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Increased melatonin in oral mucosal tissue of oral lichen planus (OLP) patients: A possible link between melatonin and its role in oral mucosal inflammation

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ABSTRACT

Objective: The existence of extra-pineal melatonin has been observed in various tissues. No prior studies of melatonin in human oral mucosal tissue under the condition of chronic inflammation have been reported. The aim of this study was to investigate the presence of melatonin in oral mucosal tissue of patients with oral lichen planus (OLP) which was considered as a chronic inflammatory immune-mediated disease causing oral mucosal damage and ulcerations.
Materials and methods: Sections from formalin-fixed and paraffin-embedded oral mucosal tissue of OLP patients (n = 30), and control subjects (n = 30) were used in this study. Immunohistochemical staining was performed and the semiquantitative scoring system was used to assess the levels of arylalkylamine-*N*-acetyltransferase (AANAT: a rate-limiting enzyme in the biosynthesis pathway of melatonin), melatonin, and melatonin receptor 1 (MT1) in oral mucosa of OLP patients and normal oral mucosa of control subjects.
Results: AANAT, melatonin, and MT1were detected in oral mucosal tissue of OLP patients and control subjects. Immunostaining scores of AANAT, melatonin, and MT1 in oral mucosal tissue of OLP patients were significantly higher than those in control subjects ($p = 0.002$, $p < 0.001$, and $p = 0.031$, respectively).
Conclusions: Increased levels of AANAT, melatonin, and MT1 in the inflamed oral mucosal tissue of OLP patients imply that chronic inflammation may induce the local biosynthesis of melatonin via AANAT, and may enhance the action of melatonin via MT1.

1. Introduction

Melatonin (*N*-acetyl-5-methoxytryptamine) is mainly produced by the pineal gland, and considered as the major chronobiotic hormone (Hardeland, 2008; Johnston & Skene, 2015). The biosynthesis of pineal derived melatonin is regulated by the suprachiasmatic nuclei-driven sympathetic innervation of the pineal gland, leading to the activation of arylalkylamine-*N*-acetyltransferase (AANAT) which is a rate-limiting enzyme in the melatonin synthesis pathway (Klein, 2007). Melatonin has two major mechanisms of actions: receptor-mediated and receptor-independent pathways (Reiter, Tan, & Galano, 2014). There are two membrane-bound G-protein related melatonin receptors: MT1 and MT2 (Dubocovich, & Markowska, 2005). Melatonin also binds other molecules such as quinone reductase 2 (Calamini, Santarsiero, Boutin, & Mesecar, 2008), and nuclear receptors (Smirnov, 2001). Besides its influence on circadian regulation, melatonin has other biological functions such as anti-oxidant activity (Tan, Manchester, Esteban-Zubero, Zhou, & Reiter, 2015); anti-inflammatory activity (Mauriz, Collado, Veneroso, Reiter, & Gonzalez-

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Gallego, 2013); and immunomodulation (Carrillo-Vico, Lardone, Alvarez-Sanchez, Rodriguez-Rodriguez, & Guerrero, 2013). The role of melatonin in the innate and acquired immune response were addressed in several review articles (Cardinali, Esquifino, Srinivasan, & Pandi-Perumal, 2008; Calvo, Gonzalez-Yanes, & Maldonado, 2013). It was reported that melatonin and its related molecules were produced by activated immune cells in the inflamed tissue (Markus, Cecon, & Pires-Lapa, 2013).

In addition to the pineal gland, the existence of melatonin-synthesizing enzymes, melatonin, and melatonin receptors has been demonstrated in many tissues (for a review, see Acuna-Castroviejo et al., 2014). It was suggested that extra-pineal melatonin might help protecting cells from oxidative and inflammatory damage (Acuna-Castroviejo et al., 2014). Information on melatonin and its receptors in human oral tissue is limited. Melatonin and melatonin-synthesizing enzymes were observed in epithelial cells of striated ducts of submandibular glands, whereas MT1 was undetected (Shimozuma et al., 2011). It was reported that MT1 was detected in ameloblasts and odontoblasts of dental tooth germs (Kumasaka et al., 2010). No prior studies of melatonin in human oral mucosal tissue under the condition of chronic inflammation have been reported.

Oral lichen planus (OLP) is a chronic inflammatory immune-mediated disease of oral mucosal tissue, and is usually found in middle-aged adults with more female predilection (Alrashdan, Cirillo, & McCullough, 2016). Clinical characteristics of OLP consist of redness and white lines on oral mucosa, called Wickham striae (Scully & Carrozzo, 2008). The manifestation of OLP can be classified as reticular, papular, plaque, atrophic, erosive, and bullous (Gupta & Jawanda, 2015). The lesions with atrophic and erosive types usually cause mucosal damage and oral ulcerations which interfere patients' quality of life (Rana, Kanatas, Herzberg, Gellrich, & Rana, 2015; Tadakamadla, Kumar, & Johnson, 2015). Histopathological findings of OLP usually demonstrate intense subepithelial infiltration of T-lymphocytes, degeneration of basal keratinocytes, and epithelial basement membrane disruption (Gupta & Jawanda, 2015; Scully & Carrozzo, 2008). OLP has been considered as a potentially malignant disorder associated with an increased risk for oral cancer (Gandolfo et al., 2004; Warnakulasuriya, Johnson, & van der Waal, 2007; van der Waal, 2009). The exact etiology of OLP is not fully understood. Most studies concerning the pathogenesis of OLP have focused on the immunological aspects (Chaiyarit et al., 1999; Kurago, 2016; Payeras, Cherubini, Figueiredo, & Salum, 2013; Sugerman et al., 2002). Accumulated data support the involvement of cell-mediated immune dysfunction in the development of OLP (Firth et al., 2015; Wang, Zhou, Fu, Wang, & Zhou, 2015; Zhang et al., 2015). However, data of melatonin in oral mucosa from OLP patients have never been reported. Therefore, we hypothesized that chronic inflammation might affect the production of melatonin in the inflamed oral mucosal tissue of OLP patients. The present study was conducted to test the hypothesis by comparing the levels of AANAT, melatonin, and MT1 in the inflamed oral mucosal tissue from OLP patients with those in the normal oral mucosa of control subjects, using an immunohistochemical method.

2. Materials and methods

2.1. Selection of oral mucosal tissue specimens

This study was approved for the use of tissues from human subjects by the institutional human ethics committee of Khon Kaen University (HE572236). Thirty formalin-fixed and paraffin-embedded biopsy specimens from OLP patients and 30 normal oral mucosal (NOM) specimens from control subjects were obtained from the archives of Oral Pathology Division, Faculty of Dentistry, Khon Kaen University. The inclusion criteria to select tissue biopsy specimens were based on OLP patients's recorded data as follows: (1) oral lesions clinically demonstrated redness and white lines defined as Wickham striae; (2) OLP patients had no history of systemic diseases, smoking cigarettes, receiving medications or being pregnant; (3) selected biopsy specimens of OLP patients were histopathologically confirmed by oral pathologists. The histopathological criteria of OLP were defined as: lymphocytic infiltration in the subepithelial layer and degeneration of basal epithelial cells. The exclusion criteria were as follows: (1) OLP patients had history of systemic diseases, cigarettes smoking, receiving medications, or being pregnant; (2) OLP patients received topical or systemic steroid for treatment of OLP in the past 3 months. Systemic diseases were defined as infectious or non-infectious diseases which were reported in the patient's dental chart such as heart diseases, hematologic diseases, lung diseases, GI tract diseases, liver disease, kidney diseases, and endocrine disorders. Medications were defined as any drugs that the patient had taken according to his or her systemic diseases reported in the patient's dental chart.

According to the ethical limitations in collecting normal tissue samples with the same sites of surgical biopsy and with age and gender matching to those in the OLP group, tissue biopsy specimens in the control group were alternatively obtained from retromolar area of the surgical procedure of tooth extraction from healthy individuals. The subjects had no history of systemic diseases, cigarettes smoking, receiving medications or being pregnant. Selected tissue biopsy specimens in the control group were clinically and histopathologically diagnosed as normal oral mucosa. Demographic and clinical data of OLP patients and control subjects were summarized in Table 1.

2.2. Immunohistochemistry

The sections were cut with 5 micrometer thickness from each biopsy specimen, placed on glass slides, and left in hot air oven at 60 °C for 24 h. The sections were deparaffinized in xylene, rehydrated through graded alcohols. Microwave-based antigen retrieval was done in 1 mmol/L sodium citrate buffer at pH of 6.0 for 10 min. The sections were left at room temperature for 10 min, and washed with phosphate buffered saline (PBS) twice for 5 min. Endogenous peroxidase activity was quenched by Peroxo-Block™

Table 1
Demographic and clinical data of OLP patients and control subjects.

	OLP patients	Control subjects
Gender (cases)		
male	4	10
female	26	20
Age* (years)		
mean ± SD	55.67 ± 9.62	22.67 ± 7.08
range	34–86	18–57
Biopsy sites (cases)		
buccal mucosa	26	–
gingival mucosa	2	–
labial mucosa	2	–
retromolar area of buccal mucosa	–	30
Clinical diagnoses (cases)		
reticular OLP	1	–
atrophic OLP	15	–
erosive OLP	14	–
normal oral mucosa with impacted teeth	–	30

* Significant differences in age between OLP patients and control subjects were observed ($p = 0.001$).