EDITORIAL

Part II

As a continuation of Part I, herein Garry Buettner [1] addressed the critical role of manganese superoxide dismutase, MnSOD - the enzyme that we cannot live without. MnSOD is a central player in the redox biology of cells and tissues [1]. It is critical for establishing the appropriate balance in redox circuitry of the mitochondria. Thus, there has been an increasing demand to develop powerful SOD mimics. In the studies where MnSOD was overexpressed, the increased levels of H_2O_2 were found, which suggests that H_2O_2 has a major role in metastases. The loss of MnSOD is likely an early event in tumor progression allowing for further propagation of the tumorigenic phenotype resulting from the steady state increases in free radical production [2]. Garry Buettner contribution, and manuscripts by Irwin Fridovich [3] and Lee Ann MacMillan Crow and John Crow [4], as well as from Melendez [2] and St. Clair [5] groups addressed the possible, and still controversial origin of the increased peroxide as a direct or indirect consequence of MnSOD overexpression. More work is needed to gain a profound insight into the dichotomous role of MnSOD as a tumor suppressor or oncogene [2]. Similarly, much is to be learned about redox-based compounds developed originally as SOD mimics. Major classes of such compounds are addressed in this Issue: Mn porphyrins, metallotexaphyrins, Mn salens, metallocorroles, nitroxides, nitrones, and quinones. Many were shown to exert anticancer effects, acting as tumor suppressors as exemplified with Mn porphyrins in contribution from Keir *et al*., [6], corroles by Zeev Gross group [7], texaphyrins by Jonathan Sessler group [8], nitrones by Robert Floyd *et al*., [9], and quinones by Pedro Buc Calderon group [10]. However, the mechanism of action of those compounds is not yet fully understood.

Cationic Mn(III) *N*-substituted pyridylporphyrins are potent SOD mimics and protect SOD-deficient *E. coli* when it grows aerobically. Ines Batinic-Haberle and Ludmil Benov groups showed that in the presence of cellular reductant ascorbate, which is abundant *in vivo*, Mn porphyrins suppressed *E. coli* growth *via* cytotoxic H_2O_2 production. Under milder conditions and in a rich growing medium, over time the adaptive response of *E. coli* was observed, whereby *oxyR* regulon was induced and endogenous antioxidants - peroxide-removing enzymes, peroxidases and catalases upregulated [11]. The data exemplify how a pro-oxidative event could exert antioxidative effects [11]. Such data caution us to differentiate between the nature of the actions of synthetic antioxidants and the type of the effects observed.

A manuscript by Robert Floyd *et al*., [9] discusses the potent anticancer effects of nitrones observed in three experimental cancer models: (1) the rat choline-deficiency liver cancer model; (2) the rat C6 glioma model; and (3) the mouse $APC^{Min/+}$ colon cancer model. Originally, nitrones were developed as spin traps for free radicals. The two mostly studied nitrones are α-phenyl-*tert*-butylnitrone (PBN) and its derivative, 2,4-disulfophenyl-*tert*-butylnitrone (OKN-007, formerly known as NXY-059 and developed earlier for stroke therapy [9]). The ionic PBN derivative was shown to cause shrinkage of fully formed tumors in the rat C6 glioma model. The extensive human safety studies, showing that it is a safe compound to use, combined with its demonstrated potency to decrease the size of tumors, makes it an ideal candidate for clinical trials, which Robert Floyd is currently pursuing. Again, the mechanism of action has not yet been fully understood. The decreased $^{\cdot}NO$ production due to the PBN-mediated suppression of iNOS expression, S-nitrosylation of critical proteins such as caspases and Bcl-2, as well as free radical scavenging, may play a role. Nitrones were previously shown to exert general anti-inflammatory effects. The exacerbated inflammatory cellular signaling processes are suppressed by their administration [9]. The inhibition of NF-κB master transcription factor was reported with PBN, and needs to be tested with OKN-007 [9]. Mn porphyrin, MnTnHex-2-PyP^{5+} has been shown in this Issue [6] to exert anticancer effect in mouse glioma study. Both Mn porphyrin and nitrones have been shown to affect cellular transcriptional activity as they inhibit NF-kB activation [6,9]. Both also scavenge reactive species [6,9]. It may be challenging, from therapeutic and mechanistic perspective, to compare Mn porphyrin action to the anticancer effect of nitrones in the same glioma cell line.

The other papers in Part II of this Issue relate to three different classes of redox-active compounds developed originally as SOD mimics. The metal-containing compounds are Mn(III) corroles developed by Zeev Gross group [7], and Mn(III) salen derivatives developed by Susan Doctrow group [12]. The third group comprises the nitroxides, described by Ryan Davies *et al*., [13].

Susan Doctrow group reported the therapeutic potential of Mn(III) salen derivatives as mitigators of normal tissue injury of kidney, lung, skin and oral mucosa resulting from ionizing radiation [12]. The data obtained with EUK-134, EUK-189 and EUK-207 are presented. Among these Mn salen compounds, EUK-207 is of particular interest, as its cyclic structure affords additional stability to the molecule. A significant efficacy was exerted by EUK-207 in a mitigation of thoracic and renal radiation injuries. For example, a full normalization of breathing rate frequencies at 28 weeks was observed when EUK-207 administration of 8 mg/kg sc for 14 weeks started 1 hour after 10 Gy thoracic irradiation. DNA damage was significantly reduced as were the levels of collagen and hydroxyproline; the latter are indicators of lung fibrosis [12].

Zeev Gross group is designing and exploring the therapeutic potential of modified porphyrins – corroles [7]. With one less *meso* position, Mn site is coordinated with tri-anionic corrole ligand, and is thus more electron rich and stabilized in a higher oxidation state than it is in a complex with porphyrin. Thus, the metal complexes are extremely stable with respect to demetallation process. Consequently, the optimization of a corrole-based drug requires a different approach than of Mn porphyrins. Thus far, the most prospective corrole for clinical development is anionic compound with two sulfonato groups in pyrrolic positions and three pentafluorophenyl groups in three *meso* positions. The Fe and Mn complexes are aimed for treating diseases characterized by the excessive production of reactive species as they possess superoxide and peroxynitrite scavenging capacity [7]. The fluorescent Ga analogue, though, has cytotoxic properties and is thus perspective as an anticancer agent [7]. In their contribution, the authors report data on cellular uptake and organ accumulation of Ga corrole [7]. When conjugated to protein with tumor-targeting and cell-internalization motif, this corrole accumulates in tumors.

While not potent SOD mimics, nitroxides are scavengers of a variety of other reactive species such as peroxynitrite and its progeny, $CO_3^{\cdot-}$ and $^{\cdot}NO_2$ [13]. Due to their paramagnetic nature they have been used as spin probes for electron paramagnetic resonance imaging as well as contrast agents for magnetic resonance imaging. *In vivo* they undergo the reduction to hydroxylamine. The rate of reduction is dependent upon the redox status of the cell, thus they bear potential for differential imaging of tumor *vs* normal cell. Recently, they have been explored as radioprotectors of normal tissue due to the higher levels of Tempol and 3-carbomyl proxyl (3-CP) found in healthy relative to tumor tissue [13].

All compounds addressed in this Issue have been either developed originally or tested later as SOD mimics and/or peroxynitrite scavengers. To act as SOD mimics they must be able to fairly equally reduce and oxidize superoxide, $O_2^{\cdot-}$. That means that they can fairly easily accept and donate electrons and consequently be involved in the reduction and oxidation of the biological molecules, reactive species,

proteins, lipids, etc. Most of those compounds can interfere with transcription factors, such as NF-κB, which action has been shown, at least with Mn porphyrins, to be oxidative in nature - glutathionylation of p60 subunit of NF-κB was suggested [14]. Glutathionylation has been recently reported as a major protein modification involved in signaling processes [15,16].

As clearly shown, under reducing conditions here [11] and elsewhere, MnPs could *in vivo* catalyze H_2O_2 production. By yet not fully understood pathways, MnSOD overexpression reportedly [1] results in increased H_2O_2 levels. Is this a coincidence, or a consequence of a common mechanism operative with both enzyme (MnSOD) and its mimic?

In summary, the manuscripts presented in this Issue show that further studies are needed to comprehend the normal and diseased cell physiology, in order to successfully treat human diseases. The understanding of the redox biology of *in vivo* systems, and the chemistry and biology of synthetic redox-active compounds will remain a challenge and a motivation for our future endeavors.

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