Reactive nitrogen species; devastating intracellular players and melatonin as a defender

Ahmet Korkmaz¹,², Lucien C. Manchester¹

¹Department of Cellular and Structural Biology, UT Health Science Center, San Antonio, Texas, USA.
²Department of Physiology, Gulyhane Military Medical Academy, Ankara, Turkey.

It is widely accepted that reactive oxygen species (ROS) readily cause cellular damage (i.e., oxidative stress) and play an important role in the pathogenesis of a variety of human diseases and sequelae [1-3]. Since the first seminal free radical studies by Harman in 1950’s [4], oxidative stress has gained much attention and many studies have confirmed that free radicals especially those that are oxygen-based such as the superoxide anion (•O₂⁻), hydroxyl radical (•OH), and non-radical hydrogen peroxide (H₂O₂) are harmful molecules intracellularly. However, H₂O₂ for example, functions as an important second messenger at physiological levels and involves a vast number of pathways within cells [5, 6]. Therefore, the general belief can be summarized as “oxygen-based free radicals are harmful when they are produced at high levels within a certain time period”.

Since cells are well equipped with an antioxidative defense system both enzymatically and non-enzymatically, oxidative stress can only appear if the amount of ROS produced in the cells exceeds the neutralizing capacity of anti-oxidative defense system. In this case, supporting cells overwhelmed by the ROS with exogenous non-enzymatic antioxidants such as vitamins (i.e., vitamin A, C, and E), n-acetylcysteine, and selenium appear as a choice [7]. Although numerous experimental studies have documented that exogenous antioxidants scavenge ROS and prevent cellular damage, in prospective clinical studies, however, they have basically failed to exert beneficial effects in terms of preventing many human diseases such as diabetes type 2, cardiovascular events, hypertension and cancer [8-10]. These outcomes have raised controversy that oxidative stress is not sufficient to explain cellular damage, and therefore, conventional antioxidants have been proven to be less useful than previously expected.

This controversy can be partly explained by the involvement of excess nitric oxide (•NO), a vital signaling molecule at physiological levels, in the oxidative stress battleground [11]. •NO, although a nitrogen radical, plays a crucial role in numerous physiological mechanisms, involving oxidative stress. However, excessive amounts of •NO can also be sequestered by the •O₂⁻ and causes peroxynitrite (ONOO⁻) production. •NO is the only known biological molecule that reacts faster with •O₂⁻ and is produced in such high concentrations that it out-competes the endogenously derived superoxide dismutase (SOD); hence, the creation of the “devil’s triangle” [12]. The resulting product, ONOO⁻, is not a radical but a substrate for downstream reactive nitrogen species (RNS) and changes the circumstance from oxidative stress to nitro-oxidative stress which is more hostile for living cells [11-13]. Therefore, nitro-oxidative stress is now considered as the fundamental harmful mechanism instead of oxidative stress which may explain, at least in part, the failure of clinical studies performed with antioxidants especially those that can literally do nothing against RNS.

Prof. Hardeland from Germany admirably reviewed the molecular interactions of RNS and its consequential roles in the current issue of Journal of Experimental and Integrative Medicine [14]. He emphasized that the basal or moderately enhanced •NO levels are unquestionably vital and beneficial for living cells, excess amount, however, can readily trigger the production of RNS under oxidative stress conditions. Emerging role of RNS in the pathophysiological mechanisms of cardiovascular, neurodegenerative, inflammatory and metabolic diseases drive scientific attention to scavenge and/or decompose the RNS in particular ONOO⁻. Among the various candidates with RNS scavenging/decomposition properties such as metalloporphyrins, ebselen, desferrioxamine, mercaptoalkylguanidines, amide derivatives and several marketed drugs including cabergoline, nebivolol, and acetaminophen none of which has yet offered promising outcomes [15].
As a second part of the paper, Hardeland outlined melatonin and its derivatives as a multi-tasking family against nitro-oxidative stress. Melatonin has been known as a powerful antioxidant since 1993 and its antioxidant efficacy has been demonstrated in numerous experimental and clinical studies [16-18]. Several features of melatonin make it a unique antioxidant among its counterparts. It directly scavenges all ROS [19], and influences several intracellular enzymatic antioxidants such as SOD and glutathione peroxidase (GSH-Px) [20]. The latter effect is not only derived from ROS scavenging property of melatonin; but it possesses genomic actions that up-regulates the expression of SOD and GSH-Px [20, 21]. Unlike classical antioxidants, melatonin has virtually no pro-oxidant property; therefore, it is classified as a suicidal or terminal antioxidant [22, 23]. Finally it is free of toxicity and teratogenicity at both physiological and pharmacological doses [24-26]. Another supremacy of melatonin comes from the fact that there is clear coexistence of nitro-oxidative stress and inflammation in the pathogenesis of human diseases. In addition to the multi-tasking antioxidative features of melatonin and its metabolites, they show anti-inflammatory actions which were also indicated by Hardeland. Consistently, melatonin has been shown to be more effective than vitamin E [27-29], β-carotene [30, 31] or vitamin C [30, 32, 33] against a variety of oxidative and inflammatory conditions. Are these features enough to make melatonin a unique antioxidant? Even though it may be so, in the writings of Hardeland, you will see melatonin and its derivatives in a never-ending series of interaction with RNS [34].

After its discovery as a pineal product by Lerner et al in the 1950s [35], much attention has been given to melatonin as a master regulator of seasonal and circadian rhythms and pathophysiological consequences of disrupted melatonin rhythm [36-38]. Further studies have also revealed that, this indolamine was ubiquitous and can be synthesized by virtually all cells with nucleus. The so-called “extra-pineal melatonin” has no role in regulating seasonal or circadian rhythms; rather it has several other roles as mentioned above and in Hardeland’s paper. Therefore, melatonin is much more crucial than glutathione, known as the most important intracellular, non-enzymatic antioxidant [34]. We can now coin the term “the best intracellular defender” for “melatonin and its derivatives” against nitro-oxidative stress and possible harmful molecules that appear as intermediaries [39].

References
32. Gültekin F, Delibas N, Yasar S, Kilinc I. In vivo changes in antioxidant systems and protective role of melatonin and a combination of vitamin c and vitamin e on oxidative damage in erythrocytes induced by chlorpyrifos-ethyl in rats. Arch Toxicol 2001; 75:88-96.

Key words:
Melatonin;
Nitric oxide;
Peroxynitrite;
Reactive nitrogen species

Correspondence:
A. Korkmaz
Gulhane Askeri Tip Akademisi,
Fizyoloji Anabilim Dali,
06010 Etilik, Ankara, Turkey.
drkorkmaz@gmail.com

Received: March 9, 2011
Accepted: March 18, 2011
Published online: March 27, 2011