EDITORIAL

Part I

The Special Issue titled "***SOD enzymes and their mimics in cancer: pro- vs anti-oxidative mode of action"*** aimed at challenging our thoughts on what is the true mechanism behind the beneficial effects we observe with redox-active compounds, both endogenous and exogenous, in different *in vitro* and animal models of diseases, which could help us in treating human diseases more successfully.

SOD enzymes were proved essential for the functioning of our body in numerous publications since their mechanism of action had been established nearly half a century ago by McCord and Fridovich in a seminal *J Biol Chem* paper on Cu,ZnSOD [1]. Moreover, the mitochondrial Mn form of superoxide dismutase, MnSOD, is absolutely essential to our lives; the MnSOD-knockout mice survive only days after birth. It is therefore no wonder that therapeutic strategies targeting mitochondria have been sought. The review on MnSOD in this Issue was prepared by Daret St. Clair who has dedicated herself to understanding the critical role of this enzyme under physiological and pathological conditions and most specifically to the role of mitochondria in cancer [2].

Through the years a substantial body of evidence has emerged from a number of groups for an anticancer role of MnSOD. While this is true for many cancers Melendez and coworkers, in their chapter, discuss the strong connection between high level MnSOD expression and redox-signaling in metastatic disease. The effects were ascribed to the increased levels of H_2O_2, that was thought to be a direct consequence of overexpression of MnSOD. However the possibility is allowed, and elaborated by Melendez group in this Issue, that MnSOD overexpression could modify the cellular redox environment to indirectly enhance the production of $O_2^{\cdot-}$ and H_2O_2 [3]. Several possible mechanisms of peroxide increase as a consequence of MnSOD overexpression, other than that resulting directly from MnSOD action, were proposed by Irwin Fridovich [4]. Further, Lee Ann MacMillan-Crow and John Crow reported that in their hands overexpression of MnSOD did not lead to increased levels of peroxide [5]. MacMillan-Crow and John Crow also noted that both peroxynitrite and peroxide behave similarly with respect to the effects that they produce in assays which we routinely use for measurements of reactive species. Hardly an assay exists that is specific to a particular reactive species. Thus, what we attribute to the increased levels of peroxide might indeed relate to the increased peroxynitrite levels. Based on their experiments, the authors illustrate how MnSOD overexpression could lead to peroxynitrite formation.

Recently it became obvious that both the activity of redox-active proteins and their expression must be studied in order to fully comprehend their *in vivo* actions and their role in cellular signaling processes. For example as shown by Terry Oberley, the thioredoxin levels may be upregulated, while its activity suppressed due to the excessive oxidation of thioredoxin SH groups [6]. The references listed in the contribution from the Melendez group in this Issue have shown that such scenario may be operative for MnSOD also [3].

Understanding the impact of SOD enzymes on our health has resulted in a desire to mimic their activity. A number of compounds have been developed and used in different models of diseases with varying success over the last three decades: metalloporphyrins, Mn(III) salens, Mn(II) cyclic polyamines, nitroxides, different metal oxides [reviewed in 7], quinones and others. A number of naturally occurring antioxidants have been tested also. For a long time, all of those compounds were viewed as antioxidants. To be as successful SOD mimic as the enzyme itself, the compound must reduce and oxidize $O_2^{\cdot-}$ with equal rate constants. For that to happen, the metal-centered reduction potential must be positioned at the midway between the potential for the oxidation and reduction of $O_2^{\cdot-}$ (~+300 mV *vs* NHE); that fact immediately tells us that powerful SOD mimics are equally good pro-oxidants as they are antioxidants. Years ago, Barry Halliwell wrote an article questioning the actions of polyphenols [8]. The role of ascorbate was questioned too [9], particularly in the presence of metals and metal complexes [10]. Are such compounds pro- or antioxidants? The same question holds true for the metalloporphyrins also. The Mn(III) cationic *N*-substituted pyridylporphyrins are very active in the dismution of $O_2^{\cdot-}$ [11]. Further, it has been shown that under certain circumstances in the presence of ascorbate, the pro-oxidative action of Mn porphyrins is enhanced. In such a scenario metal complexes (or other redox-active compounds) catalyze the ascorbate-driven peroxide production, as evidenced by us and others [12, 13], and proposed by Chen *et al*., to result in anticancer effects [14, 15]. An ascorbate/menadione system has been approved by FDA for the treatment of metastatic, locally advanced, inoperable transitional cell carcinoma of the urothelium (stage III and IV bladder cancer). The ascorbate/ menadione system is discussed by Pedro Buc Calderon group in this Issue [16]. Ines Batinić-Haberle and Ludmil Benov groups (Part II) have recently shown that Mn porphyrins clearly act as SOD mimics in allowing SOD-deficient *E. coli* to grow aerobically, but become cytotoxic in the presence of ascorbate. Depending upon the circumstances, such action provoked an adaptive response and we observed the upregulation of endogenous antioxidative defenses [12]. Under stringent conditions, at high concentrations and longer exposure to proxidants, the cells were not able to adapt and died [12]. With redundant peroxide–removing systems (catalases, glutathione peroxidases, glutathione reductases, glutathione transferases, thioredoxins, peroxyredoxins) such pro-oxidative action of Mn porphyrins would not create a problem to a normal cell. Tumors are frequently under oxidative stress and also have compromised reactive species-removing systems, particularly an inadequate ratio of superoxide- to peroxide-removing systems. Thus they may not be able to tolerate the additional oxidative burden and would perish. Therefore, under particular circumstances and in the presence of cellular reductants such as ascorbate and glutathione, and at inadequate ratio of superoxide- to peroxide removing systems, metalloporphyrins could act as powerful cytotoxic agents and kill tumor *via* excessive production of reactive species.

This Issue covers the first report by Stephen Keir, Mark Dewhirst, John Kirkpatrick, Darell Binger and Ines Batinić-Haberle of the antitumor properties of lipophilic Mn porphyrin, MnTnHex-2-PyP^{5+} as a single agent in adult and pediatric brain tumors [17]. Data were obtained on intracranial and subcutaneous xenografts implanted to Balb/c nu/nu mice. It is also the first report where a redox-active metal complex has been used in glioma therapy. The possible mechanisms of actions, anti- or pro-oxidative with respect to reactive species, transcription factors and porphyrin localization, were discussed in details [17].

When a redox-active metal site of a metalloporphyrin is replaced by a redox-inactive Zn, photosensitizers are produced. They kill cancer cells *via* increased production of singlet oxygen [18]. Their actions and therapeutic potential were described by Ludmil Benov group in this Issue.

Given the abundance of ascorbate and other cellular reductants *in vivo,* any compound with a reduction potential of > +50 mV *vs* NHE could oxidize ascorbate and thus modulate the cellular redox poise. Pedro Buc Calderon showed that even compounds with highly negative potentials such as quinones are able to couple with ascorbate; such events were explained on kinetic grounds [16]. The modified porphyrins, called texaphyrins produce anticancer effects *via* pro-oxidative action, as shown by Jonathan Sessler group [19]. Texaphyrins are now in development as anticancer agents and for the treatment of the neurodegenerative disorder - amyotrophic lateral sclerosis [20]. Newer texaphyrin-based applications associated with targeted drug delivery are described also [19].

The topic covered in this Issue stresses once again the complexity of *in vivo* systems with respect to the therapeutic strategies we apply to modify them under pathological conditions. A number of factors determines the nature of the action/s of endogenous and exogenous antioxidants which result in favorable therapeutic effects: levels of reactive species and level of oxygen, levels of endogenous antioxidants, enzymes and low molecular-weight compounds, levels of synthetic redox-active compounds and their cellular and subcellular accumulation and the mutual encounters of all of those.

The complexity of the *in vivo* systems may be best exemplified with actions of Mn porphyrins with respect to the NF-κB transcription factor. Margaret Tome provided evidence that the Mn porphyrin, MnTE-2-PyP^{5+}, known for decades as a SOD mimic, glutathionylates p65 of NF-κB [21, 22]. Glutathionylation and consequently inactivation of mitochondrial proteins has recently been reported by Enrique Cadenas as a major consequence of oxidative stress, facilitated by GSSG trapped in mitochondria. This modification was observed primarily with the ATP synthase-α subunit and succinyl-CoA transferase [23]. Earlier, it was suggested by Piganeli and Tse that several Mn porphyrins, can oxidize the p50 subunit of NF-κB (presumably its cys 62) thus preventing NF-κB activation [24, 25]. At our present knowledge, the experimental data related to either p65 or p50 subunits of NF-κB, could only be explained on the basis of the pro-oxidative actions of a Mn porphyrin. The vastly enhanced anticancer effects of glucocorticoids provided by Mn porphyrin were observed in the former case [21, 22]. In the latter case, the Mn porphyrins were able to attenuate diabetes and protect human islet cells during isolation; the NF-κB inhibition by Mn porphyrin was shown to suppress excessive inflammatory and immune responses [24, 25]. While the antioxidant effects of Mn porphyrins were observed in diabetes-related studies [24, 25], their true action was pro-oxidative. Such data warn us to differentiate between the actual mechanisms of action of redox able compounds, and the effects we observe.

In summary, the complexity of *in vivo* systems on one side, and the complex redox reactions of redox-active therapeutics on the other side, and in particular with respect to mitochondria, makes our research even more challenging. Further work is needed to understand the true mechanism of action/s of redox active compounds under specific conditions that result in effects we observe. I hope that the papers in this Issue would stimulate our motivation and enthusiasm for deepening our knowledge of the redox-based pathways in cellular metabolism under normal and pathological conditions, which would help us to be more successful in treating diseases.

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