J. Pharm. Pharmacol. 1999, 51: 783–790
Received December 12, 1998
Accepted March 1, 1999
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# Effect of Fatty acids on the Permeation of Melatonin across Rat and Pig Skin In-vitro and on the Transepidermal Water Loss in Rats In-vivo

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**Abstract**

Transdermal delivery of melatonin would be advantageous in the treatment of sleep disorders considering the short biological half-life of melatonin and its variable bioavailability via the oral route. This study looked at suitable penetration enhancers for the transdermal permeation of melatonin.

The permeation of melatonin was enhanced by all saturated and unsaturated fatty acids across both rat and porcine skin. There was a parabolic relationship between the carbon chain length of saturated fatty acids and the enhancement of melatonin permeation across rat and porcine skin. For rat skin, the maximum flux was observed with undecanoic acid ($45{\cdot}33\,\mu g\,cm^{-2}\,h^{-1}$) which enhanced the flux of melatonin 8·6 times compared with the control, whereas lauric acid produced the maximum flux of melatonin ($24{\cdot}98\,\mu g\,cm^{-2}\,h^{-1}$; 4·7 times) across porcine skin. An increase in the number of double bonds in *cis*-9-octadecanoic acid increased the flux of melatonin across rat skin. In contrast, with porcine skin, the flux of melatonin decreased as the number of double bonds increased, although the flux values were not statistically significant. Treatment of rats with undecanoic acid, oleic acid and linolenic acid for 3 h using Hill top chamber enhanced the transepidermal water loss significantly. The maximum transepidermal water loss was observed with undecanoic acid and linolenic acid among saturated and unsaturated fatty acids, respectively. Nonanoic acid and myristic acid did not cause a significant change in the transepidermal water loss.

The enhancement effect of saturated fatty acids on the permeation of melatonin was dependent on the chain-length of the fatty acid in both rat and porcine skin. While an increase in the number of double bonds in the fatty acid increased the flux of melatonin in rat skin, no significant difference in the flux was observed with porcine skin. The permeation enhancement of melatonin by saturated and unsaturated fatty acids across rat skin was significantly higher than that of porcine skin. A positive correlation was observed between the permeation enhancement effect of the fatty acids across rat skin in-vitro and the transepidermal water loss in rats in-vivo, suggesting that there is a similarity in the mechanism by which fatty acids enhance the permeation of melatonin and in the enhancement of transepidermal water loss.

We conclude that saturated fatty acids such as undecanoic acid or lauric acid which showed maximum permeation across rat and porcine skin, respectively, may be used as potential penetration enhancers in the development of a transdermal delivery system for melatonin.

Melatonin is an *N*-acetyl, 5-methoxy tryptamine produced by the pineal gland. Day and night cycle is an important environmental factor controlling the circadian rhythm of melatonin. Melatonin is produced more in the night than during the day, hence it is referred to as the chemical expression of darkness. This internal 'Zeitgeber' is involved in the timing and control of a number of rhythmic functions such as reproduction and sleep patterns. It has been reported that the jet lag in aircrew and cosmonauts is a consequence of the disturbances in melatonin circadian rhythm. Melatonin has been reported to alleviate jet lag following long flights.

The pharmacokinetics of oral melatonin administration is influenced by marked first-pass hepatic metabolism resulting in a very short half-life of 45 min (Mallo et al 1990). Hence novel delivery

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systems utilizing transdermal (Lee et al 1994), mucosal (Benes et al 1997) and oral (Lee et al 1996) routes have been developed for the controlled delivery of melatonin. The transdermal route would be the most advantageous of all, owing to patient compliance and its ability to avoid first-pass metabolism.

To simulate the endogenous melatonin levels in plasma at night, a rapid-in, followed by a steady state and a rapid-out (square wave pattern) is required. A previous study on various vehicles demonstrated that there is a need to use penetration enhancers to eliminate the lag time and to enhance the permeation of melatonin through the skin. These studies also indicated that a water:ethanol ratio of 40 : 60 was a promising vehicle for the permeation of melatonin through rat skin (unpublished data). Saturated fatty acids and unsaturated fatty acids have been widely used in the enhancement of transdermal permeation of various drugs (Ogiso & Shintani 1990; Tanojo et al 1997a). The enhancement of transdermal permeation of drugs by fatty acids appears to involve disruption of the densely packed lipids that fill the extracellular spaces of the stratum corneum (Aungst 1989).

It has been established that transdermal absorption of drugs in humans is different from that of commonly used laboratory animals (Wester & Maibach 1987). Pig skin has been reported to have the characteristics most close to that of human skin (Monteiro-Riviere 1986). The enhancement effect of skin penetration enhancers across different animal species and humans has not been studied thoroughly. The effect of a few penetration enhancers has been compared in some animal species and in human skin (Okamoto et al 1987; Priborsky & Muhlbachova 1990). This study has compared the effect of a series of saturated and unsaturated fatty acids on the permeation of melatonin across excised rat skin and porcine skin.

The measurement of transepidermal water loss, i.e. the outward diffusion of water through the skin, has become increasingly important in studies evaluating the stratum corneum barrier function (Maibach et al 1984). Skin lipids have been postulated to be intimately involved in maintaining barrier function (Michaels et al 1975). Transepidermal water loss has also been used to elucidate the effect of skin penetration enhancers (Tanojo et al 1997b). It appears that both transepidermal water loss and skin permeation enhancement effect of fatty acids are associated with the involvement of lipids of stratum corneum. One of the objectives of this study was to determine the relationship between the transepidermal water loss and skin permeation enhancement caused by fatty acids.

Therefore, the objectives of the present study were: to investigate the effects of various saturated and unsaturated fatty acids on the permeation of melatonin across excised rat skin; to study the effect of carbon chain length and number of double bonds in the fatty acid on the permeation enhancement of melatonin; to compare the enhancement effects of various fatty acids in excised rat and porcine skin; and to examine the relationship between the enhancement effect of fatty acids on the permeation of melatonin across rat skin in-vitro and on the transepidermal water loss in rats in-vivo.

## Materials and Methods

### *Materials*

Fatty acids and melatonin were procured from Sigma Chemical Co. (St Louis, MO, USA) Ethanol USP was obtained from Florida Distillers Co. (Lake Alfred, FL, USA). Water, methanol (both HPLC grade) and nylon filters were obtained from Fisher Scientific (Atlanta, GA, USA). All chemicals were of reagent grade and were used as received from the suppliers without further purification. Hill top chambers were obtained from Hill Top Co. (Cincinnati, OH, USA). A mixture of water and ethanol (40 : 60 mix) was used as a vehicle in all studies.

### *Skin samples*

The skin samples were obtained from the dorsal surface of male Sprague-Dawley rats, 150–250 g (Harlan Sprague Dawley, Inc., Indianapolis, USA). Hair on the dorsal surface of the animals was removed with an electrical clipper 24 h before the experiment. The animals were killed just before the experiment and the dorsal skin (full thickness) was obtained after careful excision and cleaning from the fat and subcutaneous tissue. Porcine skin from pinna was obtained from the local slaughter house and dermatomed to 500 $\mu$m before use.

### *Solubility of melatonin*

To investigate the effect of different fatty acids on the solubility of melatonin in the vehicle, the saturation solubility of melatonin was determined at 32°C in vehicle alone and in the presence of various saturated and unsaturated fatty acids. A 5·0% w/v of each enhancer was added to the vehicle in a round-bottomed flask. An excess amount of melatonin was added to this solution and equilibrated in an environmental shaker maintained at 32°C for 24 h. After equilibration, the liquid with excess melatonin was filtered through a 0·45 $\mu$m

nylon filter and the filtrate was analysed by UV spectrophotometry at 223 nm. The solubility studies were performed in triplicate.

*Preparation of drug solutions*

An excess amount of melatonin was added to the vehicle containing 5·0% w/v of penetration enhancer and the mixture was placed in an environmental shaker at 32°C for 24 h to obtain a saturated solution of melatonin. The melatonin solution was filtered through a 0·45-$\mu$m nylon filter and 1 mL of this solution was placed in the donor compartment of the Franz diffusion cell assembly.

*In-vitro skin permeation studies*

The excised skin was mounted between the donor and receptor compartments of a Franz diffusion cell (Permegear Inc., Riegelsville, PA, USA) with the stratum corneum facing the donor compartment. Each Franz cell had a diffusional surface area of 0·636 $cm^2$ and the receptor volume was 5 mL. The temperature of the receptor compartment was maintained at 32 ± 0·1°C with an external, constant-temperature circulator water bath. The receptor compartment was filled with phosphate buffer, pH 7·4, which was stirred continuously with a magnetic bar. The drug solution (1·0 mL) was placed in the donor compartment and the donor surface was occluded with a sheet of aluminium foil and sealed with parafilm. At predetermined time intervals, samples (0·5 mL) were taken from the receptor compartment, and the cell was refilled with the same volume of fresh buffer solution. Samples were kept in a freezer (−20°C) until analysed by HPLC. All the experiments were performed in triplicate.

*HPLC analysis*

The analysis of melatonin was carried out using the HPLC method of Konsil et al (1995) with a slight modification. A Beckman System Gold HPLC was used with an ODS-AQ column (5 $\mu$m, 3 × 150 mm; YMC Inc., USA). The HPLC system consisted of an autosampler 507e, double pumps model 125, and an UV detector 166. A Guard Cartridge (ODS-AQ S-5 120A; YMC Inc., USA) was connected to the analytical column. A combination of methanol and water (50 : 50) at a flow rate of 0·5 mL $min^{-1}$ was used as the mobile phase. The concentration of melatonin was analysed with the UV detector set at a wavelength of 223 nm. The retention time of melatonin was 5·6 min. The whole system was interfaced with an IBM 433 DX/Si computer and was run by the Beckman GOLDV810 software.

*Measurement of transepidermal water loss in-vivo*

The transepidermal water loss studies were performed in male Sprague-Dawley rats (150–250 g). Hair on the dorsal surface of the rats was removed 24 h before the experiment using an electrical clipper. The temperature of the room was maintained at 20–25°C and the humidity was maintained at 35–45% relative humidity. The rats were anaesthetized with pentobarbitone sodium (35 mg $kg^{-1}$) during the fixation of the Hill top chamber and during the transepidermal water loss measurements. In each animal the test area was divided into A and B regions. The vehicle (300 $\mu$L) containing 5% w/v enhancer was placed in a Hill top chamber and adhered onto region A. Region B was affixed with the Hill top chamber containing no solution (control) to study the effect of occlusion. The intimate contact of the chambers on the skin was ensured during the movement of the rats by the application of water-proof tape (Johnson and Johnson Inc., NJ, USA). The Hill top chamber was removed after 3 h, and the test and control area was gently washed with 3 × 500 $\mu$L of the vehicle, followed by 3 × 500 $\mu$L of distilled water using a soft tissue and the area was blotted dry. Transepidermal water loss was measured at various time intervals until a stable reading was displayed (approx. 1 min ) using the Tewameter (Courage + Khazaka, Koln, Germany).

*Data analysis*

The cumulative amount of the drug diffused through the skin was plotted as a function of time. The steady-state flux ($\mu g\ cm^{-2}\ h^{-1}$) was calculated from the slope of the linear portion of the plot. Since the transepidermal water loss value of control site (applied with Hill top chamber containing no solution) was significantly different among different rats, the control value was measured in each rat at various time intervals. The enhancement ratio (transepidermal water loss after the treatment of enhancer solution to that of control) was determined and was plotted against time. Students, *t*-test was performed to determine the level of significance. The data was considered to be significant at $P < 0.05$.

## Results and Discussion

*Solubility of melatonin*

The solubility of melatonin in the vehicle (40% water : 60% ethanol) was determined to be 113·66 ± 3·54 mg $mL^{-1}$. The addition of 5% w/v of fatty acids did not change the solubility of melatonin significantly (data not shown). The thermo-

dynamic activity of melatonin in the vehicle was assumed to be similar in the presence of different fatty acids.

*Permeation enhancement of melatonin through rat skin*

*Saturated fatty acids.* The effect of various saturated fatty acids on the permeation profiles of melatonin across rat skin is shown in Figure 1. All saturated fatty acids enhanced the permeation of melatonin through rat skin. A sharp increase in the permeation of melatonin was observed when the fatty acid chain length was increased from 9 to 10 carbons. A further increase in the permeation of melatonin was observed when the chain length was increased to 11 carbons. However, the permeation of melatonin decreased when the chain length was increased from 11 carbons. Table 1 shows the steady-state flux values and enhancement factors of melatonin with different saturated fatty acids across excised rat skin. It can be observed that the flux values of melatonin have a parabolic relationship with the chain length of the saturated fatty acids.

Melatonin is a slightly lipophilic drug. The log octanol:water partition coefficient of melatonin determined in our laboratory was 1·198 ± 0·0012 (n = 3). In the present study, the permeation of melatonin was enhanced several fold by all saturated fatty acids. The mechanism by which fatty acids increase skin permeability appears to involve

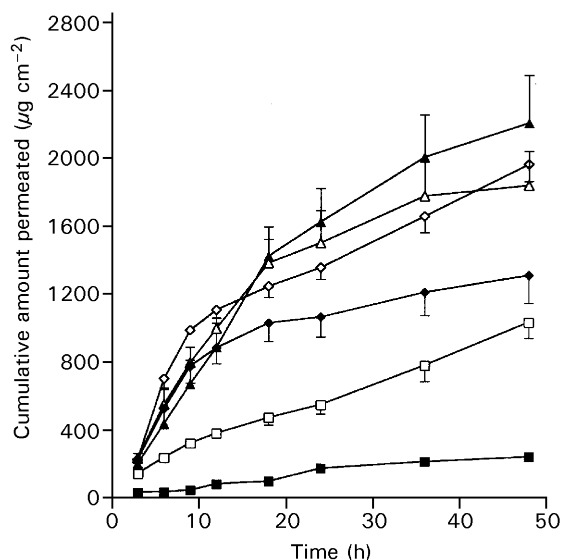


Figure 1. Effect of saturated fatty acids (5%w/v) on the permeation profile of melatonin through rat skin. ■ Control, ◆ nonanoic acid, △ decanoic acid, ▲ undecanoic acid, ◇ lauric acid, □ myristic acid. Control is the permeation profile of melatonin from the vehicle without enhancer. Data are means ± s.e. (n = 3).

Table 1. Steady-state flux values ($\mu$g cm$^{-2}$ h$^{-1}$) of melatonin with different saturated and unsaturated fatty acids across rat and porcine skin. The enhancement factors* are given in parenthesis.

| | Rat | Pig |
|---|---|---|
| Control | 5·29 ± 0·21 (1·00) | 5·34 ± 0·59 (1·00) |
| Saturated fatty acids | | |
| Nonanoic acid | 20·31 ± 1·92 (3·83) | 7·77 ± 0·55 (1·45) |
| Decanoic acid | 34·38 ± 2·43 (6·49) | 18·79 ± 1·59 (3·52) |
| Undecanoic acid | 45·33 ± 2·72 (8·57) | 23·70 ± 2·64 (4·44) |
| Lauric acid | 32·30 ± 1·70 (6·11) | 24·98 ± 1·45 (4·67) |
| Myristic acid | 18·75 ± 0·68 (3·54) | 17·29 ± 1·31 (3·24) |
| Unsaturated fatty acids | | |
| Oleic acid | 28·32 ± 2·09 (5·35) | 11·52 ± 1·29 (2·16) |
| Linoleic acid | 34·83 ± 1·95 (6·58) | 9·14 ± 0·65 (1·71) |
| Linolenic acid | 39·88 ± 1·47 (7·54) | 10·23 ± 0·52 (1·91) |

*Enhancement factor = flux of melatonin in presence of penetration enhancer/flux of melatonin from the vehicle without enhancer.

disruption of the densely packed lipids that fill the extracellular spaces of the stratum corneum (Aungst 1989). The change in the physical structure of stratum corneum lipids has been assessed using differential scanning calorimetric and infrared spectroscopic techniques (Golden et alet al 1986). It has been reported that C-10 to C-12 fatty acids possess an optimal balance between partition coefficient or solubility parameters and affinity to skin (Ogiso & Shintani 1990). By increasing their own permeation across the stratum corneum, the saturated fatty acids simultaneously increase the drug (melatonin) permeation. However, increasing the chain length of the hydrocarbon group beyond 11 carbons, increases the lipophilicity of the molecule, which results in their increased affinity towards stratum corneum lipids. This retards the permeation of fatty acids, which in turn reduces the permeation of melatonin. Fatty acids having less than 10 carbon atoms in their structure are not adequately lipophilic and their partitioning through skin is decreased, thereby decreasing the permeation of melatonin.

A similar correlation has been reported in the literature. Ogiso & Shintani (1990) examined the effects of a series of fatty acids (C-6 to C-18) on the flux of propranolol across rabbit skin and a parabolic relation between enhancer efficacy and enhancer alkyl chain length was observed. Tanojo et al (1997a) and Aungst et al (1986) observed a similar relationship between the enhancement effect and alkyl chain length. Additionally, C-10 to C-12 chain length corresponds to the dimensions of the cholesterol skeleton, which leads to the hypothesis that disruption of ceremide–cholesterol, or cholesterol–cholesterol, interaction is an

important factor in permeability modification by the C-10 to C-12 fatty acids (Brain & Walters 1993).

*Unsaturated fatty acids.* The penetration enhancement effect of oleic acid (a mono unsaturated fatty acid) and linoleic and linolenic acids (polyunsaturated fatty acids) was studied at 5% w/v concentration (Figure 2). As the number of double bonds increased there was a slight increase in the permeation of melatonin. The steady-state flux of melatonin with linolenic acid was significantly higher than that with oleic acid ($P < 0{\cdot}05$) (Table 1). However there was no significant difference in the flux values of linoleic acid and linolenic acid ($P > 0{\cdot}05$).

Among unsaturated fatty acids, oleic acid has been reported to be an effective skin penetration enhancer for polar and non-polar drugs (Barry 1987). *cis*-Unsaturated fatty acids (for example oleic acid, linoleic acid and linolenic acid) have been reported to form separate domains within stratum corneum lipids which effectively decrease either diffusional path length or the resistance (Ongpipattanakul et al 1991; Tanojo et al 1997a). The presence of double bonds in the structure has been proposed to cause the formation of kinks in the lipid structure to allow water permeation across the skin (Potts & Francoeur 1990). In the present study, an increase in the number of double bonds in the unsaturated fatty acids increased the flux of melatonin linearly in rat skin, possibly by causing more kinks in the lipid structure of skin.

Increasing the number of the double bonds in *cis*-octadecenoic acid from one to two or three also increased the flux of naloxone through human skin (Aungst et al 1986). However the flux of naloxone in the presence of linolenic acid (three double bonds) was less than that of the flux in the presence of linoleic acid (two double bonds). A similar enhancement pattern was also observed by Carelli et al (1992) and Tanojo et al (1997a). In contrast, Morimoto et al (1996) reported that the skin permeation enhancement of indomethacin by unsaturated fatty acids (C-18 chain) was not affected by their differences of position and number of double bonds.

*Permeation enhancement of melatonin through porcine skin*

*Saturated fatty acids.* The effect of saturated fatty acids on the permeation of melatonin through porcine skin is presented in Figure 3. All saturated fatty acids enhanced the permeation of melatonin through porcine skin. Further, as the chain length of the fatty acid increased, there was an increase in the permeation of melatonin up to C-12 (lauric acid). A further increase in the chain length to C-14 (myristic acid) decreased the permeation of melatonin. Table 1 shows the steady-state flux values and enhancement factors of melatonin with various saturated fatty acids. As observed with rat skin, a

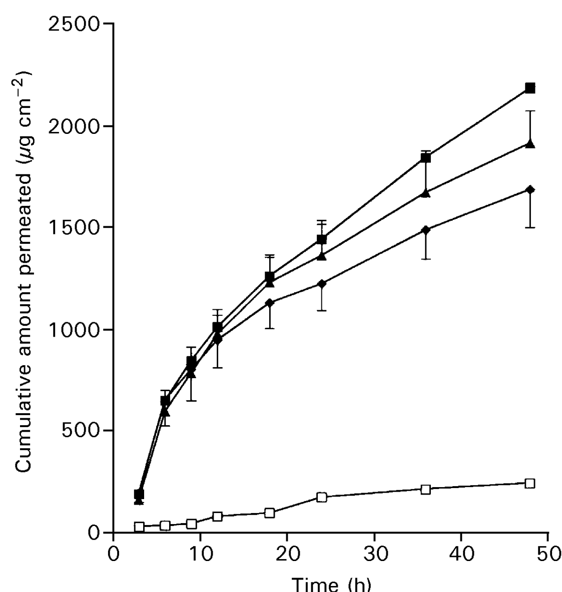


Figure 2. Effect of unsaturated fatty acids (5%w/v) on the permeation profile of melatonin through rat skin. □ Control, oleic acid, ▲ linoleic acid, ■ linolenic acid. Control is the permeation profile of melatonin from the vehicle without enhancer. Data are means ± s.e. (n = 3).

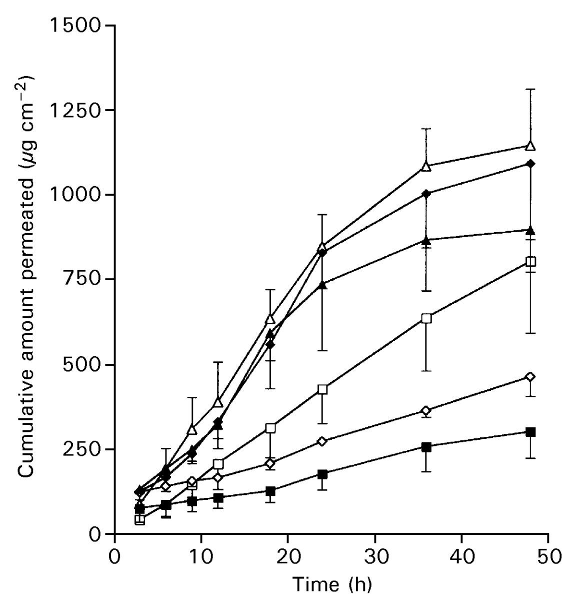


Figure 3. Effect of saturated fatty acids (5%w/v) on the permeation profile of melatonin through porcine skin. ■ Control, ◇ nonanoic acid, ▲ decanoic acid, ◆ undecanoic acid, △ lauric acid, □ myristic acid. Control is the permeation profile of melatonin from the vehicle without enhancer. Data are means ± s.e. (n = 3).

parabolic relationship between chain length of fatty acid and the flux of melatonin was seen with porcine skin. However, lauric acid (C-12) showed the maximum flux of melatonin across porcine skin, although it was not statistically different from that of undecanoic acid (C-11) ($P > 0{\cdot}05$).

*Unsaturated fatty acids.* The permeation profiles of melatonin with unsaturated fatty acids across porcine skin are presented in Figure 4. In contrast to rat skin, an increase in the number of double bonds in cis-9-octadecanoic acid (oleic acid) decreased the flux of melatonin both with linoleic acid (two double bonds) and linolenic acid (three double bonds). However the flux values of linoleic acid and linolenic acid were not significantly different from that of oleic acid ($P > 0{\cdot}05$) (Table 1).

### *Comparison of enhancement effects of fatty acids in rat and porcine skin*

Skin permeation enhancers have been reported to act differently in different skin species (Aungst et al 1990). In general, hairless mouse skin has been reported to be a very poor model for human skin in terms of studies with penetration enhancers (Bond & Barry 1988). Priborsky & Muhlbachova (1990) studied the in-vitro permeation enhancement effect of *N*-methyl-2-pyrrolidone in several laboratory animals (rat, mouse, new-born pig, rabbit and guinea-pig) and man with a model compound, brilliant blue and quantitative differences were observed in various species compared with man.

The histological characteristics of pig and human skin have been reported to be comparable with similarities existing for epidermal thickness and composition, pelage density, lipid content and general morphology (Monteiro-Riviere 1986). Further, pig-ear skin is reported to be the better anatomical region presenting a reliable supply of skin that has been used by other researchers (Dyer & Aziza 1989) and its permeation characteristics were found to be close to those of human skin.

The results of the present study indicate that the permeation enhancement of melatonin by saturated and unsaturated fatty acids across porcine skin was much lower than that of rat skin. The thickness of stratum corneum, epidermis and whole skin in pigs is significantly higher than that of rat skin (Bronaugh et al 1982). The differences in skin morphology and composition may be the reason for the variation in the enhancement effect of fatty acids across rat and pig skin. Saturated fatty acids such as undecanoic acid and lauric acid which showed maximum permeation across rat and porcine skin, respectively, may be used as potential penetration enhancers in the development of a transdermal delivery system for melatonin.

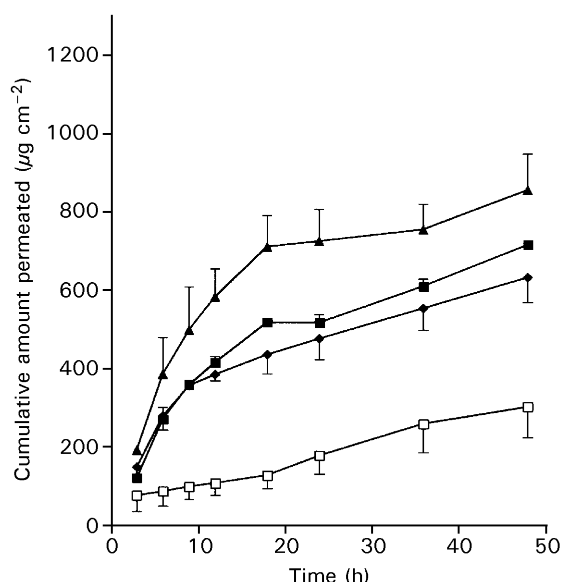

Figure 4. Effect of unsaturated fatty acids (5%w/v) on the permeation profile of melatonin through porcine skin. □ Control, ▲ oleic acid, ◆ linoleic acid, ■ linolenic acid. Control is the permeation profile of melatonin from the vehicle without enhancer. Data are means ± s.e. (n = 3).

### *Effect of enhancers on transepidermal water loss*

The transepidermal water loss after the application of melatonin solution containing selected penetration enhancers was measured in rats in-vivo and the results are presented in Figure 5. Interestingly, there was a correlation between the transepidermal water loss and the enhancement effect of different penetration enhancers across rat skin (Figures 1, 2 and 5). For saturated fatty acids, as the carbon chain length increased from C-9 (nonanoic acid) to C-11 (undecanoic acid), there was a substantial increase in the transepidermal water loss. However, the transepidermal water loss decreased below the level of control (vehicle) as the chain length was increased to C-14 (myristic acid). Similarly, for unsaturated fatty acids, there was a positive correlation between transepidermal water loss and permeation enhancement across rat skin (Figures 2 and 5 ). Linolenic acid, with 3 double bonds, produced a higher transepidermal water loss than oleic acid which has only one double bond. For undecanoic acid, oleic acid and linolenic acid, the transepidermal water loss increased up to about 24 h, and thereafter gradually decreased to the initial level in 72 h. Overall, linolenic acid produced the highest transepidermal water loss of $49{\cdot}17\,g\,m^{-2}\,h^{-1}$ at 24 h, which was 5·8 times that of the normal

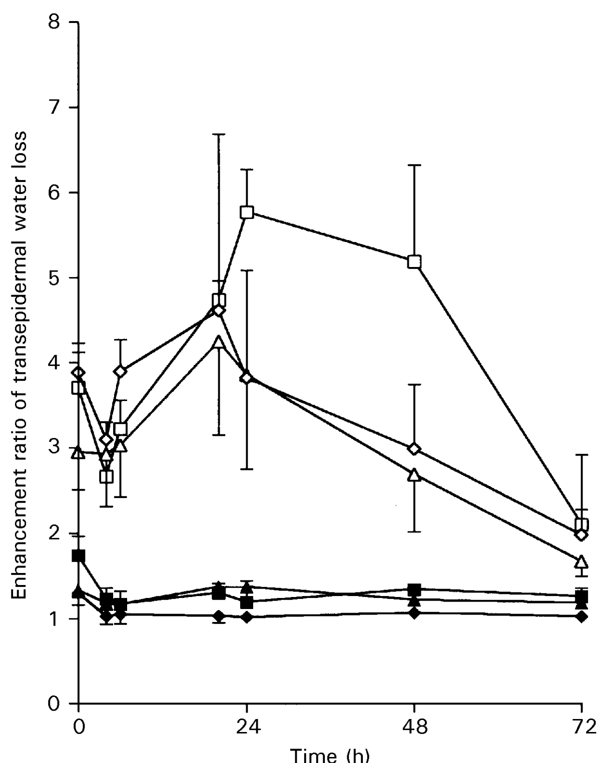


Figure 5. Effect of penetration enhancers on the transepidermal water loss in rats in-vivo. ■ Vehicle, ▲ nonanoic acid, ◇ undecanoic acid, ◆ myristic acid, △ linolenic acid. Data are means ± s.e. (n = 3).

transepidermal water loss value of about 8·53 $g\,m^{-2}\,h^{-1}$ at the control site. Nonanoic acid and myristic acid did not produce a significant change in transepidermal water loss compared with control (vehicle). The treatment sites (control or test) did not show any apparent skin reactions such as eczema or erythema.

Skin penetration enhancers have been reported to increase transepidermal water loss (Yosipovitch et al 1996). Gao & Singh (1997) reported that treatment of porcine epidermis for 2 h with terpene enhancers (carvone, cineole and thymol) increased the transepidermal water loss in-vitro. Tanojo et al (1997b) studied the effect of occlusive treatment of oleic acid in propylene glycol on the transepidermal water loss in human volunteers and the transepidermal water loss was reported to be enhanced by a factor of two. The authors also reported that the increase in transepidermal water loss caused by unsaturated fatty acids was greater than that of straight-chain fatty acids having 6–12 carbon atoms, in volunteers (Tanojo et al 1998). The results of this study in rats are in agreement with that of Tanojo et al (1998). However in the present study, the increase in transepidermal water loss among unsaturated fatty acids was higher with linolenic acid, whereas oleic acid showed the highest enhancement in the study of Tanojo et al (1998). Further, in the present study, a positive correlation was observed between the permeation enhancement effect of fatty acids in-vitro across rat skin and transepidermal water loss in rats in-vivo, both with saturated fatty acids and unsaturated fatty acids (Figures 1, 2 and 5). These results suggest that there is a similarity in the mechanism by which saturated and unsaturated fatty acids enhance the transdermal permeation of melatonin and in the enhancement of transepidermal water loss. Further studies at microscopic level would be necessary to explain this phenomenon. Currently studies in this direction are in progress in our laboratory.

*Acknowledgements*

The authors acknowledge the financial support provided by NASA, Ames Center (Grant No. NCC 2-1005).

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