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Skin permeation enhancement effect and skin irritation of saturated fatty alcohols

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Abstract

Though the skin permeation enhancement effect of chemical penetration enhancers has been studied extensively, their skin irritation potential has not been adequately investigated. The objective of this study was to evaluate the skin permeation enhancement effect and skin irritation of saturated fatty alcohols using melatonin as a model compound. A saturated solution of melatonin in a mixture of water and ethanol (40:60) containing 5% w/v of saturated fatty alcohol was used in the skin permeation studies using Franz diffusion cells. For skin irritation studies, 230 μl of fatty alcohol solution was applied on the dorsal surface of the hairless rats using Hill top chamber®. The skin irritation was evaluated by visual scoring method and bioengineering methods such as measurement of transepidermal water loss (TEWL) and skin blood flow. The flux of melatonin across hairless rat skin was found to be dependent on the carbon chain length of the fatty alcohols, with decanol showing the maximum permeation of melatonin. All fatty alcohols increased the TEWL and skin blood flow significantly compared with the vehicle. The fatty alcohols (decanol, undecanol and lauryl alcohol), which showed greater permeation of melatonin, also produced greater TEWL, skin blood flow and erythema. Tridecanol and myristyl alcohol showed lower permeation enhancement effect but caused greater skin irritation. Octanol and nonanol may be the most useful enhancers for the transdermal delivery of melatonin considering their lower skin irritation and a reasonably good permeation enhancement effect. However, further studies are needed to ascertain their safety as skin penetration enhancers. Skin permeation and skin irritation in experimental animals such as rats are generally higher compared with human skin. Further studies in human volunteers using fatty alcohols at the concentrations of 5% or lower may provide useful information on the utility of these fatty alcohols as permeation enhancers.

Keywords: Saturated fatty alcohols; Skin permeation enhancement; Transepidermal water loss; Skin blood flow; Erythema

1. Introduction

Transdermal drug delivery offers several advantages over the conventional dosage forms such as tablets and injections, including elimination of first

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pass metabolism, minimization of pain, and possible sustained release of drugs. However, the highly organized structure of the stratum corneum forms an effective barrier to the permeation of a diverse range of substances, which must be modified if poorly penetrating drugs are to be administered. The use of chemical penetration enhancers would significantly increase the number of candidates suitable for transdermal delivery. The practical use of chemical penetration enhancers requires careful balancing of their benefits and risks, i.e. penetration rates and irritation. Though the skin permeation enhancement effect of chemical penetration enhancers has been studied extensively in the last two decades (Walter and Hadgraft, 1993), their skin irritation potential have not been investigated adequately. The correlation between the skin permeation enhancement effect and irritation of chemical penetration enhancers has not been fully established. Clearly, further studies are needed in the areas of evaluation of skin permeation enhancement vis-a-vis skin irritation in order to chose penetration enhancers which possess optimum enhancement effect with no skin irritation.

Several non-invasive techniques based on different physical principles have been developed to investigate skin irritation and have been used for patch test assessment. The measurement of transepidermal water loss (TEWL) and skin blood flow using evaporimeter and laser Doppler velocimetry (LDV), respectively, is widely used in the evaluation of skin irritation. The advantages of these bioengineering methods are represented by the possibility of collecting data with objectivity and by monitoring readings and values on a linear scale with recording devices. TEWL has been used in relation to the assessment of either the irritation (Agner and Serup, 1990; Loffler et al., 2001) or the effects of penetration enhancers (Green et al., 1988; Tanojo et al., 1998).

Measurement of skin blood flow by LDV has been employed by several researchers as a non-invasive technique to evaluate the skin irritation (Agner and Serup, 1990; Tanojo et al., 1998). The use of LDV has proven more accurate than visual scoring in the scaling and differentiation of irritation effects (Berardesca and Maibach, 1988) and it has also been employed to measure the effects of enhancers on the percutaneous absorption of nicotinates (Ryatt et al., 1986).

Melatonin is a good candidate for transdermal delivery considering its variable oral absorption, short biological half-life (45 min) and a favorable octanol: water partition coefficient (15.8). Lee et al. (1994) studied the transdermal delivery of melatonin in human volunteers using a solution of melatonin in pH 6.1 phosphate buffer containing propylene glycol. The authors concluded that melatonin can be delivered transdermally in human volunteers, although an inter-subject variability was noted. In another study, Benes et al. (1997) compared the delivery of melatonin from transmucosal, oral controlled-release and transdermal administration in human volunteers. It was observed that transdermal administration of melatonin showed a significant lag time and a gradual decline in drug delivery after patch removal possibly due to deposition of melatonin in the skin. Some of these problems can be overcome at least in part by using a more effective penetration enhancer and by applying the patch a few hours before the required time of onset of action.

The skin penetration enhancement effect of saturated fatty alcohols has been demonstrated in many in vitro studies (Aungst et al., 1986; Kanikkannan et al., 2000). However, the skin irritation of saturated fatty alcohols has not been studied systematically. The present study was undertaken to investigate the skin permeation enhancement effect and skin irritation of a series of saturated fatty alcohols in hairless rats using melatonin as a model compound. Though hairless rat skin is not a precise model for human skin for percutaneous absorption and skin irritation studies, it can be used to gain insight into the general pattern and mechanisms. The absence of hair on the skin would avoid introduction of artifacts due to the methods used for hair removal in regular rats. In addition to the visual scoring method, bioengineering techniques such as measurement of TEWL and skin blood flow were also employed for the evaluation of skin irritation.

2. Materials and methods

2.1. Materials

Melatonin, fatty alcohols [octanol, nonanol, decanol, undecanol, lauryl alcohol, tridecanol and myristyl alcohol] were procured from Sigma Chemical Co. (St. Louis, MO). Water, methanol (HPLC grade) and nylon filters were obtained from Fisher Scientific (Atlanta, GA, USA). Hill top chambers® were obtained from Hill Top Co., Cincinnati, OH.

2.2. Preparation of melatonin solutions

An excess of melatonin was added to the vehicle (mixture of water and ethanol, 40:60) (WE) containing 5% w/v of fatty alcohol and the mixture was shaken in an environmental shaker at 37 ± 0.1 °C overnight to obtain a saturated solution of melatonin. Addition of 5% fatty alcohol did not change the solubility of melatonin in the vehicle significantly ($P > 0.05$). The melatonin solution was filtered through a 0.45 μm nylon filter and 1 ml of this solution was placed in the donor compartment of the Franz diffusion cell assembly.

2.3. In vitro skin permeation studies

CD®(SD) hrBi hairless rats (250–300 g; Charles River Laboratories) were sacrificed and the full thickness skin was excised and mounted between the donor and receptor compartments of the Franz diffusion cells (Permegear Inc., Riegelsville, PA). Each cell had a diffusional surface area of 0.636 cm^2. The skin was mounted between the donor and receptor compartments of a Franz diffusion cell with the stratum corneum facing the donor compartment. The melatonin solution (1 ml) containing 5% w/v fatty alcohols was placed in the donor compartment and the receptor compartment was filled with 5 ml of phosphate buffer pH 7.4. The vehicle used in the preparation of melatonin solution (60% alcohol) may act as a preservative for the skin tissue. There was no visible indication of growth of microorganisms (turbidity) in the receptor compartment during the experiment. The temperature of the receptor compartment was maintained at 37 ± 0.1 °C with an external, constant temperature circulator water bath. At predetermined time intervals, samples (0.5 ml) were taken from the receptor compartment and the cell was refilled with an equivalent amount of fresh buffer solution. The samples were analyzed by HPLC method using UV detector (Kandimalla et al., 1999).

2.4. Measurement of TEWL

The protocol for skin irritation studies with CD®(SD) hrBi hairless rats (250–300 g; Charles River Laboratories) was approved by the Animal Care and Use Committee of the Florida A and M University. CD®(SD) hrBi hairless rats have normal immune response mechanisms similar to regular rats (e.g. Sprague–Dawley rats) but they are free from hair on the skin.. The protocol for the measurement of TEWL followed published guidelines (Pinnacoda et al., 1990). The room temperature and humidity were maintained at 22 ± 1 °C and 35–45% RH, respectively. The rats were anaesthetized with pentobarbitone sodium (35 mg/kg) during the measurements of TEWL and skin blood flow. The control and treatment sites were marked as circular area (~ 3 cm^2) with a felt tip marker on the dorsal surface of the rat (one treatment site and one control site). WE (230 μl) or WE containing 5% w/v of enhancer was placed in the Hill top chamber® (surface area 1.04 cm^2) and affixed on the dorsal surface of the skin. The control sites were affixed with a Hill top chamber® without any solution in order to evaluate the occlusive effects of the chamber. The intimate contact of the chambers on the skin was ensured by the application of a waterproof tape (Johnson and Johnson, Inc., NJ, USA). The Hill top chamber® was removed after 3 h, and the treatment area was gently wiped with Kimwipes to remove the residual liquid on the skin surface. Measurements of TEWL and skin blood flow were taken before application and at 0, 1, 2, 4, 6, 24, 48, 72, and 96 h after removal of the chamber. The TEWL was measured using Tewameter TM 210 (Courage + Khazaka, Cologne, Germany). The probe of the Tewameter was placed perpendicular

to the surface of the skin and a stable reading of TEWL was reached in about 60 s.

2.5. Measurement of skin blood flow by LDV

The skin blood flow was measured on the control and treated sites using the DRT4 Laser Doppler monitor system (Moore Instruments, Devon, England). The measuring probe contains a diode laser with a wavelength of 780 nm. The instrument was allowed to warm up for 15 min after being turned on. The probe was held gently on the skin to avoid vascular compression using an adhesive pad. The readings (arbitrary units, a.u.) were performed after stabilization of the output signal.

2.6. Skin irritation

The skin irritation (erythema) was evaluated by visual scoring using a modified method of Draize et al. (1944). The scores were given from 0 to 4 depending on the degree of erythema as follows: no erythema—0, slight erythema (barely perceptible-light pink)—1, moderate erythema (dark pink)—2, moderate to severe erythema (light red)—3, severe erythema (extreme redness)—4.

2.7. Data analysis

The cumulative amount of melatonin permeated per cm^2 of skin was calculated and plotted as a function of time. The slope of the curve was estimated as the flux of melatonin. The correlation coefficients were calculated by regression analysis using Graph Pad Prism^{TM}. The data were considered significant at $P < 0.05$.

3. Results and discussion

3.1. Skin permeation of melatonin across hairless rat skin in vitro

The effect of saturated fatty alcohols on the permeation of melatonin across hairless rat skin is presented in Fig. 1. All saturated fatty alcohols enhanced the permeation of melatonin through hairless rat skin and the permeation of melatonin was found to be related to the carbon chain length of the fatty alcohols. The flux values of melatonin across hairless rat skin are presented in Fig. 2. An increase in the permeation of melatonin was observed when the fatty alcohol chain length increased from eight to ten carbons. However, the permeation of melatonin decreased when the chain length was increased beyond ten carbons. It can be observed that the flux values of melatonin have a parabolic relationship with the carbon chain length of the saturated fatty alcohols. Decanol showed the maximum permeation of

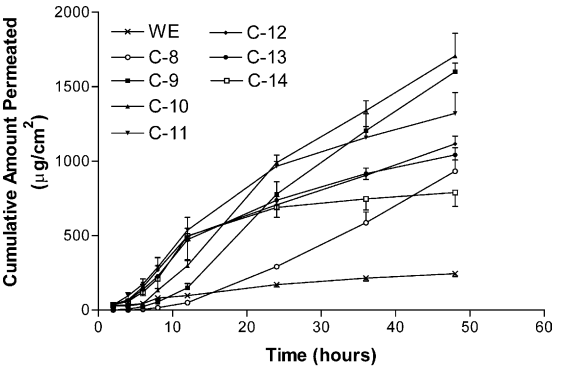

Fig. 1. Effect of saturated fatty alcohols [octanol (C-8), nonanol (C-9), decanol (C-10), undecanol (C-11), lauryl alcohol (C-12), tridecanol (C-13) and myristyl alcohol (C-14)] on the permeation of melatonin across hairless rat skin in vitro. Data are mean ± S.D. of three to four determinations.

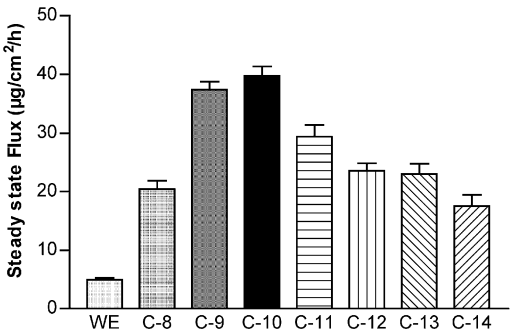

Fig. 2. Effect of saturated fatty alcohols [octanol (C-8), nonanol (C-9), decanol (C-10), undecanol (C-11), lauryl alcohol (C-12), tridecanol (C-13) and myristyl alcohol (C-14)] on the steady state flux of melatonin across hairless rat skin in vitro. Data are mean ± S.D. of three to four determinations.

melatonin, which was approximately 7-fold greater compared with WE.

Long-chain fatty alcohols have been shown to be the effective penetration enhancers for a variety of drugs. Aungst et al. (1986) studied the influence of saturated alcohols (C-8 to C-18) on the flux of naloxone in propylene glycol through the human skin. The permeation of naloxone was found to have a parabolic relationship with the carbon chain length, with decanol and lauryl alcohol being the most effective. The mechanism by which fatty alcohols increase skin permeability appears to involve disruption of the densely packed lipids, which fill the extra cellular spaces of the stratum corneum. It may be significant that the most effective carbon chain lengths (ten to 12) correspond to the chain length of the steroid nucleus of cholesterol, suggesting that these may act by disrupting ceramide–cholesterol or cholesterol–cholesterol interaction (Brain and Walters, 1993). A parabolic relationship between carbon chain length of fatty alcohol and permeation enhancement was also observed for other drugs including tegafur (Lee et al., 1993) and theophylline (Sloan et al., 1998).

Table 1 presents the reported values of physicochemical parameters (molecular weight, melting point, water solubility and octanol–water partition coefficient) of the saturated fatty alcohols (Meylon and Howard, 1995; Meylon et al., 1996). As the carbon chain length of the fatty alcohols increased, their molecular weights, melting points and log octanol–water partition coefficients also increased linearly. The lower permeation enhancement effect of fatty alcohols having over 11 carbon atoms may be attributed to the decreased mobility of fatty alcohols within the skin layers due to the combined effect of their higher molecular weight, melting point and octanol–water partition coefficient. It can be observed that there is a significant decrease in the permeation rate of melatonin with lauryl alcohol (C-12), tridecanol (C-13) and myristyl alcohol (C-14). This may be attributed to the decreased enhancer concentration possibly by the precipitation of enhancer in the donor compartment. The melting point of saturated fatty alcohols increases as the carbon chain length is increased (see Table 1). The melting point of lauryl alcohol, tridecanol and myristyl alcohol is higher than room temperature. Secondly, since the donor compartment was exposed to room temperature (~ 23 °C), a portion of the melatonin might have been precipitated (saturated melatonin solution was prepared at 37 °C). In all experiments, melatonin crystals were seen in the donor compartment when observed at the end of the experiment. Though excess melatonin was presumably present in the donor compartment, since there was no stirring in the donor compartment, there could be a depletion of melatonin in the microenvironment above the skin surface. The decrease in the permeation rate of melatonin observed with most of fatty alcohols beyond 24 h may be partly attributed to the depletion of enhancer and melatonin in the microenvironment above the skin surface.

3.2. Transepidermal water loss

Fig. 3 shows the effect of fatty alcohols (5% w/v) on the TEWL in hairless rats. The error bars have not been depicted in Fig. 3 for better clarity of the

Table 1
Physicochemical properties of saturated fatty alcohols

Fatty alcohol	Molecular weight	Melting point (°C)	Water solubility at 25 °C (mg/l)	log P (octanol–water)
Octanol	130.23	−15.5	540.0	3.00
Nonanol	144.26	−5.0	140.0	3.77
Decanol	158.29	6.9	37.0	4.57
Undecanol	172.31	19.0	19.1	4.28
Lauryl alcohol	186.34	24.0	4.0	5.13
Tridecanol	200.37	32.5	1.36	5.26
Myristyl alcohol	214.39	39.5	0.191	6.03

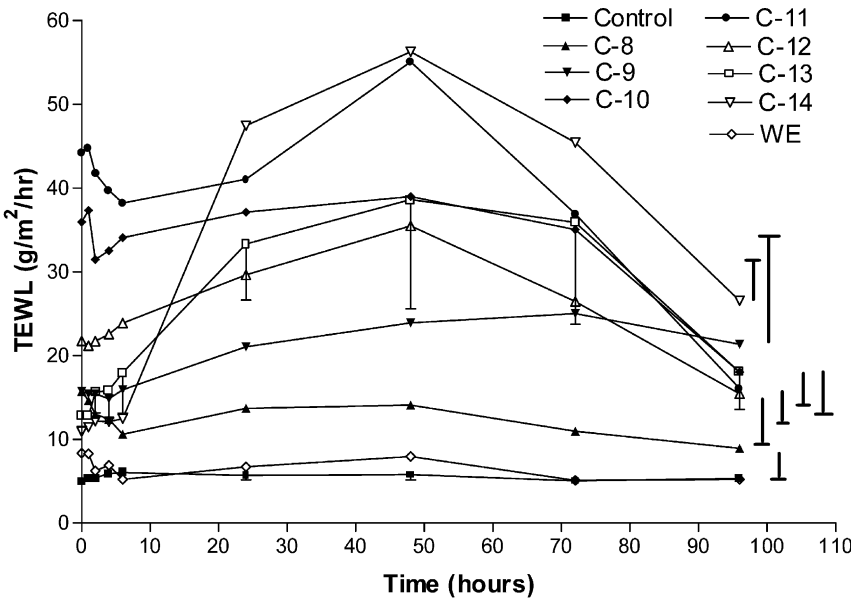

Fig. 3. Effect of saturated fatty alcohols [octanol (C-8), nonanol (C-9), decanol (C-10), undecanol (C-11), lauryl alcohol (C-12), tridecanol (C-13) and myristyl alcohol (C-14)] on the TEWL in hairless rats. Data are means of three to four determinations. The error bars for 96 h time point are shown at the side of each graph.

graphs. However, the error bars for 96 h point have been provided at the side of each graph to give some idea of the size of the experimental errors involved in the measurements. All fatty alcohols increased the TEWL significantly compared with the control (chamber containing no solution). Treatment with WE did not show a significant change in the TEWL compared with the control. As the carbon chain length of fatty alcohol increased from eight to 11, there was a steady increase in the TEWL. However, when the carbon chain length increased to 12, there was a sharp decrease in the TEWL. Surprisingly, tridecanol and myristyl alcohol showed a significantly different TEWL profiles compared with other fatty alcohols. There was a low and constant TEWL level in the initial period (upto 8 h) followed by a sharp increase in the TEWL. The TEWL shown by myristyl alcohol was the least among all fatty alcohols at 0 h after the removal of patches, but at 48 h, the TEWL was the highest of all fatty alcohols. In general, the TEWL continued to increase up to 48 h with all fatty alcohols, and then started decreasing.

In an attempt to determine the correlation between the flux values of melatonin and the TEWL in hairless rats, the area under the curve (AUC) of the plot of TEWL versus time was calculated. The correlation between the flux of melatonin and the AUC of the plot of TEWL versus time with fatty alcohols from octanol through myristyl alcohol was poor and statistically insignificant ($r = 0.6642$; $P > 0.05$). However, when the regression analysis was performed with fatty alcohols from octanol through lauryl alcohol, there was a significant correlation observed between the flux of melatonin and the AUC of the plot of TEWL versus time ($r = 0.8472$; $P < 0.05$). This may be attributed to the major shift in the TEWL profiles of tridecanol and myristyl alcohol compared with other fatty alcohols. The different behavior of tridecanol and myristyl alcohol compared with other fatty alcohols may be due to their higher melting points and octanol–water partition coefficients (Table 1). However, tridecanol and myristyl alcohol increased the TEWL substantially after 8 h and continued to increase up to 48 h.

It is known that drug penetration across the skin increases with decreased barrier function, mostly

due to the damage of skin. A high TEWL indicates defects in the barrier function of the skin. As the skin barrier function is believed to be primarily located in the intercellular domains (Elias, 1981), the lipid phase acts as a barrier against water loss. It is logical to correlate the increase in TEWL with the perturbation of the intercellular lipids of the skin by fatty alcohols. Monteiro-Riviere et al. (2001) studied the effects of selective lipid extraction and tape stripping on TEWL at three body regions in Yorkshire pigs. Extraction of lipids across all body sites of the pig increased TEWL to a level similar to that seen with repeated tape stripping and there was a correlation observed between the presence of total lipid and TEWL.

Skin penetration enhancers can improve the penetration of other substances by perturbing the barrier function of the stratum corneum. Chemical penetration enhancers such as fatty acids (Tanojo et al., 1998; Jiang et al., 2000) and terpenes (Gao and Singh, 1997; Yosipovitch et al., 1996) have been reported to increase the TEWL through the skin of experimental animals and human volunteers. Wilhelm et al. (1991) studied the effect of pretreatment of sodium lauryl sulfate (SLS) on the TEWL in hairless guinea pigs and in vitro percutaneous absorption of drugs with diverse physicochemical properties (hydrocortisone, indomethacin, ibuprofen and acitretin). SLS pretreatment resulted in moderate irritant dermatitis in all animals and increased TEWL 4.5 times. The flux was increased with all the four chemicals compared with the control. Tanojo et al. (1998) studied the effects of a series of fatty acids on the skin barrier function and LDV in human volunteers. The saturated fatty acids only caused a slight increase in TEWL and LDV compared with the unsaturated fatty acids that produced a significant increase in TEWL and LDV. In the present study, saturated fatty alcohols, which have lower melting points than their corresponding acids, showed significant increase in TEWL compared with the control.

3.3. Skin blood flow

Fig. 4 shows the effect of treatment of skin with fatty alcohol solutions for 3 h on the skin blood flow in hairless rats. The error bars have not been depicted in Fig. 4 for better clarity of the graphs. All fatty alcohols increased the skin blood flow significantly compared with the control. Treatment with WE did not show a significant change in the skin blood flow compared with the control (chamber containing no solution). Lauryl alcohol produced the highest skin blood flow value at 0 h after removal of the patches. However, the skin blood flow decreased steadily as the time progressed. In general, the skin blood flow values decreased after the 0 h measurements with most of the fatty alcohols except undecanol where the skin blood flow values remained high upto 72 h and then decreased. Surprisingly, decanol and lauryl alcohol decreased the skin blood flow significantly after 24 h and the skin blood flow with decanol was the lowest of all fatty alcohols at 72 and 96 h. The application site of decanol appeared dry, thick and reddish after about 72 h of removal of the patches. Probably the measurement of skin blood flow was not accurate in this case because of the interference due to the abnormal condition of the skin (thick, dry and reddish).

LDV was initially used along with visual scoring in the differentiation of skin irritation. A positive relationship was found between the dose of SLS applied to the skin and blood flow values (Agner, 1992). The use of LDV for the skin permeation study was employed initially to measure the penetration of methyl nicotinates across the stratum corneum/epidermis to exert erythema as a result of vasodilatation on cutaneous capillary blood vessels yielding increase in skin blood flow (Guy et al., 1983). Subsequently, the vasodilatation capability of nicotinates was used to measure the penetration enhancement of pyrrolidine derivatives (Ryatt et al., 1986) and fatty acids (Green et al., 1988; Tanojo et al., 1998, 1999). In this study, LDV was used to quantify the degree of irritation after the application of saturated fatty alcohols. Tanojo et al. (1998) studied the effect of saturated and unsaturated fatty acids on the LDV in human subjects. Application of saturated fatty acids resulted in a low irritation index compared with unsaturated fatty acids. In the present study with saturated fatty alcohols, which have lower melting points than their corresponding fatty acids

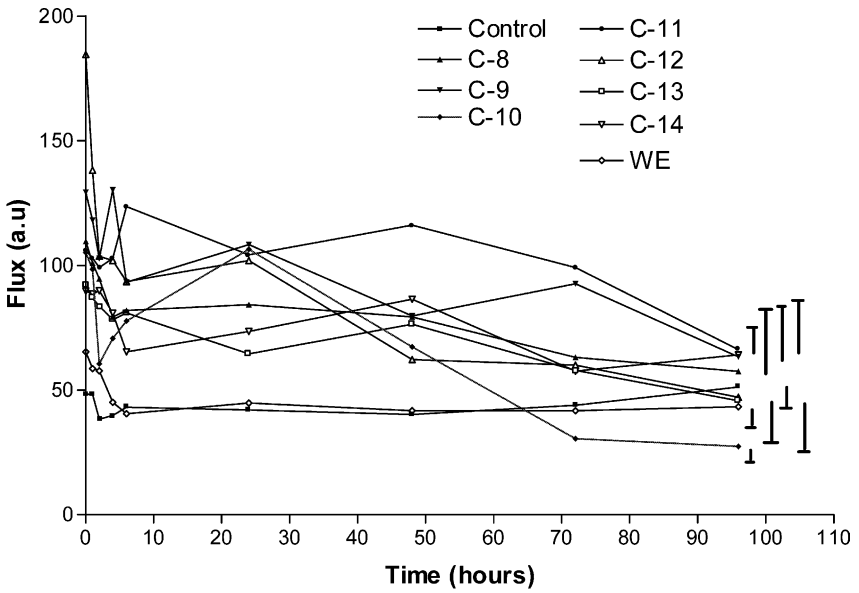

Fig. 4. Effect of saturated fatty alcohols [octanol (C-8), nonanol (C-9), decanol (C-10), undecanol (C-11), lauryl alcohol (C-12), tridecanol (C-13) and myristyl alcohol (C-14)] on the skin blood flow as measured by LDV in hairless rats. Data are means of three to four determinations. The error bars for 96 h time point are shown at the side of each graph.

showed a significant increase in the LDV in hairless rats. Green et al. (1988) studied the effect of oleic acid and lauric acid on the TEWL and skin permeation of methyl nicotinate in human volunteers. Pretreatment of skin with oleic acid and lauric acid caused a significant increase in the TEWL and permeation of methyl nicotinate as assessed by LDV. The present study demonstrates a significant linear relationship between the flux of melatonin in vitro and the skin blood flow with saturated fatty alcohols.

3.4. Skin irritation (erythema)

The erythema scores upon exposure of hairless rat skin to fatty alcohols have been presented in Fig. 5. The error bars have not been depicted in Fig. 5 for better clarity of the graphs. Decanol and undecanol caused erythema rapidly and the erythema level increased further up to 72 h. Though the erythema caused by lauryl alcohol was less compared with decanol and undecanol up to 24 h, lauryl alcohol produced the highest erythema from 48 h onwards. Tridecanol and myristyl alcohol took a long time to show erythema. However, as the time progressed, the erythema levels increased significantly and the values were comparable to that of undecanol at 72 and 96 h. Table 2 presents the TEWL, skin blood flow and erythema values at 48 h (normalized with respect to the corresponding WE value) after the treatment. These results indicate that there is no good correlation between

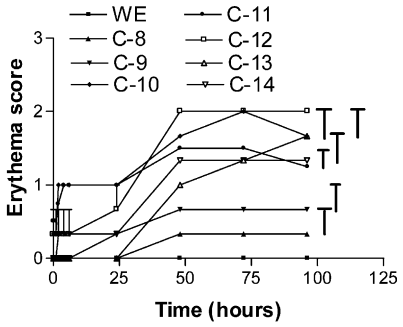

Fig. 5. Skin erythema upon exposure to fatty alcohols [octanol (C-8), nonanol (C-9), decanol (C-10), undecanol (C-11), lauryl alcohol (C-12), tridecanol (C-13) and myristyl alcohol (C-14)] in hairless rats. Data are means of three to four determinations. The error bars for 96 h time point are shown at the side of each graph.

Table 2
Effect of fatty alcohols on the TEWL, skin blood flow and erythema in hairless rats at 48 h (normalized with respect to the corresponding WE value) after the treatment

Fatty alcohol score	TEWL (g/m^2 per h)	Skin blood flow (a.u)	Erythema
C-8	6.2	37.7	0.3
C-9	16.03	6.2	0.7
C-10	31.0	23.7	1.7
C-11	47.2	72.6	1.5
C-12	27.6	18.7	2.0
C-13	30.7	32.9	1.0
C-14	48.4	42.9	1.3

the skin permeation enhancement effect (Fig. 2) and skin irritation of saturated fatty alcohols.

Because of their hydrophobic nature, tridecanol and myristyl alcohol can easily partition in to stratum corneum but the diffusion through the aqueous viable epidermis and dermis may be hindered. The delayed irritation with tridecanol and myristyl alcohol may be due to the long time taken to partition into the aqueous viable epidermis from stratum corneum. Octanol and nonanol showed the least erythema compared with other fatty alcohols. Decanol, which showed the highest erythema next to lauryl alcohol, showed the least LDV value at 72 and 96 h. Previous studies have shown that LDV is useful in discriminating between the negative and positive reactions, but fails to quantify strong positive reactions (Andersen and Staberg, 1985; Blanken et al., 1986). The results of the present study are in agreement with the above reports. LDV rather underestimates the severe skin irritation as observed in the case of decanol. Furthermore, our observations support the findings of the previous reports that visually indistinguishable skin irritation reactions (see erythema scores in Fig. 5) can induce significantly different changes in TEWL (Fig. 3) and skin blood flow (Fig. 4) in the case of mild irritants such as octanol and nonanol.

4. Conclusions

The results of the present study demonstrate that enhancers, which showed greater permeation enhancement effect, can also cause significant skin irritation. Fatty alcohols (decanol and undecanol), which showed greater enhancement in the permeation of melatonin, also caused higher TEWL, skin blood flow and erythema. Octanol and nonanol may be the most useful enhancers for the transdermal delivery of melatonin considering their lower skin irritation and a reasonably good permeation enhancement effect. However, further studies are needed to ascertain their safety as skin penetration enhancers. Permeation of drugs and chemicals through hairless rat skin is generally higher compared with human skin. The greater irritation in hairless rats may be due to the increased absorption of fatty alcohols into hairless rat skin. Further studies in human volunteers using fatty alcohols at the concentrations of 5% or lesser may provide useful information on the utility of these fatty alcohols as permeation enhancers. The present study demonstrates that penetration enhancers can cause severe skin irritation and there is no good correlation between their skin permeation enhancement effect and skin irritation.

Acknowledgements

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References

Agner, T., 1992. Noninvasive measuring methods for the investigation of irritant patch test reactions. A study of patients with hand eczema, atopic dermatitis and controls. Acta Derm. -Venereol. (Stockh.) Suppl. 178, 3–26.

Agner, T., Serup, J., 1990. Sodium lauryl sulfate for irritant patch testing—a dose response study using bioengineering methods for determination of skin irritation. J. Invest. Dermatol. 95, 543–547.

Andersen, K.E., Staberg, B., 1985. Quantitation of contact allergy in guinea pigs by measuring changes in skin blood flow and skin fold thickness. Acta. Derm. Venereol. 65, 37–42.

Aungst, B., Rogers, N., Shefter, E., 1986. Enhancement of naloxone penetration through human skin in vitro using fatty acids, fatty alcohols, surfactants, sulfoxides and amides. Int. J. Pharm. 33, 225–234.

Benes, L., Claustrat, B., Horriere, F., Geoffriau, M., Konsil, J., Parrott, K.A., Degrande, G., McQuinn, R.L., Ayres, J.W., 1997. Transmucosal, oral controlled-release and transdermal drug administration in human subjects: a crossover study with melatonin. J. Pharm. Sci. 86, 1115–1119.

Berardesca, E., Maibach, H.I., 1988. Bioengineering and the patch test. Contact Dermatitis 18, 3–9.

Blanken, R., Vander Valk, P.G.M., Nater, J.P., 1986. Laser Doppler flowmetry in the investigation of irritant compounds on human skin. Dermatosen 34, 5–9.

Brain, K.R., Walters, K.A., 1993. Molecular modeling of skin permeation enhancement by chemical agents. In: Walters, K.A., Hadgraft, J. (Eds.), Pharmaceutical Skin Penetration Enhancement. Marcel Dekker, New York, pp. 389–416.

Draize, J., Woodard, G., Calvery, H., 1944. Methods for the study of irritation and toxicity of substances topically applied to skin and mucous membranes. J. Pharmacol. Exp. Ther. 82, 377–390.

Elias, P.M., 1981. Lipids and the epidermal permeability barrier. Arch. Dermatol. Res. 120, 95–117.

Gao, S., Singh, J., 1997. Mechanism of transdermal transport of 5-fluorouracil by terpenes: carvone, 1,8-cineole and thymol. Int. J. Pharm. 154, 67–77.

Green, P.G., Guy, R.H., Hadgraft, J., 1988. In vitro and in vivo enhancement of skin permeation with oleic and lauric acids. Int. J. Pharm. 48, 103–111.

Guy, R.H., Wester, R.C., Tur, E., Maibach, H.I., 1983. Noninvasive assessments of the percutaneous absorption of methyl nicotinate in humans. J. Pharm. Sci. 72, 1077–1079.

Jiang, S.J., Hwang, S.M., Choi, E.H., Elias, P.M., Ahn, S.K., Lee, S.H., 2000. Structural and functional effects of oleic acid and iontophoresis on hairless mouse stratum corneum. J. Invest. Dermatol. 114, 64–70.

Kandimalla, K., Kanikkannan, N., Singh, M., 1999. Optimization of a vehicle mixture for the transdermal delivery of melatonin using artificial neural networks and response surface method. J. Control. Release 61, 71–82.

Kanikkannan, N., Kandimalla, K., Lamba, S.S., Singh, M., 2000. Structure-activity relationship of chemical penetration enhancers in transdermal drug delivery. Curr. Med. Chem. 7, 593–608.

Lee, C., Uchida, T., Noguchi, E., Kim, N., Goto, S., 1993. Skin permeation enhancement of tegafur by ethanol/panasate 800 or ethanol/water binary vehicle and combined effect of fatty acids and fatty alcohols. J. Pharm. Sci. 82, 1155–1159.

Lee, B., Parrott, K.A., Ayres, J.W., Sack, R.L., 1994. Preliminary evaluation of transdermal delivery of melatonin in human subjects. Res. Commun. Mol. Pathol. Pharmacol. 85, 337–346.

Loffler, H., Pirker, C., Aramaki, J., Frosch, P.J., Happle, R., Effendy, I., 2001. Evaluation of skin susceptibility to irritancy by routine patch testing with sodium lauryl sulfate. Eur. J. Dermatol. 11, 416–419.

Meylon, W.M., Howard, P.H., 1995. Atom/fragment contribution method for estimating octanol–water partition coefficients. J. Pharm. Sci. 84, 83–92.

Meylon, W.M., Howard, P.H., Boethling, R.S., 1996. Sources and estimations of octanol–water partition coefficients and water solubilities. In: Ostrander, G.K. (Ed.), Techniques in Aquatic Toxicology. CRC Lewis Publishers, Boca Raton, pp. 395–404.

Monteiro-Riviere, N.A., Inman, A.O., Mak, V., Wertz, P., Riviere, J.E., 2001. Effect of selective lipid extraction from different body regions on epidermal barrier function. Pharm. Res. 18, 992–998.

Pinnacoda, J., Tupker, R.A., Agner, T., Serup, J., 1990. Guidelines for transepidermal water loss (TEWL) measurement. Contact Dermatitis 22, 164–178.

Ryatt, K.S., Stevension, J.M., Maibach, H.I., Guy, R.H., 1986. Pharmacodynamic measurement of percutaneous penetration enhancement in vivo. J. Pharm. Sci. 75, 374–377.

Sloan, K.B., Beall, H.D., Taylor, H.E., 1998. Transdermal delivery of theophylline from alcohol vehicles. Int. J. Pharm. 171, 185–193.

Tanojo, H., Boelsma, E., Junginger, H.E., Ponec, M., Bodde, H.E., 1998. In vivo human skin barrier modulation by topical application of fatty acids. Skin Pharmacol. Appl. Skin Physiol. 11, 87–97.

Tanojo, H., Boelsma, E., Junginger, H.E., Ponec, M., Bodde, H.E., 1999. In vivo human skin permeability enhancement by oleic acid: a laser Doppler velocimetry study. J. Control. Release 58, 97–104.

Walter, K.A., Hadgraft, J., 1993. Pharmaceutical Skin Penetration Enhancement. Marcel Dekker, New York.

Wilhelm, K.P., Surber, C., Maibach, H.I., 1991. Effect of sodium lauryl sulfate-induced skin irritation on in vitro percutaneous absorption of four drugs. J. Invest. Dermatol. 96, 963–967.

Yosipovitch, G., Szolar, C., Hui, X.Y., Maibach, H.I., 1996. Effect of topically applied menthol on thermal, pain and itch sensations and biophysical properties of the skin. Arch. Dermatol. Res. 288, 245–248.