Non-Enzymatic Antioxidants

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Oxidative stress has long been considered one of the pathophysiological mechanisms involved in numerous diseases, which has led to the investigation of the antioxidant systems as a promising therapy more than two decades ago. A useful antioxidant must meet specific characteristics; it must be capable of interacting with biologically relevant oxidants and free radicals; its reaction by-products should be harmless; and finally, it must reach a sufficiently high concentration in the tissue and cell compartments to ensure its activity is quantitatively relevant.

Oxidative Stress   Antioxidants   Non-Enzymatic

1. Introduction

An imbalance of reactive species (RS) production and reactive intermediates detoxification generates oxidative stress. Oxidative stress affects cellular function by targeting nucleic acids, lipids, and proteins, all of which are constituents of cell organelles [1]. RS are generated by biological processes within the cell in normal and stressful conditions [2]. RS have a double and opposed function on cell fate. Under physiological conditions, a moderate RS increase plays an essential role in promoting cell proliferation and survival. However, when RS exceed baseline levels, surpassing the cells' antioxidant capacity, cell metabolic processes are affected. Usually, antioxidant defenses counteract oxidative stress; antioxidants along with the turnover of oxidated macromolecules and organelles, leading to cell survival. If oxidative stress persists, it can trigger cell death, including neuronal cell death or permanent cell damage producing cellular transformation [3]. To protect itself from this damage, the cell has evolved and developed a complex antioxidant defense system, that includes various antioxidant enzymes such as copper/zinc and manganese-dependent superoxide dismutase (SOD), iron-dependent catalase (CAT), selenium-dependent glutathione peroxidase (GPx), and glutathione reductase (GR) [4]. In addition, the antioxidant scavenging system includes several non-enzymatic antioxidants (**Figure 1**), which are easy to obtain and modify the administration dose, including mitochondria-targeted antioxidant (MitoQ) [5], coenzyme Q10 [6], and carnosine [7], which are targeted to the mitochondria, the main source of free radicals; some endogenous antioxidants precursors like inosine, which is metabolized into uric acid (UA) [8], and N-acetylcysteine (NAC) [9], a glutathione (GSH) precursor; GSH itself [10], known as "the master antioxidant"; and vitamins C and E [11].
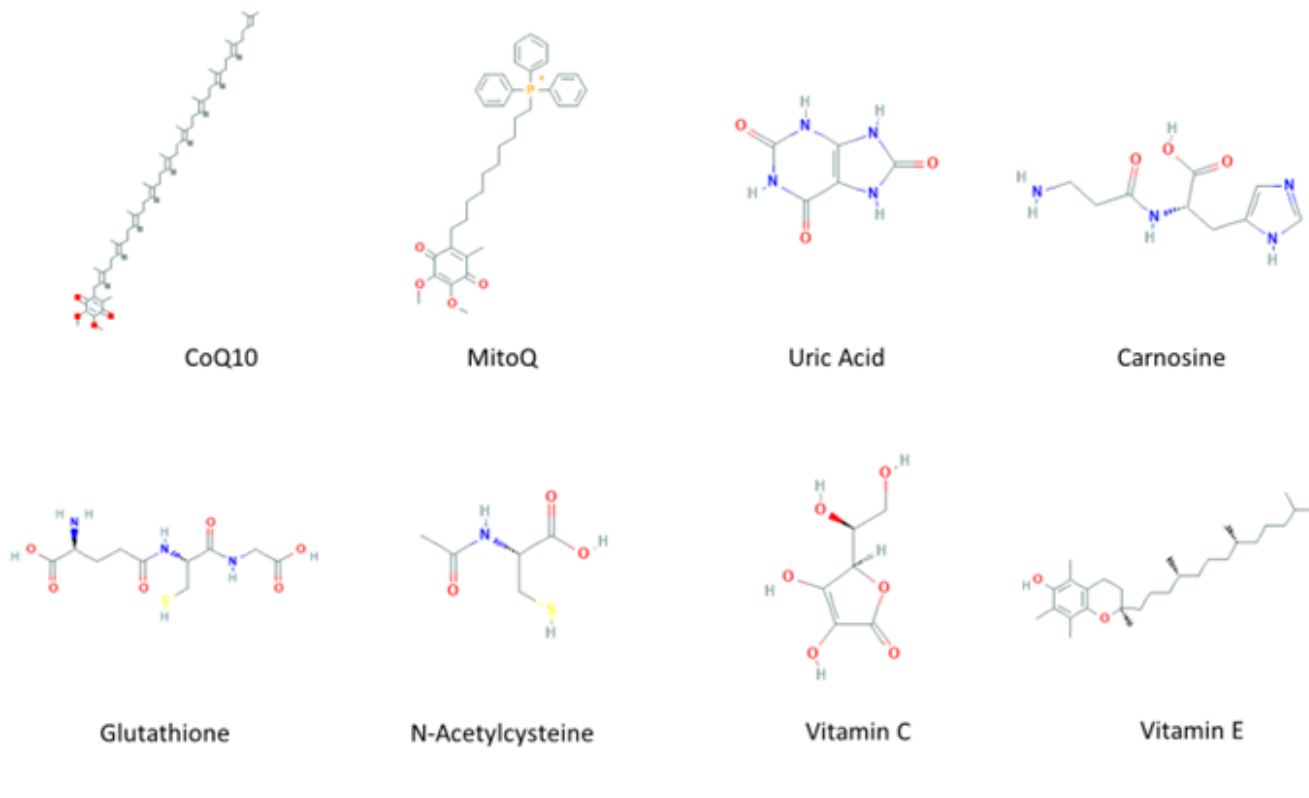
Figure 1. Molecular structures of non-enzymatic antioxidants.

2. Mitochondria-targeted antioxidants

2.1 Coenzyme Q10

Coenzyme Q10 (CoQ10) is a parabenzoquinone (also known as ubiquinone) ubiquitous in virtually all cells; it is a crucial component of the oxidative phosphorylation process in the mitochondria but is not just an agent for energy transduction. It is also well located close to the membranes unsaturated lipids, acting as a primary scavenger of free radicals to avoid lipid peroxidation [6], protecting biological membranes and DNA from oxidative damage [12].

The chemical structure of CoQ10 is very similar to vitamins; however, it is not considered one of them because it is the only lipid-soluble antioxidant that can be synthesized *de novo* by animal cells [13].

Ubiquinone endogenous levels depend on the production and consumption rates within the organism. The dietary intake of CoQ10 is minimal with daily contributions of around 3-5 mg [14], which explains why supplementation with CoQ10 has been recommended in cases of deficiency. Despite this, oral administration of CoQ10 has a poor absorption efficiency due to its hydrophobic nature. Therefore, various formulations have been created to improve its bioavailability [15].

2.2 Mitochondria-Targeted antioxidant (MitoQ)

An orally available derivative of mitochondrial-targeted coenzyme Q10 is a therapeutic compound termed Mitoquinone (MitoQ), which is an ubiquinone synthesized from the union of its oxidized (mitoquinone) and reduced (mitoquinol) form and a covalent bond to a lipophilic thriphenylphosphonium cation through an aliphatic carbon chain [5].

The MitoQ oral formulations have shown suitable pharmacokinetics behavior; doses of 1 mg/kg reach a maximum plasma concentration of 33.15 ng/ml after one hour of administration [16]. MitoQ is rapidly cleared from plasma and accumulates in the heart, skeletal muscle, liver, and brain; its accumulation came to a steady-state after 7 to 10 days of administration [17].

MitoQ quickly crosses the blood-brain barrier and cell membranes and concentrates on mitochondria because of its high membrane potential across the inner mitochondrial membrane [18]. MitoQ is rapidly absorbed by the mitochondria, driven by the membrane potential. Once inside, almost all the accumulated MitoQ is adsorbed to the inner membrane's matrix surface, where it is reduced to the active antioxidant ubiquinol by complex II in the respiratory chain. MitoQ scavenges peroxyl radicals (ROO$^\bullet$), ONOO$^-$ and O$_2^{\bullet-}$ and protects mitochondria against lipid peroxidation. It is also a poor substrate for complex I and has no reactivity with complex III, so MitoQ remains in its reduced form ubiquinol, being more efficient than CoQ10 [19].

2.3 Carnosine

Carnosine (β-alanine-L-histidine) is anendogenous dipeptide of excitable tissues (skeletal muscle, heart, and brain); it is highly hydrophilic, penetrates the blood-brain barrier easily, and has significant antioxidant properties. Carnosine is electrochemically active as a reducing agent; it shows peroxyl radical-trapping activity; inhibits deoxyguanosine oxidative hydroxylation induced by copper ions; and acts as a metal ion chelator, quenching singlet oxygen and binding hydroperoxides [7].

Carnosine concentration in human brain is currently lacking in the literature. Homocarnosine (γ-aminobutyryl-L-histidine) is a novel alternative imidazole peptide with structural similarity to carnosine. Homocarnosine concentration in human brain is quite high (0.4-1.0 μM). Both, carnosine and homocarnosine are synthesized by carnosine synthase and degraded by carnosinase [20].

3. Inosine

Inosine, also known as hypoxanthosine or panholic-L, belongs to the organic compounds known as purine nucleosides. It is an intermediate in the degradation of purines and purine nucleosides to UA and purine salvage pathways.

Inosine is a urate precursor, which has an antioxidant effect *in vitro* [8] and *in vivo* [21]. UA represents about 60% of total plasma antioxidant capacity [22]; it scavenges singlet oxygen ($^1O_2$), OH$^\bullet$, H$_2O_2$, and ONOO$^-$ [23]. However, its effect is not limited to eliminating free radicals. UA-mediated neuroprotection has been improved by high K$^+$-

induced depolarization through a mechanism involving Ca^{2+} increasing and extracellular signal-regulated kinase 1/2 (ERK1/2) activation, which is involved in neuronal survival [24]. UA also interacts and stabilizes other antioxidant systems, including SOD [25]. UA has metal-complexing properties, too; it chelates iron by forming stable complexes with Fe^{3+} and blocking iron-dependent oxidation reactions [26].

4. Cysteine-based antioxidant

4.1 Glutathione

GSH is a tripeptide (cysteine, glycine, and glutamic acid) found in high concentrations in most cells' mitochondrial and cytoplasmatic compartments. It is synthesized in the cytoplasm by the sequential addition of cysteine to glutamic acid, followed by glycine addition. GSH functions as an antioxidant, a free radical scavenger, and a detoxifying agent; it is a GSH peroxidase cofactor, acts as a substrate for GSH S-transferase, and maintains exogenous antioxidants, such as vitamins C and E, in their reduced (active) forms [27][28][29]. The cysteine's sulfhydryl group is essential for GSH function as it participates in the reduction, oxidation, and conjugation reactions [30]. This antioxidant exists in two states: reduced (GSH) and oxidized (GSSG); in the reduced state, the cysteine's thiol group can donate an electron to unstable molecules such as ROS; by donating this electron, the GSH oxidizes and react with another GSH to form GSH disulfide (GSSG). Therefore, the ratio of GSH/GSSG determines the cells' redox status [30].

4.2 N-Acetylcysteine (NAC)

Acetylcysteine is a synthetic N-acetyl derivative of the endogenous amino acid L-cysteine, a GSH precursor (PubChem). NAC has been used for more than 50 years in the clinic to replenish hepatic GSH after acetaminophen overdose [31], as a mucolytic in lung diseases [32], and as a disease modifier in infectious diseases [33]. NAC's antioxidant activity is attributed to its rapid reaction with $OH^•$, $NO^•_2$, CO_3, and thiyl radicals, as well as to the detoxification of semiquinones, hypochlorous acid (HOCl), nitroxyl (HNO), and heavy metals [9].

5. Vitamins

5.1 Vitamin C

Vitamin C (Vit C), also known as ascorbic acid, ascorbate, or L-ascorbate, is a natural water-soluble vitamin that plays significant roles as a free radical scavenger and as a cofactor of several enzymes reactions, including catecholamine synthesis [34]. Its effect as an antioxidant comes from its actions as a non-enzymatic reducer of $O_2^{•-}$, hydroxyl ($HO^•$), alkoxyl ($RO^•$), peroxyl ($ROO^•$), and other radicals [35]. Vit C also reacts with the radical tocopheroxyl, which results from Vitamin E oxidation when it scavenges free radicals in lipid membranes, regenerating Vitamin E (Vit E) [36].

Unlike most molecular low-weight compounds, the absorption, distribution, and metabolism of Vit C are complex. Its uptake in tissues and distribution mainly occurs through the sodium-dependent vitamin C transporter family of proteins [37]. These transporters' differential expression between tissues leads to nonlinear pharmacokinetics of Vit C under physiological conditions, and its distribution is highly compartmentalized [38]. Vit C distribution pattern has a wide range of concentrations in the different tissues ranging from 0.2 mM in muscle and heart to 10 mM in the adrenal glands and brain [39].

5.2 Vitamin E

Vit E is the generic term for eight substances or trocochromanols, four tocopherols, four tocotrienols [40], and the major lipid-soluble antioxidant [41]. As this vitamin cannot be synthesized in the human body, it must be supplied by the diet. The antioxidant effects exerted by each Vit E isoform are complicated, and their mechanisms are still not well understood. However, researchers theorize that Vit E may protect key cell components by reducing free radicals and breaking lipid peroxidation chain reaction. Thus, cell membranes are protected by lipid repair and replacement [42].

As mentioned above, the antioxidant effects of Vit E and its mechanism of action are not well understood. Vit E absorption efficiency varies from 10 to 33% [43] and is affected by several factors including the food matrix, genetic factors, and metabolic fate, altering its bioavailability [44].

6. Conclusions

Oxidative stress has long been considered one of the pathophysiological mechanisms involved in numerous diseases, which has led to the investigation of the antioxidant systems as a promising therapy more than two decades ago. A useful antioxidant must meet specific characteristics; it must be capable of interacting with biologically relevant oxidants and free radicals; its reaction by-products should be harmless; and finally, it must reach a sufficiently high concentration in the tissue and cell compartments to ensure its activity is quantitatively relevant (**Figure 2**). Antioxidant bioavailability is related to the antioxidant structure, interaction with other molecules, half-life, and delivery efficiency into the brain. Increasing the antioxidant dosage not necessary would increase its tissue-specific concentration; it may produce adverse effects. To improve the antioxidant half-life, tissue-specific delivery, and bioavailability, antioxidants can be incorporated in a biocompatible substrate to surpass the administration limitations and increase its therapeutic effect.

Recently, antioxidants delivery and transportation systems were developed for specific subcellular targeting [45] by encapsulation into liposomes or linkage to nanoparticles including nanovesicles, solid lipid nanoparticles, nanostructured lipid carriers, nanoemulsions, and polymeric nanoparticles to make them more stable [46][47]. These are up-and-coming systems to be evaluated in preclinical and clinical studies to obtain the most significant antioxidants benefits, with the least toxicity and side effects.
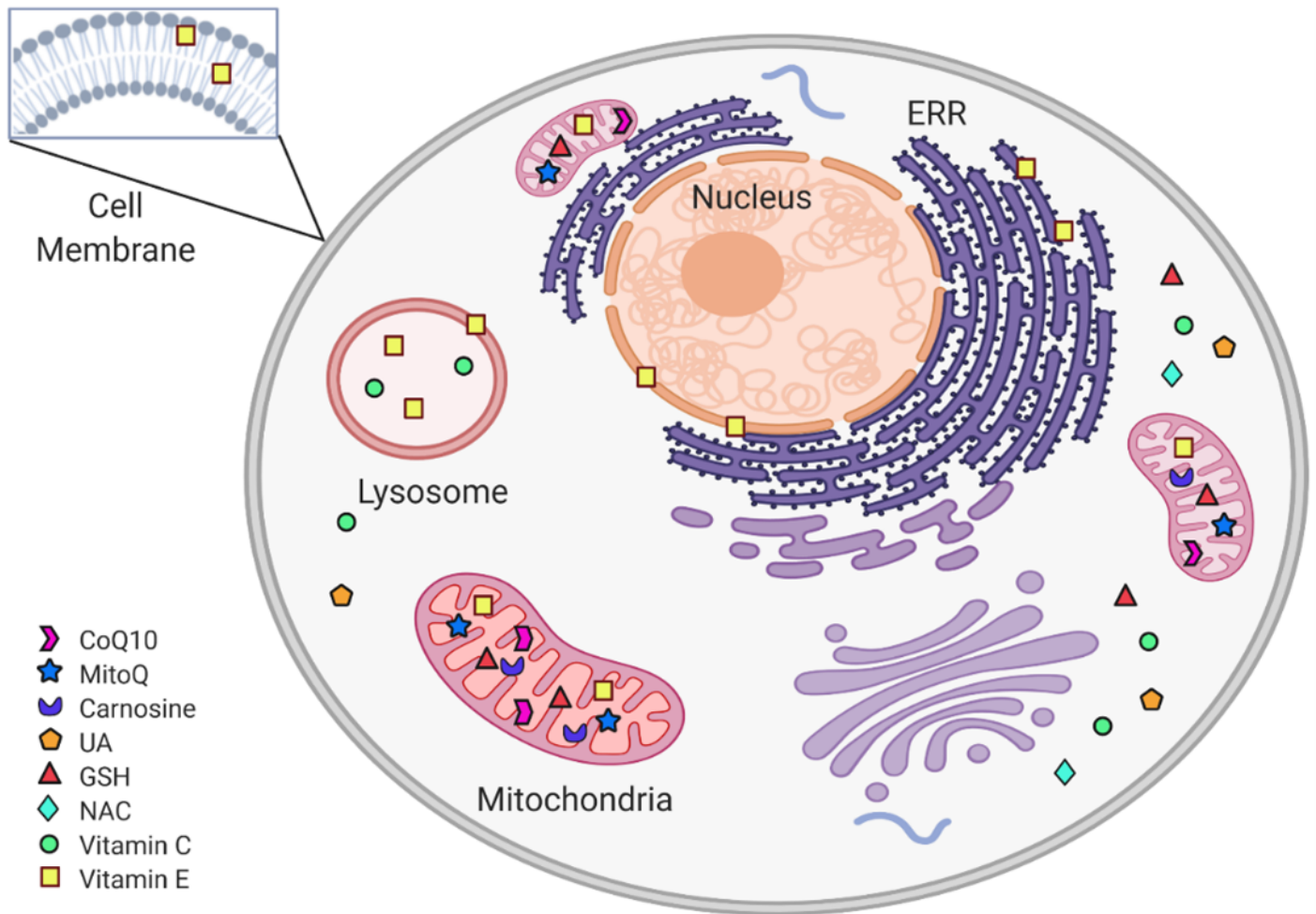
Figure 2. Subcellular non-enzymatic antioxidants targets. Within the cell, non-enzymatic antioxidants are distributed according to their subcellular target. The mitochondria are protected from oxidative stress by CoQ10, MitoQ, carnosine, and Vit C. In the cytoplasm, the antioxidant system includes GSH, NAC, and UA. Vit E is found in cytoplasmic, ERR, and mitochondrial membranes, as well as in lysosomes. (Figure created in BioRender.com)

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