

# Preface

The inauguration of this book marks ~ half a century since the inception of the field of Free Radicals in Biology and Medicine with the seminal discovery of the major endogenous antioxidative defense, Cu,Zn superoxide dismutase enzyme (Cu,ZnSOD), by Irwin Fridovich and Joe McCord in 1969 [1].

The discovery of Cu,ZnSOD was followed by discoveries of mitochondrial (MnSOD) and extracellular (Cu,ZnSOD) isoforms. The subsequent studies of Babior et al. [2] supported the biological relevance of superoxide ( $O_2^{\bullet-}$ ) demonstrating that this radical is formed, as a part of antibacterial strategy, by the action of NADPH oxidases in white blood cells. Over years it has been demonstrated that a number of enzymatic systems, oxidases, oxygenases, nitric oxide synthases, complexes I and III of mitochondrial respiration, and others produce  $O_2^{\bullet-}$  (intentionally or not) and subsequently and rapidly  $H_2O_2$  (enzymatically or not) under physiological and pathological conditions. Throughout half a century of research, immense knowledge has been collected on free radicals and other reactive species demonstrating their critical roles in redox biology of healthy, metabolically stressed and neoplastic cells. This in turn has inspired numerous studies that explore therapeutic approaches, many of which are covered in this book, to normalize physiological redox status in normal but diseased cells and induce apoptosis of cancer cells.

Ten years after the discovery of the SOD enzyme, the first study on an SOD mimic was reported by Pasternack and Halliwell [3]. The authors demonstrated the SOD-like activity of an Fe porphyrin. In the early 1980s Archibald and Fridovich [4] showed that Mn salts, such as Mn(II) lactate, possess high SOD-like activity thereby justifying the existence of organisms that accumulate mM levels of Mn to overcome their lack of an SOD enzyme. The first report on the Mn salen class of SOD mimics (EUK-8) appeared in 1993 [5]. One of those, EUK-134, is in use as an active ingredient in sunscreen products. Meanwhile Irwin Fridovich's group embarked on decades-long development of porphyrin-based SOD mimics. A highly efficacious Mn porphyrin-based SOD mimic was reported in 1997 by Ines Batinić-Haberle [6], setting the stage for the design of multiple redox-active metalloporphyrins on the basis of structure-activity relationships. In parallel with Mn porphyrin-based SOD mimics, works by Dennis Riley's group [7] gave rise to the

Mn(II) cyclic polyamine (aza-crown ethers) class of potent SOD mimics. Compound leads from both porphyrin and polyamine classes of SOD mimics are presently in clinical trials as radioprotectors of normal tissue. While not SOD mimics, in the early 1990s the redox-active (non-metal based) nitrones and nitroxides have been developed as therapeutics [8]. The OKN-007 nitrone (also known as NXY-059) went through clinical trials for stroke and is presently in a clinical trial as an anti-cancer therapeutic for recurrent malignant glioma. The redox-active quinone-based compound, MitoQ, has been in clinical trials for Parkinson's disease and for chronic hepatitis C and is presently used for skin care. In the late 2000s "shrunk" porphyrins, metallocorroles, emerged as prospective SOD mimics and therapeutics [9]. Numerous other redox-active metal complexes and SOD mimics were developed as therapeutics by different groups, some of which are addressed in this book. The wealth of data collected thus far demonstrates that structure-activity relationships, initially developed for Mn porphyrins, are valid for different classes of SOD mimics. The photosensitizing porphyrin ligand,  $H_2TM-4-PyP^{4+}$ , is being incorporated into nano-scaffolds to form nano-phototheranostics for diagnosis and treatment of various cancers. As alternatives to such photosensitizers, quantum dots made of semiconductor metal-containing materials (such as CdSe, CdTe, and InAs) conjugated or not to Zn(II) porphyrins have been explored [10].

Since the discovery of SOD enzymes, tremendous progress has been made on the chemistry of small endogenous reactive species and enzymes which maintain the balanced redox environment of a normal cell; the perturbed balance results in a pathological condition known as oxidative stress. Different classes of SOD mimics were initially developed and anticipated to be specific to  $O_2^{\bullet-}$ . It might have been obvious from the very beginning that such small molecules (relative to a protein-structured enzyme), with biologically compatible reduction potentials, would react with numerous reactive species. Yet, the wealth of knowledge on the chemistry of those species was not available to allow for such "free" thinking. Moreover the biological importance of numerous species such as nitric oxide, peroxynitrite, nitroxyl, carbon monoxide, reactive sulfur, and selenium species and their cross-talk has not yet fully emerged. In turn, not until the end of the 1990s and early 2000s did the rich reactivity of SOD mimics and other redox-active therapeutics towards species other than  $O_2^{\bullet-}$  surfaced.

Researchers have often incorrectly assigned the effects of redox-active drugs to particular reactive species. The unselective chemistry of such compounds, the multitude of reactive species involved, and the biological milieu are too complex to define the mechanism of action with certainty. The use of genetically modified animals or microorganisms has allowed progress. While insight into the redox biology of a cell and redox-active compounds is expanding, the actions of compounds are still often incorrectly singularly attributed to the dismutation of either  $O_2^{\bullet-}$  or/and  $H_2O_2$ , when neither a true SOD mimic nor a functional catalase mimic is used. A recent comprehensive study pointed out that the majority of metal complexes (various Mn or Fe porphyrins, Mn salen EUK-8 or Mn(II) cyclic polyamines such as M40403) are not catalase mimics [11]. Only Fe(III) corroles have modest catalase-like activity, and its biological relevance awaits further exploration. Another

important issue with any drug is its purity. Caution needs to be exercised, as commercial suppliers have frequently sold impure compounds, which in turn have hindered correct discussions of the effects observed.

It was not until the mid-2000s that evidence was provided to demonstrate the interaction between redox-active therapeutics and transcription factors, such as HIF-1 $\alpha$ , AP-1, SP-1, and NF- $\kappa$ B. Effects on transcription factors have been found with different classes of therapeutics, both synthetic and natural, such as Mn porphyrins, nitroxides, Mn salen derivatives, sulforaphane, flavonoids, and polyphenols. Initially, it was speculated that the effects were due to the ability of Mn porphyrins to rapidly remove reactive species, produced upon oxidative stress, which would have otherwise activated one or more transcription factors and in turn transcription of a group of genes. It took nearly a decade before it became clearer that at least one of the major mechanisms involves protein thiols. This learning process required joint efforts of chemists, biochemists, pharmacologists, and biologists.

For years, an obvious fact was overlooked. Catalysis of O<sub>2</sub><sup>•-</sup> dismutation is efficacious *ONLY* if a mimic (or SOD enzyme) is an equally good oxidant and antioxidant, i.e., it equally well oxidizes O<sub>2</sub><sup>•-</sup> to oxygen and reduces it to H<sub>2</sub>O<sub>2</sub>. Thus an SOD mimic and/or SOD enzyme can act in vivo both as an antioxidant and (pro)oxidant. Moreover, the reduction of O<sub>2</sub><sup>•-</sup> by an SOD mimic gives rise to an oxidant—H<sub>2</sub>O<sub>2</sub>. At that point it was a common understanding that H<sub>2</sub>O<sub>2</sub> cannot accumulate in the cell as it is eliminated by numerous (redundant) enzymatic systems such as catalase, glutathione peroxidases (GPx), and peroxiredoxins. Only recently has it emerged that those systems may be downregulated and/or inactivated during disease, which would in turn lead to H<sub>2</sub>O<sub>2</sub> accumulation—a frequent scenario in cancer. While the SOD enzyme is a tumor suppressor in healthy cells, under disease conditions, the SOD enzyme may become a tumor promoter. Such reports coincide with the conclusion that indeed an SOD mimic, with redox properties similar to SOD enzyme, can function as either a pro- or antioxidant depending upon the local environment.

Jon Piganelli was the first to suggest that, in diabetes models, Mn porphyrin can act as an oxidant, possibly oxidizing the p50 subunit of NF- $\kappa$ B in nucleus [12]. The notion was supported by a pharmacokinetic study on macrophages which showed that Mn porphyrin accumulates threefold more in the nucleus than in cytosol. A crucial study on lymphoma cells by Margaret Tome and her colleagues [13] furthered Piganelli's notion in helping understand which reactions are likely involved in the suppression of NF- $\kappa$ B transcriptional activity. Tome showed that H<sub>2</sub>O<sub>2</sub> and GSH are indispensable in the actions of a redox-active Mn porphyrin in oxidatively modifying—S-glutathionylating—p65 and p50 subunits of NF- $\kappa$ B. Once glutathionylated, NF- $\kappa$ B cannot bind to DNA to initiate gene transcription. S-glutathionylation was then demonstrated by Tome's group to occur with other thiol-bearing proteins including mitochondrial complexes I, III, and IV. The inactivation of complexes I and III resulted in the suppression of ATP production. An effect of Mn porphyrin on glycolysis was also seen, possibly involving S-glutathionylation reactions. Importantly, no toxicity was observed with normal lymphocytes. Mitochondrial accumulation of Mn porphyrins and comprehensive aqueous chemistry on cysteine oxidase and/or GPx-like activity of Mn porphyrins by Batinić-Haberle and her

colleagues supported such observations. Studies point to a major role of  $H_2O_2$  in the actions of Mn porphyrins and agree well with a growing recognition of the critical role of this species in cell biology. A similar mechanism of action for other redox-active drugs is highly likely as several other compounds such as Mn salens, nitroxides, and polyphenols also inhibit NF- $\kappa$ B.

Studies as such have challenged how small molecule redox-active SOD mimics, originally considered as selective antioxidants, are viewed. Current data point to the activation of Nrf2 by different redox-active drugs including curcumin, sulforaphane, nitroxides, and Mn porphyrins, presumably via oxidation (or *S*-glutathionylation) of the cysteines of its Keap1 unit, reminiscent of the NF- $\kappa$ B story described above. Such action would result in upregulation of endogenous antioxidative defenses. The data provided by Thambi Dorai et al. [14] on rat kidney ischemia/reperfusion injury showed that Mn porphyrin, rather than acting as SOD mimic in its own right, may activate Nrf2. Activation of Nrf2 would upregulate numerous endogenous antioxidative defenses including mitochondrial and extracellular SOD enzymes. Recent preliminary data from Daret St. Clair's group suggest that the activation of Nrf2 by Mn porphyrin may, though, not occur in cancer. Indeed the activation of Nrf2 by Mn porphyrin was not seen with malignant hematopoietic stem cells derived from patients with myelodysplastic syndrome, but Nrf2 was activated in normal hematopoietic stem cells.

In addition to favorable redox properties, redox-active therapeutics MUST reach a site of action in the body without imposing significant toxicity. Significant efforts have been invested towards understanding how structural properties of Mn porphyrins affect their toxicity and biodistribution in organs, cells, and subcellular organelles. Similar studies on other redox-active drugs are needed.

This book provides a comprehensive, up-to-date source of information on the molecular design and mechanistic, pharmacological, and medicinal aspects of redox-active therapeutics. The first two sections of the book discuss the role of SOD enzymes under physiological and pathological conditions and address multiple classes of redox-active drugs. The basic aspects of the chemistry and biology of redox-active drugs and the brief overview of the redox-based pathways involved in cancer and the medical aspects of redox-active drugs are provided assuming little in the way of prior knowledge. The third and fourth sections of the book deal with the therapeutic approaches towards different diseases. Among therapeutic effects obtained with redox-active drugs, the radioprotection of normal tissues is of particular interest as there is no efficacious and nontoxic radioprotector of normal tissue available for human use. Such effects gave rise to ongoing clinical trials on the radioprotective effects of two efficacious SOD mimics (Mn porphyrin-based BMX-001 and Mn cyclic polyamine-based GC4419)—a critical step forward in the development of a redox-active SOD mimic as a human therapeutic. Up to 50 % of all cancer patients undergo some type of radiotherapy; thus there is a substantial need to protect normal tissue during tumor irradiation. While protecting normal tissue, it is essential that the radioprotector does not protect tumor tissue. Multiple studies now show that an SOD mimic can simultaneously function as a radioprotectant for normal tissue and as a radiosensitizer for tumor tissue. Only recently has a possible explanation for such apparent dichotomy emerged. It relies on differential redox

environments of normal vs. cancer tissue and differential accumulation of Mn porphyrin in those tissues. This drives vast suppression of antiapoptotic NF- $\kappa$ B in the cancer cell while there is only moderate suppression in normal cells; in turn, tumor cells undergo apoptosis while survival pathways are upregulated in normal cell.

In recent years there has been a rapid advance in developing and exploiting redox-active therapeutics towards a wide range of diseases and pathophysiological states other than cancer, such as inflammation, diabetes, cardiovascular, and neurodegenerative diseases. Contrary to classical therapeutic approaches that target directly specific proteins, enzymes, or macromolecules, redox-active therapeutics constitute a novel strategy whose target is the organism redox network, the so-called *Redoxome*. Mimics of redox enzymes and compounds that are able to reestablish the physiological levels of reactive species in normal tissues (demonstrated as a suppression of oxidative stress-dependent immune responses and secondary inflammatory processes), or selectively increase the oxidative burden in some targeted cells and tissues (such as tumor), are being heavily sought as experimental therapeutics. The adventure into fully understanding the effects obtained with metal-based therapeutics in inducing subtle changes, which would be observed as the “healing” of a diseased or death of a cancerous tissue, is still in its infancy; yet remarkable therapeutic effects are driving research in this relatively new interdisciplinary area linking chemistry, biochemistry, biology, and medicine.

The abundance of new information and the paradigm shift in our understanding of the mechanism of how the redox-active drugs work in a wide variety of diseases impose a need for a book to synthesize the current state of knowledge regarding the role of redox-active compounds in healthy and diseased tissue. Thus, this appears to be the first book fully dedicated to addressing the critical role that redox processes play in the development of different classes of therapeutics from the bench to the clinic and stressing awareness of these concepts for the treatment of disease. The chapters in this book describe the progresses in defining the central role of redox biology in many disease processes including inflammation, immunology, and neoplasia. This sets the foundation for the development of new classes of small redox-active molecules with unique effects in control of redox biology through modulation of key transcription factors at the core of inflammation, immunity, and neoplasia. The next decade shows a promise for the translation of this body of knowledge into new, transformative human therapeutics.

Durham, NC, USA  
João Pessoa, PB, Brazil  
Durham, NC, USA

Ines Batinić-Haberle  
Júlio S. Rebouças  
Ivan Spasojević

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