Urine 6-sulfatoxymelatonin levels are inversely associated with arterial stiffness in post-menopausal women

Jee-Yon Lee, Duk-Chul Lee*

Department of Family Medicine, Severance Hospital, Yonsei University, College of Medicine, Yonsei 50, Seodaemun-gu, Seoul 120-752, Republic of Korea

A R T I C L E   I N F O

Article history:
Received 10 January 2014
Received in revised form 23 March 2014
Accepted 26 March 2014

Keywords:
Melatonin
6-Sulfatoxymelatonin
Arterial stiffness
Atherosclerosis
Menopause

A B S T R A C T

Object: The secretion of melatonin, a pleiotropic hormone mainly synthesized by the pineal gland, typically decreases with age and may be associated with the development of aging-related pathologic conditions such as cardiovascular disease. Atherosclerosis is an aging-related disease, the pathogenesis of which involves chronic inflammation and increased oxidative stress. Since melatonin has both anti-oxidant and anti-inflammatory properties, it may be associated with atherosclerosis. Therefore, we investigated the relationship between urine concentrations of 6-sulfatoxymelatonin (aMT6s) and arterial stiffness in post-menopausal women.

Methods: A total of 66 post-menopausal women participated in the study. Melatonin secretion was estimated by measuring aMT6s levels in first morning urine samples. The cardio-ankle vascular index (CAVI) was used as an indicator of arterial stiffness.

Results: Estimated mean CAVI decreased gradually with increasing aMT6s quartiles. The multivariate logistic regression analysis showed that the fourth aMT6s quartile was associated with a high CAVI with an adjusted odds ratio of 0.02 (95% confidence interval, 0.01–0.47).

Conclusion: Our study revealed an inverse relationship between urine aMT6s and arterial stiffness as determined by CAVI. Although it is impossible to determine causality, our results suggest that melatonin may have a beneficial role in the pathogenesis of atherosclerosis. Further prospective studies are required to establish the clinical significance of our study.

© 2014 Elsevier Ireland Ltd. All rights reserved.

1. Introduction

Cardiovascular disease (CVD) is a leading cause of death in the world, and the prevalence of CVD is rapidly increasing after menopause in women [1]. Atherosclerosis, a chronic inflammatory vascular disease characterized by the development of arterial plaques, is known to increase the risk of CVD including coronary artery disease and stroke, as well as mortality in the elderly [2,3]. The pathogenesis of atherosclerosis is a complicated process involving inter-related biological, hormonal, and immunological mechanisms [4]. Among them, increased oxidative stress and inflammatory cytokines associated with aging are thought to play important roles in the disease process [5,6].

Melatonin, a neuro-hormone produced mainly in the pineal gland, is a pleiotropic molecule with diverse biological functions. In addition to its well-known role as a circadian rhythm regulator, melatonin is also a highly potent anti-oxidant [7] as well as an effective anti-inflammatory agent [8]. Endogenous secretion of melatonin decreases with age, especially during menopause in women [9]. Melatonin secretion can reduce the risks associated with various aging-related diseases and conditions, including dementia [10], cancer [11], and insulin resistance [12]. Furthermore, the beneficial roles of melatonin on CVD have been reported recently. Low endogenous melatonin levels have been associated with increased risk of CVD including hypertension [13] and coronary artery disease [14]; conversely, melatonin administration significantly reduced blood pressure [15,16] and ischemic/reperfusion coronary injury [17]. Although the precise role of melatonin with respect to CVD has not been elucidated, the anti-oxidative and anti-inflammatory properties of melatonin are thought to be involved [4].

Because atherosclerosis is one of the most common aging-related diseases associated with oxidative stress and inflammation and increases in prevalence after the onset of menopause in women [18], reduced melatonin secretion may be associated with the risk of atherosclerosis in post-menopausal women; however, there are currently no published clinical studies that evaluate this. In the first examination of its kind, we investigated the
relationship between first morning urine levels of 6-sulfatoxymelatonin (aMT6s, a major metabolite of melatonin) and arterial stiffness (a predictor of CVD, often associated with atherosclerosis) in 67 post-menopausal Korean women.

2. Methods

2.1. Ethics statement

All subjects participated in the study voluntarily, and written informed consent was obtained from each participant. The study complied with the Declaration of Helsinki and the Institutional Review Board of Severance Hospital (4-2011-0695) approved this study.

2.2. Study participants

This study was performed as part of the Yonsei Menopause Study (YMS) [19], which is designed to investigate factors related to the health of peri- and post-menopausal women in Korea. Total 50 peri-menopausal and 100 post-menopausal women between ages of 45 and 65 years who were apparently healthy participated in the study from July 2013 to October 2013. Participants were recruited via advertisements posted by the Department of Family Medicine at Severance Hospital. Post-menopausal status was defined as having had no menstrual periods for 12 consecutive months without any biological or physiological cause.

We excluded women with medical conditions, including those with a history of chronic liver disease, chronic renal disease, coronary artery occlusive disease, cerebro-vascular disease, or cancer. Women with a history of hysterectomy or hormone therapy use were also excluded.

Anthropometric measurements, lifestyle questionnaire and biochemical blood test were performed in all participants. Among them, only 80 postmenopausal women completed urine melatonin measurement.

Additionally, arterial stiffness measurements were performed on November 2013. One researcher made a phone call to every participant and informed them about the arterial stiffness measurements. 79 participants who agreed visited the outpatient clinic one more time and completed arterial stiffness measurement. Among 79 participants, our study finally included 68 women with menopause.

The diabetes group was defined as women with fasting blood glucose ≥126 mg/dL or the use of insulin or other hypoglycemic medication.

2.3. Measurements

All subjects were asked to complete a lifestyle questionnaire that included questions about tobacco and alcohol use as well as exercise habits. Tobacco use was defined as current smoking, and alcohol use was defined as drinking ≥70 g of alcohol/day or more frequently than once a week. Regular exercise was defined as physical exercise or work performed more than three times a week for 30 min.

Anthropometric measurements were performed by one well-trained family medicine doctor. Blood pressure (BP, measured with subject seated, following a 10-min resting period), body mass index (BMI, weight divided by height squared [kg/m²]), and waist circumference (cm, measured at the umbilicus level with subject standing) were measured. Blood samples were collected after a 12-h overnight fasting period. Fasting glucose, high sensitivity C-reactive protein (hs-CRP), total cholesterol, high-density lipoprotein cholesterol (HDL-cholesterol), and triglycerides levels were measured with an ADVIA 1650 chemistry system (Siemens Medical Solutions, Tarrytown, NY, USA). The inter-assay coefficients of variance for fasting glucose, hs-CRP, total cholesterol, HDL-cholesterol, and triglycerides were 1.58 ± 4.90%, 3.76 ± 0.39%, 0.9 ± 0.94%, 1.37 ± 0.47%, and 1.01 ± 0.92%, respectively. The Friedewald equation was used to calculate low-density lipoprotein cholesterol levels [LDL-cholesterol = Total cholesterol – (HDL-cholesterol + Triglycerides)/5]. Fasting insulin was measured by electrochemiluminescence using an Elecsys 2010 immunoassay analyzer (Roche, Indianapolis, IN, USA). The inter-assay coefficient of variance was 2.55 ± 0.53%. The homeostatis model assessment of insulin resistance (HOMA-IR) was used to estimate insulin resistance (HOMA-IR index: [insulin (μIU/mL) × fasting blood glucose [mg/dL]/18])/22.5). Serum adiponectin was measured with an enzyme immunoassay kit (Mesdia, Seoul, South Korea), and the inter- and intra-assay coefficients of variance were 4.6 ± 1.4% and 4.5 ± 0.6%, respectively.

Study participants that had systolic BP ≥140 mmHg or diastolic BP ≥90 mmHg or who were taking anti-hypertensive medications were classified as having hypertension. Study participants that had a fasting blood glucose level ≥126 mg/dL or who were taking insulin or other hypoglycemic medications were classified as having diabetes.

2.4. Measurement of urinary aMT6s levels

The first morning urine was collected from each study participant. An enzyme-linked immunosorbent assay (Genway Biotech Inc., San Diego, CA, USA) was used to measure urine aMT6s levels. The inter- and intra-assay coefficients of variance were 5.1–14.9% and 5.2–12.2%, respectively.

2.5. Measurement of arterial stiffness

CAVI was measured with a VaSera VS-1000 instrument (Fukuda Denshi Co. Ltd., Tokyo, Japan) using previously described methods [20]. Subjects were examined in supine position after 10 min of bed rest. Electrocardiogram electrodes were placed on both wrists and a phonocardiogram was placed on the right sternum border of the second intercostal space. Cuffs were applied around both upper arms and ankles to detect brachial and ankle pulse waves. Pulse wave velocity was measured by dividing pulse wave length by the time needed for the pulse wave to propagate from the aorta, through the femoral artery, to the tibial artery of the ankle. CAVI was calculated as follows:

\[
\text{CAVI} = a \left[ \left( \frac{2 \rho}{\Delta P} \right) \times \ln \left( \frac{P_s}{P_d} \right) \right] + b,
\]

where \(P_s\) = systolic pressure, \(P_d\) = diastolic pressure, \(\Delta P = P_s - P_d\), \(\rho = \text{blood density}, \text{a and b are constants. We recorded mean values of right and left CAVI.}\)

2.6. Statistical analysis

Normally distributed data were expressed as means ± standard deviations (SD), and non-normally distributed data were expressed as medians and interquartile ranges. Non-normally distributed data including aMT6s, fasting glucose, fasting insulin, triglycerides, and hs-CRP levels, as well as HOMA-IR, were logarithmically transformed to reduce the skewness of the distribution. The aMT6s urine concentration quartiles (Q1–Q4) were categorized as follows, and were compared using linear trend analysis: Q1: <2.48 ng/mL, Q2: 2.48–5.72 ng/mL, Q3: 5.72–8.88 ng/mL, Q4: >8.88 ng/mL. Pearson correlation analysis was performed to evaluate relationships between aMT6s levels and other clinical variables. Estimated mean CAVIs corresponding to each of the aMT6s quartiles were calculated by analysis of covariance (ANCOVA). Multiple linear regression
analysis was used to identify factors contributing to arterial stiffness. For this analysis, variables with \( p < 0.05 \) (as determined by Pearson correlation analysis) and clinically important variables including age, BMI, alcohol and tobacco use, and exercise habits were accounted for. If there was significant correlation (\( r > 0.8 \)) between two variables, only one was entered into the model. The odds ratios and 95% confidence intervals (CIs) for the prevalence of high CAVI were calculated after adjusting for confounding variables across aMT6s quartiles using multivariate logistic regression analysis. High CAVI was defined as a value greater than the cutoff of 75th percentiles. In model 2 analysis, clinically important variables (age, BMI, alcohol and tobacco use, and exercise habits) were selected as confounding factors. In model 3 analysis, variables with \( p < 0.05 \) (as determined by univariate logistic regression analysis = systolic BP, HOMA-IR, HDL-cholesterol) and clinically important variables (age, BMI, alcohol and tobacco use, and exercise habits) were selected as confounding factors. We performed all statistical analyses using the Statistical Package for the Social Sciences, version 19/8.0 (SPSS Inc., Chicago, IL, USA). Statistical significance was defined as \( p < 0.05 \).

### 3. Results

Table 1 shows the clinical characteristics of the 66 women, summarized according to aMT6s quartile. Mean fasting insulin, hs-CRP, and CAVI were significantly higher (\( p < 0.05 \)) in patients who had aMT6s urine concentrations >8.88 ng/mL (Q4). In a simple correlation, CAVI was positively correlated with age (\( r = 0.32, p = 0.01 \)), BMI (\( r = 0.28, p = 0.02 \)), waist circumference (\( r = 0.36, p < 0.01 \)), systolic BP (\( r = 0.29, p = 0.02 \)), fasting glucose (\( r = 0.28, p = 0.02 \)), and HOMA-IR (\( r = 0.28, p = 0.02 \)) and negatively correlated with HDL-cholesterol (\( r = -0.25, p = 0.04 \)) and adiponectin (\( r = -0.28, p = 0.02 \)) (data not shown). Fig. 1 illustrates the inverse relationship between aMT6s concentration and CAVI (\( r = -0.53, p < 0.01 \)). The mean log CAVI decreased gradually with increasing aMT6s concentration after adjusting for age, tobacco and alcohol use, exercise habits, systolic BP, HOMA-IR, and HDL-cholesterol (Fig. 2).

In a linear multiple regression analysis, age, fasting glucose, and urine aMT6s were identified as significant explanatory variables for CAVI, accounting for 32% of the variance in CAVIs (Table 2).

Table 3 shows the prevalence of high CAVI according to the aMT6s quartiles. The multivariate-adjusted odds ratio (95% CI) for the fourth versus the first quartiles was 0.03 (0.01–0.47) after adjusting for age, BMI, tobacco and alcohol use, exercise habits, HOMA-IR, and HDL-cholesterol.

### 4. Discussion

Our cross-sectional study showed an inverse relationship between urine aMT6s levels and arterial stiffness in 66 postmenopausal women. This association was statistically significant after adjusting for confounding factors that may affect an individual’s risk of developing atherosclerosis.

Melatonin has diverse functions within the human body, and reduced endogenous melatonin is likely related to several aging-associated pathologic conditions including CVD [4,10,11,12]. Although atherosclerosis is one of the most common aging-related diseases and increases the risk of CVD [2,3], the relationship between melatonin levels and atherosclerosis is not yet well understood. Ours is the first clinical study to investigate the relationship between endogenous melatonin secretion and atherosclerosis.

We measured overnight melatonin secretion by collecting an early morning urine sample from each study participant and measuring the concentration of aMT6s, a major metabolite of melatonin. aMT6s has correlated well with serum and salivary melatonin levels in previous studies [21,22] and is known to accurately reflect overnight plasma melatonin concentrations [23,24]. Therefore, first morning urine aMT6s concentration could be a reliable measure of overnight melatonin secretion. In addition, arterial stiffness, which is often a feature of atherosclerosis, was estimated using CAVI. Brachial-ankle peak wave velocity (baPWV) was widely used in the measurement of arterial stiffness before the development of CAVI because of its simple, non-invasive technique and reproducible results. baPWV, however, can be influenced by blood pressure fluctuations during measurement [25]. CAVI, a recently-developed method that estimates arterial stiffness by integrating PWV and blood pressure information, is independent of blood pressure fluctuations [26] and shows high reliability in cardiovascular...
risk prediction [27,28]. Recent studies have reported the superiority of CAVI to baPWV in measuring arterial stiffness and predicting of coronary artery disease [29,30]. Furthermore, CAVI showed significant agreement with carotid–femoral PWV, which is considered the gold standard of arterial stiffness measurement but is a more invasive test than baPWV [31]. Wang et al. reported that both cPWW and CAVI results were positively correlated with homocysteine levels in vascular disease patients; additionally, cPWW and CAVI results showed a linear positive correlation independent of homocysteine levels [32]. These findings collectively suggest that CAVI can be a reliable index for measuring arterial stiffness.

The precise reasons behind the relationship between urine aMT6s concentration and arterial stiffness remain unclear; here, we present three possible explanations. The first involves the antioxidative properties of melatonin. Lipoprotein oxidation is the main cause of atherosclerosis, and oxidized lipids are present in human atherosclerotic plaques [33]. LDL particles are known to be oxidized by various types of free radicals in the arterial intima [6], and research has demonstrated that increased levels of free radicals (including superoxide) impair endothelial function [34]. Melatonin and its metabolites detoxify free radicals directly by scavenging, and also up-regulate anti-oxidant enzymes and inhibit oxidizing enzymes [35,36]. Melatonin also has been shown to effectively inhibit the oxidation of LDL particles in vitro [37]. Although it is not possible to definitively establish causality based on the results of our cross-sectional study, our findings and those of other researchers collectively suggest that melatonin may act protectively with respect to atherosclerosis by reducing oxidative stress.

The second possible explanation involves melatonin’s role as an anti-inflammatory agent. Atherosclerosis is a chronic inflammatory vascular disease, and inflammatory cascades play an important role in its pathogenesis [5]. Once arterial wall tissue is damaged, inflammatory cascades are triggered and pro-inflammatory cytokines facilitate atherosclerotic plaque formation [38]. Melatonin is known to reduce inflammation by modulating the immune system [39,40], and may selectively block cyclooxygenase 2 and downstream inflammatory moderators [41]. Six weeks of melatonin administration to hypertensive rats reduced renal cell inflammation by decreasing nuclear factor kappa-light-chain-enhancer
activity [42]. Similarly, our study revealed decreased hs-CRP levels, a chronic inflammatory marker, according to increased aMT6s quartiles. Hence, the anti-inflammatory properties of melatonin may partially explain the relationship between urinary aMT6s and arterial stiffness.

The hypolipidemic or “lipid-lowering” role of melatonin is the third possible explanation for melatonin’s association with arterial stiffness. High levels of LDL-cholesterol and triglycerides are associated with atherosclerosis [43], whereas HDLs help protect against the disease via removal of cholesterol from macrophages [44]. In rats, melatonin administration decreased serum free cholesterol and increased cholesterol esterification; in human mononuclear leukocytes, incubation with melatonin decreased cholesterol synthesis and lowered the LDL fraction of total cholesterol [45,46]. Furthermore, Tamura et al. reported that one month of melatonin supplementation significantly elevated HDL-cholesterol levels in peri- and post-menopausal women [47]. These findings suggest that the hypolipidemic properties of melatonin may protect against the development of atherosclerosis. Further prospective and experimental studies are needed to elucidate the precise mechanisms underlying the role(s) of melatonin in the pathogenesis of atherosclerosis.

Despite compelling results, our study has some limitations which should be considered when designing any further studies on this topic. First, the cross-sectional design of our study precludes determination of a causal relationship between aMT6s levels and arterial stiffness. Second, the small sample size (all from within a single hospital) does not allow for extrapolation to the population at large. Third, although we adjusted for several metabolic parameters that may influence melatonin secretion and atherosclerosis, it is possible that unidentified mediating factors influenced our study results.

In conclusion, urinary aMT6s levels were independently associated with the prevalence of arterial stiffness in post-menopausal Korean women. These findings suggest that endogenous secretion of melatonin may be associated with the pathogenesis of atherosclerosis in post-menopausal women, and although finding causality from this study is impossible, melatonin administration may show promise for use in the prevention of atherosclerosis. Further prospective studies are needed to fully understand the clinical significance of our findings.

### Competing interests

The authors declared no conflict of interest.

### Funding

The authors have received no funding for this article.

### Ethical approval

The study was approved by the Institutional Review Board of Severance Hospital.

### References