Melatonin is not a carbonyl scavenger

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Abstract

Objectives: In our previous study on the human skin accumulation of AGEs and lifestyle behaviors, as well as smoking and alcohol drinking, lack of sleep clearly enhanced AGE accumulation. We hypothesized that melatonin may participate in this phenomenon. Here, we investigated the carbonyl scavenging capacity of melatonin in human serum albumin (HSA)/glucose model in attempt to reveal an as yet undiscovered function of melatonin.

Methods: AGE-derived fluorescence intensity was measured by fluorescence spectroscopy at excitation 370 nm and emission 440 nm. Pentosidine, 3-deoxyglucosone (3-DG), glyoxal (GO), and methylglyoxal (MGO) were analyzed by high performance liquid chromatography (HPLC). A system composed of glucose and HSA was employed as an in vitro model in our study. Aminoguanidine was used as positive control.

Results: Melatonin neither inhibited fluorescent AGE formation in both HSA/glucose and collagen/glucose models nor inhibited the generation of AGE intermediates such as 3-DG, GO, and MGO in HSA/glucose model. In contrast, aminoguanidine showed a dose-dependent inhibition of formation of these AGEs and carbonyl compounds.

Conclusion: The present study compelled us to conclude that melatonin has no direct influence on AGE generation. In the phenomenon showing that a lack of sleep in the lifestyle behavior plays a role in promoting skin AGE accumulation, it is likely that a mechanism other than melatonin is involved.

KEY WORDS: melatonin, aminoguanidine, carbonyl compounds, human serum albumin

Introduction

Melatonin is a somewhat mysterious substance. In animals, melatonin is secreted from the pineal gland during the night. It acts as a hormone, functioning as a circadian mediator for time information over the course of each day, and it is also able to eliminate free radicals (reactive oxygen species). Melatonin also exists in higher plants (edible plants), and is inadvertently obtained from daily meals.

Glycation intermediates and the two reactive carbonyl groups, namely the reactive carbonyl species (RCSs), are of great importance for the cytotoxicity of advanced glycation endproducts (AGEs). The toxic AGE precursors, such as 3-deoxyglucosone (3-DG), glyoxal (GO), and methylglyoxal (MGO), are characteristic of individuals susceptible to diabetic complications. Although the chemical reactions leading to AGE formation are nonenzymatic, the RCS levels are ultimately controlled by enzymatic mechanisms involving glyoxalase, MG and 3-DG reductase. The RCS intermediates act as potent crosslinkers. Protein crosslinking results in aggregates that form intracellular protease-resistant and ubiquitin-proteasome-resistant deposits, and consequently affect specific protein functions. Therefore, the formation of RCSs is considered the key event in the intermediate stage of protein glycation.

AGEs are not only markers but also principal causative factors for the pathogenesis of diabetes complications, including neuropathy, nephropathy, retinopathy, cataract, and other health disorders, such as atherosclerosis, Alzheimer’s disease, and normal aging. Thus, the discovery of AGE inhibitors can offer a promising therapeutic approach for the prevention of diabetes and other pathogenic complications. Both natural compounds and synthetic compounds have been evaluated as inhibitors of AGEs formation. The synthetic AGE inhibitors discovered to date can be divided into three classes: (I) carbonyl-trapping agents that attenuate carbonyl stress; (II) metal ion chelators, which suppress glycoxidation; and (III) cross-link breakers that reverse AGE cross-links.

Aminoguanidine, a hydrazine-like molecule, is a nucleophilic agent that traps reactive carbonyl intermediates.
such as 3-DG, GO, and MGO to form relatively nontoxic adducts. However, the drug was not ultimately approved for commercial production due to side effects observed in diabetes patients in phase III clinical trials; such effects may be related to the sequestration of pyridoxal, resulting in vitamin B6 deficiency. Despite AG’s limitations, proof-of-concept studies indicated that inhibition of AGE formation by trapping reactive carbonyl species could be a reasonable therapeutic approach for the treatment of diabetes complications.

Free radicals are generated during the early stage of glycation. Schiff bases are prone to oxidation, generating free radicals and reactive carbonyl groups, and the glycated protein can also catalyze the generation of free radicals. Therefore, the generation of reactive carbonyl or dicarbonyl groups is related to oxidative metabolism. At an early stage, anti-glycation strategies involve scavenging of hydroxyl radicals and superoxide radicals to alleviate oxidative stress, and reducing the generation of reactive carbonyl or dicarbonyl groups. Thus, these strategies inhibit glycation. Melatonin’s functions as an anti-oxidant include: I), direct free radical scavenging, II), stimulation of anti-oxidative enzymes, and III), augmenting the efficiency of other anti-oxidants. Many anti-oxidants can protect against free radicals generated during glycation.

In our previous results of the skin accumulation of AGEs, which was detected as specific fluorescence, we examined the relation to lifestyle behaviors, such as smoking and alcohol drinking, lack of sleep clearly, and promoted AGE accumulation. We hypothesized that melatonin may participate in this phenomenon. In this regard, because of the direct free radical scavenging capacity of melatonin, and as part of revealing the undiscovered function of melatonin, we investigate whether it has reactive carbonyl scavenging capacity or not.

Methods

Chemicals and instrument

Aminoguanidine, phosphoric acid, 40% glyoxal solution, sodium bicarbonate (NaHCO₃), acetonitrile (ACN) and ethanol were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Melatonin and MGO solution was purchased from Sigma-Aldrich (St. Louis, MO, USA). 2, 3-diaminonaphthalene (DAN) and 3-DG were purchased from Dojindo Laboratories, Kumamoto, Japan. Analytical high performance liquid chromatography (HPLC) was carried out using a LC-20AT system equipped with SPD-20A UV/VIS detector and LC-solution software (Shimadzu, Kyoto, Japan).

Evaluation of the inhibitory effect of 3-DG, GO and MGO formation

In order to evaluate the effect of melatonin on the formation of 3-DG, GO and MGO, 3 concentrations (10 mg/mL, 1 mg/mL and 0.1 mg/mL) were prepared by dissolving in ethanol. The effect of aminoguanidine on the formation of 3-DG, GO and MGO was also analyzed as a reference compound to compare the effect of melatonin. As advanced glycation end product intermediates 3-DG, GO and MGO were measured by HPLC (LC-20AT) equipped with a SPD-20A UV/VIS detector and LC-solution software (Shimadzu).

Results

Melatonin did not demonstrate an anti-glycation effect in the in vitro glucose HSA reaction model, i.e., an inhibitory effect on the formation of fluorescent AGEs (Fig. 1). At the concentration of 1 mg/mL and 10 mg/mL of melatonin, % inhibition of fluorescent AGE formation was 7.4% and 30.1% respectively. On the contrary, aminoguanidine, as a positive control, showed a dose-dependent inhibitory effect.

Carbonyl trapping activity of melatonin was examined also in the in vitro glucose HSA reaction model (Fig. 2). However, melatonin showed no activity, while aminoguanidine dose-dependently reduced the formation of carbonyl compounds, 3-DG, GO and MGO.

Discussion

Effects of melatonin on fluorescent AGE formation

The fluorescent AGE inhibition activity of melatonin has been investigated in HSA/glucose model along with aminoguanidine as a reference compound. Fluorescent AGEs inhibition activity of aminoguanidine increases dose dependently. At a concentration of 10 mg/mL, aminoguanidine inhibits fluorescent AGE formation by more than 95%. On the other hand, no noticeable fluorescent AGE inhibition activity has been observed with melatonin. Similar results have also been found in the in vitro collagen I/glucose reaction model (data not shown).
**Fig. 1.** Effect of melatonin and aminoguanidine on fluorescent AGE formation.
Concentrations of melatonin and aminoguanidine were 0.1 mg/mL, 1 mg/mL and 10 mg/mL. Results are expressed as means ± standard deviation, n = 3. AGE, advanced glycation endproduct.

**Fig. 2.** Carbonyl trapping activity of melatonin.
a) 3-DG inhibition rate (%), b) GO inhibition rate (%), c) MGO inhibition rate (%). Concentrations of melatonin and aminoguanidine were 0.1 mg/mL, 1 mg/mL and 10 mg/mL (n = 2). 3-DG, 3-deoxyglucosone; GO, glyoxal; MGO, methylglyoxal.
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Inhibitory effects on the formation of 3-DG, GO and MGO

The reactive carbonyl species (RCS) such as 3-DG, GO, and MGO act as potent crosslinkers. Formation of RCSs is considered the central event in the intermediate phase of protein glycation.² The inhibitory activity of aminoguanidine on the generation of 3-DG, GO, and MGO increases dose-dependent manner. At a concentration of 10 mg/mL, aminoguanidine inhibits the reactive carbonyl species by more than 90%. Historically, aminoguanidine, a hydrazine-like small molecule, was the first AGE inhibitor explored in clinical trials. It is a nucleophilic agent that traps reactive carbonyl species such as 3-DG, GO, and MGO to form relatively nontoxic adducts.¹⁶ No inhibitory activity of melatonin against any reactive dicarbonyl has been found at any concentration. Our findings suggest that there is no direct role of melatonin in AGE formation.

Conclusion

The present study revealed that melatonin neither inhibit fluorescent formation nor inhibit the generation of reactive carbonyl intermediates such as 3-DG, GO, and MGO.

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Conflict of Interest Statement

The authors state that the performance of this study entailed no issues representing a conflict of interest.

References