항산화제로서 Melatonin

김 석 중
한국식품개발연구원

Melatonin as an Antioxidant

Seok Joong Kim[a] and Russel J. Reiter[b]
[a]*Korea Food Research Institute,* [b]*The University of Texas Health Science Center*

Abstract

Melatonin, a chemical mediator produced in the mammalian pineal gland and several other organ, is a ubiquitously acting antioxidant. It has been shown to scavenge the hydroxyl radical (· OH), singlet oxygen ($^1O_2$) and the peroxynitrite anion ($ONOO^-$). In addition, melatonin reportedly stimulates a number of antioxidative enzymes including glutathione peroxidase, glutathione reductase and glucose-6-phosphate dehydrogenase.

Antioxidative effect of melatonin in pharmacological and physiological level was investigated using hepatocarcinogen 2-nitropropane (2-NP) and pinealectomized (Px) rats, respectively. Lipid peroxidation (LPO) as indicated by malondialdehyde and 4-hydroxyalkenals and DNA damage as indicated by 8-hydroxydeoxyguanosine (8-OH-dG) induced by 2-NP were prevented by melatonin. The degree of LPO and DNA damage in Px rats were higher than those of intact old and young ones suggesting the removal of pineal gland resulted in higher accumulation of oxidative damage.

Introduction

Since melatonin, N-acetyl-5-methoxytryptamine, was first identified in bovine pineal extracts on the basis of its ability to aggregate melanin granules and thereby lighten the color of frog skin[1], there are now many evidences that melatonin may have a role in the biologic regulation of circadian rhythms, sleep, mood, and perhaps reproduction, tumor growth, and aging[2]. One of its most attractive functional talents recently, however, may be its role as a free radical scavenger and antioxidant because free radical is well known to relate various diseases and aging etc.[3]. In this proceeding, we summarize current knowledge about melatonin as an antioxidant, and also show melatonin's ability in pharmacological level to prevent oxidative damage induced by toxic chemical (2-NP) and the importance of melatonin in *in vivo* level using Px rats.

Pineal gland and melatonin

In human, the pineal gland producing melatonin lies in the center of the brain, behind the third ventricle and consists of two types of cells: pinealocytes, which predominate and produce both indolamines (mostly melatonin), and peptides (such as arginine vasotocin), and neuroglial cells. The synthesis and release of melatonin are stimulated by darkness and inhibited by light. In humans, melatonin secretion increases soon after the onset of darkness, peaks in the middle of the night (between 2 and 4 a.m.), and gradually falls during the second half of the night. The peak nocturnal concentrations which are highest (average, 325 pg/ml) at the age of one to three years decline gradually as man is getting older. In normal young adults, the average daytime and peak nighttime values are 10 and 60 pg/ml, respectively[4].

In the biosynthesis of melatonin, tryptophane is first converted by tryptophane hydroxylase to 5-hydroxytryptophan, which is decarboxylated to serotonin. The synthesis of melatonin from serotonin is catalyzed by two enzymes (arylalkylamine N-acetyltransferase ans hydroxyindole -O-methyltransferase) that are largely confined to the pineal gland[5,6]. Melatonin is rapidly metabolized, chiefly in liver, by hydroxylation (to 6-hydroxymelatonin) and, after conjugation with sulfuric or glucuronic acid, excreted in the urine. The urinary excretion of 6-sulfatoxymelatonin (the chief metabolite of melatonin) closely parallels serum melatonin concentrations[7].

Melatonin as a free radical scavenger

The bane of the use of oxygen (dioxygen, $^3O_2$, O_2) by aerobic organisms is the fact that a small percentage (up to 4%) of the O_2 utilized is converted to agents, free radicals and active oxygen species such as the superoxide anion radical (O_2^- ·), $ONOO^-$, hydrogen peroxide (H_2O_2) and · OH, that have toxic effects in organisms[8]. Fortunately, there are cellular defenses against the damage inflicted by free radicals or reactive oxygen species, by either enzymatic means such as superoxide dismutase, catalase and glutathione peroxidase or low-molecular antioxidants that directly detoxify, neutralize or scavenge free radicals.

Examples of these include vitamins C and E, β-carotene, and glutathione. Despite of these protections, however, OH once generated is still very harmful because it reacts with virtually all biological targets at rates exceeding 109 $M^{-1}s^{-1}$ and is not enzymatically inactivated. This · OH could be scavenged by melatonin very effectively[9-11] and Tan et al.[9] showed that melatonin scavenged · OH 5 times better than glutathione and 15 times better than mannitol. Additionally, melatonin also detoxify the $ONOO^-$[12], to neutralize $^1O_2$[13], and to scavenge the peroxyl radical (LOO ·), which is generated during the oxidative deterioration of lipids[14]. In addition to its direct scavenging property, melatonin also metabolizes H_2O_2 to non-toxic products by stimulating two important antioxidative enzymes: glutathione peroxidase and glutathione reductase[15].

Melatonin also inhibits the pro-oxidative enzyme nitric oxide synthase[16]. This combination of activities establishes melatonin as an effective antioxidant and an excellent candidate to assist cells in combatting with oxidative challenges.

Melatonin as an antioxidant

The oxidation of macromolecules by free radicals and reactive oxygen species, when the damage goes unrepaired, is involved in a variety of diseases[8], cell death[17] and aging[18]. Melatonin has been tested for its ability to resist oxidative damage; the bulk of these studies have been concerned with melatonin's ability to curtail LPO and DNA damage[3]. LPO is characterized by a well-defined sequence of reactions which leads to the breakdown of fatty acids. Free radical scavengers can either prevent the initiating step in this process or they can interrupt the chain reaction by scavenging the ROO · . Melatonin can both prevent the initiating step in the process of LPO by scavenging highly toxic radicals and it can interrupt the process by detoxifying the ROO · . From various studies, it is apparent that pharmacologically melatonin is effective both *in vitro* and *in vivo* in reducing the breakdown of lipids induced by agents or procedures that promote free radical generation[3]. Relatively few comparative studies have been performed on the efficacy of melatonin in reducing oxidative damage to lipids compared to the better known antioxidant, e.g., vitamin E. In one *in vitro* study, melatonin proved less effective than vitamin E in reducing the formation of thiobarbituric acid reactive substances. What is needed in this area are *in vivo* studies which take into consideration the absorption, uptake, and metabolism of vitamin E and melatonin as well as their efficacies as free radical scavengers and antioxidants.

Oxidatively damaged DNA has been an endpoint in a variety of studies where melatonin has been tested as an antioxidant. Using a variety of different methods to detect damaged DNA products, melatonin invariably reduced oxidative mutilation of the nuclear genetic material. These findings suggest that melatonin readily gets into the nuclei of cells; this is consistent with the immunocytochemical studies of melatonin's subcellular distribution[3].

Protective effect of melatonin against 2-NP[19]

The secondary nitroalkane, 2-NP, which is widely used as an intermediate in chemical syntheses and in formulation of inks, paints, varnishes, adhesives and other coatings, and also found in cigarette smoke is known to be an acute hepatotoxicant and a potent hepatocarcinogen in rodents. 2-NP induced LPO and the formation of 8-OH-dG in liver, lung and kidney, and increased sorbitol dehydrogenase (SDH) activity in serum as parameters of hepatotoxicity. The induction of LPO was significantly reduced in lung and kidney when melatonin (2.5, 5 or 10 mg /kg) was given prior to 2-NP administration. The elevation in serum SDH and 8-OH-dG content caused by 2-NP was also reduced when melatonin was given. These findings show that pharmacological

levels of melatonin can reduce the toxicity of this hepatocarcinogen.

Accelerated accumulation of oxidatively damaged lipid and DNA in long-term melatonin-deficient rats[20]

To investigate the role of melatonin in physiological level, the survival, the degree of LPO and the content of 8-OH-dG of Px rats (the pineal gland was removed) after 24 months were compared to those of intact 3 months and 24 months animals. The level of LPO in liver, lung, kidney, testes, pancreas, small intestine, muscle and hippocampus, and the content of 8-OH-dG in liver, kidney and pancreas in Px rats were higher than those of intact young and old rats. The survival of Px rats was 67% of intact rats after 24 months. These results show that melatonin in physiological level can also prevent the oxidative damages accumulated naturally, which may result in death finally.

Conclusion

Since the discover of · OH-scavenging property of melatonin, many studies demonstrated melatonin was highly effective in reducing the damage normally inflicted by a variety of free radical generating agents in pharmacological doses. In a small number of studies, even physiological levels of melatonin were found to the protective against oxidant induced molecular damage. Our results in Px experiment also show melatonin may be critical in preventing the accelerated accumulation of free radical damage that occurs during aging. As the damage to essential molecules by reactive oxygen species which occurs throughout life has a significant impact on the ability of cells and organs to perform their vital functions, postponing this damage with melatonin, or other antioxidants, might reasonably promote longevity and forestall some age-related diseases.

In addition to melatonin's ability to scavenge free radicals and to affect enzymatic system, another feature to make melatonin a better antioxidant than others may be its lipid-[21] and water-solubility[22], which seems to make melatonin readily distribute to all tissue throughout both the lipid and aqueous portion of cells. Kim et al.[23] showed recently melatonin prevent efficiently the oxidation of both water-soluble protein and lipid at the same time but glutathione and tocopherol didn't.

Acknowledgement

References

1. Lerner, A.B., Case, J.D., Takahashi, Y., Lee, T.H. and Mori, W.: Isolation of melatonin, the pineal gland factor that lightens melanocytes. *J. Am. Chem. Soc.*, **80**, 2587 (1958).

2. Brzezinski, A.: Mechanism of disease. Melatonin in humans. *New Eng. J. Med.*, **336**, 186-195 (1997).

3. Reiter, R.J., Tan, D.X., Kim, S.J. and Qi, W.: Melatonin as a pharmacological agent against oxidative damage to lipids and DNA. Proc. West Pharmacol. Soc., in press (1998).

4. Reiter, R.J.: The pineal gland. In *Principle of Medical Biology*, vol 10A, JAI Press Inc., p. 145-164 (1997).

5. Axelrod, J. and Weissbach, H.: Enzymatic O-methylation of N-acetylserotonin to melatonin. *Science*, **131**, 1312-1313 (1960).

6. Coon, S.L., Roseboom, P.H., Baler, R., Weller, J.L., Namboodiri, M.A., Koonin, E.V. and Klein, D.C.: Pineal serotonin N-acetyltransferase: expression cloning and molecular analysis. *Science*, **270**, 1681-1683 (1995).

7. Lynch, H.J., Wurtman, R.J., Moskowitz, M.A., Archer, M.C. and Ho, M.H.: Daily rhythm in human urinary melatonin. *Science*, **187**, 169-171 (1975).

8. Reiter, R.J.: Oxidative processes and antioxidative defense mechanisms in the aging brain. *FASEB J.*, **9**, 525-533 (1995).

9. Tan, D.X., Chen, L.D., Poeggeler, B., Manchester, L.C. and Reiter, R.J.: Melatonin: a potent, endogeneous hydroxyl radical scavenger. *Endocr. J.*, **1**, 57-60 (1993).

10. Matuszak, Z., Reszka, K. and Chignell, C.F.: reaction of melatonin and related indoles with hydroxyl radicals: EPR and spin trapping investigations. *Free Rad. Biol. Med.*, **13**, 367-372 (1997).

11. Stasica, P., Ulanski, P. and Poriak, J.M.: Melatonin as a hydroxyl radical scavenger. *J. Pineal Res.*, **25**, 65-66 (1998).

12. Gilad, F., Guzzocrea, S., Zingarelli, B., Salzman, A.L. and Szab, C.: Melatonin is a scavenger of peroxynitrite. *Life Sci.*, **60**, 169-174 (1997).

13. Cagnoli, C.M., Atabay, C., Kharlamova, E. and Manev, H.: Melatonin protects neurons from singlet oxygen-induced apoptosis. *J. Pineal Res.*, **18**, 222-228 (1995).

14. Marshall, K.A., Reiter, R.J., Poeggeler, B., Aruoma, O.I. and Halliwell, B.: Evaluation of the antioxidant activity of melatonin *in vitro*. *Free Radic. Biol. Med.*, **21**, 307-315 (1996).

15. Pablos, M.I., Reiter, R.J., Ortiz, G.G., Guerrero, J.M., Agapito, M.T., Chuang, J.I. and Sew erynek, E.: Rhythm of glutathione peroxidase and glutathione reductase in the brain of chic ks and their inhibition by light. *Neurochem. Int.*, **32**, 69-75 (1998).

16. Bettahi, I., Pozo, D., Osuna, C., Reiter, R.J., Acuna-Castroviejo, D. and Guerrero, J.M.: Physiological concentrations of melatonin inhibit nitric oxide synthase activity in the rat

hypothalamus. *J. Pineal Res.*, 20, 205-210 (1996).

17. Ceballos-Picot, I.: The role of oxidative stress in neuronal death. Landes Bioscience, Austin (1997).

18. Harmon, D.: Free Radicals and aging. Birkh user, Boston (1992).

19. Kim, S.J., Reiter, R.J., Garay, M.V.R., Qi, W., El-Sokkary, G.H. and Tan, D.X.: 2-Nitro-propane-induced lipid peroxidation: antitoxic effects of melatonin. *Toxicology, in press*, (1998).

20. Reiter, R.J., Kim, S.J., Garay, M.V.R., Tan, D.X., Qi, W. and Garcia, J.J.: Accelerated accumulation of oxidatively damaged lipid and DNA in long-term melatonin-deficient rats. *Proc. Natl. Acad. Sci. U.S.A.*, in preparation.

21. Costa, E.J., Lopes, R.H. and Lamy-Freund, M.T.: Permeability of pure lipid bilayers to melatonin. *J. Pineal Res.*, 19, 123-126 (1995).

22. Shida, C.S., Catrucci, A.M.L. and Lamy-Freund, M.T.: High melatonin solubility in aqueous medium. *J. Pineal Res.*, 16, 198-201 (1994).

23. Kim, S.J., Qi, W., Tan, D.X., Cabrera, J. and Reiter, R.J.: Melatonin prevents oxidative damage to protein and lipid induced by ascorbate-Fe^{3+}-EDTA: comparison with glutathione and α-tocopherol. *Free Radic. Biol. Med.*, in presentation (1998).