Advanced Drug Delivery Reviews 61 (2009) 1375–1385

Contents lists available at ScienceDirect

Advanced Drug Delivery Reviews

journal homepage: www.elsevier.com/locate/addr

Mitochondrial targeting of electron scavenging antioxidants: Regulation of selective oxidation vs random chain reactions ☆

Valerian E. Kagan [a,b,*], Peter Wipf [c], Detcho Stoyanovsky [a,b], Joel S. Greenberger [d], Grigory Borisenko [g], Natalia A. Belikova [a,b], Naveena Yanamala [e], Alejandro K. Samhan Arias [a,b], Muhammad A. Tungekar [a,b], Jianfei Jiang [a,b], Yulia Y. Tyurina [a,b], Jing Ji [f], Judith Klein-Seetharaman [e], Bruce R. Pitt [b], Anna A. Shvedova [h], Hülya Bayır [a,f]

[a] Center for Free Radical and Antioxidant Health, University of Pittsburgh, Pittsburgh, PA, 15219, USA
[b] Department of Environmental and Occupational Health, University of Pittsburgh, Pittsburgh, PA, 15219, USA
[c] Department of Chemistry, University of Pittsburgh, Pittsburgh, PA, 15260, USA
[d] Department of Radiation Oncology, University of Pittsburgh, Pittsburgh, PA, 15232, USA
[e] Department of Structural Biology, University of Pittsburgh, Pittsburgh, PA, 15261, USA
[f] Department of Critical Care Medicine, University of Pittsburgh, Pittsburgh, PA, 15201, USA
[g] Institute of Physico-Chemical Medicine, Moscow, 119992, Russia
[h] Pathology and Physiology Research Branch, Health Effects Laboratory Division, National Institute for Occupational Safety and Health, and West Virginia University, Morgantown, WV, 26505, USA

ARTICLE INFO

Article history:
Received 4 June 2009
Accepted 8 June 2009
Available online 27 August 2009

Keywords:
Cytochrome *c*
Cardiolipin
Mitochondria
Apoptosis
Triphenylphosphonium
MnSOD
Peroxidation
Nitroxides
Gramicidin S-conjugates

ABSTRACT

Effective regulation of highly compartmentalized production of reactive oxygen species and peroxidation reactions in mitochondria requires targeting of small molecule antioxidants and antioxidant enzymes into the organelles. This review describes recently developed approaches to mitochondrial targeting of small biologically active molecules based on: (i) preferential accumulation in mitochondria because of their hydrophobicity and positive charge (hydrophobic cations), (ii) binding with high affinity to an intra-mitochondrial constituent, and (iii) metabolic conversions by specific mitochondrial enzymes to reveal an active entity. In addition, targeted delivery of antioxidant enzymes via expression of leader sequences directing the proteins into mitochondria is considered. Examples of successful antioxidant and anti-apoptotic protection based on the ability of targeted cargoes to inhibit cytochrome *c*-catalyzed peroxidation of a mitochondria-specific phospholipid cardiolipin, in vitro and in vivo are presented. Particular emphasis is placed on the employment of triphenylphosphonium- and hemi-gramicidin S-moieties as two effective vehicles for mitochondrial delivery of antioxidants.

Contents

1. Introduction: selective oxidation vs random chain reactions of lipid peroxidation . . . 1376
2. ROS reactivity: specific enzyme-dependent ROS signaling vs random free radical damage . . . 1376
3. Mitochondrial peroxidation reactions — catalysis and role of the electron transport chain (ETC) . . . 1376
4. Mitochondrial targeted delivery of oxidation regulators: major principles . . . 1377
5. Mitochondrial targeted delivery of antioxidant enzymes . . . 1377
6. Chemistry of small molecule targeted delivery into mitochondria . . . 1378
   6.1. Delivery of GS-nitroxides and TPP-nitroxides . . . 1378
   6.2. TPP-aminoxyls suppress the peroxidase activity of cyt *c*/CL complexes . . . 1381
   6.3. Modulation of CL unsaturation leads to protection against apoptosis . . . 1381
   6.4. Targeted avoidance of specific compartments such as mitochondria: delivery of NBD-CL . . . 1382
7. Concluding remarks . . . 1383
Acknowledgements . . . 1383
References . . . 1383

☆ This review is part of the *Advanced Drug Delivery Reviews* theme issue on "Mitochondrial Medicine and Therapeutics, Part II".

* Corresponding author. Center for Free Radical and Antioxidant Health, Department of Environmental and Occupational Health, University of Pittsburgh, Bridgeside Point, 100 Technology Drive, Room 330, BRIDG; Pittsburgh, PA 15219-3130, USA. Tel.: +1 412 624 9479; fax: +1 412 624 9361.
E-mail address: kagan@pitt.edu (V.E. Kagan).

0169-409X/$ – see front matter
doi:10.1016/j.addr.2009.06.008

"The proletarians have nothing to lose but their chains." Karl Marx

1. Introduction: selective oxidation vs random chain reactions of lipid peroxidation

The remarkable success of chemistry in understanding the mechanisms and kinetics of chain reactions in the gas phase [1,2] and the subsequent demonstration of these ideas for chemical oxidation reactions in the liquid phase [3] created a supposition that free radical chain oxidation reactions can also take place in biological systems. This resulted in the appearance of several novel hypotheses on the free radical mechanisms of aging [4,5] and radiation injury [6] as well as their role in major chronic cardiovascular and neurodegenerative diseases and cancer [7–12]. Reactive oxygen species (ROS) or oxygen radicals, particularly superoxide radicals ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2) and subsequently formed highly reactive hydroxyl radicals, have been implicated in the oxidative modification of biological molecules and initiation of free radical chain reactions [13,14].

Logically, this has led to attempts to utilize free radical scavengers as therapeutic and/or preventive remedies. The initial optimism, however, faded over the years as many (if not most) antioxidant clinical trials have failed [15–27]. A skeptical view is that the major concept may be flawed: are there indeed (peroxidation) chain reactions in tissues and cells of our body normally or in disease conditions that develop as a random uncontrolled process? Is there solid experimental proof for hydroxyl radicals formed in vivo — based, for example, on spin trapping or other specific biomarkers [28–30]? Of course, there is a popular concept of chain breaking (sacrificial) water-soluble and lipid-soluble antioxidants such as vitamin C, vitamin E, ubiquinol, etc. But is antioxidant action their only or major function, and do they act as "random" scavengers of "random" radicals? Is it conceivable that old-fashioned classical biochemistry still works with the peroxidation reactions? If so — the attempts to use chain breaking antioxidants are destined to fail. The consequences and conclusions are simple — mechanisms of specific peroxidation reactions have to be revealed and interventions aimed at their regulation and/or inhibition have to take into consideration the compartmentalized nature of these reactions.

2. ROS reactivity: specific enzyme-dependent ROS signaling vs random free radical damage

ROS – formed during one-electron reduction of oxygen – are believed to be essential for the initiation of free radical reactions. They are commonly viewed as nonspecific oxidants capable of inducing oxidation of practically any biological molecule (proteins, lipids, DNA) via free radical pathways [31]. Yet, direct interactions of ROS (namely, $O_2^{\cdot-}$ and H_2O_2) with lipids and reactive groups of proteins are slow and inefficient. For example, the rate of the reaction of H_2O_2 with unsaturated lipids is negligible [14]; the rate of the reaction with thiols is usually below 30 $M^{-1}s^{-1}$ [32]. In addition, both species are effectively removed by antioxidant enzymes of cells — superoxide dismutases (SOD; the reaction rate with $O_2^{\cdot-}$ is ~10^9 $M^{-1}s^{-1}$ [33]), catalase (the reaction rate with H_2O_2 is ~10^7 $M^{-1}s^{-1}$) and glutathione peroxidases (the reaction rate with H_2O_2 is ~10^7 $M^{-1}s^{-1}$) [34–36].

To solve this conundrum, the role of ROS in direct oxidations, proposed chemical mechanisms that often include chain reactions catalyzed by redox active metal ions via the generation of highly reactive O-, S- and C-centered radicals [13,14]. However, detection of these radicals in vivo and in cells appears to be difficult. The generation of O- and S-centered radicals has been documented in cultured cells (but not in whole organisms) following exposures to high doses of toxicants under conditions incompatible with normal physiology. Experiments with a combination of primary and secondary radical-traps – dimethyl sulfoxide/α-(4-pyridyl-1-oxide)-N-*tert*-butylnitrone (POBN) – provided some evidence in favor of the formation of hydroxyl radicals in vivo in acute injury induced by cadmium poisoning and LPS-exposure [37,38]. Similarly, C-centered radicals were reported in vivo predominantly in acute injury (i.e., methanol intoxication, chromium poisoning, superantigen-induced toxic shock syndrome) [39–42]. Thus, the physiological relevance of random free radical reactions requires further investigation.

3. Mitochondrial peroxidation reactions — catalysis and role of the electron transport chain (ETC)

An alternative view on the ROS production and functions in cells suggests that they are involved in specific, compartmentalized and controlled catalytic reactions. What are the known major sites of radical production and oxidative stress? There are multiple possible site-specific sources of oxidizing equivalents and enzymes with high oxidizing potential that may participate in the generation of oxygen radicals. NADPH oxidases in the plasma membrane of inflammatory cells are potent producers of $O_2^{\cdot-}$ and H_2O_2. The generated ROS are believed to play a significant role in inflammation. "Friendly fire" produced by activated immune cells can induce growth arrest, apoptosis or necrotic death in off-target cells contributing to and modifying the inflammatory response [43–46]. Thus generated oxidized epitopes on cell surfaces and in the extracellular matrix enhance immune reactions and trigger autoimmune response [47–49].

Among potent catalysts of peroxidation reactions are heme-containing proteins, particularly heme-peroxidases. These enzymes can effectively utilize H_2O_2 (with rate constants in the range of 10^4–10^7 $M^{-1}s^{-1}$) and oxidize specific substrates and generate reactive intermediates at extremely high rates (up to 10^8 $M^{-1}s^{-1}$) [50]. In particular, cyclooxygenase can oxidize arachidonic acid upon reaction with peroxides at rates up to ~10^7 $M^{-1}s^{-1}$ and produce arachidonic acid hydroperoxides and endoperoxides, prostaglandins G2 and H2, which possess specific biological activity [51,52]. Neutrophil myeloperoxidase and the peroxidase activity of cyt *c* complexes with mitochondria-specific phospholipid cardiolipin (CL) in the intermembrane space of mitochondria add to the list of examples of enzymatic systems involved in the compartmentalized generation of oxidative stress.

It is commonly accepted that mitochondria and their electron transport chains (ETCs) – if and when de-regulated – act as the major source of oxygen radicals in cells. Initial estimates suggested that during the normal transfer of electrons, 2–5% of total molecular oxygen consumed by mitochondria is converted into superoxide due to its incomplete reduction and electron escape during the process coupled with oxidative phosphorylation [53]. In subsequent studies, arguments were presented that at physiological level of tissue oxygenation only 0.2% oxygen is converted to superoxide [54]. The sites of superoxide production in the mitochondrial ETC have been mostly associated with Complexes I and III in the mitochondrial inner membrane [55–59]. Since the superoxide does not freely diffuse across membranes, the location of superoxide within mitochondria is important. It has been suggested that Complex I generates superoxide within the mitochondrial matrix. In contrast, Complex III can release superoxide both into the intermembrane space and matrix [60]. Dysfunctional ETC, resulting from either genetic mutations or the action of toxic chemicals or environmental factors, may lead to enhanced production of ROS via facilitated deviation of electron flow to molecular oxygen causing its univalent reductions [61,62].

A typical example is the disruption of electron transport in cells undergoing apoptosis [63]. Until recently, the role of ROS production in the execution of the apoptotic program has not been elucidated. Establishment of the important role of oxidation of a mitochondrial-specific phospholipid, CL, in the permeability transition and release of pro-apoptotic factors [64] pointed to a possible connection of this