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Effects of vehicles and enhancers on transdermal delivery of melatonin

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Abstract

For a more effective transdermal delivery of melatonin (MT), the effects of vehicles and enhancers on its skin permeation and lag time were evaluated. Skin permeation study was conducted in Franz diffusion cells using excised hairless mouse skins. MT was analyzed by HPLC. As vehicles, ethanol (EtOH), polyethylene glycol 400 (PEG), or propylene glycol (PG) was used alone or mixed with a phosphate buffer. Binary vehicles (EtOH/buffer, PEG/buffer, PG/buffer) showed different effects on the skin permeation of MT and its lag time. Compared with the buffer alone, the PEG/buffer shortened the lag time of MT but reduced its skin permeation. EtOH/buffer significantly increased the flux of MT but prolonged the lag time with the content of EtOH. PG/buffer did not affect the lag time but slightly increased the skin permeation of MT at the higher content of PG ($\geq 80\%$). These results indicate that the composition of vehicles exerts significant influence but it per se might have limitation in modulating the transdermal delivery of MT. Next, one tested whether fatty acids could more effectively enhance the skin permeation of MT and shorten its lag time. Given the influence of vehicles on both permeation and lag time, PG was used as a vehicle for fatty acids. The permeation-enhancing effects of saturated fatty acids increased in the following order: C10 > C12 > C14 > C16 > C18. The saturated fatty acid, however, did not significantly shorten the lag time regardless of the carbon chain length. Meanwhile, similar to saturated lauric acid (C12), unsaturated oleic acid (C18) dramatically enhanced the skin permeability coefficient of MT more than 950-fold over the effect of PG alone. Moreover, oleic acid showed the shortest lag time (2.1 h). The results suggest that oleic acid in a suitable vehicle could more effectively enhance the skin permeation of MT and shorten its lag time than did the vehicles of various compositions.

Keywords: Melatonin; Transdermal delivery; Fatty acid; Enhancer; Vehicle

1. Introduction

Melatonin (MT), a neurohormone produced from the pineal gland, has been extensively studied for its therapeutic potential. MT was reported

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to be useful for the treatments of insomnia (Haimov et al., 1995) and circadian rhythm disorders manifested in jet lag and shift work (Bubenik et al., 1998). Recently, MT was also shown to be effective in the treatment of cancer-related thrombocytopenia (Lissoni et al., 1999) and the prevention of cyclosporine-induced nephrotoxicity (Kumar et al., 1999). With the new findings on the various therapeutic potentials of MT, it would be of great importance to develop the optimal pharmaceutical dosage forms of MT.

Currently, MT is administered orally. Owing to its short half-life (< 30 min) (Mallo et al., 1990), oral controlled-release formulations liberating MT for prolonged periods have been developed (Zisapel, 1996). However, orally administered MT undergoes extensive first-pass hepatic metabolism and suffers from low and variable bioavailibility (Lane and Moss, 1985).

To reduce the high hepatic extraction ratio of MT and enhance its bioavailibility, other routes of administration such as the transbuccal (Dawson et al., 1998), intranasal (Bechgaard et al., 1999), and transdermal routes (Lee et al., 1994a) have been investigated. Of the non-oral dosage forms, transdermal delivery system might have potential to avoid first-pass metabolism of MT and provide sustained plasma levels. Furthermore, the skin-protecting effect of MT indicates that MT might be a good candidate for transdermal delivery (Dreher et al., 1998). However, the impractically long lag time and relatively low skin absorption profiles of MT have limited the development of the transdermal delivery systems of MT (Benes et al., 1997).

The composition of vehicles has been reported to increase the skin permeation and shorten the lag time of several drugs. The combination of vehicles has been shown to modulate the transdermal delivery of ketoprofen (Kim et al., 1992, 1993; Goto et al., 1993) and tegafur (Lee et al., 1993). Carelli et al. (1998) reported that slight changes in vehicle composition could highly influence the skin permeation of yohimbine.

In cases of other drugs such as zidovudine (Kim and Chien, 1996) and dihydrotestosterone (Clarys et al., 1998), the addition of enhancers have been shown to be required for effective transdermal delivery from the aspect of both permeation and lag time. It has been reported that the need for enhancers might vary depending on the hydrophilicity of drugs (Lee et al., 1994b).

Thus, in this study aimed at designing a more effective transdermal delivery system of MT, one first tested whether the use of binary vehicles could effectively modulate the skin permeability of MT and its lag time. It is reported here that the composition of vehicle mixtures significantly affects the skin permeation of MT but to the limited extent. Next, the effects of fatty acids on the transdermal delivery of MT were tested. As compared with the binary vehicles, the use of fatty acids such as oleic acid dramatically enhanced the skin permeation of MT as well as shortened its lag time.

2. Materials and methods

2.1. Materials

MT was purchased from Aegis (Morton Grove, IL). Capric acid (CA), lauric acid (LA), myristic acid (MA), palmitic acid (PA), stearic acid (SA), and oleic acid (OA) were supplied from Sigma (St. Louis, MO). Female hairless mice (type SKH), 10–16 weeks old, were kindly provided from Yuhan Pharmaceutical (Seoul, South Korea).

2.2. Analysis of MT

The amount of MT was quantified by HPLC (Hitachi LTD, Tokyo, Japan) using a Nova-Pak C18 column (3.9×150 mm, 4 μm, Waters, Milford, USA). The mobile phase was a mixture of 47% 0.01 M acetate buffer (pH 5.0) and 53% methanol. The flow rate was 0.8 ml/min and the eluent was monitored at 229 nm.

2.3. Solubility test

The solubility of MT in various vehicles was determined by saturating the vehicles with MT. Excess amounts of MT were added to vehicles composed of a phosphate buffer mixed with solvents such as ethanol (EtOH), propylene glycol (PG), and polyethylene glycol 400 (PEG). The resulting suspensions were shaken for 5 days in a

water bath at 32 ± 1°C, then filtered through a membrane filter with a pore size of 0.45 μm. The amounts of MT in the filtrates were determined by HPLC.

2.4. Diffusion study

A section of abdominal skin from a female hairless mouse was mounted with epidermis uppermost in a Franz-type diffusion cell. The available diffusion area was 3.14 cm^2. The temperature of a receptor chamber was maintained at 37 ± 0.5°C. The receptor chamber was filled with degassed saline to prevent the formation of air bubbles at the skin-receptor fluid interface. Formulations containing MT (1.5 ml) were placed within the donor chamber, then the donor part was occluded with parafilm. Samples (0.2 ml) of the receptor solution were taken at designated time intervals. The volume of each sample was replaced with the same volume of saline. The amounts of MT in the samples were determined by HPLC as described above.

2.5. Penetration parameters

Cumulative amounts of MT (μg) permeated per unit area were plotted against time. Lag time (t_L, h) was determined by extrapolating the linear portion of each curve to the time axis. The steady-state flux (J_{ss}, μg/h per cm^2) was calculated from the slope. The apparent diffusion parameter (D/L^2) was calculated using Eq. (1). The permeability coefficient (K_p) was calculated using Eq. (2).

$$D/L^2 = 1/6t_L \quad (1)$$

$$K_p = J_{ss}/C_d \quad (2)$$

L represents the thickness of skin, C_d denotes the concentration of MT in the donor solution (mg/ml), and D is the diffusion coefficient.

2.6. Statistical analysis

Data are expressed as means ± S.D. ($n > 3$). Statistical differences between two mean values were evaluated using the unpaired Student's t-test. Results were taken as significantly different at $P < 0.05$.

3. Results and discussion

3.1. Solubility of MT in various vehicles

Additions of EtOH, PEG and PG to a phosphate buffer increased the solubility of MT. In all binary vehicles (EtOH/buffer, PEG/buffer, PG/buffer), the solubilities of MT increased exponentially with added organic solvents (Fig. 1). The solubility of MT in the binary vehicles also depended on the nature of the added solvent. With the same content of vehicle solvent, the solubility of MT was lowest in the PG/buffer. The EtOH/buffer and the PEG/buffer showed comparable values. For example, at 40% vehicle content, the solubility of MT was 13.9, 42.5 and 44.5 mg/ml in the PG/buffer, PEG/buffer and EtOH/buffer, respectively. The solubilities of MT were similar in pure PG (105.7 mg/ml) and EtOH (104.6 mg/ml), more than 60-fold higher than the solubility of MT in the phosphate buffer alone (1.6 mg/ml). Clearly, the solubility of MT can be effectively modulated over wide extremes by choice of vehicle. The exponentially increasing solubilities at low to modest contents of the water-miscible sol-

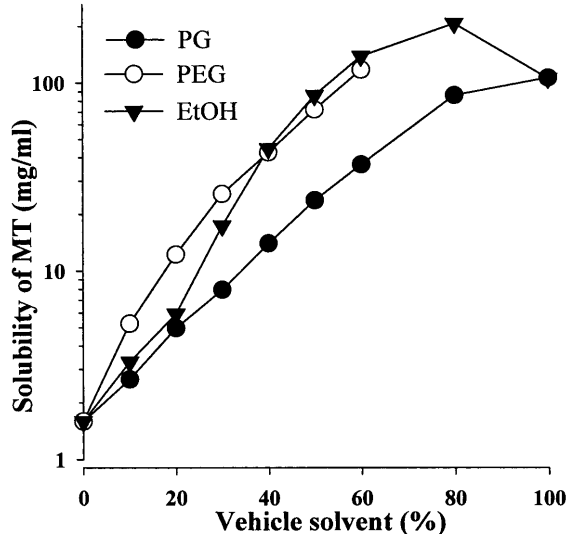

Fig. 1. Solubilities of melatonin (MT) in various vehicles. Vehicles were composed of a phosphate buffer mixed with a vehicle solvent (ethanol (EtOH), propylene glycol (PG), or polyethylene glycol 400 (PEG)). In some cases, a pure buffer or solvent was used as a vehicle.

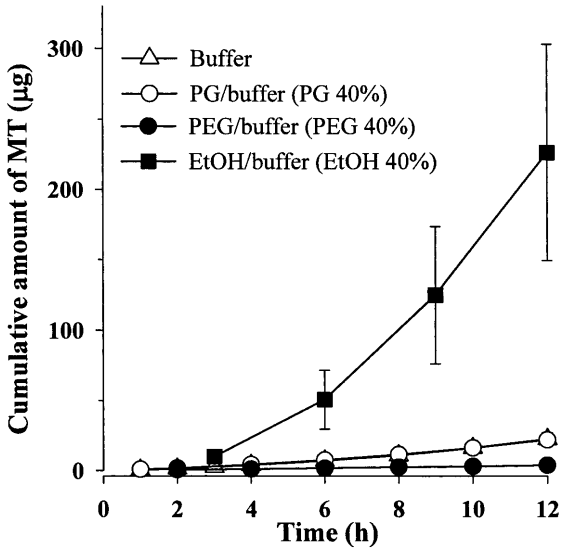

Fig. 2. Skin permeation profiles of melatonin (MT) delivered through various vehicles. Vehicles were composed of a 0.05 M phosphate buffer (pH 6.1) and 40% of a vehicle solvent (ethanol (EtOH), propylene glycol (PG), or polyethylene glycol 400 (PEG)). As a control, a 100% phosphate buffer was used as a vehicle. The amount of MT in the donor compartment was 15 mg. Data are expressed as means ± S.D. ($n = 4$).

vents establish the fact that MT is a relatively hydrophobic compound.

3.2. Effect of vehicles on the skin permeation of MT

Although EtOH, PG and PEG all increased the solubility of MT, they showed different effects on the skin permeation profiles of MT. Compared with the phosphate buffer alone, the EtOH/buffer system containing 40% EtOH greatly increased the skin permeation of MT (Fig. 2). The PG/buffer vehicle (40% PG) showed a skin permeation profile similar to that of the phosphate buffer alone. The 40% PEG-containing binary vehicle significantly reduced the skin permeation of MT. At 12 h of incubation, the cumulative amounts of MT permeated per unit area were 226.0 μg in the EtOH/buffer, 22.4 μg in the buffer, 21.8 μg in the PG/buffer, and 3.7 μg in the PEG/buffer (Fig. 2).

The apparent diffusion parameters of MT were affected by the composition of vehicles to different extents (Fig. 3A). Compared with the buffer, the PG/buffer binary vehicles did not significantly alter the apparent diffusion parameters (D/L^2) of MT while the EtOH/buffer gradually decreased them with the content of EtOH. The PEG/buffer increased the apparent diffusion parameters about 2-fold over the effect of buffer alone.

The skin permeability coefficients (K_p, cm/h) of MT were the highest in the EtOH/buffer followed by the PG/buffer and the PEG/buffer at each concentration of organic solvents (Fig. 3B). At 40% of vehicle solvents, K_p (cm/h) was 19.7 ×

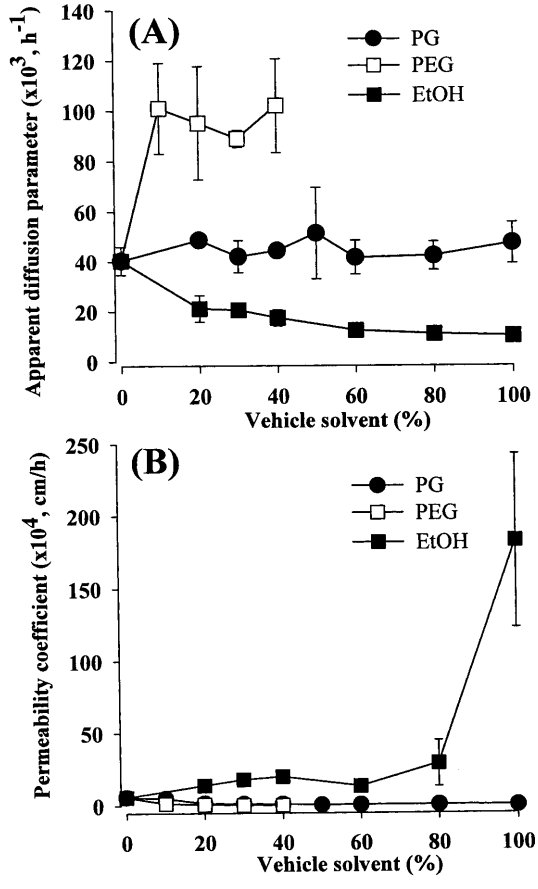

Fig. 3. Effect of vehicles on skin penetration parameters of melatonin (MT) through a hairless mouse skin. Effects of vehicles on the apparent diffusion parameters (A) and permeability coefficients of MT (B) were measured. Vehicles were composed of a phosphate buffer and a vehicle solvent (ethanol (EtOH), propylene glycol (PG), or polyethylene glycol 400 (PEG)). In some cases, a 100% buffer or solvent was used as a vehicle. Data are expressed as means ± S.D. ($n = 4$).

10^{-4} in the EtOH/buffer, 0.84×10^{-4} in the PG/buffer and 0.11×10^{-4} in the PEG/buffer. K_p values of MT gradually decreased with the contents of PEG, and was immeasurable in the PEG/buffer with more than 60% PEG because the amounts penetrated were below the detection limit of MT (4 ng/ml). In contrast, the EtOH/buffer enhanced the skin permeability of MT. The increase of skin permeability in EtOH/buffer was limited to 2–3-fold relative to the phosphate buffer. When pure EtOH was used as vehicle, the permeability coefficient of MT was greatly increased.

The increased K_p of MT observed at the binary vehicles with EtOH might be explained by the enhancer functions of EtOH. EtOH was reported to function as a solvent-type enhancer which permeates through the skin and increases the partition of a drug into skin (Knutson et al., 1990). In addition, the dramatic increase of K_p observed at pure EtOH might have resulted from the formation of a pore in the stratum corneum, conformational changes of keratinized protein and partial lipid extraction of EtOH (Ghanem et al., 1987).

In line with the permeability coefficients, the flux of MT were the highest in the EtOH/buffer, followed by the PG/buffer and the PEG/buffer (Fig. 4A). The flux of MT from the PEG/buffer with more than 60% PEG could not be obtained due to the aforementioned analytical limitations.

Fig. 4B indicates that the compositions of vehicles can affect the lag time of MT. EtOH highly prolonged the lag time whereas PEG reduced it. In contrast, PG did not show significant impact on the lag time. Such various effects of vehicles on the lag time might be attributed to their different impacts on the apparent diffusion parameters of MT (Fig. 3A).

Recently, Kandimalla et al. (1999) reported that the ternary mixture of water, EtOH and PG would be the best vehicle for transdermal delivery of MT in terms of flux and lag time. The results agree with their report in that the vehicle compositions might be an important factor to affect the skin absorption and lag time of MT. However, unlike the finding on the lag time (Fig. 4B), the lag time-increasing effect of ethanol was not observed in their report. The discrepancy appears to be contributed by the different skin samples. They used the skin samples obtained from the Sprague–Dawley rat, whereas we utilized those from the hairless mouse.

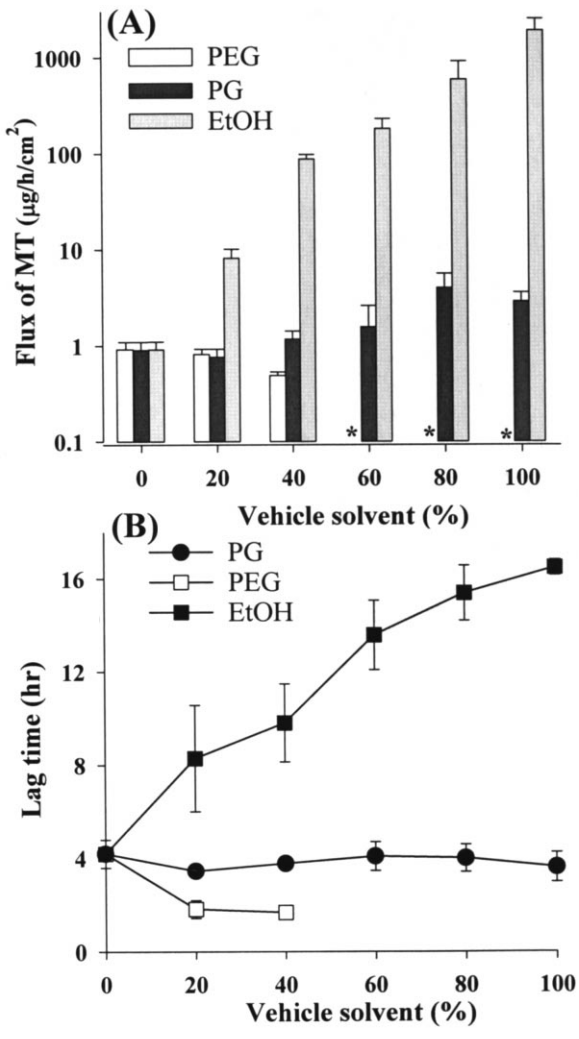

Fig. 4. The flux and lag time of melatonin (MT) delivered through various vehicles. (A) The flux of MT delivered through various vehicles. * The flux of MT at polyethylene glycol 400 (PEG)/buffer vehicles with PEG more than 60% could not be determined due to analytical limitations. The detection limit of HPLC was validated as 4 ng/ml. (B) The lag time of MT delivered through various vehicles. Lag time was determined from the plot of the cumulative amount of MT vs. time. Data are expressed as means ± S.D. ($n = 4$).

3.3. Effect of fatty acids on the skin permeation of MT

Although the composition of vehicles influenced the skin absorption and lag time of MT, the transdermal delivery of MT might have limitation by simple modulation of vehicle compositions. EtOH/buffer binary vehicles showing the highest flux suffered from the longer lag time than other vehicles. PEG/buffer that shorten the lag time reduced the skin permeation of MT as compared with buffer alone. Regardless of the contents of PG, PG/buffer showed the similar lag times, but slightly increased the flux of MT at the higher content of PG ($\geq 80\%$). These results indicate that enhancers need to be added for more effective transdermal delivery of MT.

Among enhancers, various fatty acids have been widely used for transdermal delivery of compounds. However, the enhancing effects of various fatty acids depended on the physicochemical natures of active compounds to deliver. Also, a recent report indicated that the permeation-enhancing effects of fatty acids were affected by changes of their alkyl chain length (Morimoto et al., 1996). Here, one tested whether fatty acids could enhance the transdermal delivery of MT more effectively than the binary vehicles. Furthermore, one studied if the enhancing effect of fatty acids was affected by the carbon chain numbers and the existence of a double bond.

Based on the vehicle data, PG was chosen as a vehicle for dissolving fatty acids since PG did neither interfere the skin permeation of MT as observed in PEG (Fig. 4A) nor delayed the lag time as shown in EtOH (Fig. 4B). Moreover, unlike EtOH that might irritate the skin during long-term use (Fyrand and Jakobsen, 1986), PG has been known as a relatively inert vehicle. Of PG-based vehicles, 100% PG was chosen in that it slightly increased the flux of MT as shown in Fig. 4A and increased the solubility of fatty acids in the vehicle.

The apparent diffusion parameters of MT were affected by the existence of double bond in the fatty acids but not by their alkyl chain length (Fig. 5A). As compared with pure PG as a control (D/L^2, 46.5×10^{-3}/h), saturated fatty acids of various carbon numbers (C10–C18) did not significantly alter the apparent diffusion parameters. In contrast, the unsaturated fatty acid with a double bond, OA, enhanced the parameter (79.8×10^{-3}/h) about 1.7-fold over the effect of PG.

Saturated fatty acids with smaller carbon chain lengths more effectively enhanced the skin permeation of MT. In saturated fatty acids, the permeation-enhancing effects decreased in the order of CA (C10), LA (C12), MA (C14), PA (C16) and SA (C18). CA with the smallest carbon chain length, demonstrated the maximum increase in the permeability coefficient of MT (Fig. 5B). CA increased the value as high as 1267-fold (507.0×10^{-4} cm/h) relative to the control vehicle, PG (0.4×10^{-4} cm/h). The strongest enhancing effect of CA might be partly attributed to the fact that CA could also increase the skin permeation rate of PG (Aungst et al., 1990).

In the fatty acids with the same carbon number, the existence of a double bond resulted in a dramatic change in the skin permeability of MT. In contrast to SA (C18) which did not show any enhancing effect, the unsaturated fatty acid of C18 (OA) showed dramatic increase in skin permeability of MT. The permeation-enhancing effect of OA was similar to LA, showing that K_p increased more than 950-fold compared with the effect of PG. This result might be explained by the different lipid packing properties of OA, forming a sharp kink at the double bond (Small, 1984). OA was reported to function by partitioning into the lipid regions of the stratum corneum, disrupting the structure and lipid fluidity of the stratum corneum (Kim et al., 1993). The result revealing that OA increased the diffusion of MT through the skin (Fig. 5A) appears to be in agreement with the reported mechanism by which OA enhanced the permeability of a drug.

Although the carbon number of saturated fatty acids showed a correlation with the skin permeation-enhancing effect, Fig. 5C shows that it does not affect the lag time of MT. LA (C12) showed a similar lag time to SA (C18). CA with the highest K_p showed the longest lag time. Although OA provided the second highest skin permeation

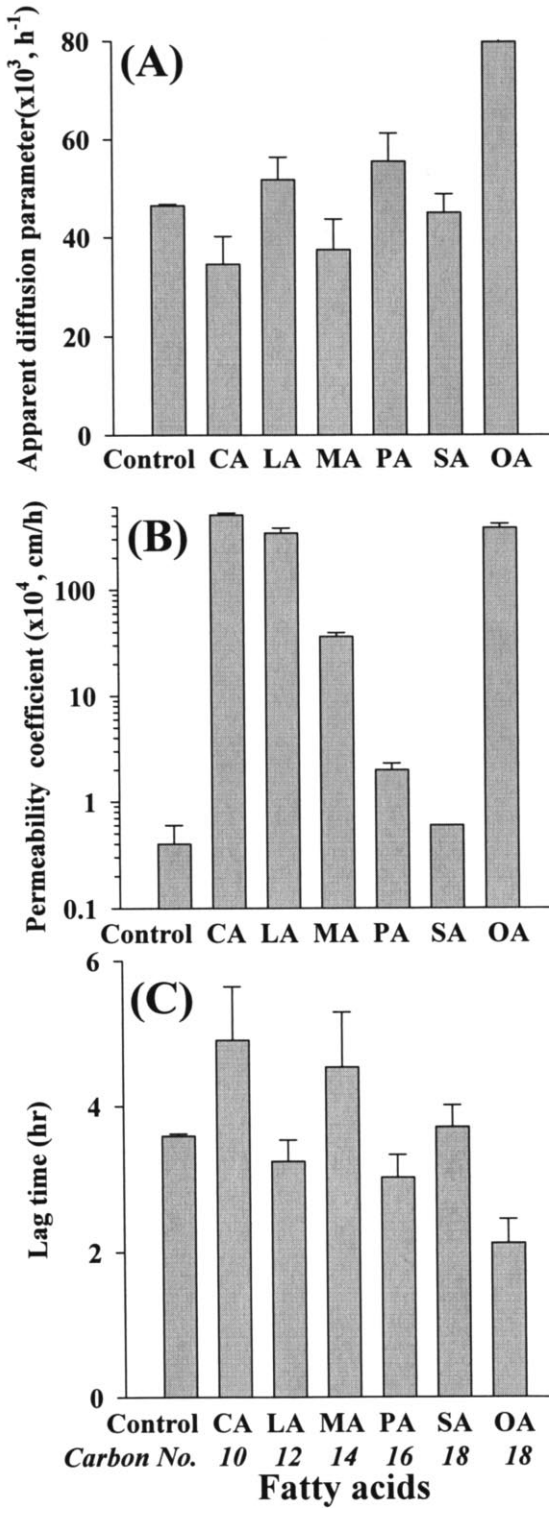

Fig. 5.

among fatty acids, it offered the shortest lag time. The shortest lag time observed in OA appears to be contributed by the increased diffusion of MT by OA. In the aspect of both skin permeation-enhancing effects and lag time, OA might be the most effective enhancer among the fatty acids tested in this study.

The recent report of Kandimalla et al. (1999) indicated that their best vehicle composed of water, EtOH and PG offered a lag time of 5.26 h. In this study, OA-containing PG showed a lag time of 2.12 h, almost 3 h shorter than the previously reported lag time of the ternary vehicle mixture.

A recent clinical study indicated that 0.5 mg of MT in oral dose was effective in alleviation of jet lag (Suhner et al., 1998). Given that the oral bioavailability of MT is approximately 15% (DeMuro et al., 2000), a high μg-level of MT absorption might be enough to exert pharmacological effect. The flux data shows that 100% PG vehicle allowed 2.89 μg/h per cm^2 MT to be absorbed through the skin (Fig. 4A). Provided that the flux is proportional to K_p, the remarkable 950-fold increase of K_p by the use of OA might allow a high-μg level flux of MT. Thus, the optimal formulation (PG-based vehicle with OA as an enhancer) employed in the present study may have a potential to deliver pharmacologically effective amounts of MT in clinical setting.

In conclusion, the results indicate that the composition of vehicles could significantly influence the skin permeation of MT and its lag time but to the limited extent. The use of the effective enhancer and suitable vehicle such as OA-containing PG could improve the skin permeation of MT and shorten its lag time much more effectively than the vehicles of various compositions.

Fig. 5. Effects of fatty acids on skin penetration parameters and lag time of melatonin (MT). Effects of fatty acids on the apparent diffusion parameters (A), permeability coefficients of MT (B), and lag time (C) were measured. Fatty acids (5%) were dissolved in propylene glycol (PG). A control PG was used as a single vehicle of MT. Lag time was determined from the plot of the cumulative amount of MT vs. time. Data are expressed as means ± S.D. ($n = 3$).

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