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Percutaneous Absorption and Model Membrane Variations of Melatonin in Aqueous-based Propylene Glycol and 2-Hydroxypropyl-β-cyclodextrin Vehicles

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Percutaneous absorption and model membrane variations of melatonin (MT) in aqueous-based propylene glycol and 2-hydroxypropyl-β-cyclodextrin vehicles were investigated. The excised hairless mouse skin (HMS) and two synthetic ethylene vinyl acetate (EVA) and microporous polyethylene (MPE) were selected as a model membrane. The solubility of MT was determined by phase equilibrium study. The vertical Franz® type cell was used for diffusion study. The concentration of MT was determined using reverse phase HPLC system. The MT solubility was the highest in a mixture of PG and 2-HPβCD. The percutaneous absorption of MT through excised HMS increased as the solubility increased. However, the permeability coefficient decreased and then slightly increased in a mixture of PG and 2-HPβCD. On the other hand, both flux and permeability coefficient through EVA membrane decreased as the solubility increased. No MT was detected over 12 h after starting diffusion through MPE membrane. The flux of MT was dependent on the type of membrane selected. Flux of MT was greatest in excised HMS followed by EVA and MPE membrane. Flux of MT through EVA membrane was 5~20 times lower when compared to excised HMS. Interestingly, volumes of donor phase when MPE membrane was used, significantly increased during the study period. The HMS might be applicable to expect plasma concentration of MT in human subjects based on flux and pharmacokinetic parameters as studied previously. The current studies may be applied to deliver MT transdermally using aqueous-based vehicles and to fabricate MT dosage forms.

Key words : Melatonin, Percutaneous absorption, Model membrane variations, Aqueous-based vehicles, Propylene glycol, 2-Hydroxypropyl-β-cyclodextrin

INTRODUCTION

Transdermal delivery is one of considering routes to deliver various drugs for a desired length of time (Kydonieus and Berner, 1987; Hadgraft and Guy, 1989). Melatonin (MT) as a model drug is an indole amide neurohormone secreted by the pineal gland in a circadian rhythm (Waldahuser and Dietzel, 1985). It was reported that sustained release dosage form that mimics the endogenous circadian rhythms of MT in a physiological fashion could be effective in resetting circadian rhythm disorders of MT such as sleep disorder jet-lag, shift work syndrome and seasonal affective disease in humans (Haimov *et al.*, 1995; Garfinkel *et al.*, 1995; Lee *et al.*, 1995). For these reason, oral, transmucosal and transdermal delivery system for MT has been widely investigated due to its short half-life (Benes *et al.*, 1993; Lee *et al.*, 1994; Konsil *et al.*, 1995; Lee *et al.*, 1995; Lee *et al.*, 1996; Lee and Min, 1996; Lee *et al.*, in press).

We previously reported that Hilltop chamber® containing MT in PG 40% solution could be delivered transdermally in human subjects (Lee *et al.*, 1994). The effect of fabrication techniques on the MT release through EVA membranes with different ratios of vinyl acetate content was also investigated (Konsil *et al.*, 1995). Recently, we reported that low aqueous solubility of MT significantly increased in a mixture of propylene glycol (PG) and 2-hydroxypropyl-β-cyclodextrin (2-HPβCD), motivating enhanced percutaneous absorption of MT as useful vehicles (Lee *et al.*, 1997). PG and cyclodextrin derivatives have been used in the transdermal delivery of various drugs (Kydonieus

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and Berner, 1987; Hadgraft and Guy, 1989; Lee *et al.*, 1994; Loftsson *et al.*, 1989; Chang and Banga, 1996). However, the percutaneous absorption of MT has not been widely investigated.

The purpose of this work was to investigate percutaneous absorption of MT as a model drug in aqueous-based PG and 2-HPβCD vehicles. The excised hairless mouse skin (HMS) and synthetic ethylene vinyl acetate (EVA) and microporous polyethylene (MPE) were selected as a model membrane.

MATERIALS AND METHODS

Materials

Melatonin (MT) was purchased from Morepen (New Delhi, India). Ten week old, male hairless mice (body weight 20~25 g, 4 weeks age, HRS/J strain) were purchased from Charles River (Wilmington, MA, USA). Propylene glycol (PG) was obtained from J.T. Baker (Phillisburg, NJ, USA). 2-hydroxypropyl-β-cyclodextrin (2-HPβCD) was provided by courtesy of Richwood (Seoul, Korea). Ethylene vinyl acetate (EVA) with 9% vinyl acetate, a 3 M CoTran™ controlled caliper film #9702 and microporous polyethylene (MPE), a 3 M CoTran™ microporous film #9710 membrane were provided by 3 M company (St. Paul. MN, USA). All chemicals used were of reagent grade. Deionized water was used throughout the study.

Equilibrium solubility of MT

Excess amounts of MT were added to 0.05 M phosphate buffer (pH 6.1) containing various concentrations (w/w %) of PG and 2-HPβCD expressed as weight percentages. The resulting mixtures were placed in a 25± 0.1°C constant temperature water bath. Parafilm was used to cover the top to prevent evaporation. Equilibrium solubility was reached within 24 h. Samples were centrifuged at 10,000 g for 15 min and the clear supernatant was collected and diluted with phosphate buffer. MT concentration was determined by HPLC as previously reported (Lee and Min, 1996).

Diffusion studies

After two weeks' acclimation period, the hairless mice were sacrificed by cervical dislocation. Full thickness abdominal HMS was excised and kept for 15 min in 0.9% physiological saline equilibrated at 30°C, and then mounted immediately on the diffusion cell.

Skin and synthetic membrane samples were mounted between the donor and receptor phase in vertical Franz® type diffusion cells (3.14 cm^2). Vehicle solutions (1.5 ml) containing saturated MT, based on the its solubility were applied to donor chamber using a pipette. Receptor phase filled with 0.9% physiological saline was stirred by a motor-driven magnetic stirring star bars and maintained at 32°C (approximate skin temperature) with a circulating water bath during the entire experiment. Samples (150 μl) from the receptor chamber were collected using a pipette attached with teflon tube through the sampling port of the diffusion cell at a given sampling interval for 12 h. The receptor phase was replenished with an equal volume of 0.9% physiological saline after each sample were collected. Sink conditions were maintained in the receptor. Analysis of each subsequent sample was corrected for all previous samples that had been removed for analysis. The collected samples were centrifuged at 7000 g for 10 min to remove any insoluble residue. MT concentration was determined by HPLC as reported previously.

Data analysis

The cumulative amount of MT (μg/cm^2) penetrated was calculated based on the MT concentration and volume in the receptor phase. The flux (μg/h/cm^2) of MT vehicles was obtained from the slope and the intercept of the plot of cumulative amount of MT (μg/cm^2) penetrated at steady state against time using linear regression analysis. According to the Fick's equation, the flux is expressed as follows.

$$\text{Flux} = \frac{1}{S}\frac{dQ}{dt} = \frac{DK}{h}C_s\, t = P\, C_s\, t$$

where Q, amount of drug penetrated through membrane with thickness (h) at time (t), S, area of membrane exposed, D, diffusion coefficient, K, partition coefficient, C_s, concentration of donor phase, and P, permeability coefficient, respectively.

All values are expressed as mean (standard deviation of at least three determinations.

RESULTS AND DISCUSSION

The cumulative amount of MT penetrated in various aqueous-based PG and 2-HPβCD vehicles through excised HMS is shown in Fig. 1. The flux and permeability coefficient of MT as a function of MT solubility in various aqueous-based vehicles through excised HMS are also compared in Fig. 2. The observed MT solubility in buffer, PG 20%, PG 40%, 2-HPβCD 30% and PG 40%/ 2-HPβCD 30% was 6.5, 12.1, 41.4, 78.1 and 87.9 mM, respectively. MT solubility was the highest in a mixture of PG 40% and 2-HPβCD 30%, resulting in increased flux when compared to vehicle alone. The percutaneous absorption of MT through excised HMS increased as the solubility increased. However, the permeability coefficient decreased and then slightly increased in a mixture of PG and 2-HPβCD. MT solubility in PG 40%/2-HPβCD 30

% increased about 13 times when compared to buffer but flux of MT only twice increased. Flux of MT may be more compensated by the decreasing partitioning coefficient of MT and the increasing MT solubility. It was also noted that although the solubility of MT was highest in a mixture of PG 40%/2-HPβCD 30% solution, the solubilization efficiency was reduced because the observed total solubility of MT was not equivalent to the sum of MT solubility in PG and 2-HPβCD individually (Lee *et al.*, 1997). PG and 2-HPβCD may be effective to improve MT permeability through excised HMS. PG as a cosolvent, acts on the skin to enhance permeation of the drug. The enhancing effects of 2-HPβCD on MT flux may result from the increase of MT solubility by complexation as well as direct membrane disruption due to extraction of lipids from the skin (Loftsson *et al.*, 1989). However, it was also noted that percutaneous absorption of hydrocotisone using iontophoresis was reduced by complexation with cyclodextrins (Chang and Banga, 1996).

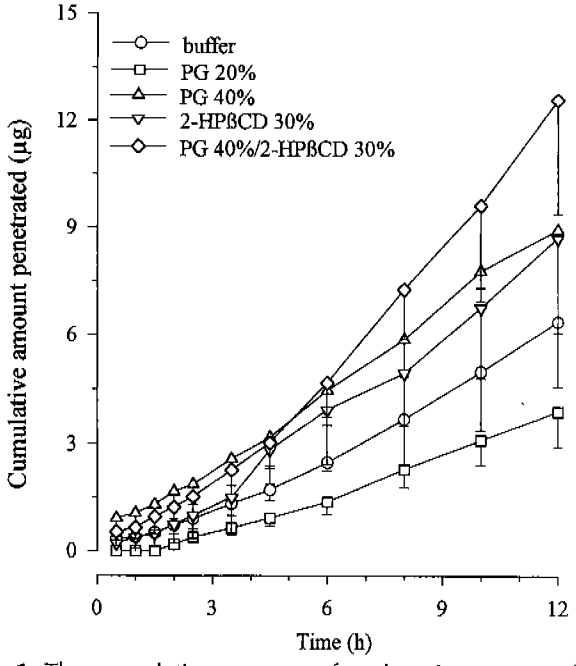

Fig. 1. The cumulative amount of melatonin penetrated in various aqueous-based propylene glycol and 2-hydroxypropyl-β-cyclodextrin vehicles through excised hairless mouse skin. Values are expressed as mean±standard deviation (n=5).

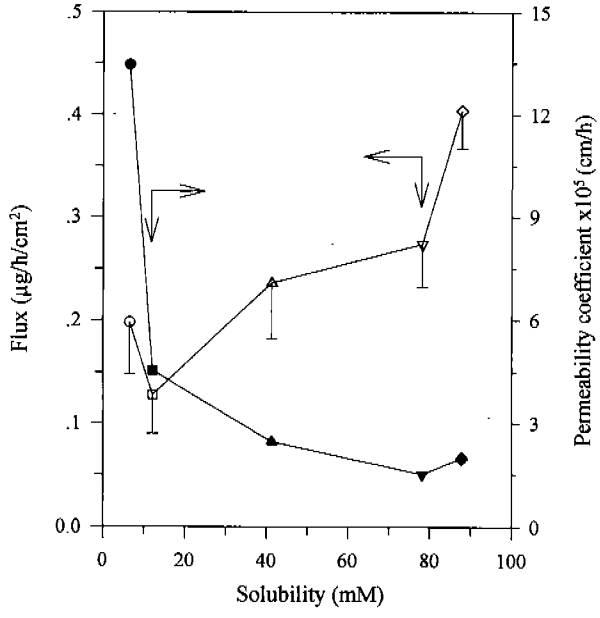

Fig. 2. Comparison of flux (open symbols) and permeability coefficient (closed symbols) of melatonin as a function of MT solubility in various aqueous-based buffer (○), propylene glycol 20% (□), propylene glycol 40% (△), 2-hydroxypropyl-β-cyclodextrin 30% (▽) and a mixture of propylene glycol 40% and 2-hydroxypropyl-β-cyclodextrin 30% (◇) vehicles through excised hairless mouse skin.

Many polymeric membranes have also been used to control drug diffusion and fabricate transdermal delivery devices. Permeability of MT through two commercially available 3 M CoTran™ films, EVA and MPE membranes as a component of transdermal delivery device was evaluated in a mixture of PG and 2-HPβCD vehicles.

The cumulative amount of MT penetrated in various aqueous-based PG and 2-HPβCD vehicles through EVA membranes is shown in Fig. 3. The flux and permeability coefficient of MT through EVA membrane decreased when a vehicle of PG /2-HPβCD was used. PG and 2-HPβCD appeared to be less efficient to improve permeability of MT through this membrane.

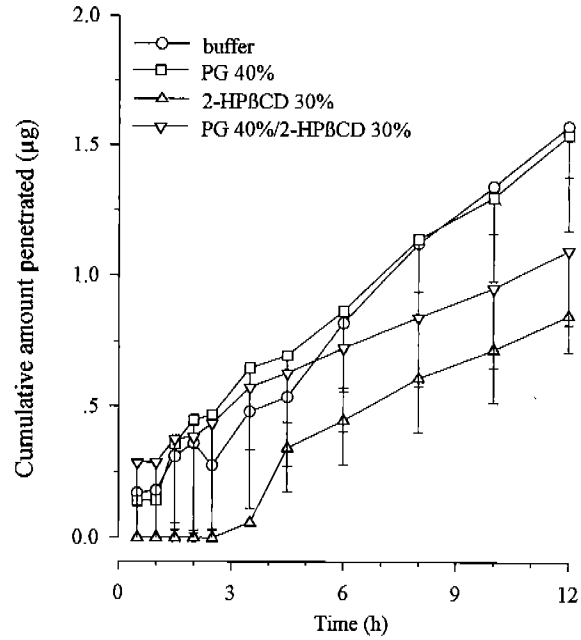

Fig. 3. The cumulative amount of melatonin penetrated in various aqueous-based propylene glycol and 2-hydroxypropyl-β-cyclodextrin vehicles through EVA membranes. Values are expressed as mean±standard deviation (n=5).

The flux and permeability coefficient of MT as a function of MT solubility in various aqueous-based vehicles through EVA membrane is also compared in Fig. 4. Unlike the excised HMS as studied previously, flux of MT through EVA membrane had a tendency to decrease as MT solubility increased. On the other hand, no MT diffused through the MPE membrane over 12 h no matter which vehicles were used. Flux of MT through MPE membrane was so low or negligible that they could not be used as a rate controlled membrane. It was observed that the release rate of drug from adhesive polymer matrix was governed by the drug solubility and diffusion coefficient in polymer (Roy *et al.*, 1996). Although MT solubility was increased by complexation with 2-HPβCD, permeation of MT might be hindered in case of synthetic membranes due to its low solubility and permeability.

This finding was verified by mass balance studies of MT in two synthetic membranes. The mass balance studies of MT in a vehicle of PG 40% and 2-HPβCD 30% saturated with MT through synthetic membranes at 12 h after MT diffusion are shown in Table I. The amount of MT penetrated through EVA membrane was so low. The vinyl acetate contents in EVA membranes were very important to control the permeability of MT as previously studied in our group, showing increased flux as the vinyl acetate contents increased (Konsil *et al.*, 1995). However, PG/2-HPβCD vehicles were ineffective for the penetration of MT through MPE membranes.

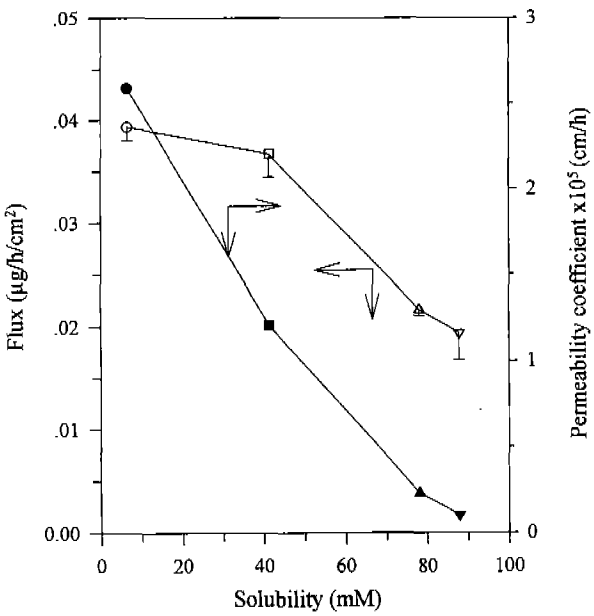

Fig. 4. Comparison of flux (open symbols) and permeability coefficient (closed symbols) of melatonin as a function of MT solubility in various aqueous-based buffer (○), propylene glycol 40% (□), 2-hydroxypropyl-β-cyclodextrin 30% (△) and a mixture of propylene glycol 40% and 2-hydroxypropyl-β-cyclodextrin 30% (▽) vehicles through EVA membrane.

Table I. Mass balance studies of MT in a vehicle of PG 40% and 2-HPβCD 30% through synthetic membranes at 12 h after MT diffusion (n=4)

Applied (mg)		Recovered		
		Donor (mg)	Membrane (μg)	Receptor (μg)
EVA	22.17	21.98±0.45	ND[a]	1.08±0.37
MPE	22.17	22.07±0.39	ND	ND

[a]Not detectable.

On the other hand, volume changes of donor phase containing MT in a vehicle of PG 40% and 2-HPβCD 30% through two synthetic membranes at 12 h after diffusion study are also noted in Table II. With the EVA membrane, no significant volume changes of donor phase occurred except small evaporation of solvent. However, the volumes of donor phase applied were markedly increased in case of MPE membrane. If solvent transport across membranes is involved in drug permeability, volume changes of donor phase may affect MT flux through the membranes. This reverse solvent flux may also prevent the drug from migrating to the receptor phase. However, the volume changes were not affected by the surface of membranes exposed to donor phase. The detailed phenomena needs to be further validated, based on the membrane structure, diffusion property and osmotic behavior.

The flux of MT with the same vehicle compositions was clearly dependent on the type of membrane selected by affecting penetration parameters in Fick's equation. Flux of MT was greatest in excised HMS, followed by the EVA and MPE membrane. Flux of MT through the EVA membrane was 5~20 times lower when compared to excised HMS (see also Fig. 2 and Fig. 4). Is the flux of MT in aqueous-based PG and 2-HPβCD vehicles enough to reach the endogenous plasma concentration? When the percutaneous absorption is treated as intravenous infusion assuming

Table II. Volume changes (%) of donor phase containing MT in a vehicle of PG 40% and 2-HPβCD 30% through synthetic membranes at 12 h after diffusion study

Membrane[a]	Surface[a]	Applied (ml)[b]	Recovered (ml)[c]	%[d]
EVA	G	1.5	1.43±0.035	-5.0±2.4
EVA	NG	1.5	1.38±0.035	-8.3±2.4
MPE	G	1.5	3.70±0.14	146.7±9.4
MPE	NG	1.5	3.73±0.11	148.3±7.1

[a]Membrane surface exposed to donor phase, G, glassy, NG, non-glassy.
[b]Total volume (ml) initially applied to donor phase.
[c]Total volume (ml) recovered from donor phase after diffusion for 12 h.
[d]Volume changes (%)=(recovered volume-applied volume)÷applied volume×100.

no skin metabolism and degradation is occurred, the flux (about 0.24 μg/h/cm^2) in PG 40% vehicles gives about 25 pg/ml, based on pharmacokinetic parameters of MT (Lee *et al.*, 1995). It was reported that MT could be delivered transdermally when PG 40% vehicles only having 0.24 μg/h/cm^2 of flux was used in human subjects, giving plasma concentration in the range of 20~100 pg/ml (Lee *et al.*, 1994). Therefore, the HMS might be useful to expect plasma concentration of MT in human subjects. Furthermore, the percutaneous absorption of MT using other penetration promoters through HMS is widely under investigation. However, the flux through EVA membrane was so low that it could be used as a rate controlling membrane if the percutaneous absorption of MT was highly increased using enhancers. The MPE membranes may be used as backing materials because no MT was penetrated over 12 h. The combination of artificial membranes for biphasic or circadian rhythmic release of drugs may be utilized but it needs to be further validated in the future.

In conclusion, flux of MT was dependent on the aqueous-based formulation vehicles containing PG and 2-HPβCD, and the type of model membranes selected. The current studies may be applied to deliver MT transdermally using aqueous-based vehicles and to fabricate MT dosage forms.

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