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Review Article

Oxidative Stress Targeting Therapy-from Bench to Clinical Application

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Abstract

Oxidative stress has been widely studied in experiments and well recognized as an important mechanism involving in aging and various diseases, such as cancers, cardiovascular diseases, and degenerative diseases. Based on the supportive findings of experimental research, many trials with clinically feasible therapy targeting for oxidative stress have been conducted. Even clinical evidence based on meta-analyses keeps mounting. The scope of this article is to review the mechanisms based on basic experiments and available evidence about the clinical application of oxidative stress-targeting therapeutic strategies. In addition, specific ways for patients undergoing maintenance hemodialysis, such as electrolyzed-reduced water (ERW) for dialysis water, are mentioned.

Keywords: Anti-Oxidant; Electrolyzed Reduced Water; Enzymatic Anti-Oxidative Capacity; Non-Enzymatic Anti-Oxidative Capacity

Abbreviations: ROS: Reactive Oxygen Species; ETC: Electron Transportation Chain; MPO: Myeloperoxidase; HOCl: Hypochlorous Acids; NDI1: Dehydrogenase Internal 1; ERW: Electrolyzed Reduced Water

Introduction

Oxidative stress is an imbalance between reactive oxygen species (ROS) and anti-oxidative capacity in living organisms. Excessive ROS directly react with biologically essential molecules, such as proteins, lipids, carbohydrate and nucleic acids, causing structural and functional damage in cells and inducing consequent diseases. ROS also act as second messengers triggering the caspase cascade in apoptosis [1]. Thus, to reduce or inhibit overwhelming production of ROS is one of two main domains of the oxidative stress targeting therapy. The other domain is to counter-balance or scavenge ROS through anti-oxidative therapy with refreshment or augmentation of anti-oxidative capacity. The representative targets of anti-oxidative therapy based on experimental mechanisms are listed in Table 1.

Table 1: Targets based on experimental mechanisms for oxidative stress.

Production of Reactive Oxygen Species (ROS): To reduce or inhibit overwhelming production of ROS
Electron transportation chain reactions
Xanthine oxidase
Nicotinamide adenine dinucleotide phosphate oxidase (NADPH oxidase)
Myeloperoxidase (MPO)
others
Anti-Oxidative Capacity in the Body: To counter-balance or scavenge ROS with refreshment or augmentation of anti-oxidative capacity
Enzymatic Anti-Oxidative Capacity

Superoxide dismutase (SOD)
Catalase (CAT)
Glutathione peroxidase (GSHPx)
others
Non-Enzymatic Anti-Oxidative Capacity
Vitamin C (ascorbic acid)
Vitamin E (tocopherols)
Glutathione (GSH)
N-acetylcysteine (NAC)
others

Production of Reactive Oxygen Species (ROS)

ROS are not only evocable from various exogenous resources but also endogenously generated in our own bodies. Some dominant, endogenous sources of ROS production are reviewed. Normally, almost 90% of cellular ROS are the by-products of electron transportation chain (ETC) reactions, major sites of premature electron leakage to oxygen, in the mitochondria [2]. ROS are also produced by xanthine oxidase, involving the catabolism of purines. In stimulated neutrophil white blood cells, NADPH oxidase (nicotinamide adenine dinucleotide phosphate oxidase), an enzyme complex in the membranes of phagosomes, exaggerates ROS to kill engulfed microorganisms. As well, myeloperoxidase (MPO) is abundantly expressed and produces hypochlorous acids (HOCl) to carry out the antimicrobial activity.

Strategies to Reduce ROS Production

To reduce ROS production in mitochondria, some specific interventions to modify the reactions of ETC have been proposed [3]. While induction random mutagenesis of ETC subunits is not viable, using RNAi technology to reduce the concentration of respiratory complexes is easily achieved in invertebrate models. Regarding substituting the complex enzymes producing damage with more efficient enzymes, it has been explored in experimental methods using alternative enzymes to bypass Complexes III and IV [4] or using NADH dehydrogenase internal 1 (NDI1) to bypass Complex I [5]. Yet, no results of clinical trials have been reported. Widely used in daily practice, xanthine oxidase inhibitors include purine analogues like allopurinol, and others, like febuxostat. Allopurinol, as a structural isomer of hypoxanthine (a naturally occurring purine in the body), competitively inhibits xanthine oxidase.

Febuxostat works by non-competitively blocking the molybdenum pterin center, the active site of xanthine oxidase. Not only in basic researches but also in many clinical trials [6,7] the beneficial effects of the pharmaceutical inhibitions of xanthine oxidase have been shown in various disease conditions involving oxidative stress. Regarding inhibition of the family of NADPH oxidase (NOX), the reasonable approaches include inhibition of assembly, subcellular translocation, post-transductional modifications, calcium entry/release, electron transfer, and genetic expression [8]. Small molecule inhibitors, like sulfhydryl-modifying reagents, offer a powerful tool in pathological conditions because it completely inhibits superoxide production due to their high affinity towards targets [9]. Membrane channel blockers, such as felodipine and amolodipine [10] also act as NOX inhibitors through blocking the activation of calcium channels and NOX-derived ROS production by angiotensin II. The effects of peptide inhibitors have also been experimentally explored [11]. Although there have been some experimental data suggesting NOX inhibitors, via reducing ROS-mediated stress, be beneficial in various malfunctions and diseases, these compounds have not been used clinically, perhaps concerning about safety, selectivity, toxicity, bioavailability and significant side effects. The results of a clinical trials of GKT137831, an inhibitor of NOX isoforms, are pending [12]. For inhibition of MPO, two possible general mechanisms have been considered to intervene [13]. The first is developing substrate blocking H_2O_2 from accessing active site, heme center of the enzyme, and rendering enzyme inactive in heme-dependent peroxidase and HOCl formation.

Another mechanism involves competition between inhibitory compound and enzyme substrate for the active site of the enzyme. The effects of both reversible and irreversible blockers have been demonstrated in many experiments [14,15]. Nevertheless, evidence showing potency and safety of MPO inhibitors in human studies is lack yet. The clinical trials with irreversible 2-thioxanthine MPO suicide substrate, (S)-3-((tetrahydrofuran-2-yl)methyl)-2-thioxo-1,2,3,7-tetrahydro-6H-purin-6-one (AZD5904) was tested in Phase I clinical trials for a treatment of chronic obstructive pulmonary disease and multiple sclerosis by AstraZeneca were, however, discontinued [16]. Another randomized trials with irreversible MPO inhibitor, AZD342, with structural formula of the compounds is 1-(2-Isopropoxyethyl)-2-thioxo-1,2,3,5-tetrahydropyrrolo(3,2-d) pyrimidin-4-one, were also discontinued due to lack of efficacy [17].

Anti-Oxidative Capacity in the Body

Anti-oxidative capacity consists of enzymatic and non-enzymatic (or anti-oxidants) components. Large molecular Anti-oxida-

tive enzymes, such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSHPx), catalyze ROS to less detrimental substrate and prevent them from attacking essential molecules. SOD dismutates O_2·- into H_2O_2 to avoid accumulation to toxic levels. Three isoforms of SOD are found: cytosolic copper–zinc-dependent form (CuZnSOD, SOD1), the mitochondrial manganese-dependent form (manganese-dependent SOD [MnSOD], SOD2), and the extracellular copper-zinc-dependent form (extracellular SOD [ecSOD], SOD3). CAT, as a peroxisomal protein in mammalian cells, converts H_2O_2 into H_2O and O_2·-. GPx, located in the mitochondria and cytosol, detoxifies H_2O_2 and hydroperoxides (ROOH) into H_2O and alcohols (ROH), respectively. Small-molecule antioxidants, such as vitamin C, vitamin E, glutathione (GSH), N-acetylcysteine (NAC), and other antioxidants, scavenge ROS by neutralize them directly. Vitamin C (ascorbic acid), water-soluble, mainly scavenges $ONOO^-$, NO, and HOCl but also quenches ·OH, and O_2·-. Through the ascorbate peroxidase reaction, it reduces H_2O_2 to H_2O. In addition, ascorbic acid also helps to restore or rescue other small molecules such as α-tocopherol, GSH, urate and β-carotene [18]. Vitamin E, an effective lipid soluble antioxidant, protects cell membranes against lipid peroxidation by directly scavenging lipoperoxyl radicals [19]. Glutathione, γ-glutamylcysteinylglycine, is the major non-enzymatic antioxidant, is a ubiquitous tripeptide, either in reduced (GSH) or oxidized form (GSSG), regulating intracellular redox homeostasis. GSH scavenges H_2O_2, O_2·- and ·OH directly. Besides, GSH can restore ascorbic acid via the ascorbate-GSH cycle [18]. NAC not only provides cysteine for synthesis of glutathione, but also has the ability to scavenge ROS directly.

Strategies on Enzymatic Anti-Oxidative Capacity

In both prokaryotes and eukaryotes, SODs have shown to play a role in protecting enzymes and proteins against oxygen toxicity [20]. A meta-analysis has concluded that measurement of blood SOD, especially in erythrocytes, could potentially be used as a diagnostic and monitoring marker in patients with gastric cancer [21]. The therapeutic effects of supplement of SOD have also been shown in animal models [22]. The primary results are promising in a phase 1b/2a trial about intravenous infusion of SOD mimetic GC4419 for alleviation of chemoradiotherapy-induced oral mucositis [23]. However, before supplement of SOD could be accepted as a standard therapy, more evidence based on more clinical trials is necessary. CAT plays an important role in cell defense against oxidative damage by H_2O_2, which forms other ROS through the Fenton reaction and acts as a second messenger involving many biological processes including changes of morphology, proliferation, NF-κB signaling, apoptosis and so on [24].

In addition to the dominant 'catalatic' activity (decomposition of H_2O_2), CAT can also decompose peroxynitrite, oxidize nitric oxide to nitrite [25]. Many studies have demonstrated that CAT expression is altered in cancer cells, and CAT is proposed as a future therapeutic target using pro-oxidant approaches [26]. However, there is no clinical trial showing the therapeutic effects of supplement or augmentation of CAT yet. Glutathione peroxidase 4 (GPX4) constitutively control ferroptosis, a form of regulated cell death characterized by iron-dependence and lipid hydroperoxides accumulation. And, it was proposed directly targeting glutathione peroxidase 4 may be more effective than disrupting glutathione on ferroptosis-based cancer therapy [27]. Although the theoretical mechanisms have been proposed, there is no clinically feasible intervention directly targeting GPX yet.

Strategies on Non-Enzymatic Enzymatic Anti-Oxidative Capacity

The molecular mechanisms of anti-oxidative effects of vitamin C have been widely recognized. Considering its ready availability, water solubility, and safety even with high dose as 3-10g per day, vitamin C, dietary supplement or intravenous, has been tested in many clinical trials and many meta-analyses have been reported [28,29]. However, no definite clinical benefits have been proved in certain conditions, such as various cancers [30] and cardiovascular diseases [31]. Based on the registration database of the Clinical Trials.gov., there are still plenty of clinical trials are ongoing. Vitamin E actually includes four tocopherols and four tocotrienols. Out of four isoforms of tocopherols, α-tocopherol is the most abundant. The molecular mechanisms of anti-oxidative effects of vitamin E have also been widely clarified. Regarding its fat solubility, vitamin E sometimes are tested with water-soluble vitamin C for synergic effects or just for comparisons. There are also many meta-analyses in the literature [32]. However, evidence supporting the clinical benefits of vitamin E supplementation for various conditions is still controversial. Even all-cause mortality might be increased with high-dose vitamin E (≥400 units/day) [33]. Based on the registration database of the ClinicalTrials.gov., there are also plenty of clinical trials are ongoing. Both of glutathione and its precursor, NAC, have been recognized as effective antioxidants in various experimental models. Intact form of glutathione, used as dietary nutrient supplement, is absorbed somewhat in the intestines, but it must be metabolized to form L-cysteine before being taken up into cells. In contrast, NAC provides L-cysteine efficiently at a lower financial cost than glutathione. Actually, NAC is inexpensive and fairly safe. Even an extremely high dose of N-acetylcysteine, totally 1,330mg per kilogram body weight orally in three days, is the FDA-approved protocols for the treatment of acute acetaminophen ingestion [34]. Therefore, supplement of NAC, rather than glutathione, has been adopted in many clinical trials. The clinical benefits of NAC supplement for various diseases have been supported by abundant evidence based on the clinical trials and even meta-analyses [35,36] Based on the registration database of the ClinicalTrials.gov., there are also plenty of clinical trials are ongoing.

Specific Anti-Oxidative Stress Strategies for Hemodialysis Patients

As introduced above, supplement of anti-oxidants is easier and more feasible for clinical use than interventions directly targeting the enzymes involving production or scavenging processes of ROS. Therefore, it is not surprising many clinical trials with supplement of various anti-oxidative substances have been conducted and are ongoing. However, most of the anti-oxidants are taken orally or administrated intravenously. During an ordinary 4-hour hemodialysis session, the neutrophils stimulated in the extracorporeal circuit release ROS, and oxidative stress is exaggerated, determined by checking ROS in blood. Thus, vitamin C infusion and vitamin E coated-dialyzer have been tried to reduce the hemodialysis-related oxidative stress [36]. Besides, hemodialysis with electrolyzed reduced water (ERW) has been shown to reduce hemodialysis-induced oxidative stress and subsequent adverse influences in patients undergoing hemodialysis [37-39]. The long-term beneficial effects of ERW for hemodialysis patient are based on two features: ERW contains active atomic hydrogen which have reactive oxygen species (ROS) scavenging ability; the patients are treated with 120 L anti-oxidative dialysis water thrice a week, more than 25 times of normal water intake (2 L per day and 7 days a week).

Conclusion

The rationales of oxidative stress targeting therapy are based on broad knowledge of the detrimental effects of oxidative stress and supported by extensive evidence from abundant experiments. In comparison to the possible interventions to reduce or inhibit overwhelming production of ROS via experimental modification of intracellular enzymatic pathways, the methods regarding counter-balancing or scavenging ROS through refreshment or augmentation of anti-oxidative capacity, especially oral supplement of anti-oxidants, are more practical and have been widely applied to clinical conditions. Beyond traditional supplement pathways, such as orally or parentally, using ERW for dialysis water is a specific way for patients undergoing hemodialysis.

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