Arch Pharm Res Vol 25, No 3, 392-396, 2002

Archives of Pharmacal Research
http://apr.psk.or.kr

Effect of Vehicles and Enhancers on the *In Vitro* Permeation of Melatonin through Hairless Mouse Skin

Hye Sun Gwak, Seung Ung Kim, and In Koo Chun

Laboratory of Pharmaceutics, College of Pharmacy, Dongduk Women's University, Seoul 136-714, Korea

(Received March 7, 2002)

The effects of vehicles and penetration enhancers on the *in vitro* permeation of melatonin through dorsal hairless mouse skin were investigated. Propylene glycol laurate (PGL), isopropyl myristate (IPM), propylene glycol monolaurate (PGML) and propylene glycol monocaprylate (PGMC) showed high permeation fluxes and PGL, PGML and PGMC decreased lag time significantly. In both of the binary co-solvents of diethylene glycol monoethyl ether (DGME)-PGL and DGME-IPM, the highest fluxes were achieved at 20% of DGME, which were 10.5 ± 1.5 and 9.1 ± 2.4 $\mu g/cm^2/h$, respectively. Among fatty acids used as a permeation enhancer, capric acid and oleic acid in DGME-PGL (80 : 20 v/v) showed relatively high enhancing effects. Capric acid also shortened the lag time of melatonin from 2.4 ± 0.7 to 1.3 ± 0.2 h. Oleic acid, however, failed to shorten the lag time. Therefore, for effective solution formulations in terms of permeation flux and lag time, capric acid-containing DGME-PGL (80 : 20 v/v) could be used to enhance the skin permeation of melatonin.

Key words: Vehicles, Penetration enhancers, Melatonin, Permeation fluxes, Lag time

INTRODUCTION

Melatonin (MT, Fig. 1) is an indole amide neurohormone produced in the pineal gland (Lerner *et al.*, 1958). The production and secretion of MT are mediated largely postganglionic retinal nerve fibers, and the neuronal system is activated by darkness and suppressed by light (Fung, 1987; Pangerl., 1990). In humans, melatonin secretion increases soon after the onset of darkness, peaks in the middle of the night, and gradually falls during the second half of the night (Brzezinski, 1997). Exogenous MT has been proved to affect the speed of falling asleep, as well as the duration and quality of sleep and have hypnotic effects (Vollrath *et al.*, 1981; Lieberman *et al.*, 1984). MT, however, is rapidly metabolized chiefly in the liver, by hydroxylation and, after conjugation with sulfuric or glucuronic acid, is excreted in the urine (Lynch *et al.*, 1975). The bioavailability of orally administered MT varies widely (Waldhauser *et al.*, 1984).

Transdermal delivery has been recognized to have advantages of avoiding the first-pass metabolism by oral administration, and maintaining a relatively constant plasma drug concentration. Lee *et al.* (1994) demonstrated that transdermal delivery of MT was feasible. However, the long lag time and relatively low skin absorption rate limited its development (Bénès *et al.*, 1997).

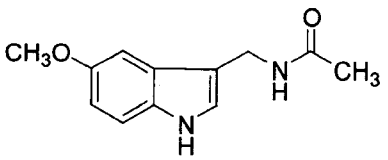

Fig. 1. Chemical structure of melatonin (N-acetyl-5-methoxytryptamine)

Thus, in this study, we examined the effect of pure solvents, co-solvents and penetration enhancers on the *in vitro* permeation of MT from solution formulation through dorsal hairless mouse skin to design a more effective transdermal delivery system of MT.

MATERIALS AND METHODS

Materials

MT and methyl parahydroxybenzoate (methyl paraben) were purchased from Sigma Chemical Co. (St Louis, MO, USA). Propylene glycol monolaurate (PGML, Lauroglycol®

Correspondence to: In Koo Chun, College of Pharmacy, Dongduk Women's University, Seoul 136-714, Korea
E-mail: ikchun@dongduk.ac.kr

90), propylene glycol laurate (PGL, Lauroglycol® FCC), propylene glycol monocaprylate (PGMC, Capryol® 90), diethylene glycol monoethyl ether (DGME, Transcutol® P), caprylocaproyl macrogol-6 glycerides (LBS, Labrasol®), oleoyl macrogol-6 glycerides [LBF 1944, Labrafil® (LBF) M 1944 CS], polyethylene glycol-8 glyceryl linoleate (LBF 2609 LBF WL 2609 BS) (Gattefossé, Gennevilliers Cedex, France), and propylene glycol dicaprylate/dicaprate (Captex® 200) and glyceryl dicaprylate/dicaprate (Captex® 300) were obtained from Karlshamns Corp. (Janesville, WI, USA). Polyethylene glycol 400 (PEG 400), propylene glycol (PG), ethanol, and *n*-octanol were of analytical grade. Isopropyl myristate (IPM), oleyl alcohol (OAl), lauric acid, oleic acid (OA), linoleic acid, capric acid, and caprylic acid were purchased from Sigma Chemical Co. (St Louis, MO, USA). Acetonitrile and methanol used were of HPLC grade. Other reagents were of analytical grade.

Animals

Male hairless mice aged 7~8 weeks were purchased from Samtako Bio Korea Co., Ltd. (Osan, Korea).

Analysis

Samples from solubility, partition coefficient and permeation studies were analyzed by high-performance liquid chromatography (HPLC). The HPLC system consisted of a pump (Series 310, Perkin Elmer, USA) with a detector (Model LC 90, Perkin-Elmer, USA) set at 229 nm and an integrator (Model 4290, Varian, USA). An ODS column (μ Bondapak C18, Waters, USA) equipped with a C18 Radial Pak insert was used. The mobile phase was composed of MeOH and water (50 : 50 v/v), and delivered at a flow rate of 1.2 ml/min. The injection volume was 20 μl. The internal standard used was methyl paraben. A calibration curve was constructed based on peak area measurements.

Solubility determination

An excess amount of MT was added to the various pure solvents, cosolvents or McIlvaine buffered solutions (pH 2.10-8.05), and shaken at 32°C for more than 48 h. The supernatant was filtered by 0.45 μm membrane filter (PVDF, Whatman). The filtered was assayed by HPLC after appropriate dilution.

Determination of *n*-octanol/water partition coefficient (P_c)

n-Octanol and water were saturated with each other for 24 h before the experiment. MT solution (1000 μg/ml) was prepared with water saturated with *n*-octanol. One milliliter of this solution was then transferred to 10 ml centrifuge tube containing 1 ml of *n*-octanol saturated with water. The tube was vortexed for 3 min and centrifuged at 3000 g for 3 min. After centrifugation, 100 μl was withdrawn from water phase and *n*-octanol phase, respectively, and the intrinsic P_c was determined by HPLC.

Preparation of test solutions

To determine the effects of various vehicles and enhancers on the permeation of MT, appropriate amounts of MT were dissolved in pure solvents or co-solvents. For the preparation of saturated solutions, an excess amount of MT was added to pure solvents or co-solvents, and shaken at 32 °C for 24 h.

Procedure for skin permeation *in vitro*

After sacrificing with ether, the dorsal skin of each hairless mouse was excised, and mounted on a side-by-side permeation system; the dermal side was in contact with the receptor compartment. Receptor compartment cells were filled with 3 ml of 40% PEG 400 in saline and the media were stirred by a Teflon-coated magnetic bar to keep them well mixed. Three milliliters of MT test solutions in various pure solvents or co-solvents were added to donor compartments. The permeation media were maintained at 32°C. At predetermined time intervals, 100 μl of receptor solutions were withdrawn, and the permeated amount of MT was determined by HPLC.

Data analysis

As described by Barry (1983), the steady-state flux (J_s), lag time (T_L), diffusion coefficient (D), and skin / vehicle partition coefficient (K), are defined by equations 1-2.

$$J_s = (dQ/dt)_{ss} \cdot 1/A = DKC/h \quad (1)$$

$$D = h^2/6T_L \quad (2)$$

A: the effective diffusion area
h: the thickness of skin
C: the constant concentration of the donor solution
$(dQ/dt)_{ss}$: the steady-state slope

RESULTS AND DISCUSSION

Effect of pure solvents

Roy and Flynn (1988) suggested that the transdermal transport of a drug is related to its physicochemical characteristics. Even though MT has relatively suitable conditions for transdermal delivery compared to ketoprofen, which is known to have high permeability, the skin absorption profiles of MT were known to be relatively

low (Bénès *et al.*, 1997); the molecular weight, melting point, intrinsic solubility in water, and intrinsic P_c of MT and ketoprofen were 232.3 and 254.3, 117 and 94°C, 2.1 ± 0.1 and 0.29 ± 0.008 mg/ml, and 14.1 ± 0.83 and 5.2 ± 0.01, respectively (Cordero *et al.*, 1997). Thus, appropriate vehicles or vehicle mixtures with/without enhancers were tested to design a more effective transdermal delivery system of MT.

It has been suggested that vehicles can enhance drug permeation by increasing the thermodynamic activity of the drug or changing the barrier property of the skin (Møllgaard and Hoelgaard, 1983). Cho and Choi (1998) described the relationship between the solubility of a drug in the vehicle and the thermodynamic activity of the drug in the vehicle. They suggested that if the solubility of a drug in the vehicle is high, then the thermodynamic activity of the drug in the vehicle is low, which results in low percutaneous absorption rate unless the vehicle alters the barrier property of the skin. We investigated the effects of five pure vehicles at 10 mg/ml drug concentration on the permeation of MT. Without any actions of the vehicles on the skin, the linear relationship between the ratio of concentration to the solubility of the drug in the vehicle and the permeation flux should be obtained (Cho and Choi, 1998). As illustrated in Fig. 2, the ratio of donor concentration to the solubility of MT increased in the rank order of DGME, PG and LBF 1944, and linearity (r^2 = 0.9569) was achieved between the ratio and the permeation flux. However, PGML and PGMC showed much higher permeation rates deviating from the linearity; the ratio of concentration to the solubility and permeation flux of MT in PGML and PGMC were 28 and 48, and 8.84 and 9.45 μg/cm²/h, respectively. Thus, it was found that PGMC or PGML, ester-compound of PG, has

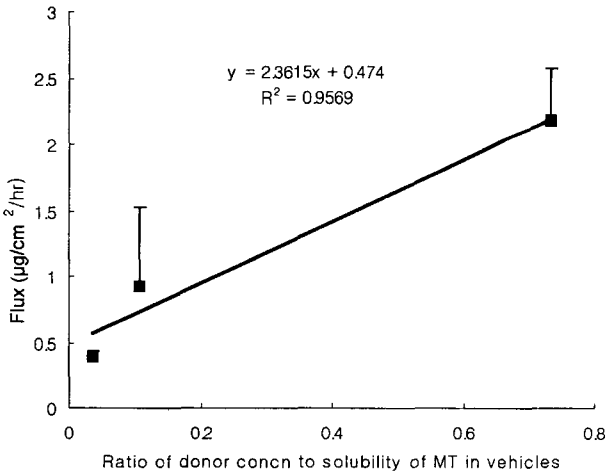

Fig. 2. Linear relationship between the ratio of concentration to the solubility of MT and the permeation flux (n = 3). The ratio increased in the rank order of DGME, PG and LBF 1944.

Table I. Permeation parameters of MT from 10 mg/ml and saturated solutions in various pure solvents across hairless mouse skin

Vehicle	Drug concentration	J_s (μg/cm²/h)	T_L (h)	Solubility (mg/ml)
Phosphate buffer (pH 6.02)	saturated	0.66 ± 0.10	4.15 ± 2.57	1.12 ± 0.08
PG	10 mg/ml	0.93 ± 0.60	4.57 ± 0.22	93.0 ± 2.17
DGME	10 mg/ml	0.38 ± 0.05	2.86 ± 0.31	269.2 ± 11.4
OAI	saturated	5.06 ± 4.02	2.75 ± 0.22	2.80 ± 0.30
PGL	saturated	8.86 ± 2.47	1.95 ± 0.58	12.6 ± 0.82
PGML	10 mg/ml	8.84 ± 3.23	1.66 ± 0.14	28.0 ± 0.71
PGMC	10 mg/ml	9.45 ± 0.47	1.33 ± 0.11	41.7 ± 0.49
IPM	saturated	6.98 ± 4.04	2.58 ± 0.85	0.71 ± 0.12
LBF 1944	10 mg/ml	2.19 ± 0.39	2.70 ± 0.26	13.6 ± 0.09
LBF 2609	saturated	4.37 ± 1.37	2.57 ± 0.38	27.9 ± 0.41
Captex 200	saturated	9.94 ± 1.14	3.04 ± 0.10	1.93 ± 0.12
Captex 300	saturated	3.12 ± 0.09	2.02 ± 0.44	2.04 ± 0.05

Data were expressed as the mean ± S.D. (n = 3).

an effect on the skin although the mechanism of their effects is unclear.

Compared to phosphate buffer, other vehicles used showed marked enhancing effects in the study using saturated drug concentration as shown in Table I. Especially, IPM, PGL and Captex 200 showed high permeation fluxes. The higher permeation fluxes using saturated drug concentration over fixed drug concentration was expected due to its maximized thermodynamic activity, however, PGML and PGMC with fixed drug concentration below solubility showed very high permeation rates.

As described earlier, the long lag time has been problematic for the development of the transdermal delivery system of MT. In the study of transdermal delivery of MT, Oh *et al.* (2001) showed that the addition of EtOH to phosphate buffer prolonged the lag time whereas PEG reduced it. PG did not show significant effect on the lag time of MT. In the present study, the lag time of MT in phosphate buffer was around 4 h, which was similar to the findings from their study. Some vehicles used dramatically shortened the lag time; PGL, PGML and PGMC had a lag time below 2 h possibly due to the decreased D or K.

The solubility of MT at 32°C in various vehicles decreased in the rank order of DGME > PEG 400 ≫ PG > PGMC > PGML ≅ LBF 2609 > LBF 1944 > PGL > water > OAI > Captex 300 = Captex 200 > phosphate buffer > IPM.

Effect of co-solvents

To design the appropriate transdermal delivery system, it is necessary to have a vehicle that shows high permeation flux and short lag time, and dissolves enough

Table II. Permeation parameters of MT from saturated solutions in various ratios of DGME-PGL and DGME-IPM binary co-solvents.

Vehicle		J_S (μg/cm²/h)	T_L (h)	D (×10⁴, cm²/hr)	K	Solubility (mg/ml)
DGME :	0 : 10	8.86 ± 2.47	1.95 ± 0.58	2.15 ± 0.67	0.15 ± 0.08	12.6 ± 0.82
PGL	2 : 8	21.5 ± 7.34	2.44 ± 0.67	0.94 ± 0.11	0.79 ± 0.38	34.9 ± 3.72
	4 : 6	8.62 ± 1.85	2.88 ± 0.89	1.00 ± 0.10	0.49 ± 0.28	78.1 ± 5.79
	6 : 4	6.15 ± 0.21	2.95 ± 0.10	1.18 ± 0.22	0.24 ± 0.04	126.8 ± 1.62
	8 : 2	3.00 ± 0.66	3.42 ± 0.28	1.06 ± 0.12	0.13 ± 0.02	218.8 ± 11.1
	10 : 0	0.38 ± 0.09	2.86 ± 0.31	1.26 ± 0.13	0.01 ± 0.01	269.2 ± 11.4
DGME :	0 : 10	6.98 ± 2.04	2.58 ± 0.85	1.53 ± 0.79	2.62 ± 2.27	0.71 ± 0.12
IPM	2 : 8	9.09 ± 2.38	0.92 ± 0.57	4.70 ± 3.41	0.10 ± 0.04	11.4 ± 4.33
	4 : 6	7.78 ± 2.57	1.20 ± 0.11	3.00 ± 0.67	0.13 ± 0.05	54.1 ± 8.32
	6 : 4	6.78 ± 2.44	1.73 ± 0.92	1.76 ± 0.97	0.20 ± 0.15	120.9 ± 11.0
	8 : 2	5.40 ± 1.70	2.38 ± 0.95	1.29 ± 0.18	0.23 ± 0.05	204.9 ± 8.36
	10 : 0	0.38 ± 0.09	2.86 ± 0.31	1.26 ± 0.13	0.01 ± 0.01	269.2 ± 11.4

Data were expressed as the mean ± S.D (n = 3)

amount of drug for therapy. Based on the findings from the above pure solvent study, PGL and IPM were chosen for their enhancing effects and relatively short lag time. However, they have a limitation due to the low solubility. To improve the solubility, DGME was added. It has been suggested that DGME itself may not have a profound effect on the structural integrity of the skin, and it just eases the partition of a compound by increasing the solubility of the compound in the skin (Cho and Choi, 1998).

The effects of co-solvents containing DGME-PGL and DGME-IPM on the MT permeation were investigated. Table II shows the fluxes of MT permeated across hairless mouse skin from saturated solutions with various ratios of DGME-PGL and DGME-IPM co-solvents. Both of the two co-solvents showed the highest fluxes at 20% of DGME, which were 21.5 ± 12.7 and 9.1 ± 2.4 μg/cm²/h, respectively. Although the solubility of MT in the two co-solvents increased as the concentration of DGME increased, these fluxes were much greater than that of PGL, IPM or DGME alone.

From the calculated data in Table II, the high permeation fluxes in DGME-PGL and DGME-IPM at the 20 : 80 ratio were attributable to the high K and relatively high D, respectively.

Effect of enhancers

It has been suggested that fatty acids increase lipid fluidization in the stratum corneum by interacting with phospholipids at the boundary lipid layer (Kitagawa *et al.*, 1985). We investigated the effect of various fatty acids on the MT permeation. Five fatty acids at 5% concentration were added to DGME-PGL (20 : 80 v/v) co-solvent: three were saturated fatty acids - C_8 (caprylic acid), C_{10} (capric acid) and C_{12} (lauric acid); and two were unsaturated fatty acids - C_{18} with one double bond (OA), and C_{18} with two double bonds (linoleic acid). Gwak and Chun (2002) reported that the most effective saturated fatty acid was C_{12} chain length for tenoxicam permeation enhancement. As depicted in Fig. 2, capric acid and OA showed relatively high enhancing effect among fatty acids used. However, C_8 and C_{12}, rather, retarded the skin permeation of MT.

OA has been widely used for a permeation enhancer. Many studies have shown that the skin permeation enhancing effect of OA is greatest with PG vehicle (Cooper *et al.*, 1985; Yamada *et al.*, 1987). The mechanism of OA was thought that it melts the lipid chain portion buried within the bilayer structure, together with some non-polar material. It also breaks associations between lipid polar groups together with disruption of cholesterol-stiffened regions. It was proposed that the *cis*

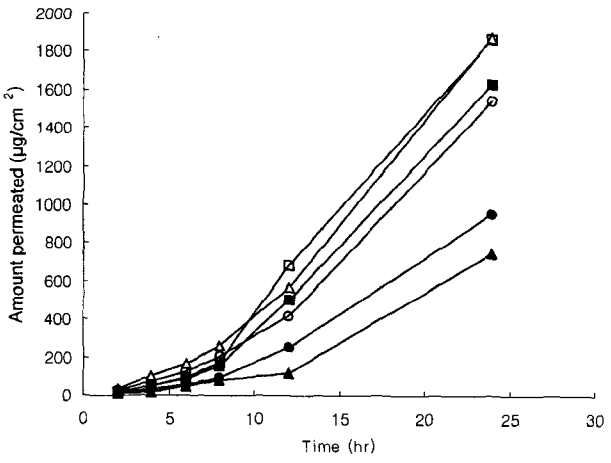

Fig. 3. Effect of fatty acids on the permeation of MT from saturated solution (n = 3). ○: No enhancer, ● : caprylic acid, △: capric acid, ▲ : lauric acid, □: OA, ■ : linoleic acid.

double bond at C_9 on OA causes a kink in the alkyl chain, which is likely to disrupt the ordered array of the predominantly saturated straight chain skin lipids and increase the fluidity of the lipid regions (Barry 1987; Golden *et al.*, 1987). A recent study demonstrated that the addition of OA to PG showed 950-fold increase in skin permeability, and decrease in lag time of MT from 5.3 to 2.1 h (Oh *et al.*, 2001). They attributed these results to the increased D of MT by OA. In this study, however, even though OA showed enhancing effect, it was not remarkable; its enhancement factor was only 1.4. Also, the enhancing effect was not due to the increased D, but increased K. K increased from 0.79 ± 0.38 to 1.67 ± 0.13, and D rather decreased from 0.94 ± 0.11 to 0.53 ± 0.02 ($\times 10^4/cm^2/hr$), compared to DGME-PGL (80 : 20 v/v) only. This discrepancy was thought to be partly due to the difference of vehicle used.

The lag time of MT in OA-containing DGME-PGL (80 : 20 v/v) was 2.6 h, which was similar to the result from the above study using OA-containing PG. Other enhancers failed to shorten the lag time of MT except capric acid. Capric acid shortened lag time from 2.4 ± 0.7 to 1.3 ± 0.2 h compared to DGME-PGL (80 : 20 v/v).

In conclusion, our data indicated that capric acid-containing DGME-PGL (80 : 20 v/v) could be used to enhance the skin permeation and shorten the lag time of MT.

REFERENCES

Barry, B.W., Mode of action of penetration enhancers in human skin. *J. Controlled Rel.*, 6, 85-97 (1987).

Bénès, L., Claustrat, B., Horrière, F., Geoffriau, M., Konsil, J., Parrott, K., DeGrande, G., McQuinn, R. L. and Ayres, J. W., Transmucosal, oral controlled-release, and transdermal drug administration in human subjects: a crossover study with melatonin. *J. Pharm. Sci.*, 86, 1115-1119 (1997).

Brzezinski, A., Mechanisms of disease: melatonin in humans. *N. Eng. J. Med.*, 336, 186-195 (1997).

Cho, Y. J. and Choi, H. K., Enhancement of percutaneous absorption of ketoprofen: effect of vehicles and adhesive matrix. *Int. J. Pharm.*, 169, 95-104 (1998).

Cooper, E.R., Merritt, E.W. and Smith, R.L., Effect of fatty acids and alcohols on the penetration of acyclovir across human skin *in vitro*. *J. Pharm. Sci.*, 74, 688-689 (1985).

Cordero, J. A., Alarcon, L., Escribano, E., Obach, R. and Domenech, J., A comparative study of the transdermal penetration of a series of nonsteroidal anti-inflammatory drugs. *J. Pharm. Sci.*, 86, 503-508 (1997).

Fung, B. K., 1987. Transducin: structure, function, and role in phototransduction. In: Osborn NN, Chader GJ, eds. Progress in retinal research, Vol. 6.; Pergamon Press: Oxford, England, pp. 151-177.

Golden, G.M., McKie, J.E. and Potts, R.O., Role of stratum corneum lipid fluidity in transdermal drug flux. *J. Pharm. Sci.*, 76, 25-28 (1987).

Gwak, H. S. and Chun, I. K., Effect of vehicles and penetration enhancers on the *in vitro* percutaneous absorption of tenoxicam through hairless mouse skin. *Int. J. Pharm.*, 236, 57-64(2002).

Kitagawa, S., Endo, J. and Kametani, F., Effects of long-chain *cis*-unsaturated fatty acids and their alcohol analogues on aggregation of bovine platelets and their relation with membrane fluidity change. *Biochem. Biophys. Acta*, 818, 391-397 (1985).

Lee, B., Parrott, K. A., Ayres, J. W. and Sack, R. L., Preliminary evaluation of transdermal delivery of melatonin in human subjects. *Res. Commun. Mol. Path. Pharmacol.*, 85, 337-346 (1994).

Lerner, A. B., Case, J. D., Takahashi, Y., Lee, T. H. and Mori, W., Isolation of melatonin, the pineal gland factor that lightens melanocytes. *J. Am. Chem. Soc.*, 80, 2587 (1958).

Lieberman, H. R., Waldhauser, F., Garfield, G., Lynch, H. J. and Wurtman, R. J., Effects of melatonin on human mood and performance. *Brain Res.*, 323, 201-207 (1984).

Lynch, H. J., Wurtman, R. J., Moskowitz, M. A., Archer, M. A. and Ho, M. H., Daily rhythm in human urinary melatonin. *Science*, 187, 169-171 (1975).

Møllgaard, B. and Hoelgaard, A., Vehicle effects in topical drug delivery I. influence of glycols and drug concentration on skin transport. *Acta. Pharm. Suec.*, 20, 433-442 (1983).

Oh, H. J., Oh, Y. K. and Kim, C. K., Effects of vehicles and enhancers on transdermal delivery of melatonin. *Int. J. Pharm.*, 212, 63-71 (2001).

Pangerl, B., Pangerl, A. and Reiter, R. J., Circadian variations of adrenergic receptors in the mammalian pineal gland: a review. *J. Neural. Transm. Gen. Sect.*, 81, 17-29 (1990).

Roy, S.D. and Flynn, G.L., Solubility and related physicochemical properties of narcotic analgesics. *Pharm. Res.*, 5, 580-586 (1988).

Vollrath, L., Semm, P. and Gammel, G., Sleep induction by intranasal application of melatonin. *Adv. Biosci.*, 29, 327-329 (1981).

Waldhauser, F., Waldhauser, M., Lieberman, H. R., Deng, M. H., Lynch, H. J. and Wurtman, R. J., Bioavailability of oral melatonin in humans. *Neuroendocrinology*, 39, 307-313 (1984).

Yamada, M., Uda, Y. and Tanigawara, Y., Mechanism of enhancement of percutaneous absorption of molsidomine by oleic acid. *Chem. Pharm. Bull.*, 35, 3399-3406 (1987).