , VEGF, NADPH oxidase NOX4, 3-nitrotyrosine, and 8-OH-dG, was observed (137).

When combined with RT, the effect was seen when a lower dose (6 mg/kg) of MnP was injected subcutaneously three times, 12 h apart, either before or after 5 Gy radiation. Suppression of tumor growth and the reduction in HIF-1 α and VEGF were demonstrated (118). Explanation offered was similar to the one provided by St. Clair's team for skin carcinogenesis: removal or RS removed the signal for the activation of HIF-1 α .

In a mouse sc D-245 MG glioma xenograft model, the similar tumor radio- and chemosensitizing properties of MnTnHex-2-PyP⁵⁺ and MnTnBuOE-2-PyP⁵⁺ were demonstrated (24, 187, 188). Tumors were analyzed on gene expression in collaboration with Piganelli's group (24). MnP/RT *versus* RT treatment downregulated metastatic pathways (*ctss*, *catepsin L*, *becn1*, *beclin 1*), antiapoptotic and NF- κ B pathways (*Nfkb1*, *Bcl211*, *Bcl2*), and PI3kinase and mTOR (*Rsp6kb1*). Changes in protein translation machinery were also implicated (*EIF5b* and *Rsp6kb1*) (24, 122).

A cellular lymphoma study by Tome's group supported the MnP-driven inhibition of NF- κ B, which was accompanied by cell death (see Section II.D.) (83–85).

FIG. 4. Mn porphyrins affect activities of several transcription factors. The inhibition of NF- κ B by MnP was implicated in numerous injuries of normal tissues [such as diabetes and stroke (131, 144, 146, 178) and in cancer (63, 83, 84, 171)]. The impact of MnP/RT on HIF-1 α was found in several lung radioprotection (76, 77) and cancer studies (117, 118, 137). Yet, it has been reported that NF- κ B controls HIF-1 α (125, 162, 179, 200). Thus, the inhibition of HIF-1 α by MnP/RT might have been mediated by the inhibition of NF- κ B activity (31, 75–77, 117, 118, 135, 137, 161). The effect of MnPs on MAPK was seen in several reports on tumor cells (63, 147, 166). The activation of Nrf2 by MnP was recently seen in the study on hematopoietic stem cells [Zhao *et al.* (195)]. All of those proteins (phosphatases, MAPK, Keap1, NF- κ B, *etc.*) reportedly have exposed cysteines. Their oxidation/S-glutathionylation modifies the activities of related proteins (162, 199). The activities are mostly inhibited except in the case of Keap1, which oxidation would activate Nrf2 (see Sections V.B.1 and V.B.2). Based on recent report, the cross talk between Nrf2, NF- κ B, MAPK, and phosphatases is probably implicated (see also Fig. 12 and Table 2) (36, 98, 126, 147). Finally in cancer, based on redox proteomic data (see also Table 2), MnP, in the presence of the source of H₂O₂, S-glutathionylates numerous endogenous antioxidative defenses, consequently modifying their activities. Such data do not exist on normal cells/tissues. For details, see Section V. AP-1, activator protein 1; HIF, hypoxia inducible factor; Keap1, Kelch-like ECH-associated protein 1; MAPK, mitogen-activated protein kinases; NF- κ B, nuclear factor κ B; Nrf2, nuclear factor E2-related factor 2; PTEN, tumor suppressor phosphatase and tensin homolog; RT, radiation therapy; SP-1, specificity protein 1; VEGF, vascular endothelial growth factor. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

D. From the catalysis of O₂^{•-} dismutation to the catalysis of protein cysteine oxidation/S-glutathionylation

1. Historical overview. Toward the end of the first decade of a third millennium, several teams of biochemists/biologists contributed to our understanding of the interactions of MnPs with proteins. In a diabetes model, Piganelli *et al.* pointed to the possible oxidation of NF- κ B cysteines with MnP. Such observation implicated for the first time the pro-oxidative action of an SOD mimic (131). In a collaborative study on several MnPs of different structures, charges, and $E_{1/2}$, his group has later verified such data (22, 178). The cationic MnP-

based SOD mimics with favorable $E_{1/2}$ were able to affect the activity of p50 subunit of NF- κ B in an electrophoretic mobility shift assay (22). The anionic and SOD-inactive MnTBAP³⁻ [Mn(III) *meso*-tetrakis(4-carboxylatophenyl) porphyrin] was not efficacious due to its inferior thermodynamics and kinetics as negative charges repelled this compound from the negatively charged deprotonated protein cysteines. In another report, we demonstrated the vast impact that electrostatics has; the MnBr₈T-2-PyP⁺ with no charges and MnTE-2-PyP⁵⁺ with four positive charges on the periphery of the molecule have identical thermodynamics of the Mn site ($E_{1/2}$ = +219 vs. +220 mV, respectively) but catalyze O₂^{•-} dismutation with rate constants that are two orders of magnitude apart (152). Most of the RS, including cysteines, are predominantly anionic at physiological pH. Their anionic charges facilitate reactions with cationic MnPs (78). Most recent aqueous chemistry studies demonstrated further that MnTBAP³⁻ can neither catalyze H₂O₂ dismutation nor mimic GPx activity (both on thermodynamic and electrostatic/kinetic grounds) and therefore is not able to interact with protein cysteines (Table 1 and Fig. 2) (37, 166, 170).

A few years later, in a lymphoma cellular study, Tome's group provided unambiguous evidence that MnTE-2-PyP⁵⁺ and MnTnBuOE-2-PyP⁵⁺ S-glutathionylate NF- κ B. She demonstrated that cysteine of the p65 was oxidized to a larger extent than that of p50 subunit; the oxidation happened only if both H₂O₂ and GSH are available. When either GSH synthesis was inhibited or catalase was overexpressed, no S-glutathionylation occurred (84). While we have not identified which cysteines are oxidized, the literature data suggest that cys62 of p50 (132) and cys38 of p65 subunit (130) are most likely the culprits [for review see also Gloire and Piette (78)]. The S-glutathionylation was enhanced when dexamethasone was coadministered with MnP (84). A subsequent article from the same group showed that MnTE-2-PyP⁵⁺ also S-glutathionylates and subsequently inactivates complexes I and III of the mitochondrial respiration. Proteins involved in glycolysis seem also to be S-glutathionylated by MnP (83). Importantly, such actions promoted apoptotic processes in lymphoma (67) but not in normal lymphocytes; such differences are due to differences in their redox environment and different MnP distribution—for details see Section V. (66).

Our most recent study (166) provided evidence in support of S-glutathionylation-based actions of MnPs. Both MnTE-2-PyP⁵⁺ and MnTnBuOE-2-PyP⁵⁺ (but not redox-inert MnTBAP³⁻) increased levels of total S-glutathionylated proteins when these MnPs were combined with sources of H₂O₂ (RT and ascorbate) in a 4T1 cellular and flank mouse tumor study (166); levels of S-glutathionylation reflected the changes in glutathione disulfide (GSSG)/GSH ratio. Consequently, a decrease in cell viability and tumor growth was demonstrated (166).

In parallel, aqueous chemistry provided strong evidence that the compounds, which are powerful SOD mimics and bear cationic charge, do indeed have the proper thermodynamics and kinetics necessary to oxidize protein cysteines. Such oxidation can involve either (O₂)Mn^VP/Mn^{III}P or O = Mn^{IV}P/Mn^{II}P redox couple (16, 17, 24, 25, 27, 28, 37, 63, 115, 166, 170, 172). Our most recent redox proteomic study of 4T1 breast cancer cells treated with MnTE-2-PyP⁵⁺/ascorbate points also to NF- κ B as a major S-glutathionylated

FIG. 5. Therapeutic effects of Mn(III) N-alkylsubstituted pyridylporphyrins. Listed are the therapeutic effects of MnPs on normal and cancer tissues. R is either ethyl, or n-hexyl or n-butoxyethyl. Most of the data have been discussed in our earlier reviews (17, 22–25, 27, 172), except the most recent ones discussed herein (6–8, 30, 41, 42, 51, 55, 83, 92, 100, 123, 164, 166, 169, 171, 187, 188, 195). ALS, amyotrophic lateral sclerosis; AT, ataxia telangiectasia; I/R, ischemia/reperfusion; PK, pharmacokinetics; SAH, subarachnoid hemorrhage; TBHP, tert-butyl hydroperoxide.

protein (Table 2) (169). The proteomic study and the data from St. Clair's and Dorai's groups indicate also that Mn porphyrin *S*-glutathionylates the cysteines of Keap1, which event in turn activates Nrf2.

Collectively, those studies provide substantial evidence that modification of the cellular transcriptional activity occurs *via* oxidation of signaling protein cysteines rather than *via* direct elimination of RS by MnP. However, NF- κ B inactivation or Nrf2 activation would indirectly result in decreased levels of RS. Tome's group provided another clue to the whole story—clear evidence that MnP does not increase steady-state levels of H_2O_2 during *S*-glutathionylation (84). This was later corroborated with the works of Filipovic and Ivanovic-Burmazovic (in preparation). The H_2O_2 produced by MnP under experimental conditions was utilized to oxidize MnP to higher valent oxo MnP species which would in turn *S*-glutathionylate protein cysteines.

2. Mechanism of *S*-glutathionylation. The explanation that fits best the experimental data is as follows. Reactions [3]–[5] relate to the reduction of $Mn^{III}P$ to $Mn^{II}P$ with ascorbate and its reoxidation to $Mn^{III}P$ with either $O_2^{\bullet-}$ [5] or with more abundant oxygen [4], giving rise to H_2O_2 . The HA^- is the dominant ascorbate species at physiological pH [$pK_a(H_2A) = 4.2$]. H_2O_2 is then reused by $Mn^{III}P$ (Eqs. [6] and

[7]) or by $Mn^{II}P$ (Eqs. [8] and [9]) in a two-electron process mimicking the first step of a catalase-like activity of MnPs (170), giving rise to high-valent and highly potent oxidants— $Mn(IV)$ or $Mn(V)$ oxo porphyrins. Subsequently, a thiyl radical is produced *via* reactions [6] to [9], which then *via* reactions [11]–[14] gives rise to *S*-glutathionylated protein, RSSG. The process mimics GPx-like activity of MnPs (25, 37). Different Mn porphyrins and other classes of redox-active compounds were also tested on their GPx-like activity (Table 1 and Fig. 2) (37). As already demonstrated, the most potent SOD mimics are the most potent mimics of GPx (Fig. 2). Thiyl radical can be also produced *via* one-electron reduction of $Mn^{III}P$ (Eq. [10]) (25) as implicated in reports by Tong et al. (165) and Batinic-Haberle et al. (22).

The set of reactions listed here may still represent a simplified version of cellular events, given that $Mn^{III}P$ can be reduced to $Mn^{II}P$ also with GSH (22) and tetrahydrobiopterin (18), then reoxidized with concomitant formation of H_2O_2 . While these reactions occur with low rate constants, the high GSH concentration may support such way of thiyl radical formation. Furthermore, $ONOO^-$ and hypochlorite (ClO^-) can also oxidize MnPs to high-valent oxo species (38, 66, 68). Such reactions seem less likely as *S*-glutathionylation cannot occur in the absence of H_2O_2 (84). Given the highly biocompatible redox properties of MnPs

FIG. 6. MnPs are efficacious in reducing normal tissue/cell injury only when given before injury becomes devastating. Despite being radioprotective when rat rectum was irradiated with 20–30 Gy protons (A), MnTE-2-PyP⁵⁺ actually enhanced rat rectal injury when irradiated with 35 Gy RT (B) (7). Data relate to 220 (A) and 100 (B) days post-RT. (C) In a streptozotocin mouse model of diabetes, MnP induced kidney damage when the mouse treatment started once diabetes already progressed (2). Under those conditions, the high oxidative stress in normal and cancer tissues may be comparable in magnitude, resulting in similar therapeutic outcomes. ROS, reactive oxygen species. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

and extremely complex cellular redox biology, numerous as-of-yet unexplored reactions of MnPs with biomolecules are possible.

III. Biodistribution, PK, and Safety/Toxicity Profiles of Mn Porphyrins

A. PK of Mn porphyrins

1. General PK. We have looked into the PK of three most frequently explored MnPs in the context of their therapeutic effects in mice, rats, and most recently in dogs: hydrophilic MnTE-2-PyP⁵⁺ and two equally lipophilic, but structurally very different, MnPs, MnTnHex-2-PyP⁵⁺ and MnTnBuOE-2-PyP⁵⁺ (Fig. 1). MnTnHex-2-PyP⁵⁺ has lipophilic alkyl chains, whereas MnTnBuOE-2-PyP⁵⁺ has equally long but polar alkoxyalkyl chains. The alkoxyalkyl chains of MnTnBuOE-2-PyP⁵⁺ suppressed the toxicity and reduced the liver accumulation while slightly reducing its accumulation within the brain, relative to MnTnHex-2-PyP⁵⁺. Comprehensive PK of these three MnPs was conducted *via* intraperitoneal, intravenous, subcutaneous, and oral routes: MnTE-2-PyP⁵⁺ and MnTnHex-2-PyP⁵⁺ (72, 154,

TABLE 2. REDOX PROTEOMICS OF 4T1 CELLS TREATED WITH MnTE-2-PyP⁵⁺/ASCORBATE

Protein	Modified Cys	Fold change
Keap1	C288	3.73
NF- κ B p105 subunit	C59	1.39
p38MAPK	C306	1.36
p38 α (MAPK14)	C162	6.84
PKC δ	C208(δ)	1.39
PKC ι	C190(ι)	2.74
Protein phosphatase 2A, subunit A and B	C174(A)	1.51(A)
	C476(B)	1.40(B)
Heat shock protein 60	C237	4.26
Glutaredoxin, Grx 3	C231(3)	1.85(3)
Grx 5	C63	2.62(5)
Glutathione S-transferase ω 1 (Gsto1)	C191	1.64
Isocitrate dehydrogenase-IDH 1	C73	1.87
IDH 2	C418	1.68
IDH 3	C351/259	2.46
Peroxiredoxins Prdx 4	C54	2.26
Prx5	C96	1.99
Prx6	C67	1.47
Thioredoxin 1	C73	1.72
Thioredoxin domain containing protein, Txndcs 5	C233, C240	1.36
Txndcs 6	C135	2.46

Shown are modified cysteines of different proteins and fold changes in cells treated with MnP/ascorbate relative to nontreated controls (169). All *p*-values are $<10^{-20}$. Cells were treated for 4 h in a growth medium with 5 μ M MnP and 1 mM ascorbate. Such treatment affected 3605 peptidyl cysteines (Cys) in total, out of which 1577 were oxidized 1.3-fold or higher compared with control, untreated samples. Distribution analyses of MnP/Asc-induced oxidation of peptidyl Cys show that >50% of oxidized peptidyl Cys bear 1.3- to 2-fold oxidation level.

Keap1, Kelch-like ECH-associated protein 1; MAPK, mitogen-activated protein kinases; NF- κ B, nuclear factor κ B; p38, p38 mitogen-activated protein kinase; PKC, protein kinase C; Prx, peroxiredoxin.

185), and MnTnBuOE-2-PyP⁵⁺ (8, 27, 73, 158). Thus far, subcutaneous is the safest route with highest body exposure.

Surprisingly high levels of MnTnBuOE-2-PyP⁵⁺ were found in lymph nodes of dogs post-sc injections (32). MnTnBuOE-2-PyP⁵⁺ was injected for 3 weeks at 0.25 mg/kg three times per week. Tissues were harvested 48 h after last injection. Drug wet tissue levels were highest in peripheral lymph nodes (prescapular, submandibular, popliteal), ranging between 4 and 6 μ M; the levels are the lowest in brain tissue. Levels in the kidney and liver were 2.5 and 2 μ M (32). The remarkable efficacy of MnP/dexamethasone in lymphoma and multiple myeloma cellular studies demonstrated by Tome's group (83, 84) and the preferential accumulation of MnTnBuOE-2-PyP in lymph nodes strongly support its use as adjuvant therapy in lymphoma and many other types of cancers that invariably invade lymph nodes (190).

2. Brain PK. The very first insight into brain levels of MnPs was done as a part of MCAO ischemic rat stroke model. The brain and plasma PK were done under same experimental conditions used for the efficacy assessment of MnTnHex-2-PyP⁵⁺ in an MCAO ischemic stroke injury. The

data showed that ~ 25 nM levels in the brain afford improvement in neurologic score and reduction in infarct size (144).

Comprehensive brain PK of MnPs in the olfactory bulb, brainstem, cerebellum, thalamus, hippocampus, and cortex was recently reported and is highly relevant to the ongoing clinical trial in radioprotection of normal brain in glioma patients by MnTnBuOE-2-PyP⁵⁺ (100). After 10 days of sc administration at 2×1.5 mg/kg/day, the brain levels of lipophilic MnTnBuOE-2-PyP⁵⁺ and MnTnHex-2-PyP⁵⁺ were between 15 and 160 nM. Much lower brain levels of MnTE-2-PyP⁵⁺ were found. The highest accumulation of those MnPs was found in the liver and kidney; the highest being of MnTnHex-2-PyP⁵⁺ (185).

3. Head and neck PK. Relevant to ongoing clinical trial on the radioprotection of salivary glands and mouth mucosa in head and neck cancer patients, MnTnBuOE-2-PyP⁵⁺ accumulation in salivary glands and tongue followed initially the plasma profile ($C_{\max} = 0.5 \mu$ M at $T_{\max} = 2$ h). The 24 h PK study was done at 10 mg/kg sc dosing of MnP. The slow elimination profile was subsequently observed in the liver and kidney (27).

4. PK versus efficacy versus redox properties of Mn porphyrins. Researchers have frequently wondered if we have reliable evidence that redox ability of MnPs is indeed essential for drug efficacy. The answer would be "yes" as long as the drug reaches a target site to a significant extent. Recent study corroborated such statement. MnTnHex-2-PyP⁵⁺ was the only lipophilic MnP that reduced stroke injury in a rat MCAO model when given sc, while equally able redox-active MnTE-2-PyP⁵⁺ and MnTnBuOE-2-PyP⁵⁺ were inefficacious (144). Most recently, in a well-designed work, Warner and Sheng (private communication) compared MnTE-2-PyP⁵⁺, MnTnBuOE-2-PyP⁵⁺, and MnTnHex-2-PyP⁵⁺ in an MCAO stroke model. These MnPs are equally potent SOD mimics yet differ in several orders of magnitude with regard to lipophilicity. The authors injected these MnPs at identical dose intracerebroventricularly—that is, at the place of injury. Consequently, all three MnPs exhibited very similar neuroprotective effects. Data point to the key therapeutic role of the redox properties of MnPs if they reach the targeted site.

B. Accumulation of Mn porphyrins in tumor versus normal tissues

Several different MnPs accumulate up to 10-fold more in tumor than in normal tissue (137, 171, 191). The data for two of those MnPs are provided in Section IV.B.2. These differences, when coupled with higher tumor H₂O₂ levels, contribute to the massive inactivation of NF- κ B with subsequent enhancement of tumor apoptotic processes (24, 83, 84, 147). While no study was done on normal and cancer tissue in the same animal, the reported data allow us to postulate that suppression of NF- κ B demonstrated in the normal tissue of the cancer-bearing animal will be moderate and will in turn allow for survival pathways.

C. Oral availability of Mn porphyrins

A large scattering of the data was observed during the early time points when mice were orally gavaged after they had been anesthetized. We wondered whether the drug, delivered to oral cavity, might have entered blood *via* the respiratory system (128). This in turn might have given rise to reportedly

FIG. 7. Control of normal cell progression toward cancer by the imbalance between the levels of RS and anti-oxidative defenses. If the normal cell gets exposed to excessive oxidative stress (excessive RS), or has mutations, it would progress into precarcinogenic and ultimately to carcinogenic state while undergoing metastases. Normal cells use RS for signaling, while cancer cells utilize RS for proliferative and metastatic pathways. Yet the cancer cell is not well equipped to handle additional oxidative stress and eventually undergoes apoptosis/necrosis. This has been utilized in anticancer therapy: RT, chemotherapy, therapy with metal complexes given as single agents or in combination with RT, chemotherapy, or ascorbate. With active and abundant antioxidative systems, the surrounding normal cells/tissues are better able to handle the stress imposed on neighboring cancer cells/tissues. Depending on the magnitude of oxidative stress imposed on cancer tissue, the neighboring normal tissue suffered moderate to large damage, which usually limits the extent of anticancer therapy.

FIG. 8. 4T1 mouse breast cancer flank model—tumor growth suppression by MnTE-2-PyP⁵⁺ (MnP) increases as the levels of H₂O₂ increase. In its own right, MnP cycles with endogenous reductants (predominantly ascorbate) producing H₂O₂. Under such conditions, MnP must be injected at a very high dose of 15 mg/kg/day to inflict modest tumor growth suppression. The effect was increased when additional source of H₂O₂, such as exogenous ascorbate, was added—its cycling with MnP produces large amounts of H₂O₂. Further increase in tumor growth suppression was seen when tumors were also irradiated. Irrespective of the amount of MnP (0.2 or 2 mg/kg), similar tumor growth suppression was seen with either RT or ascorbate, which strongly supports the catalytic role of Mn porphyrin once H₂O₂ is abundant (see also Figs. 9 and 10).

FIG. 9. 4T1 mouse breast tumor growth suppression by redox-active MnPs but not M40403. Only redox-active cationic MnTE-2-PyP⁵⁺ and MnTnBuOE-2-PyP⁵⁺ (MnBuOE) sensitize tumor to RT and ascorbate (166). The tumor suppression is due to (i) their ability to catalytically oxidize ascorbate (giving rise to H₂O₂); (ii) catalyze protein S-glutathionylation in a GPx manner; and (iii) preferentially accumulate in tumor. Shown here is the anticancer effect of MnBuOE (A); lack of the effect of MnTBAP³⁻ (B); and M40403 (C); and the accumulation of MnPs in relevant tissues (D). Much higher tumor accumulation of MnTBAP³⁻ than MnBuOE and MnE cannot overcome its redox inertness. The effect observed with mice treated with MnTBAP³⁻/RT/Asc was entirely due to the effect of RT as no significant differences were found between MnTBAP³⁻/RT and MnTBAP³⁻/RT/Asc treatment groups *versus* RT and RT/Asc treatment groups. A very similar behavior to MnTBAP³⁻ was seen with M40403, none of which was able to either oxidize ascorbate catalytically or act as GPx mimics (Table 1) (166, 169, 171). In relevant treatment groups, the sc injections of 2 mg/kg/day of MnP and the ip injections of ascorbate (5 days at 4 and 1 g/kg/day thereof) were given to flank tumor-bearing mice starting 24 h before RT (171). RT was given for 3 days with 2.5 Gy per day. Fifteen mice per group were analyzed (166). Tumor was implanted on the muscle of one leg, while the muscle of another leg was used as a corresponding normal tissue. ip, intraperitoneal; M40403, Mn(II) pentaazamacrocyclic; sc, subcutaneous.

high oral availability of MnPs. We thus revisited the oral availability of three cationic MnPs and designed experiments to assure that the drug was intentionally targeted into either the respiratory system or mouth or stomach. The results confirmed the validity of our doubts. Once the PK curve was re-evaluated in the range of 2–24 h, the oral availability of all three cationic MnPs dropped significantly (in single digits). Data, however, taught us that MnPs are highly bioavailable *via* the inhalation route. Such knowledge is important for the clinical development of MnPs.

D. Skin availability

Topical administration of MnTE-2-PyP⁵⁺ and MnTnBuOE-2-PyP⁵⁺ in a mouse model of allergic dermatitis reduced itch

and inflammation (160). Data implicated good resorption of MnPs *via* skin and agree well with the impressive anticancer effects demonstrated in a skin cancer study, where MnTE-2-PyP⁵⁺ was applied to a small patch of skin in a very low amount of 5 ng/mouse 5 days per week for 14 weeks (196).

E. Liposomal delivery of Mn porphyrins

MnTE-2-PyP⁵⁺ and MnTnHex-2-PyP⁵⁺ were incorporated into low temperature-sensitive liposomes (96, 110, 121). Study showed a larger release of lipophilic MnTnHex-2-PyP⁵⁺ than hydrophilic MnTE-2-PyP⁵⁺ from liposomes (Park *et al.*, unpublished data). Thus, 90% of MnTnHex-2-PyP⁵⁺ was released into 0.9% saline within 20 s at the phase transition temperature, *T_m*, found to be within the range of hyperthermia,

40–42°C. Under identical conditions, only 40% of MnTE-2-PyP⁵⁺ was released over the course of 2 h.

F. Distribution of Mn porphyrins into cellular organelles

Collectively, our data demonstrate that MnPs distribute in all organs and cellular organelles explored thus far (24, 27). The zinc (Zn) analogs have nearly identical structures as Mn analogs. The fluorescent properties of Zn porphyrins provide us with additional tool to visualize the widespread biodistribution of cationic metalloporphyrins (1, 64). The magnitude of the biodistribution of either MnPs or ZnPs is proportional to their lipophilicity. Highly relevant from a biological perspective is the preferential accumulation of MnPs in the mitochondria over cytosol. Both high positive charge and lipophilic alkyl chains direct cationic MnPs into mitochondria (24, 27, 101, 155). Mouse studies showed that MnTE-2-PyP⁵⁺, MnTnBuOE-2-PyP⁵⁺, and MnTnHex-2-PyP⁵⁺ accumulate in heart mitochondria where they mimic MnSOD (10, 15, 41, 59, 116, 141, 195, 196). The mitochondria/cytosol ratios were found to be 1.6:1 for ethyl and ~3:1 for both lipophilic MnPs (27, 155, 186). Lipophilic MnPs also accumulate in brain mitochondria. The cytosol/mitochondria ratio in the brain was found to be ~2:1 for MnTnBuOE-2-PyP⁵⁺ and MnTnHex-2-PyP⁵⁺, whereas MnTE-2-PyP⁵⁺ was not found in mouse brain mitochondria, precluding the evaluation of its mitochondria/cytosol ratio (157, 186). The triply negative charge of MnTBAP³⁻ should preclude its accumulation in mitochondria, as it would require transport across the anionic phospholipids of the membrane. Yet one report claims its mitochondrial localization (103).

G. Safety/toxicity studies of Mn porphyrins

1. MnTE-2-PyP⁵⁺ Safety/toxicity was reported for MnTE-2-PyP⁵⁺ and MnTnBuOE-2-PyP⁵⁺ (72, 73). Investigational new drug (IND) approval by the Federal Drug Administration (FDA) was completed for clinical trials for both drugs. MnTE-2-PyP⁵⁺ has been the least toxic Mn porphyrin thus far developed, in part, due to its very low ability to cross the blood/brain barrier. Negative Ames test was found with MnTE-2-PyP⁵⁺. In mice, the no observed adverse effect level (NOAEL) for MnTE-2-PyP⁵⁺ was determined to be 10 mg/kg/dose after 18 days of intravenous, iv, treatment followed by 17 days of recovery. Mice were more sensitive to this drug than monkeys. With cynomolgus monkeys, after 14-day daily iv injections and 14-day recovery period, the NOAEL of 5 mg/kg/day and a histopathologically based NOEL of 15 mg/kg/day were identified.

2. MnTnBuOE-2-PyP⁵⁺ Mixed results for genotoxicity of MnTnBuOE-2-PyP⁵⁺ were seen with the weight of evidence indicating that it poses no genotoxic risk in humans. Only a marginally positive Ames test was found with MnTnBuOE-2-PyP⁵⁺. After 5-week daily sc injections, NOEL in mice was identified as 12 mg/kg per sc loading dose and 2 mg/kg per maintenance dose. In a study on cynomolgus monkeys, MnTnBuOE-2-PyP⁵⁺ was given 3 days/week *via* sc injection for 5 consecutive weeks followed by a 2-week recovery period. Based on the absence of adverse effects, the NOAEL for monkeys was determined to be 6 mg/kg/dose for a loading dose and 2 mg/kg/dose for maintenance dose.

3. MnTnHex-2-PyP⁵⁺ The most apolar and lipophilic MnP with long alkylpyridyl substituents, MnTnHex-2-PyP⁵⁺, distributes at highest levels in all organs. Although this does cause dose-dependent toxicity, MnTnHex-2-PyP⁵⁺ has been efficacious at extremely low doses of 0.05 and 0.1 mg/kg in pulmonary radioprotection studies of rats and nonhuman primates, respectively [(77); no toxicity was observed under such conditions (51)]. In a mouse sc study, the TD₅₀ dose of MnTnHex-2-PyP⁵⁺, which causes shivers and hypotonia (in part due to the blood pressure drop), was found to be of 12.5 mg (133, 185).

Based on the efficacy dose of 0.05 mg/kg, MnTnHex-2-PyP⁵⁺ (12.5/0.05=250) has 16.3-fold (250/15.3) wider therapeutic window than MnTE-2-PyP⁵⁺ with TD₅₀ of 91.5 mg and efficacy dose of ~6 mg/kg (91.5/6=15.3) (76). At the end of twice sc daily injections of 0.5–2.5 mg/kg for 4 weeks, we have seen acute degeneration of hippocampal neurons, mild subcutaneous inflammation, degeneration and regeneration of subcutaneous muscles, and pigment accumulation in Kupffer cells. However, all mice recovered with no overt pathological changes 4 weeks after the injections of MnTnHex-2-PyP⁵⁺ (100, 185).

IV. Therapeutic Effects of Mn Porphyrins

MnPs suppress oxidative stress-mediated injuries, supporting the survival of the normal tissue, while promoting apoptotic processes in tumor and in those normal tissues under overwhelming oxidative stress (Fig. 5) (8, 9, 24, 25, 30, 83, 166, 187, 188, 192).

At first glance, such divergent effects of MnPs looked controversial, almost contradictory, and it took a while for a plausible explanation to emerge. The first clue was unearthed with two studies in which MnPs enhanced the already existing large oxidative stress of normal tissues (2, 7). When rat rectum was radiated with 20–30 Gy, MnTE-2-PyP⁵⁺ radioprotected it, but enhanced the injury when radiation of >30 Gy was applied (Fig. 6) (7). In another study, when the treatment started at the onset of diabetes at 24 h after streptozotocin (STZ) injection and continued throughout the study, MnTM-2-PyP⁵⁺ [Mn(III) *meso*-tetrakis(*N*-methylpyridinium-2-yl)porphyrin] suppressed diabetes-induced oxidative stress, decreased mortality, and markedly increased the life span of a diabetic rat (29). However, when therapy was started when diabetes was already advanced, MnTM-2-PyP⁵⁺ induced kidney damage (Fig. 6) (2).

Such data demonstrate that it is not the type of the tissues, but rather the extent of oxidative stress that controls the outcome of the treatment with MnP. In other words, MnPs promote same type of apoptotic processes in normal tissues, which is experiencing overwhelming oxidative stress, as in cancer.

If cell experiences perturbed balance between RS and endogenous antioxidants, and/or extensive mutations, the increase in oxidative stress would ensue (17, 19, 23, 37, 57, 62, 74, 93, 166, 171, 172), forcing the cell into the pre-carcinogenic stage. One well-known mutation is *K-ras* mutation in pancreatic cancer resulting in an increased O₂^{•-} production presumably *via* NF-κB-mediated activation of NADPH oxidases (82, 134, 182). Cell is though unable to cope with overwhelming oxidative stress; it therefore progresses toward the carcinogenic stage and eventually undergoes metastasis (Fig. 7).

Anticancer radiation and chemotherapies take advantage of the inability of tumor to cope with excessive levels of RS by further increasing its oxidative stress. Recently, combined with radio- and/or chemotherapy, ascorbate progressed to clinical trials with pancreatic (NCT02905578), brain (NCT02344355), and lung cancer patients (NCT02420314) (3, 60, 189). The oxidation of ascorbate, catalyzed by metal complexes, gives rise to large quantities of H_2O_2 (Eq. [4]) (47, 156, 166, 171, 191). The yield of H_2O_2 and in turn the efficacy of cancer therapy will be controlled by the availability of ascorbate and oxygen and the redox ability of a metal complex. In the absence of metal-based drug of finely tuned redox properties, the oxidation of ascorbate may be catalyzed by endogenous metal complexes, such as cytochrome P450 enzymes, although less efficiently (47).

A. Mn porphyrins suppress normal tissue injuries

Studies on normal tissue injuries were covered in recent reviews (16, 17, 24, 25, 27, 172), except the very recent ones reviewed below, most of which are relevant to the ongoing clinical trials (30, 100, 187, 188).

1. Brain radioprotection with lipophilic MnTnBuOE-2-PyP⁵⁺ and MnTnHex-2-PyP⁵⁺. MnTnBuOE-2-PyP⁵⁺ offers long-term radioprotection to the normal brain (187, 188). The effect was evaluated at 3 and 4 months after single 10 or 8 Gy RT to the brain, respectively. MnP was given sc twice daily for a month at 1.5 mg/kg, starting 1 week before 8 Gy RT and continuing once daily for another month at 0.5 mg/kg. Thirty days afterward, the brain concentration of MnP was 31.3 nM (188). Neurocognitive and activity testing was performed at 3 months post-RT. MnP ameliorated RT-induced loss of axons and motor efficiency (rotarod and wheel running).

The follow-up study, involving temozolomide and cisplatin, was conducted under more clinically relevant conditions. MnP was given sc twice weekly for a week before 10 Gy RT at 3 mg/kg, and continued twice weekly for 4 months at 0.5 mg/kg (187) when the MnP brain concentration was found to be 13 nM. Cisplatin was injected intraperitoneal (ip) once at 6 mg/kg 24 h before RT, and temozolomide was administered ip at 5 mg/kg for 5 days starting at 24 h before RT.

MnP completely rescued the RT-induced decrease in axon density of the corpus callosum and rotarod and running wheel performance. MnP also rescued negative effects on rotarod performance of temozolomide±RT treatment. With cisplatin-treated groups, MnP did not improve the rotarod performance, but there was no negative effect seen either. The data on the accumulation of MnP in these studies agree well with comprehensive brain PK (Section III.A.1) reported in Leu *et al.* (100). The data also agree with the PK that was designed to match the rat MCAO ischemic stroke efficacy study; brain levels of MnTnHex-2-PyP⁵⁺ of ~25 nM caused significant reduction in infarct size and improvement in neurologic score (144).

In another mouse study, RT-induced expression of p21INK4a gene in the hippocampus and subventricular zone regions resulted in a decline in neurogenesis, as determined by doublecortin (Dcx) expression and 5-bromo-2'-deoxyuridine (BrdU) incorporation (97). The effect was partially restored in an Ink4a/arf-null mouse. When injected sc for 8 weeks at 0.45 mg/kg/day (starting immediately after

6 Gy cranial RT), MnTnHex-2-PyP⁵⁺ increased neurosphere formation, but had no effect on neurogenesis in the hippocampal region, despite its ability to limit RT-induced p16INK4a expression (97). The data agree with the report on the ability of MnTE-2-PyP⁵⁺ to mitigate RT-induced long-term bone marrow suppression in mice and expression of p16INK4a in hematopoietic stem cells (HSCs) (102).

The comprehensive brain PK study on MnTE-2-PyP⁵⁺, MnTnHex-2-PyP⁵⁺, and MnTnBuOE-2-PyP⁵⁺ identified MnTnBuOE-2-PyP⁵⁺ as a candidate for the efficacy study due to its use in clinical trials, good safety/toxicity profile, and good brain distribution (100). Protection of neurogenesis was seen when MnTnBuOE-2-PyP⁵⁺ was given sc a week before and a week after 5 Gy cranial RT, but not if given a week before and 4 weeks after RT at 3 mg/kg/day. MnP supported production and long-term survival of newborn neurons in the hippocampal dentate gyrus (100). Such data suggest that the limited effect of MnTnHex-2-PyP⁵⁺ demonstrated by Beausejour's group (97) is likely due to the excessive 8-week-long dosing that might have caused toxicity. In our month-long study with 0.5–2.5 mg/kg/day of MnTnHex-2-PyP⁵⁺, we saw toxicity, which disappeared once the mice were left 1 month without drug (see under Section III.G.3) (185).

2. Salivary glands, mouth mucosa, and tongue radioprotection with MnTnBuOE-2-PyP⁵⁺. MnTnBuOE-2-PyP⁵⁺ reduced RT-mediated mucositis (xerostomia) and fibrosis in salivary glands in nontumor-bearing C57BL/6 mice. The effects were assessed by a Pro-sense imaging agent that gets activated by inflammation. The dose-modifying factor for protection against xerostomia was 0.77. In this study, MnP was injected sc twice daily at 1.5 mg/kg, beginning 1 week before RT and continued for the duration of the study; single RT doses up to 15 Gy were used. Saliva was collected weekly at 2–4 weeks after RT. In a parallel study, the FaDu human epithelial cell line from squamous cell carcinoma of the hypopharynx was used. Suppression of FaDu growth in an sc xenograft mouse model was demonstrated (see Section IV.B.4) (8).

Another more clinically relevant study was conducted when MnP was given at lower doses and in conjunction with cisplatin—a common therapy for head and neck cancer patients (30). Mice received 9 Gy RT to oral cavity and salivary glands and 6 mg/kg cisplatin *via* ip injection. MnP dosing started at 24 h before RT at 0.2, 0.6, and 2 mg/kg, and continued three times per week at 0.1, 0.3, and 1 mg/kg, respectively. Oral mucositis was assessed at 11 days. Stimulated saliva production and salivary gland fibrosis were quantified at 11 and 12 weeks post-RT, respectively. MnP protected normal tissue against RT-induced damage at early and late time points. Cisplatin did not interfere with MnP-induced radioprotection and MnP did not interfere with RT/cisplatin-mediated tumor growth (30). In a cisplatin experiment, MnP sc injections started at 24 h before RT/cisplatin at 0.6 mg/kg and continued at 0.3 mg/kg three times per week for 5 weeks. MnP lessened cisplatin-induced mouth ulceration, bleeding, and moist desquamation in the irradiated areas, and protected against RT/cisplatin-induced weight loss.

3. HSPC protection with MnTnBuOE-2-PyP⁵⁺. The enhancement of a number and function of mouse HSPCs under

physiological conditions was demonstrated with MnTnBuOE-2-PyP⁵⁺ when administered to mouse primary bone marrow cells or to C57BL/6 mouse (195). Activation of intracellular Nrf2 signaling by MnP along with the induction of antioxidant enzymes, including MnSOD, catalase, and glutathione S-transferase pi 1 (GSTp1) and mitochondrial uncoupling protein 3, was observed. The same outcome was seen with MnSOD overexpression in a transgenic mouse model. Thus, MnP apparently acted in an MnSOD-like manner. Such results are similar to what was seen earlier in a rat kidney I/R model (56): instead of acting as an MnSOD enzyme, MnP upregulated MnSOD—the final therapeutic outcome (suppression of oxidative stress) being identical.

Although protective to normal HSPCs, under identical concentration, MnP had a significant killing effect on the cells derived from patients with myelodysplastic syndrome orchestrated *via* activation of AP-1 (40–42). One could hypothesize that under such conditions, MnP had not activated Nrf2 to a significant extent. Data show the potential of MnP for the treatment of pathological bone marrow cell loss and for the increase in stem cell population for bone marrow transplantation. For details please see Carroll and St. Clair's review in this Forum (40).

4. Bone marrow radioprotection with MnTE-2-PyP⁵⁺. Total body radiation induces long-term bone marrow suppression, in part, by the induction of HSC senescence through production of reactive oxygen species (ROS). Six hours after exposure to sublethal 6.5 Gy radiation, mice were treated sc with MnTE-2-PyP⁵⁺ at 6 mg/kg/day for 30 days. MnP significantly inhibited the RT-induced increase in ROS production, DNA damage in HSC, and reduction in HSC frequency and clonogenic function. Moreover, the impact on senescence was seen as MnP-reduced RT-induced expression of p16^{Ink4a} (p16) messenger RNA (mRNA) (102). Study demonstrated that the effects are mediated at least, in part, *via* NADPH oxidase, NOX4 (128), which is in turn regulated by NF-κB (177).

5. Low pelvic region radioprotection with MnTE-2-PyP⁵⁺ and MnTnBuOE-2-PyP⁵⁺

a. Radioprotection of prostate and erectile function. Oberley-Deegan *et al.* showed remarkable radioprotection of erectile function, prostate, and testes by MnTE-2-PyP⁵⁺ in a model where the low pelvic region of rat was irradiated (45, 46, 122, 123, 148). Subsequently, Koontz and Batinic-Haberle's groups showed radioprotection of erectile function and prostate by MnTnBuOE-2-PyP⁵⁺ (79) and MnTF₃Pen-2-PyP⁵⁺ in a stereotactic prostate model (173). A most recent article from Oberley-Deegan's group reported differential effects of MnP to normal *versus* cancer tissue in a same prostate cancer radiation model (46). The authors also demonstrate the beneficial effect on bladder and skin in that region (148).

b. Radioprotection of rectum. The impressive reduction in acute and chronic radiation proctitis by MnTE-2-PyP⁵⁺ in a rat model was demonstrated by Archambeau (Fig. 6) (7, 45, 46, 148). Such results facilitated the launch of the clinical trial on the radioprotection of rectal, GI, and GU systems by MnTnBuOE-2-PyP⁵⁺ with anal cancer patients.

6. Pulmonary radioprotection in nonhuman primates with MnTnHex-2-PyP⁵⁺. There is a high likelihood of lung injury with patients having malignancies of thorax when receiving higher doses of radiation to the inferior thorax. The standard of treatment for RT-induced pneumonitis is high-dose corticosteroids, but no treatment exists for RT-induced fibrosis. Pulmonary injury has emerged as a highly relevant syndrome. The lipophilic MnTnHex-2-PyP⁵⁺ was a powerful pulmonary radioprotectant in a rat study at a low dose of 0.05 mg/kg/day for 2 weeks, starting 2 h after whole thorax radiation (77).

Based on such data, the nonhuman primate study followed (51). MnTnHex-2-PyP⁵⁺ was delivered sc to the whole thorax of the rhesus *Macaca mulatta* monkey for 2 months at 2 × 0.05 mg/kg/day, starting 2 h after 10 Gy RT. MnTnHex-2-PyP⁵⁺ delayed the onset of RT-induced lung lesions and reduced elevations of respiratory rate and pathological increases in lung weight. The progression of pulmonary disease was markedly suppressed during the 2-month drug dosing, but escalated once the treatment ended. Data suggest that a larger benefit might have been achieved with improved dosing regimen.

7. Suppression of skin damage—dermatitis, itch, and inflammation. Several studies of different Mn and Fe porphyrins demonstrated impressive effects in mouse histamine-dependent and -independent itch models (Liu *et al.*, unpublished data). The data were further substantiated in a study by Stover *et al.* where MnTE-2-PyP⁵⁺ and MnTnBuOE-2-PyP⁵⁺ were tested on allergic dermatitis, itch, and inflammation (160). The beneficial effect of MnPs on skin has been seen by Oberley-Deegan's group on prostate cancer (123, 148) and by Birer *et al.*'s data on normal tissue protection from combined MnP/cisplatin treatment (see Section IV.A.2) (30). Collectively, such data enabled the progress of MnTE-2-PyP⁵⁺ into clinical trials on atopic dermatitis and psoriasis.

8. Cardiac effects of Mn porphyrins. Recently, the impressive effect of MnTnBuOE-2-PyP⁵⁺ was demonstrated by Ferrari's group (6). The authors explored the underlying changes in the extracellular matrix (ECM) architecture and valve interstitial cell (VIC) activation in aortic valve sclerosis. The angiotensin-II chronic infusion model was used to impose aortic valve thickening and remodeling. MnTnBuOE-2-PyP⁵⁺ inhibited human VIC activation and ECM remodeling and aortic valve thickening in a murine model of aortic valve sclerosis.

B. Mn porphyrins suppress tumor growth

1. Hematologic malignancies. Tome's group found that MnTE-2-PyP⁵⁺ (84) and MnTnBuOE-2-PyP⁵⁺ (Tome's private communication) enhance glucocorticoid-induced apoptosis in lymphoma, but not in normal lymphocytes (83), *via* MnP/H₂O₂/GSH catalysis of S-glutathionylation of protein cysteines. Similar effects were also seen with multiple myeloma and the activated B cell subtype of diffuse large B cell lymphoma (DLBCL) when combined with standard-of-care chemotherapies (86). In addition, DLBCL cells with increased c-Myc (regulator gene that codes for a transcription factor) and B cell lymphoma 2 (Bcl-2) (which models a DLBCL patient population that has a poor prognosis) were

FIG. 10. 4T1 breast tumor growth suppression by MnP was orchestrated through the impact on cellular redox environment by MnP/RT/Asc as described by GSSG/GSH ratio. Redox environments of cells (A) and tumor tissues (B) were described in terms of the GSSG/GSH couple. In a cellular study, the 4T1 cells were treated in DMEM with 5 μ M MnP or M40403, 1 mM ascorbate, and 10 Gy and analyzed 4 h after RT. In a mouse study, the sc injections of 2 mg/kg/day of MnTE-2-PyP⁵⁺ and ip injections of ascorbate (5 days at 4 and 1 g/kg/day thereof) were given to flank tumor-bearing mice throughout the study, starting at 24 h before RT (171). RT was given for 3 days at 2.5 Gy/day. Fifteen mice per group were analyzed (166). Only tumors implanted on the muscle of one leg were radiated; the rest of the body was protected from RT. The muscle from another leg served as corresponding normal tissue. Only the most aggressive MnP/RT/Asc treatment induced increase in oxidized GSH and only in tissue exposed to RT-tumor. Values are mean $\pm$ SEM; [#] $p < 0.05$ versus control. DMEM, Dulbecco's modified Eagle's medium; GSH, glutathione; GSSG, glutathione disulfide.

sensitive to MnTE-2-PyP⁵⁺ when used as a single agent (see also Section II.D) (83–86).

2. Breast cancer

a. 4T1 mouse breast cancer sc flank tumor model. Powerful SOD mimics (MnTE-2-PyP⁵⁺, MnTnHex-2-PyP⁵⁺, MnTnBuOE-2-PyP⁵⁺) and a redox-inert, SOD-inactive (MnTBAP³⁻) were studied with 4T1 estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2)-negative mouse breast cancer cell line (63, 117, 118, 147, 166, 169, 171). For comparison, other types of redox-active Mn complexes, Mn(III) salen, EUK-8, and Mn(II) pentaazamacrocyclic, M40403 (GC4403), were also explored in breast cancer cell and mouse models (166).

In a first study, no antitumor effect was seen when MnTE-2-PyP⁵⁺ was given sc as a single agent at a lower dose of 2 mg/kg/day. Yet, at a much higher dose of 15 mg/kg/day, modest but significant tumor growth suppression was demonstrated (137). In this model, MnP produced H₂O₂ predominantly *via* cycling with intracellular reductants such as ascorbate and thiols (Eqs. [3] and [4] for cycling with ascorbate) (Fig. 8).

In subsequent studies, on a same mouse tumor model, H₂O₂ levels and consequently the anticancer effect were significantly increased when mice were treated jointly with 2 mg/kg/day of MnP (MnE or MnBuOE), RT, and exogenous (pharmacological) ascorbate (Figs. 8 and 9) (166, 169, 171).

In a similar study design on 4T1 cells, MnTnHex-2-PyP⁵⁺ acted also as a tumor radiosensitizer (147). MnP/RT reduced cell viability, clonogenic cell survival and DNA damage repair, and synergistically increased MnP/RT-induced apoptosis of 4T1 cells when the cells were pre-treated for 3 h before 4 Gy RT with 10 μ M MnP (see also Section V.B.3). The MnP/RT

suppressed phosphorylation of several mitogen-activated protein kinases (MAPK) and inhibited NF- κ B activation. The data on MAPK and NF- κ B agree well with redox proteomics on 4T1 cells treated jointly with MnP/ascorbate (Table 2) (see also Sections V.A.2 and V.B.3).

The key mechanistic insight into differential effects of MnPs emerged when preferential accumulation of redox-active MnPs in cancer over normal tissues was demonstrated in a 4T1 mouse flank tumor study (166) (Fig. 9D). The magnitude of the tumor suppression depends therefore on the interplay between the (i) ability of MnP to oxidize ascorbate (producing H₂O₂ essential for protein S-glutathionylation) and subsequently S-glutathionylate proteins (thereby affecting GSSG/GSH ratio) and (ii) accumulate in tumor.

In other words, the preferential accumulation of MnP in tumor (Fig. 9) as well as the MnP-driven total protein S-glutathionylation in a GPx-like manner (166), and the perturbed GSSG/GSH ratio (Fig. 10) (166), collectively gave rise to anticancer effects (Fig. 9) (166). The higher tumor bioavailability of MnTnBuOE-2-PyP⁵⁺ relative to MnTE-2-PyP⁵⁺ overcame its lower ability to oxidize ascorbate; as a result, their antitumor effects were similar. As anticipated, effects on S-glutathionylation were only observed with tissues that were irradiated (tumor), but not with analogous normal tissue (Fig. 9). For details, see also Section II.D. Due to the catalytic nature of MnP-driven S-glutathionylation, the amount of MnP appears to be less critical than the amount of H₂O₂—similar effects were observed with 0.2 or 2 mg/kg of MnP [(171) and Tovmasyan *et al.*, private communication].

However a high tumor accumulation cannot compensate for the redox inertness of MnTBAP³⁻, because it can neither catalyze ascorbate oxidation nor S-glutathionylate protein cysteines (Eqs. [3]–[14]) (Table 1), and is thus neither a radio- nor chemosensitizer (Fig. 9B, D) (166). The observed

anticancer effects were due entirely to RT treatment (Fig. 9B) (166). The same is true for two other Mn complexes, M40403 and EUK-8, which cannot oxidize ascorbate or mimic GPx (for M40403 see Figs. 9C and 10A and Table 1) (see also Section VI) (166).

In a 4T1 mouse flank breast cancer study by Park's group the tumor radiosensitization by MnTnHex-2-PyP⁵⁺ was observed. MnTnHex-2-PyP⁵⁺ was injected ip at 2 mg/kg/day, throughout 15 days of study, starting when tumor volumes reached approximately 80–100 mm³. Tumors were irradiated at 5 Gy/day for 3 consecutive days, starting at day 3 post-MnP injections.

Is MnP/RT/Asc too aggressive a source of H₂O₂ for normal tissue to cope with? Most recently, we provided unambiguous evidence that this is not the case; normal tissue, unlike tumor tissue, has the means to cope with increased oxidative stress. The MnP/RT/Asc offers identical radioprotection to the prostate and neighboring penis as does MnP/RT (173). Using ascorbate as adjuvant therapy would significantly increase the anticancer effect of MnP/RT, whereas supporting the MnP-driven radioprotection of neighboring normal tissue.

b. Human breast cancer MDA-MB-231. The effect of MnPs on the suppression of metastatic pathways was demonstrated with ER, PR, and HER2-negative highly aggressive and metastatic cell line. The 5 μ M MnTnHex-2-PyP⁵⁺ and 100 nM doxorubicin reduced the chemotactic cell migration to 84% and proteolytic invasion to 77% of control untreated cells ($p < 0.05$) (70).

3. Head and neck cancer. Pronounced tumor growth suppression was seen in mice bearing FaDu flank xenografts when MnTnBuOE-2-PyP⁵⁺ was given sc at 2×1.5 mg/kg/day along with tumor RT, starting a week after tumor cell transfer and continued for 40 days; the dose modifying factor was 1.3. Tumors were radiated for FIVE consecutive daily fractions, ranging from 5 to 7 Gy; RT started when tumor reached a volume of approximately 200–300 mm³ (8).

In subsequent work, lower doses of MnP, which are compatible with those tested in clinical trial on the radioprotection of salivary glands and mouth mucosa with head and neck cancer patients, were used. The 0.6 mg/kg/day was injected sc on day 7 posttumor cell transplant and was followed by 0.3 mg/kg of MnP 3 days per week for the rest of the study. Mice were radiated for 3 days at doses ranging from 6 to 17 Gy. The radiation started when tumor volumes reached ~ 200 mm³ (30). Cisplatin was injected ip at 6 mg/kg 4 h before RT. No radiosensitization was seen with less frequent or lower dosing of MnP (30). However, MnTnBuOE-2-PyP⁵⁺ did not protect tumor against RT or cisplatin. Please see also review on head and neck cancer by Chen *et al.* in this Forum (48).

4. Glioma. Two lipophilic MnPs (MnTnHex-2-PyP⁵⁺ and MnTnBuOE-2-PyP⁵⁺) radio- and chemosensitized the D-245 MG glioblastoma multiforme in an sc patient xenograft mouse study. Once the tumors reached a volume of ~ 80 mm³, the MnPs were given at 2×1.6 mg/kg/day for the duration of the study, starting 24 h before RT and chemotherapy (see Fig. 11A) (24, 187, 188). Tumors were radiated with 1 Gy/day for 3 consecutive days, starting 24 h post-RT injections of MnPs. Temozolomide ip injections started concurrently with RT for 5 days at 5 mg/kg/day (24, 187, 188). In addition to tumor growth suppression, the impact of MnTnBuOE-2-PyP⁵⁺ on the suppression of different pathways, including NF- κ B and metastatic pathways, was seen (see also Section II.C.2) (24).

The stage of tumor growth and the dosing regimen controlled the magnitude of the anticancer effect. Similar to the head and neck cancer studies (see Section IV.B.3.), MnP becomes ineffective in an animal model with inappropriate dosing regimens (Fig. 11B).

5. Prostate cancer. Oberley-Deegan's team showed that MnTE-2-PyP⁵⁺ and MnTnBuOE-2-PyP⁵⁺ inhibit the growth of LNCAP and PC3 prostate tumors, which were orthotopically implanted into athymic mice; mice were subsequently exposed

FIG. 11. Therapeutic outcome depends on the correct dosing regimen. A comprehensive study of two lipophilic MnPs was conducted in a nude/nude Balb/c mice sc patient xenograft mouse model of glioma, D-245 MG (glioblastoma multiforme). Both MnTnHex-2-PyP⁵⁺ and MnTnBuOE-2-PyP⁵⁺ exhibited similar effects, acting as radio- and chemosensitizers, as shown in (A) for MnTnBuOE-2-PyP⁵⁺ (24, 187, 188). However, this effect was only demonstrated if MnPs injections started at an early enough stage in tumor growth, when its volume averaged ~ 80 mm³ (A) (24, 187, 188). The tumor suppressive effect was lost when the MnTnBuOE-2-PyP⁵⁺ administration started when the tumor volume was ~ 300 mm³ (Tovmasyan *et al.*, private communication) (B). RT was given for 3 consecutive days at 1 Gy per day. Twice-daily sc injections of MnP at 1.5 mg/kg started 24 h before RT and continued throughout the study. The ip injections of temozolomide started in parallel with RT for 5 days, at 5 mg/kg/day. Eight mice per group were analyzed.

to RT. While inducing oxidative stress in tumor, MnPs protect normal prostate tissue (see Section IV.A.5) (46).

6. Melanoma. In a B16 melanoma flank mouse model, Park's team showed the radiosensitizing effect of MnTnHex-2-PyP⁵⁺ (147). MnTnHex-2-PyP⁵⁺ was injected ip at 2 mg/kg/day, throughout 15 days of study, starting when tumor volumes reached approximately 80–100 mm³. Tumors were irradiated at 5 Gy/day for 3 consecutive days, starting at day 3 post-MnP injections. In a cellular study MnP/RT reduced cell viability, clonogenic cell survival, and DNA damage repair, and synergistically increased RT-induced apoptosis of B16 cells when cells were pre-treated with 10 μ M MnP 3 h before 4 Gy RT.

7. Skin cancer. St. Clair's group demonstrated the impressive anticancer effect of MnTE-2-PyP⁵⁺, which reduced the number of papillomas from 30 to 5 in a TPA mouse skin cancer model (see details in Section II.C.2) (196).

V. Mechanistic Considerations

A. NF- κ B is a key protein involved in the actions of Mn porphyrins

1. Impact of Mn porphyrins on oxidative stress injuries of normal tissues. Stroke and diabetes data have clearly identified NF- κ B as a major pathway involved in the suppression of cycling inflammation—the same type of pro-oxidative action as that demonstrated in cancer (22, 53, 131, 144, 146, 178). Recently, and related to type 2 diabetes (T2D), Piganelli's group also showed that during 12 weeks of feeding with high-fat diet, treatment with MnTE-2-PyP⁵⁺ resulted in significant improvements in mouse weight, hepatic steatosis, biomarkers of liver dysfunction, insulin sensitivity, and glucose tolerance, and led to the reduction in serum insulin and leptin levels. In turn, Mn prevented the progression to T2D by specifically improving liver pathology and hepatic insulin resistance in obesity (53). The effect is attributed to the inhibition of hepatic NF- κ B.

In another report, the redox-inert anionic Mn porphyrin, MnTBAP³⁻, ameliorates pre-existing obesity and improves insulin action—basis for such action is not straightforward, given the poor redox properties of this compound (33). Authors proposed that MnTBAP³⁻ reduced caloric intake and increased PKB phosphorylation and expression.

2. Anticancer actions of Mn porphyrins. S-glutathionylation is a major modification of protein thiols in oxidative stress injuries; among oxidized proteins are NF- κ B, MAPK, phosphatases, and Keap1 (36, 43, 126, 176). In our most recent 4T1 cellular and mouse flank tumor model, MnP/RT/Asc treatment increased levels of total S-glutathionylated proteins, which change was accompanied by changes in GSSG/GSH ratio and tumor growth delay (see also Section IV.B.2) (166). In agreement with such reports, our recent redox proteomics identified NF- κ B/TAB3/p38MAPK/p38 α (MAPK14)/heat shock protein 60 (HSP60) as a key network, which was S-glutathionylated in response to MnP/Asc treatment of 4T1 breast cancer cells (Table 2) (169). MnP/Asc—a H₂O₂ producing system—affected 1762 proteins, among them 942 proteins associated with >1.3-fold oxidized peptidyl cysteines (Cys), including NF- κ B, protein kinase C (PKC), p38 mitogen-

activated protein kinase (p38) MAPK, protein phosphatases type 2A, and Keap1 (Table 2) (169). The analyses of total thiol and GSSG/GSH and CysSS/Cys revealed a significant oxidative burden, accompanied with less negative reduction potential of MnP/Asc-treated *versus* untreated cells implying less reducing equivalents for the cellular metabolism (171).

A more recent study by Park's team on mouse 4T1 breast cancer cells demonstrated that MnTnHex-2-PyP⁵⁺ prevented RT-induced activation of NF- κ B as well as different MAPKs; cells were pre-treated with 10 μ M MnP for 3 h before 4 Gy RT (see Section V.B.3) (147).

In cellular studies on inflammatory breast cancer, SUM149, MnP/Asc suppressed activation of NF- κ B and extracellular signal-related receptor kinase (ERK) and decreased expression of X-linked inhibitor of apoptosis protein, XIAP, which is the most potent caspase inhibitor. Consequently, apoptosis-inducing factor, AIF, translocated into the nucleus where it induced apoptosis (63). Conversely, in another study, where normal cells (cardiomyocytes) were stressed with doxorubicin, MnPs prevented translocation of AIF into the nucleus (116). Similar effects were also seen in a mouse D-245 MG glioblastoma multiform tumor model (24, 187, 188), in which a gene array study showed that MnP/RT *versus* RT affected different pathways, NF- κ B being among those (see Sections II.C and IV).

B. Additional pathways involved in the actions of Mn porphyrins in oxidative stress injuries of normal tissues and cancer

1. Nrf2/Keap1—normal tissue. Although the abundant cysteines of Keap1 seem to be a likely target of the redox-active drugs, the Nrf2-related pathway as affected by redox-active drugs was only marginally addressed in studies on flavonoids, nitroxides, and MnPs (50, 80, 90, 106, 142, 149, 193, 198). In a rat kidney I/R model, MnTnHex-2-PyP⁵⁺-based formulation induced the expression of endogenous antioxidative defenses many of which are controlled by Nrf2: catalase, lactoperoxidase, GPx, peroxiredoxins 2, 3, and 5, thioredoxin reductase, MnSOD, and ECSOD (24, 52, 56, 193). Most recently, St. Clair's group showed that MnTnBuOE-2-PyP⁵⁺, when given to primary bone marrow cells or to C57B/L6 mice, significantly enhanced the number of HSPCs *via* activation of Nrf2 signaling pathway (195) [see Section IV.A.3 and the Carroll and St. Clair review in this Forum (40)].

2. Nrf2/Keap1—tumor. Activation of Nrf2 pathways in tumors has reportedly interfered with cancer therapies (80, 90, 106, 142, 149, 193, 198). Yet, the inhibition of Nrf2 activation by flavonoid wogonin was seen in a cellular head and neck cancer study (90). In a 4T1 breast cancer cell model, we showed that MnP/ascorbate induced S-glutathionylation of Keap1 (Table 2). Yet, had the oxidation of Keap1 by MnP resulted in a significant Nrf2 activation, the MnP-mediated tumor growth suppression would have been greatly reduced or absent (24, 166, 171, 187, 188).

The biology of Nrf2 is extremely complex (36, 50, 99). In addition to Keap1, Nrf2 may be controlled by Keap1-independent pathways, including its phosphorylation by various protein kinases (PKC, phosphatidylinositol 3-kinase/AKT, c-Jun-N-terminal kinase (JNK), ERK), some of which were reportedly affected by MnP-based cancer treatment (Fig. 12) (63, 147). Interaction with other protein partners,

FIG. 12. NF- κ B and Nrf2 pathways cross talk via different redox-sensitive MAPK, the oxidation of which affects activities of NF- κ B and Nrf2 (36, 126). The effect of MnP on the suppression of NF- κ B activity and phosphorylation of ERK, JNK, AKT, and p38MAPK in 4T1 breast cancer cells, mediated by H₂O₂, suggest the involvement of MnP-driven protein oxidation (147). The validity of such assumption is supported by a direct evidence provided from the redox proteomic study (169). Study was conducted on 4T1 tumor cells. H₂O₂ produced during MnP/Asc redox cycling induced major oxidative damage of proteins in NF- κ B pathway, most so p38MAPK and p50 and p65 subunits of NF- κ B (169). In the same study, oxidation of PKC kinase, Keap1, as well as of protein phosphatase type 2A was also demonstrated (Table 2) (42, 169). The reported interactions of MnP with each and all of different redox-sensitive signaling pathways make understanding of the origin of therapeutic effects an extremely difficult task. AKT, a serine/threonine kinase; ERK, extracellular signal-regulating receptor kinase; JNK, c-Jun-N-terminal kinase; PKC, protein kinase C. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

such as p21 (downstream target of tumor suppressor protein 53) and p62, which can compete with Nrf2 for binding of Keap1, and epigenetic factors (micro-RNAs-144, -28 and -200a and promoter methylation) may be involved in increased Nrf2 activation in cancer, as summarized in a review by Bryan *et al.* (36) and Leinonen *et al.* (99). The other possible ways of Nrf2 activation in cancer are the mutations in Keap1-Nrf2 pathways, with *CUL3* and *RBX1* the most common mechanisms disrupting the binding between Keap1 and Nrf2 or preventing ubiquitination and degradation of Nrf2. In addition, disruption of tumor suppressor phosphatase and tensin homolog, mutation in epidermal growth factor receptor (EGFR), deficiency in fumarate hydratase as well as mutated K-Ras (Kirsten-rat sarcoma viral oncogene homolog that acts as GTP (guanosine triphosphate)ase and is a part of RAS/MAPK pathway), c-Myc, and B-Raf (serine threonine kinase that participates in the MAPK/ERK signaling pathway) independently of Keap1 via MAPK, ERK, and JNK can upregulate Nrf2 (99). Figure 12 summarizes the impact of MnPs on MAPK with possible involvement in the cross talk between NF- κ B and Nrf2 (63, 147, 169).

3. MAPK—tumor. In several studies, MnPs inhibited activation of different MAPK (63, 147, 169). Park's group demonstrated that MnTnHex-2-PyP⁵⁺ enhanced RT-mediated production of H₂O₂ in a 4T1 tumor cell study, which was associated with reduced cell viability, clonogenic cell survival,

DNA damage repair, and increased RT-induced apoptosis and was abolished by catalase overexpression. MnTnHex-2-PyP⁵⁺/RT suppressed phosphorylation of JNK, p38, AKT, and ERK, inhibited NF- κ B activation, increased proapoptotic signaling of Bim (Bcl-2-like protein 11) and Bak (Bcl-2 homologous antagonist/killer), and decreased antiapoptotic signaling of Bcl-2 and Mcl-1 (myeloid cell leukemia protein) (147). The treatment also suppressed 4T1 and B16 flank tumor growth. Such data strongly suggest that, similar to NF- κ B (84, 169), the MnP/H₂O₂/GSH-driven S-glutathionylation of cysteines of MAPK might have been involved in their inactivation. Data agree well with redox proteomics, which demonstrated S-glutathionylation of p38 MAPK and protein kinase C, PKC, when 4T1 cells were treated with MnTE-2-PyP⁵⁺/Asc as a source of H₂O₂ (169) (Table 2). In another inflammatory breast cancer cell model, MnTE-2-PyP⁵⁺/Asc suppressed phosphorylation of ERK and NF- κ B (63).

4. Endogenous antioxidative systems—normal tissue. In an I/R rat kidney model, numerous endogenous antioxidative defenses were upregulated by the treatment with MnTnHex-2-PyP⁵⁺ (52, 56) (see details under Section V.B.1). The analysis of the levels of S-glutathionylated proteins in kidney samples has not been done, while it would have been beneficial in furthering our understanding of MnP actions.

FIG. 13. Differential impact of MnPs on normal tumor tissue. Data generated by us and others demonstrate that MnP, in the presence of H_2O_2 , drives radioprotection of normal tissue but suppression of tumor growth. Based on present studies, we came up with the following explanation. The rather simplified scheme aims to identify only major pathways that are involved in the actions of MnPs and presumably other metal-based drugs and which have been investigated in both tumor and normal tissue. The differential effects originate from the differences in the local redox environments of those tissues: that is, much larger tumor than normal cell/tissue levels of H_2O_2 (black circles) and MnPs (black stars). In the presence of H_2O_2 and GSH, MnP catalyzes *S*-glutathionylation of protein cysteines, described by Equations [3]–[14]. The reactants in both cells/tissues are the same and the underlying fundamental reactions would have the same rate constants. However, the different yields observed from these reactions derive from the different levels of those reactants. Due to its high intracellular levels, GSH is not a rate-limiting reactant. We have thus far demonstrated that NF- κ B, a master transcription factor, has been suppressed in both tumor and normal cells/tissues. We have also shown that HIF-1 α was suppressed in normal lungs exposed to RT and in sc flank 4T breast tumor model; HIF-1 α might have been regulated by NF- κ B (125, 162, 179, 200). The redox proteomic data show that NF- κ B pathway is the main pathways affected in 4T1 mouse breast cancer cells treated with MnP/Asc as a source of H_2O_2 —its massive *S*-glutathionylation was demonstrated (24, 63, 144, 146, 147, 166, 169). The suppression of NF- κ B is larger in tumor than normal cell/tissue, as a result of the higher levels of reactants (H_2O_2 , MnP), and leads to massive tumor apoptosis. With regard to other key transcription factor, Nrf2, we only have direct evidence for the effect of MnP on Nrf2 activation in the normal hematopoietic stem/progenitor cells; presumably, the oxidation of Keap1 cysteines by MnP might have been involved (40). Indirect evidence was obtained in a rat kidney I/R injury model, where treatment of rats with MnTnHex-2-PyP⁵⁺-containing preparation up-regulated numerous endogenous antioxidative defenses, including SODs, GPx, and peroxiredoxins (56). Although we expect that Nrf2 is not activated in tumor cells/tissues, we do not have unambiguous experimental data to validate this expectation; moreover, we do see *S*-glutathionylation of Keap1 when 4T1 breast cancer cells were treated with MnP/Asc (169). Yet, Nrf2 may be controlled in ways other than oxidation of Keap1 cysteines with MnP (see Section V.B.5). MAPK and phosphatases, as well as the cross talk between Nrf2 and NF- κ B, may be involved (Fig. 11). Both MAPK and phosphatases have redox-sensitive cysteines. Our redox proteomics indicate that MnP *S*-glutathionylates p38, MAPK, and PKC, as well as phosphatase type 2A (169). Subsequent data from Park's group (147) demonstrated the MnP-driven inactivation of p38, AKT, JNK, PKC, and ERK MAPK in 4T1 cells, which suggested the interaction of MnP with the cysteines of these entities as well; direct evidence on the *S*-glutathionylated cysteines of those kinases is still lacking. See review by Chong *et al.* (50) on the complex redox-sensitive pathways, some of which *via* NF- κ B may be involved in the actions of MnPs. p38, p38 mitogen-activated protein kinase.

5. Endogenous antioxidative systems—tumor. A number of cellular antioxidants, listed in Table 2, such as thioredoxin, glutaredoxin, and peroxiredoxin were *S*-glutathionylated and presumably inactivated when 4T1 breast cancer cells were jointly treated with MnTE-2-PyP⁵⁺ and ascorbate thus disabling tumor metabolism (169).

C. Why is normal tissue protected from injury by the action of Mn porphyrins, while cancer undergoes apoptosis?

NF- κ B is the only transcription factor thus far extensively studied in both normal and tumor cells/tissues. Most of the

data on other signaling proteins relate to tumor cells/tissues. Collectively, those data indicate that the interactions of MnPs with cellular targets are extremely complex. Future studies are essential to increase our understanding how the cross talk of those pathways results in suppression of tumor growth while survival of a normal cell/tissue.

As of now our understanding of the differential effects in normal *versus* tumor tissue is as follows. The rate constants for the *S*-glutathionylation of protein cysteines by MnPs should be identical in both types of cells; presently we see no reason for MnP to distinguish tumor from normal tissue. However, the yield of the protein oxidation would depend on the localization and concentration of species impacting the

cellular redox environment: MnP, H₂O₂, protein thiols, and GSH. Given its high (mM) concentration, GSH may not be a limiting factor.

The growing knowledge on the redox biology has provided evidence for the higher levels of RS (in particular H₂O₂) in cancer *versus* normal cells (49, 57, 104, 119, 140). Five, thus far tested, MnPs accumulate in tumors up to 10-fold more than in normal cells (three MnPs are shown in Fig. 9D) (137, 166, 171, 191). With high H₂O₂ and MnP levels, the yield/extent of protein oxidation in cancer cells will be high enough to induce vast inactivation of NF- κ B, thereby promoting apoptotic events (Fig. 13). In normal cells, a modest inhibition of NF- κ B by MnP, accompanied by noncompromised endogenous antioxidative defenses, will suppress excessive tissue inflammation and help restore the physiological redox environment (Fig. 13).

No cytotoxicity to several normal cell lines by the MnP/H₂O₂ system was seen under the conditions identical to those which induce apoptotic events in cancer cell [see details in Batinic-Haberle *et al.* (24)]. Furthermore, the protection of tumor tissues/cells by MnPs combined with RT and/or chemotherapy and/or ascorbate was not seen (24, 27, 167).

VI. Mn Porphyrins Versus Other Redox-Active Drugs

Many lessons learned from MnPs have improved our understanding of the chemistry and biology of other metal-based redox-active drugs, such as Mn(III) salens, Mn(II) pentaazamacrocycles (16, 17, 24, 25, 27, 28, 115, 172), and nonmetal-based compounds (nitrones, nitroxides, flavonoids) (89, 159). Different redox properties of different classes of Mn complexes arise from differences in their structures, which in turn control the type and magnitude of their reactions (Table 1).

Data from us and others clearly show that there is still a lot to be explored and clarified with regard to redox-active drugs. The remarkable therapeutic effects seen with those compounds discussed here and elsewhere (89, 159, 166), although not yet fully understood, have impelled their progress into clinical trials and thus merit future studies. For the first time in drug discovery, we have a real chance to see in clinical practice those metal complexes that target cellular redox environment.

A. Pentaazamacrocycles, GC4403 (M40403) and GC4419

GC4419, the enantiomer of M40403 (GC4403), is in a clinical trial for the same application as MnTnBuOE-2-PyP⁵⁺—radioprotection of normal tissue in head and neck cancer patients (4, 5). Both compounds are potent SOD mimics, but differ in their redox chemistry and physical properties (Table 1). For example, Mn porphyrins are pentacationic, whereas M40403 is a neutral molecule. MnTnBuOE-2-PyP⁵⁺ is stable in Mn +3 oxidation state, whereas +2 is the resting state of Mn in M40403. As a result, they operate through different redox cycles with biological molecules. MnTnBuOE-2-PyP⁵⁺ is directly reduced with ascorbate and reoxidized with oxygen, thereby producing O₂^{•−} and subsequently H₂O₂. The production of H₂O₂ is limited only by the availability of oxygen and HA[−], both of which are abundant. However, M40403 cannot be further reduced; it can only be oxidized by O₂^{•−} to the Mn

+3 oxidation state and then reduced back with HA[−] producing A^{•−} (monodeprotonated ascorbyl radical, pK_a=1.21). A^{•−} can be further oxidized with oxygen to dehydroascorbate (DHA), giving rise to O₂^{•−}, although with a low rate constant of $\sim 10^2 \text{ M}^{-1} \cdot \text{s}^{-1}$, which is ~ 3 log unit lower than A^{•−} self-dismutation (114). The reaction of A^{•−} with oxygen is further 6–7 log units slower than the reaction of A^{•−} with O₂^{•−} ($k > 10^8 \text{ M}^{-1} \cdot \text{s}^{-1}$), the latter giving rise to H₂O₂ [for the reaction constants see Ye *et al.* (191)]. In this scenario, the levels of O₂^{•−} (commonly at nM) limit the yield of oxidized M40403 and its reaction with HA[−]. While endogenous metal complexes (such as oxidases) are not tuned for the catalysis of ascorbate oxidation, they reportedly catalyze oxidation of pharmacological ascorbate, which in turn gives rise to H₂O₂ (47, 81). M40403 can possibly react with [•]NO; reactions with ONOO[−] and ClO[−] were not reported; most recent data indicate that M40403 falls apart in the presence of ONOO[−] and H₂O₂ [see data in Filipovic *et al.* (69) and Maroz *et al.* (111)].

Mn complexes may utilize H₂O₂ in the catalysis of protein cysteine oxidation/S-glutathionylation in a GPx-like manner; such actions would modulate the activity of numerous signaling proteins, such as Nrf2, NF- κ B, MAPK, and phosphatases. However, our studies demonstrated that M40403 is neither able to directly oxidize ascorbate nor mimic GPx. Consequently, no effect of M40403 on GSSG/GSH ratio was seen (Table 1 and Fig. 10A) (166). Moreover, M40403 is found to be neither a tumor radio- nor chemosensitizers in a 4T1 mouse breast cancer study (Figs. 9 and 10) (166).

M40403, as well as its analogs, is not a stable metal complex and readily loses Mn *in vivo* (166, 183). The impact of such instability on the therapeutic effects of M40403 requires exploration. Conversely, Mn porphyrins, which have Mn in +3 oxidation state, are extremely stable with regard to the loss of Mn.

Yet, significant differences exist between different Mn porphyrins, those that are SOD mimics (MnTnBuOE-2-PyP⁵⁺ and MnTE-2-PyP⁵⁺), and those that are not (*e.g.*, MnTBAP^{3−}). Only MnP-based potent SOD mimics sensitize tumors against RT and ascorbate (Fig. 9 and Table 1) (166). MnTBAP^{3−} can neither oxidize ascorbate nor mimic GPx (Table 1), which precluded its ability to enhance the anticancer effect of RT/ascorbate (166). Yet, the fairly inert and SOD-inactive MnTBAP^{3−} continuously appears efficacious in different models of normal tissue oxidative stress injuries; such studies remind us how little we know about the whole class of MnPs (13, 33, 44, 95, 105). The differences among three types of SOD mimics (M40403, EUK-8, and MnPs) have been most recently detailed in Tovmasyan *et al.* (166).

B. Mn(III) salens

The third group of Mn complexes, Mn(III) salens (such as EUK-8), has Mn in a +3 oxidation state. Nonetheless, they are still unstable and exhibit poor SOD-like activity (Table 1). EUK-8 was neither able to sensitize tumor to radiation nor to ascorbate in a mouse 4T1 breast tumor study, which is due to its poor ability to oxidize ascorbate or mimic GPx (Table 1) (166). In MnSOD-deficient *Cryptococcus neoformans*, however, only Mn(III) salen offered protection against high temperatures, whereas none of MnPs did. The data were discussed in light of Mn salen transporting manganese into the

mitochondria. Such type of action may also explain the *in vivo* efficacy of Mn(II) pentaaza macrocycle, M40403 (183). The study on *C. neoformans* reminds us of how little we understand the complex redox chemistry of metal compounds.

C. Redox-active nonmetal-based nitrones, nitroxides, and flavonoids

These classes of redox-active drugs affect some of the same pathways as metal compounds (17, 115). Nitroxides, which are not SOD mimics, get oxidized to oxo-ammonium cations by $\text{CO}_3^{\bullet-}$ (degradation product of ONOO⁻ adduct with CO_2) and then reduced back to nitroxide with $\text{O}_2^{\bullet-}$; in turn they can modulate the cellular levels of $\text{O}_2^{\bullet-}$ (yet not in SOD-like fashion) (17). Flavonoids may affect similar superoxide-mediated pathways by using one-electron hydroxo/semiquinone/quinone redox cycling (89, 159).

VII. Concluding Remarks

In conclusion, we can safely state that the differential redox environments of cancer *versus* normal cell, as well as the differential distribution of MnPs within those cells, control MnP therapeutic effects. Several tumor and normal tissue studies strongly suggest that suppression of NF- κ B pathway is at least one of the major targets of MnPs (83, 166, 178). The data demonstrate that the components of NF- κ B pathway such as p38MAPK, p38 α (MAPK14), TGF- β activated kinase 1 and MAP3K7 binding protein 3 (TAB3), and HSP60, as well as Nrf2/Keap1 and endogenous antioxidative defenses, are involved in MnP actions (Table 2) (56, 63, 82, 143, 147, 169, 178, 195). The major limitation of our insight into the actions of MnPs has been the lack of studies that explore these pathways in both tumor and normal cell/tissue in the same animal. Future studies are needed to show how the cross talk of those pathways is orchestrated to give rise to suppression of tumor growth while survival of a normal cell.

On the molecular level we have learned the following. Once inside cell, MnP has no intelligence with which to distinguish one type of tissue from another. The type of reaction will primarily depend on the type of species MnP will encounter. The yield of the reaction, that is, the amount of the product formed, will depend on the frequency/probability of collisions, that is, the relative concentrations of reactants. However, the fundamental reactions will most likely proceed with the same rate constants in both types of cells/tissues.

With much higher MnP and H_2O_2 levels in tumor *versus* normal cell/tissue (24, 49, 57, 104, 119, 137, 140, 166, 171, 191), we may safely predict a higher yield of MnP/ H_2O_2 -driven oxidation and inactivation of proteins resulting in a prevalence of apoptotic processes in tumor (24, 83, 84, 147, 166, 169). In normal tissue, however, a lower yield of MnP/ H_2O_2 -driven protein oxidation and inactivation (such as NF- κ B) will result in suppression of excessive inflammation and restoration of the tissue physiological redox environment (Fig. 13) (7, 29, 53, 144).

Neither protection of tumor nor cytotoxicity to normal cells/tissues was seen with treatments involving Mn porphyrins and H_2O_2 : MnP/radiation, MnP/chemotherapy, MnP/ascorbate (24, 25, 27, 46, 83).

Regardless of the tissue type, the major actions of Mn porphyrins occur through their pro-oxidative reactions; yet the

effects observed in normal tissue are those we traditionally regard as antioxidative. Such type of therapeutic effects has led to the common perception of these redox-active drugs acting as antioxidants. The most obvious case was the I/R kidney rat model. There, MnP induced the upregulation of numerous endogenous antioxidative defenses (such as SODs, GPx, and peroxiredoxins), presumably as a result of the oxidation of Keap1 and subsequent activation of Nrf2. In turn we observed the reduced oxidative stress that made us think MnP acted as antioxidant (56, 195). The beneficial effect of MnTnBuOE-2-PyP⁵⁺ on the activation of Nrf2 was substantiated with hematopoietic progenitor/stem cells *via* oxidative transcription profiling analysis and quantitative polymerase chain reaction on some of the Nrf2 target genes (195). Our terminology also depends on how we view those compounds: based on the effects we see or on the type of actions that result in such effects.

Presently, two redox-active metal complexes have reached Phase I/II clinical trials: an enantiomer of Mn(II) pentaazamacrocyclic, M40403 (GC4419), and Mn porphyrin, MnTnBuOE-2-PyP⁵⁺. Among other trials, both compounds are tested on a same therapeutic application—protection of normal tissue during radiation of head and neck cancer patients. These compounds are similarly potent SOD mimics. However, they exhibit markedly distinct redox-based chemistry and metal/ligand stability. MnP is extremely stable, whereas M40403 is a metal complex of modest stability (with respect to the loss of Mn) (see also under Section VI.A). Their performance in clinical trials will provide us with invaluable information on the *in vivo* biology of the whole class of metal-based redox-active drugs.

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Address correspondence to:

Dr. Ines Batinic-Haberle

Department of Radiation Oncology

Duke University School of Medicine

MSRBI, 201B

Durham, NC 27710

E-mail: ibatinic@duke.edu

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Abbreviations Used

A^{•−} = monodeprotonated ascorbyl radical,
pK_a = 1.21

AIF = apoptosis-inducing factor

AKT = a serine/threonine kinase

AP-1 = activator protein 1

Bcl-2 = B cell lymphoma 2

c-Myc = regulator gene that codes for a
transcription factor

DLBCL = diffuse large B cell lymphoma

E_{1/2} = half-wave reduction potential

ECM = extracellular matrix

Abbreviations Used (Cont.)

ER = estrogen receptor
 ERK = extracellular signal-regulated receptor kinase
 EUK-8 = Mn(III) salen complex
 GI = gastrointestinal
 GPx = glutathione peroxidase
 GSH = glutathione
 GSSG = glutathione disulfide
 GU = genitourinary
 HA⁻ = monodeprotonated form of ascorbic acid, Asc, is a major species in aqueous solution at pH 7.8, $pK_a = 4.45$
 HER2 = human epidermal growth factor receptor 2
 HIF = hypoxia inducible factor
 HSC = hematopoietic stem cell
 HSP60 = heat shock protein 60
 HSPCs = hematopoietic stem/progenitor cells
 I/R = ischemia/reperfusion
 ip = intraperitoneal
 iv = intravenous
 JNK = c-Jun-N-terminal kinase
 Keap1 = Kelch-like ECH-associated protein 1
 M40403,
 GC4403 = Mn(II) pentaazamacrocyclic; GC4419, the enantiomer of M40403
 MAPK = mitogen-activated protein kinases
 MCAO = middle cerebral artery occlusion
 MnBr₈TM-3
 (or 4)-PyP⁴⁺ = Mn(II) *beta*-octabromo-*meso*-terakis (N-methylpyridinium-3(or 4)-yl) porphyrin
 MnPs = Mn(III) porphyrins
 MnSOD = mitochondrial isoform of SOD
 MnTBAP³⁻ = Mn(III) *meso*-tetrakis(4-carboxylatophenyl)porphyrin
 MnTDE-2-ImP⁵⁺,
 AEOL150 = Mn(III) *meso*-tetrakis(N,N'-diethylimidazolium-2-yl)porphyrin
 MnTE-2-PyP⁵⁺,
 AEOL10113,
 BMX-010, MnE = Mn(III) *meso*-tetrakis(N-ethylpyridinium-2-yl)porphyrin

MnTM-2-PyP⁵⁺ = Mn(III) *meso*-tetrakis (N-methylpyridinium-2-yl)porphyrin
 MnTnBuOE-2-PyP⁵⁺,
 BMX-001,
 MnBuOE = Mn(III) *meso*-tetrakis(N-(2'-n-butoxyethyl)pyridinium-2-yl)porphyrin
 MnTnHex-2-PyP⁵⁺,
 MnHex = Mn(III) *meso*-tetrakis(N-n-hexylpyridinium-2-yl)porphyrin
 MnTnOct-2-PyP⁵⁺ = Mn(III) *meso*-tetrakis(N-n-octylpyridinium-2-yl)porphyrin
 NF- κ B = nuclear factor κ B
 NHE = normal hydrogen electrode
 NOAEL = no observed adverse effect level
 NOX4 = NADPH oxidase 4
 Nrf2 = nuclear factor E2-related factor 2
 O₂^{•-} = superoxide
 ONOO⁻ = peroxynitrite
 p38 = p38 mitogen-activated protein kinase
 PK = pharmacokinetics
 PKC = protein kinase C
 PR = progesterone receptor
 ROS = reactive oxygen species
 RS = reactive species
 RS⁻ = deprotonated protein thiol
 RT = radiation therapy
 SAR = structure/activity relationship
 sc = subcutaneous
 SMAD = SMAD proteins are homologs of both the *Drosophila* MAD, mothers against decapentaplegic protein, and the *Caenorhabditis elegans* SMA protein (from gene *sma* for small body size)
 SP-1 = specificity protein 1
 SOD = superoxide dismutase
 T2D = type 2 diabetes
 TAB3 = TGF- β activated kinase 1 and MAP3K7 binding protein 3
 TGF- β = transforming growth factor- β
 TPA = 12-O-tetradecanoylphorbol-13-acetate
 VEGF = vascular endothelial growth factor
 VIC = valve interstitial cells