In vitro Permeation of Melatonin through a Mucous Membrane Model

การซึมผ่านในหลอดทดลองของเมลาโทนินผ่านเมมเบรนเยื่อเมือก

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ABSTRACT

Melatonin, a hormone secreted by the pineal gland, shows a potent antioxidant activity which might be effective as an anti-inflammatory when topically applied in oral cavity. For this activity, melatonin has to permeate through mucus membrane so an *in vitro* permeation was investigated using Franz diffusion cells and porcine esophageal epithelia. The permeated content was analyzed by fluorescence spectrophotometry at λ_{ex} 285nm and λ_{em} 345 ± 2 nm. Theaverage rates of melatonin permeated across the esophageal epithelia at 37 ± 2°C was about 12 $\mu g \cdot cm^{-2} \cdot h^{-1}$. ATR – FTIR spectra of the membranes used in the melatonin permeation at 3 h show spectrum changes at C – O regions (range 1500 – 1000 cm^{-1}) when compared to control. It suggests that melatonin permeated across the mucosa membrane. This leads to conclude that melatonin permeated the mucosa throug the receptor solution.

บทคัดย่อ

เมลาโทนินเป็นฮอร์โมนที่หลั่งมาจากต่อมไพเนียล ที่แสดงฤทธิ์ต้านอนุมูลอิสระที่มีศักยภาพซึ่งอาจจะมีประสิทธิภาพต้านการอักเสบเมื่อใช้ ในช่องปาก การศึกษานี้เป็นการทดสอบการซึมผ่านเยื่อกั้นของเมลาโทนินในหลอดทดลองโดยใช้Franz diffusion cells และชั้นepitheliaของหลอดอาหารหมูเป็นเมมเบรน วิเคราะห์โดยfluorescence spectrophotometry ที่ความยาวคลื่น λ_{ex} 285และλ_{em} 345 ± 2 นาโนเมตร ผลการศึกษาพบว่าอัตราเฉลี่ยของเมลาโทนินสะสมที่ซึมผ่านหลอดอาหารที่อุณหภูมิ 37 ± 2 °C ประมาณ 12 $\mu g \cdot cm^{-2} \cdot h^{-1}$ ผลของ ATR - FTIRSpectra ของเยื่อกั้นที่ใช้ในการซึมผ่านเมลาโทนินที่ 3 ชั่วโมงสเปกตรัมแสดงการเปลี่ยนแปลงที่ C-O regions (ช่วง 1250 – 1000 cm^{-1}) เมื่อเทียบกับการควบคุม แสดงให้เห็นว่าเมลาโทนินสามารถซึมผ่านชั้นเยื่อกั้นmucosa ไปสู่สารละลายตัวรับ

Key Words: Melatonin, Permeation, ATR-FTIR Spectroscopy

คำสำคัญ: เมลาโทนิน การซึมผ่าน ATR-FTIR Spectroscopy

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Introduction

Melatonin, N-acetyl-5-methoxytryptamine, is an indolamineneurohormone produced by the pineal gland in a circadian 24 h cycle. Known as a panacea, melatonin is evolutionary conserved and exerts many regulatory functions by modulating cellular behavior via binding to specific receptors and intracellular targets and has been used for many purposes such as sleep disturbances, jet lag, shift work, neuropsychiatric conditions and sleep disorders, cancer, chemotherapy side effects, cardiovascular health, epilepsy, migraines, and anti-oxidant (Claustrat et al. 2005). It is a natural compound known to our body and could exert pro- and anti-inflammatory effects suggesting that it might involve with both early phase inflammation and attenuation (Radogn et al. 2010). Its potent antioxidant ability allows scavenging of oxidative stress in the inflamed tissues (Claustrat et al. 2005;Gomez – Moreno et al. 2010;Benes et al. 1997;Karbownik& Reiter. 2000). According to circadian rhythm, its diurnal oscillation of saliva levels in normal volunteers, ranging between about 7 - 24 pg /ml allows melatonin to regulate and maintain oral health benefits (Gomez – Moreno et al. 2010;Eriksson et al. 2003). Most of the dental treatment, dealt with inflammation in the oral cavity, e.g. after tooth extraction or surgical procedures, could be one of the potential applications of topical melatonin (Gomez – Moreno et al. 2010). To do so, the permeation profile of melatonin needs to be determined.

Its physicochemical parameters suggest that melatonin, practically insoluble and partially partition between an interface of oil and water, should permeate biological membrane to some extent. An *in vitro* permeation of melatonin through porcine skin suggests that its permeation profile was rather low and could be enhanced by some adjuvants (Kikwai et al. 2002). Buccal mucosa differs from the skin barrier and thus the permeation profiles should be different. It was aimed to investigate the buccal permeation of melatonin using the esophagus as the model membrane (Consuelo et al. 2009;Sudhakar et al. 2006). Quantifications were performed by using fluorescence spectroscopy and qualitative analysis of trace amount in the membrane was conducted by attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIRspectroscopy).

Materials and methods

Spcetrofluorometer(Luminescence Spectrometer LS 50 B; PerkinElmer, U.K.), amber-glass Franz diffusion cells (Crown Glass Company, INC., U.S.A.),FT – IR (FT-IR Spectrometer Tensor 27Series, Bruker Optics, U.S.A), Germanium crystal plate(The Pike Technologies. Miracle, U.S.A.), Melatonin (Sigma Chemical, U.S.A.), Vernier caliper (Mitutoyo Co., Japan), Uitrasonic cleaner (Uitrasonic cleaner SK126 OHP, Kudos,China)

In vitro permeation studies

Donor solutions

Saturate solutions of melatonin were freshly prepared by mixing an excess amount of melatonin in water for 30 mins with ultrasonic power 85%, discarding the powder by filtration.

Pre-treatment of the Barrier membrane

Fresh porcine esophagus (n = 7) were obtained from a local slaughterhouse (Muang, KhonKaen, Thailand) and transported in cool conditions for use within 24 h after sacrifices. Isotonic phosphate buffer

(pH 7.4) was used to rinse and wash the esophagus which was longitudinally cut. The mucosa was then separated from the muscular layer by cutting the loose connective fibers with a scalpel (100-150 µm thickness). To isolate the epithelia, the excised esophageal mucosa was immersed in saline solution at 60 °C for 45 min after which the membrane was carefully teased away from the underlying tissue (Eriksson et al. 2003). It was divided into 4 – 6 pieces (1.5 cm in diameter each) were cut and fully hydrated before mounting between compartments of the Franz cells.

Receptor solution

The receptor compartment was filled with a magnetic follower and 5 ml of deionized water.

Franz diffusion cells

Franz diffusion cells (diffusional surface areas of 0.786 cm^2)were assembled by filling the receptor solution into its compartment, placing the pre-treated membrane in-between the 2 compartments, clamping the 2 compartments and controlling the temperature and stirring speed of the receptor at 37 ± 2° C and 600 rpm, respectively. Sampling of these, taken at predetermined time and fresh receptor medium of the same volume was replaced, was analyzed by spectrofluorometry. Cumulative amount of melatonin permeated per area (in cm^2) of the membrane was calculated in relative to same day standards and plotted as a function of time according to Fick's First law. The used barrier membranes were analyzed by ATR-FTIR.

Quantitative analysis by spectrofluorometric analysis

Melatonin standards in water were freshly prepared and analyzed by the fluorescence spectrophotometer at λ_{ex} 285nm and λ_{em} 345 ± 2 nm. Within and between-day analysis of 6 replicates gave reliable and reproducible in a linear relationship at the concentration between 50 ng/ml – 3 µg/ml with the limit of detection of 0.14 µg/ml and limit of quantification of 0.21µg/ml and CV of 10.1% This method was thus valid for use in this study.

Sample analysis was estimated in proportions to the same range standards determined within the same day.

ATR FTIR spectrometric analysis

The permeated membranes was cut into pieces (2×2 cm), dried in a vacuum desiccator for 1 h and exposed to a single reflection Germanium (Ge) probe of the ATR-FTIR spectrometer for the spectrum recording recorded in the range of 4000–1000 cm^{-1}.

Results

Fig. 1 showed the cumulative permeated amount of melatonin detected from the receptor media was found to be linearly correlation to the time of study until about 3 - 4 h. After 4 h, the esophagus membrane would be degraded. The permeation profiles of different parts of each esophagus were insignificantly different ($p > 0.05$). This was also the case of the results from different individuals. The overall steady state flux of melatonin across porcine esophageal mucosa was averaged to be 12 $\mu g \cdot cm^{-2} \cdot h^{-1}$. Little or no lag time was estimated from these samples, suggesting that melatonin promptly permeated across

the mucosa. Representative ATR-FTIR spectra of samples of the blank esophagus (A), in Fig 2, shows two main spectral rang corresponding to 3725 – 3000 cm^{-1} and 1800 – 1000 cm^{-1}. The major peak at 3250 cm^{-1}spectrum is NH group. At 1720 cm^{-1} is Amide I and 1550 cm^{-1} is Amide II. And at 1250, 1150 cm^{-1} is C – O regions(Akkas et al. 2007).The melatonin-permeated mucosa 3 h (B) showed evident changes in the ATR-FTIR spectra on NH group of samples due to the lipid inside mucosa, denaturedfrom temperature in permeation system. The mucosa spectrum absorb at higher than melatonin at the position of NH group at wave number about 3250 cm^{-1}. However, the C – O regions at wave number 1250 – 1150 provides clearer spectra. Therefore, melatonin permeability by considering the C – O regions showed better in determining the region.

This band is a shift towards higher wave numbers (3375 cm^{-1}). At the C – O regions have a lower absorbance.

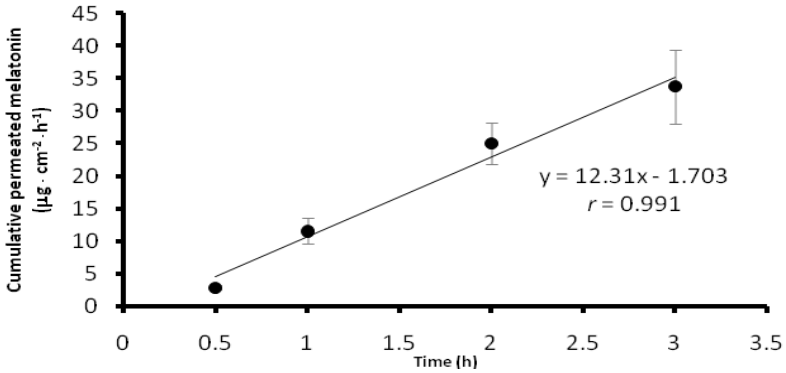

Fig.1 Cumulative amount of MT permeated (n = 32)

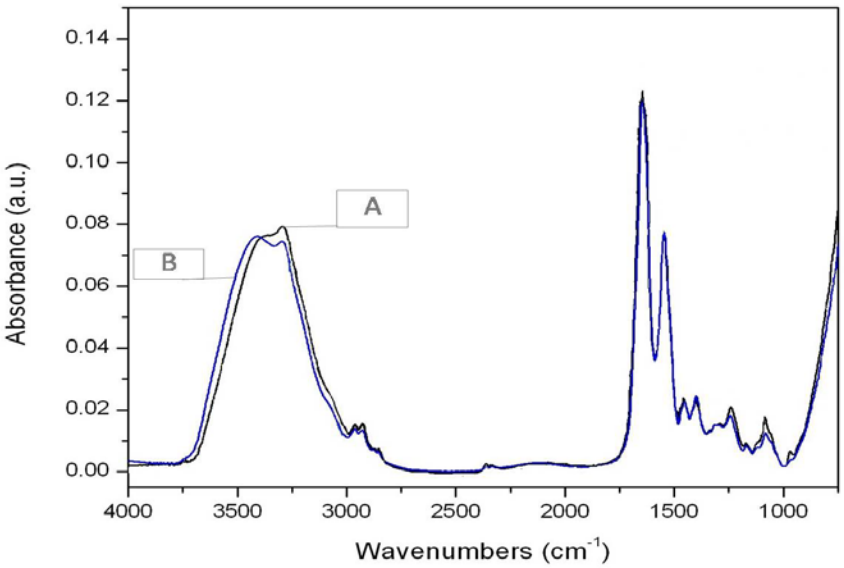

Fig. 2 ATR-FTIR spectra of the blank esophagus (A) and the melatonin-permeated esophagus(B)

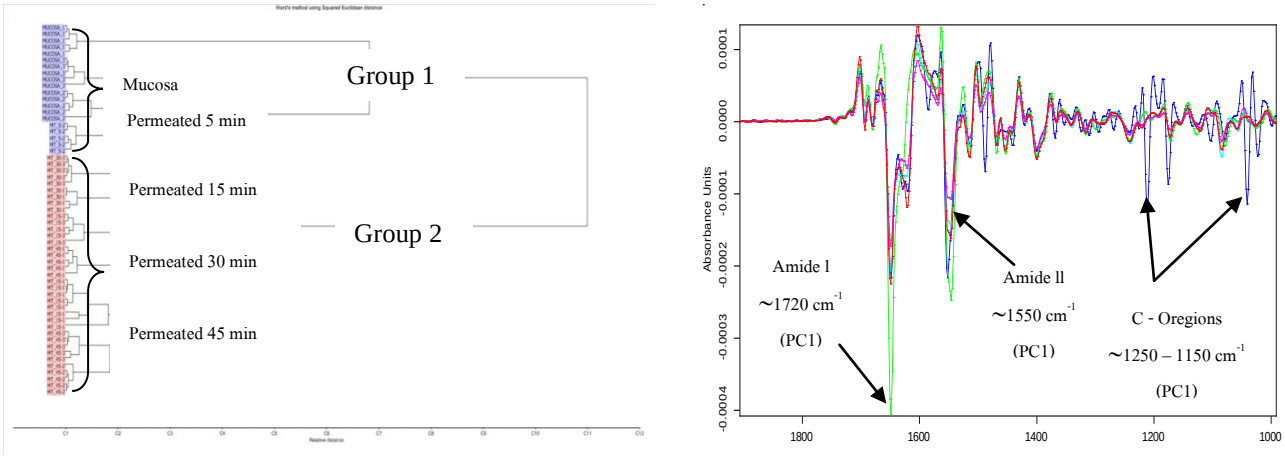

Fig. 3 (A) Cluster analysis of melatonin-permeated mucosa (EMSC andbaseline), (B) second derivativeof melatonin-permeated esophagus at 5, 15, 30, 45 min

All IR Spectrum were performed by Cluster analysis using EMSC and baseline data. From fig. 3, the samples were classified into 2 groups: Group 1 clustered blank mucosa and melatonin performed permeation for 5 min. Group 2 clustered groups of melatonin performed permeation for 15, 30 and 45 min. Thus, melatonin was found accumulative amount on the surface according to the permeation time. Moreover, from the second derivative spectrum, the permeation 5 min at 1250-1200 cm^{-1} separated from other lines. In conclusion, at the 15 and 30 minutes, melatonin permeates better than at 5 min comparing to the accumulated amount of melatonin on the surface membrane.

Conclusions

In vitro permeation of melatonin through porcine skin from an aqueous donor into phosphate buffer at pH 7.4 demonstrated a rate of about 2 $\mu g \cdot cm^{-1} \cdot h^{-1}$ (Kikwai et al. 2002). In this study the permeation rate across mucosa was about 12 $\mu g \cdot cm^{-2} \cdot h^{-1}$. It's more than permeation across porcine skin, because reasonable. When melatonin enters the body metabolism in the cell, and makes melatonin structure changes.

Topical applications exert anti-inflammatory activities in the oral cavity, particularly the buccal mucosa, requires both rapid and prolonged releases at a sufficient amount. Its saliva levels of normal volunteers of about 24 pg /ml during 23:00 – 8.00 h and 7 pg/ml at about 13:00 h (Gomez – Moreno et al. 2010;Kikwai et al. 2002). Melatonin in the saliva, very less had no effect on its use.

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