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Evaluation of physicochemical properties, skin permeation and accumulation profiles of salicylic acid amide prodrugs as sunscreen agent

Yi-Dong Yan[a], Jun Ho Sung[a], Dong Won Lee[a], Jung Sun Kim[b], Eun-Mi Jeon[b], Dae-Duk Kim[c], Dong Wuk Kim[d], Jong Oh Kim[a], Ming Guan Piao[a,e], Dong Xun Li[a,f], Chul Soon Yong[a,**], Han Gon Choi[a,d,*]

[a] College of Pharmacy, Yeungnam University, 214-1 Dae-Dong, Gyungsan, Gyungbuk 712-749, South Korea
[b] Department of Biotechnology, Dongseo University, Busan, 617-716, South Korea
[c] College of Pharmacy, Seoul National University, San 56-1, Shinlim-Dong, Kwanak-Ku, Seoul 151-742, South Korea
[d] College of Pharmacy, Hanyang University, 55 Hanyangdaehak-ro, Sangnok-gu, Ansan 426-791, South Korea
[e] Department of Pharmaceutical Sciences, College of Pharmacy, Yanbian University, Juzi Street, Yanji City, Jilin Province, 133000, China
[f] National Pharmaceutical Engineering Center for Solid Preparation in Chinese Herbal Medicine, Jiangxi University of Chinese Traditional Medicine, Nanchang, 330006, China

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ABSTRACT

Various amide prodrugs of salicylic acid were synthesised, and their physicochemical properties including lipophilicity, chemical stability and enzymatic hydrolysis were investigated. In vivo skin permeation and accumulation profiles were also evaluated using a combination of common permeation enhancing techniques such as the use of a supersaturated solution of permeants in an enhancer vehicle, a lipophilic receptor solution, removal of the stratum corneum and delipidisation of skin. Their capacity factor values were proportional to the degree of carbon–carbon saturation in the side chain. All these amides were highly stable in acetonitrile and glycerine. Amide prodrugs were converted to salicylic acid both in hairless mouse liver and skin homogenates. N-dodecyl salicylamide (C12SM) showed the lowest permeation of salicylic acid in skin compared to the other prodrugs, probably due to its low aqueous solubility. It had a high affinity for the stratum corneum and its accumulation was restricted to only the uppermost layer of skin. Thus, this amide prodrug could be a safer topical sunscreen agent with minimum potential for systemic absorption.

1. Introduction

Sunscreen products have been used for many years and are comprised of substances (commonly referred as ultraviolet (UV) filters), which are capable of absorbing UV radiation and protecting human skin from direct exposure to the deleterious wavelengths of sunlight. Sunscreen preparations are usually applied extensively over a large surface of the body and with repeated doses. Therefore, there is a concern about the systemic absorption of organic filters (Benson, 2000). An ideal sunscreen product should exhibit high skin accumulation of UV absorbers with minimal permeation to the systemic circulation (Gupta et al., 1999; Potard et al., 1999). Thus, to increase efficiency and to avoid toxicity, sunscreen should stay on the skin surface and penetrate the skin as little as possible. The desirable site of action of filters is restricted to the skin surface or within the uppermost layers of the stratum corneum (SC). These filters should have a high affinity for the stratum corneum (Walters et al., 1998; Duracher et al., 2009). UV filters are designed to remain on the uppermost layers of the skin; penetration through the skin is meant to be low (Jiang et al., 1997). For sunscreens to be effective, UV absorbers must remain in the outermost regions of the skin (Lu et al., 1999).

Salicylic acid is most commonly included as a UV-B (290–320 nm) filter (Sarveiya et al., 2004). However, salicylic acid is known to penetrate well across human and animal skin. Numerous studies have demonstrated that, following deposition onto the skin, salicylic acid will penetrate the stratum corneum and enter the systemic circulation (Birmingham et al., 1979; Taylor and Halprin, 1975). The relative bioavailabilities of salicylic acid (2%) in hydroalcoholic or cream formulation after repeated application (14 days) were determined in three different skin conditions (normal, aged and acnegenic skin). Bioavailability in normal skin types was about 58% and 44% for the hydroalcoholic and cream formulations, respectively (Davis et al., 1997). Topical application of 6% salicylic acid ointments to large body surface areas for the treatment of psoriasis and ichthyosis has been associated with high plasma concentrations of salicylates and even sporadic reports of clinical toxicities (Von Weis and Lever, 1964; Young, 1952).

* Corresponding author at: Hanyang University, College of Pharmacy, 55 Hanyangdaehak-ro, Sangnok-gu, Ansan 426-791, South Korea.
Tel.: +82 31 400 5802; fax: +82 31 400 5958.
** Corresponding author. Tel.: +82 53 810 2812; fax: +82 53 810 4654.
E-mail addresses: csyong@yumail.ac.kr (C.S. Yong), hangon@hanyang.ac.kr (H.G. Choi).

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Drug skin absorption is determined by its physicochemical properties, in particular molecular weight and lipophilicity, which play major roles in the skin permeation process. Permeation requires both lipid and aqueous solubility with an optimal log *P* (octanol–water partition coefficient); poor absorption is more likely when the log *P* is greater than 5 or lower than −1, and when the molecular weight is greater than 500 (Goldsmith, 1991; Yano et al., 1986). Many studies have highlighted the retention of lipophilic compounds with high log *P* values in the cutaneous layers (Duracher et al., 2009); the higher the log *P* value, the greater the likelihood that the permeant was retained within the skin. Unfortunately, it was reported that salicylic acid had a log *P* value of 2.06 and a molecular weight of 138.12, making it readily absorbed percutaneously and therefore rendering it unsuitable as a sunscreen agent (Johnson and Uhrich, 2009).

Recently, considerable attention has been focused on the development of prodrugs, which provides an effective strategy for improving the pharmaceutical, pharmacokinetic and pharmacodynamic characteristics of a therapeutic agent. In earlier studies on alkyl ester and amide prodrugs, their lipophilicities were found to be proportional to their carbon chain length and good linear relationships between lipophilicity and capacity factors were observed (Bhandari et al., 2007; Doh et al., 2003; Kim et al., 2005). Similarly, parabolic relationships were found between skin permeation rate and lipophilicity, indicating the possibility of designing suitably lipophilic fatty acid amides that would not be subject to systemic washout and with low skin permeation due to their lipophilicity and low aqueous solubility. Therefore, the application of an exogenous amide might result in more efficient barrier function (Feingold et al., 1990; Holleran et al., 1993; Man et al., 1993; Shah et al., 1992).

Some reports have dealt with the relationship between the structural features of a series of salicylic compounds and percutaneous absorption. Yano et al. studied in vivo percutaneous absorption in humans and found a parabolic relationship between the amount absorbed and the lipophilicity of a series of salicylic compounds (Yano et al., 1986). Our previous work also confirmed the relationship between these parameters (Im et al., 2011). However, no related data on amide prodrugs of salicylic acid have been reported so far.

In this study, to select a suitable UV protection agent among a series of salicylic acid fatty amide prodrugs such as N-octyl salicylamide (C8SM), N-decyl salicylamide (C10SM) and N-dodecyl salicylamide (C12SM), their stability in organic solvents, skin and liver homogenates was investigated. The skin penetration and accumulation of the salicylic acid amide prodrugs were evaluated using a hairless mouse model.

2. Materials and methods

2.1. Materials

The salicylic acid amide prodrugs (Fig. 1) such as N-octyl salicylamide (C8SM), N-decyl salicylamide (C10SM) and N-dodecyl salicylamide (C12SM) were kindly supplied by Medical Chemistry Laboratory at Dongseo University (Busan, South Korea). Methanol, ethanol, 2-propanol, acetonitrile, glycerine and polyethylene glycol 400 were purchased from Duksan Chemical Co. (Ansan, Korea). All other reagents were of analytical grade and used without further purification.

2.2. Determination of lipophilic index

The capacity factors (k') values of salicylic acid amide prodrugs were determined isocratically using HPLC (Hitachi, Tokyo, Japan) equipped with a Hitachi diode array detector (L-7450) at

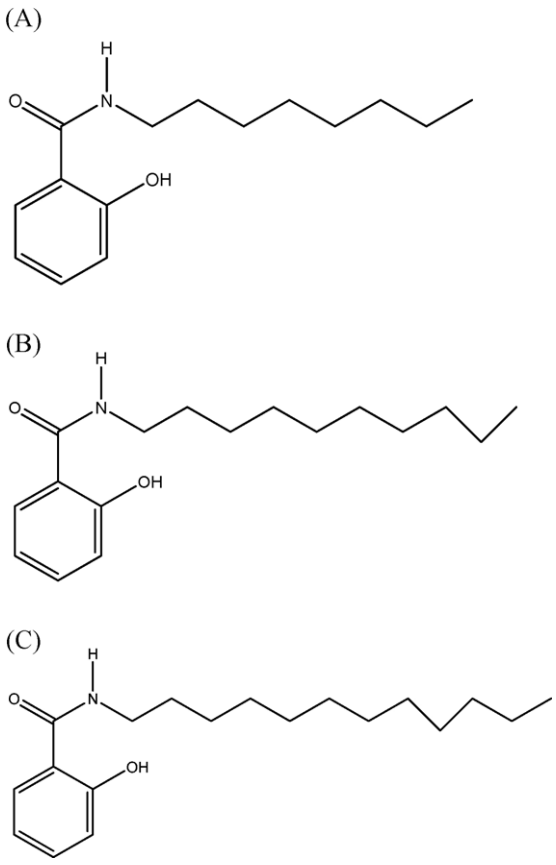

Fig. 1. Structures of amide prodrugs of salicylic acid: (A) N-octyl salicylamide (C8SM); (B) N-decyl salicylamide (C10SM); and (C) N-dodecyl salicylamide (C12SM).

225 nm and the D-7000 HSM program. HPLC separation was conducted with a 50 μl injection volume on a C8 column (Inertsil HPLC column C8-3; particle size 5 μm, 4.6 mm × 150 mm). The mobile phase was consisted of methanol, acetonitrile and distilled water (4:4:2, volume ratio) followed by adjusting to pH 4.4 with 0.1% acetic acid. The eluent was monitored at 227 nm with a flow rate of 1.0 ml/min. Retention times of each compound were measured, and the k' value was calculated from the following equation (Kim et al., 2005):

$$k\prime = \frac{t_i - t_0}{t_0}$$

where t_0 is the retention time of mobile phase and t_i is the retention time of each compound (Im et al., 2011).

Furthermore, the partition coefficient (log *P*) of salicylic acid amides was calculated by ChemBioDraw Ultra 11.0.1.

2.3. Determination of aqueous solubility

Excessive amounts of amide prodrugs were added to 5 ml of phosphate buffered solution (pH 7.4), respectively. They were shaken in a water bath at 25 °C for 10 min, centrifuged at 3000 × *g* for 10 min using a 5415 C centrifuge (Eppendorf; Hauppauge, NY, USA) and filtered through a membrane filter (0.45 μm) to obtain a clear solution. Their concentration in the resulting solution was analysed by HPLC as described below. The experiments were carried out in triplicate.

2.4. Chemical stability in organic solvents

The chemical stability of salicylic acid amide prodrugs was determined in various organic solvents such as methanol, ethanol, 2-propanol, acetonitrile, glycerine and polyethylene glycol 400 (Bhandari et al., 2007). Excess salicylic acid amides (1 mg) were added to 10 ml of each solvent and shaken at 25 °C in a water bath (Shaking water bath KMC 12055 WI, 150 rpm). At predetermined time intervals, they were centrifuged at 3000 × *g* for 10 min (Eppendorf; Hauppauge, NY, USA) and filtered through a membrane filter (0.45 μm). The concentration of the remaining amide was then analysed by HPLC as described above. Considering the initial concentration of each amide as 100%, the remaining percentage of amide in each solution was determined as a function of time.

2.5. Enzymatic hydrolysis

All animal care measures and experimental procedures were conducted according to the Guiding Principles in the Use of Animals in Toxicology, as adopted in 1989, revised in 1999 and amended in 2008 by the Society of Toxicology (SOT, 2009). The protocols for animal studies were also approved by the Institute of Laboratory Animal Resources of Yeungnam University. Skin/liver homogenates were prepared by homogenising fresh hairless mouse skin or livers with phosphate buffered saline solution (pH 7.4) to a obtain 25% (w/v) tissue suspension for 20 min in an ice bath. The supernatants were obtained after centrifugation at 4 °C for 20 min at 10,000 × *g*. One millilitre of amide prodrug solutions in glycerin (100 μg/ml) were mixed with 4 ml homogenates and shaken at 37 °C. At predetermined time intervals, the aliquots were sampled (0.25 ml), and mixed with 0.05 ml of the internal standard solution (valsartan 1 mg/ml) and 0.7 ml acetonitrile. After immediate mixing and centrifugation for 5 min at 10,000 × *g*, 0.5 ml of clear the supernatant was mixed with 0.5 ml of acetonitrile again, vortexed and centrifuged at 10,000 × *g* for 5 min. The clear supernatants were analysed by HPLC as described above. Considering the initial concentration of each amide as 100% and that of salicylic acid as 0%, the remaining percentage in each solution was determined as a function of time. Gentamycin (0.01%, w/v) was used in all media to prevent bacterial degradation of amides in the homogenates (Bhandari et al., 2007).

2.6. Preparation of stratum corneum-stripped and delipidised skins

Stratum corneum-stripped skins were obtained by stripping the intact skins with adhesive tape 30 times (Kuo et al., 1989; Shah et al., 1992). Delipidised skins were prepared from full-thickness skin using methylene chloride as an extracting solvent according to the method described by Kuo et al. (1989). Briefly, a piece of hairless mouse skin was mounted on a diffusion cell with the stratum corneum side facing the donor compartment to extract the lipids, while phosphate buffered saline (pH 7.4) containing 0.01% (w/v) gentamycin was added to the receptor compartment to maintain the moisture of the skin. The extracting solution was changed after 12 h and the extraction was performed for 24 h to achieve more favourable lipid extraction.

2.7. Skin permeation

Freshly excised hairless mouse skin was mounted in open modified Franz diffusion cells with an available diffusion area of 2.01 cm^2 and a receptor volume of approximately 13 ml (12.4–13.2 ml). The diffusion cells were placed in a temperature-controlled water bath with a constant temperature of 37 °C at the skin surface. The receptor compartments contained a mixture of glycerine and phosphate buffered saline (pH 7.4) (80:20, volume ratio) containing 0.01% (w/v) gentamycin. After a hydration period of 2 h, a finite dose of 300 μl of a saturated solution of salicylic acid amide prodrug was applied to the stratum corneum side of the membrane. Saturation was achieved by sonicating an excess of materials in glycerin at 37 °C (Doh et al., 2003; Kim et al., 2005). At appropriate time intervals, the receptor fluid (200 μl) was withdrawn and replaced with fresh temperature-controlled receptor fluid. Permeation was followed for 24 h, which had been previously shown to be sufficient (Bhandari et al., 2007). It was ensured that sink conditions were present and that the sample concentrations were well above the limit of quantification of the analytical methods. The experiments were carried out at least in triplicate. Samples were kept at 4 °C for a maximum of 2 days until HPLC analysis as described above.

Table 1
Molecular weight, capacity factor, log *P* value and solubility of amide prodrugs of salicylic acid.

Prodrugs	M.W.	Capacity factor	log *P*	Solubility
N-octyl salicylamide (C8SM)	249.35	1.82	3.7	25.4 ± 1.2
N-decyl salicylamide (C10SM)	277.40	3.52	4.53	30.8 ± 2.5
N-dodecyl salicylamide (C12SM)	305.45	7.22	5.37	36.5 ± 2.7

2.8. Skin accumulation

Accumulation the salicylic acid fatty ester prodrugs in the skin at the end of permeation study was determined by cutting the effective permeation area of the skin, which was then briefly washed with distilled water to remove any surface adhered materials (Michniak et al., 1994). The skin was then minced into small pieces and homogenised with four-fold distilled water for 15 min in an ice bath. The sample (0.45 ml) was mixed with 0.05 ml of an internal standard solution (valsartan 1 mg/ml) and 0.5 ml of acetonitrile. Then, 0.1 ml of clear supernatant, which was obtained after vortexing and centrifugation for 10 min at 10,000 × *g*, were mixed again with 0.9 ml acetonitrile, vortexed and centrifuged for 10 min at 10,000 × *g*. The supernatant was filtered through a syringe filter (0.45 μm) and analysed by HPLC for simultaneous assay of the salicylic acid amide prodrugs and the parent salicylic acid.

3. Results and discussion

3.1. Lipophilicity

The stratum corneum barrier limits drug permeation through the skin. Only some drug molecules, which present ideal physicochemical properties are actually able to cross this barrier. Since skin is lipid in nature and generally favours permeation by lipophilic drugs, lipophilicity is a very important factor for dermal permeation (Kim et al., 2005). The molecular weights of salicylic acid amide prodrugs such as N-octyl salicylamide (C8SM), N-decyl salicylamide (C10SM) and N-dodecyl salicylamide (C12SM) (Fig. 1) ranged from 249.35 to 305.45 g/mol (Table 1). The solubility of amide prodrugs in phosphate buffered saline solution (pH 7.4) ranging from 25.4 to 36.5 μg/ml was proportional to their molecular weights. However, they were lower than those of corresponding ester prodrugs with the same length of side chain (data not shown), which had a free carboxylic acid group in their molecular structures (Im et al., 2011). Their capacity factors (k') and partition coefficient (log *P*) values varied from 1.82 to 7.52 and 3.70 to 5.37, respectively. It was found that the k' values were proportional to the log *P* values, suggesting that their k' values are consistent to their lipophilicity (Bhandari

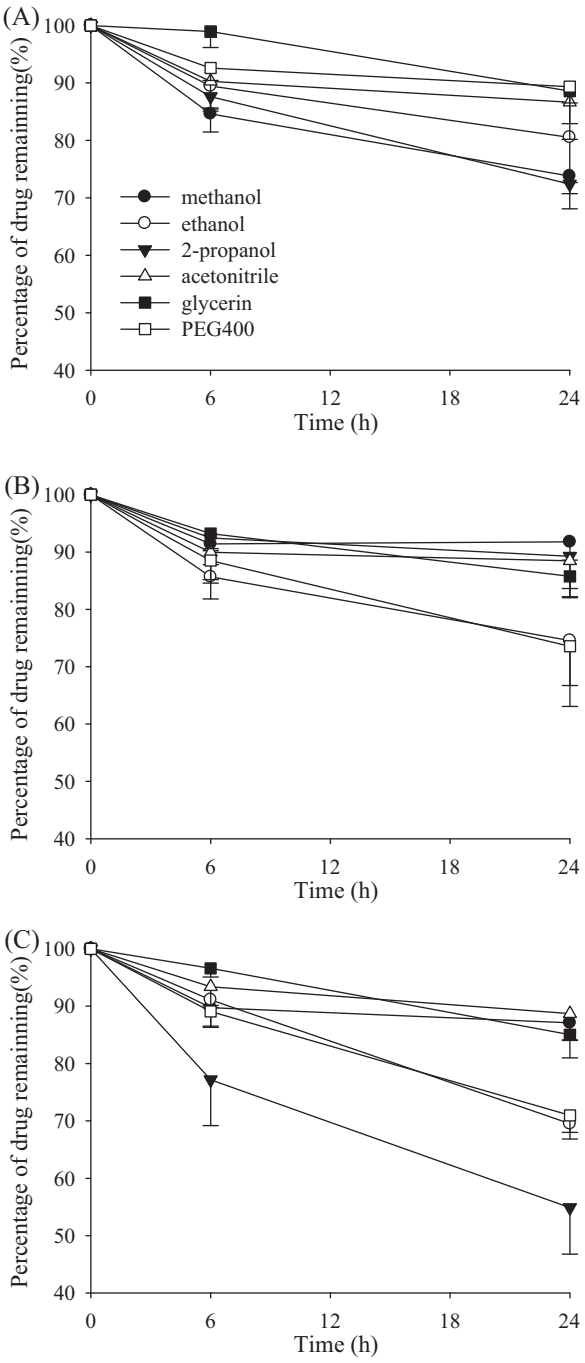

Fig. 2. Chemical stability of amide prodrugs in various organic solvents: (A) C8SM; (B) C10SM; and (C) C12SM. Each value represents the mean ± S.D. (n = 3).

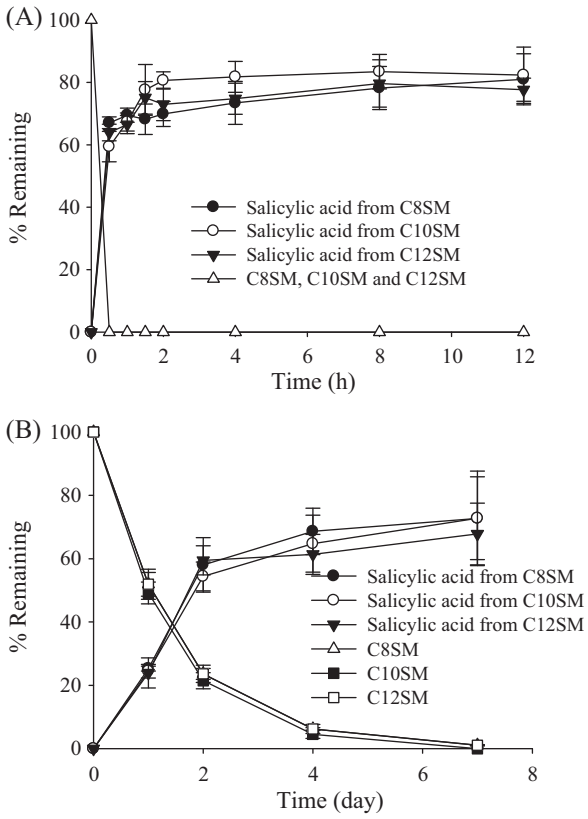

Fig. 3. Hydrolysis of amide prodrugs in hairless mouse liver (A) and skin homogenates (B). Each value represents the mean ± S.D. (n = 3).

et al., 2007). Moreover, the homologous series of amide showed k' values proportional to the number of carbon atom present (C–C) in the side chain (Im et al., 2011). Based on the capacity factor, the lipophilicity of salicylic acid amides was in the order of N-octyl salicylamide (C8SM) < N-decyl salicylamide (C10SM) < N-dodecyl salicylamide (C12SM).

3.2. Chemical stability

For the selection of solvent suitable for further study on permeation and accumulation, the chemical hydrolysis of these amide prodrugs was determined by their stability in various organic solvents such as methanol, ethanol, 2-propanol, acetonitrile, glycerin and polyethylene glycol 400 (Fig. 2). All these amides were highly stable in acetonitrile and glycerine because the remaining amide against the initial concentration was over than 85% even after 24 h. C8SM had better stability in ethanol and polyethylene glycol 400 at 24 h compared to C10SM and C12SM. However, it was also unstable in 2-propanol and methanol, which was shown by a remaining amount less than 74%. The lowest value (54.88%) was observed in 2-propanol for C12SM after 24 h, suggesting the worst stability in this solvent. Since the ester prodrug (lauroyl oxysalicylate), which had the same length of side chain, was highly stable in 2-propanol, the difference in stability was possibly associated with the linkage type in which the side chain was connected to salicylic acid entity (Im et al., 2011). Furthermore, it is worth noting that the overall stability of C12SM in various solvents was less in comparison with C8SM and C10SM. It seems that the salicylic acid amide prodrugs with longer side chains are relatively less stable in organic solvents, specially, polar solvent.

3.3. Enzymatic hydrolysis

Enzymatic hydrolysis profiles of amide produgs are shown in Fig. 3. All prodrugs were well hydrolysed in both hairless mouse skin and liver homogenates. All the prodrugs were almost completely hydrolysed in the hairless mouse liver homogenates within 0.5 h, at which point all prodrugs were also found to disappear completely; correspondingly, the newly formed salicylic acid rapidly increased to over 60% at this time point and reached a maximum level within 2 h (Fig. 3A). In the case of hairless mouse skin homogenates, all prodrugs were entirely hydrolysed within 7 days, with 50% hydrolysis in the first day (Fig. 3B). However, no significant differences were found between any of the salicylic acid amide prodrugs in terms of the hydrolysis rate in either hairless mouse skin or liver homogenates. It should be noted that only around 80% of

salicylic acid was recovered after complete hydrolysis of prodrugs, which was mostly due to the adsorption of salicylic acid to the biological matrixes. Thus, all prodrugs might be converted to salicylic acid after topical daministration, supporting their use as a UV protector in pharmaceutical formulations (Doh et al., 2003; Kim et al., 2005).

3.4. Skin permeation

In the in vitro permeation studies, the intact, stratum corneum-stripped and delipidised hairless mouse skins were used in a Franz-type diffusion cell to examine the permeation characteristics of the salicylic acid amide prodrugs. The prodrug-containing suspensions were applied as saturated solutions to maintain constant diffusion and maximum flux. After the application of prodrugs, intact salicylic acid amide prodrugs were not found in the receptor fluid, but the metabolite (active compound) was found. This was in good agreement with a previously reported study (Simonsen et al., 2002).

In the full-thickness hairless mouse skins, no drug permeation was observed until 16 h, after which salicylic acid was detected for C8SM and C10SM in a further 4 h, with a higher accumulated amount for the former (Fig. 4A). However, in the case of C12SM, salicylic acid was only observed after 24 h with a lower level compared to C8SM and C10SM. Our results showed that C12SM, which had the longest side chain among the prodrugs tested, provided the lowest level of permeation of salicylic acid. However, the differences among these permeations of three prodrugs were not statistically significant ($p > 0.05$).

Furthermore, the prodrug C8SM gave higher permeation at 24 h in SC-stripped skin (Fig. 4B) and delipidised skin compared to C10SM and C12SM (Fig. 4C). Moreover, C12SM showed almost no permeation of salicylic acid in SC-stripped skin and very poor permeation in delipidised skin. All amide prodrugs showed lower permeation of salicylic acid both in SC-stripped and delipidised skin compared to intact skin, suggesting that both stripping and delipidisation had a negative impact on the permeation profile of the parent salicylic acid from the applied amide prodrugs. However, all these differences were very small in magnitude and were not considered statistically significant. Since the penetrated amount of salicylic acid by the amide prodrug (which ranged from 0.00 to 4.25 μg/cm^2 after 24 h) was significantly lower than that of the ester prodrug observed in our previous study (Im et al., 2011), all these amide drugs were considered to be have a very low permeation ability, irrespective of the type of skin. However, the permeation of salicylic acid might be increased with time, since the hydrolysis of prodrug accumulated in skin might increase the formation of salicylic acid, which subsequently could affect the salicylic acid permeation profile.

As shown in Fig. 5, there was an obvious correlation between the permeated amount of salicylic acid and the capacity factors of the amide prodrugs in all three kinds of skin. With increasing capacity factors, less permeation of salicylic acid was found in receptor solution. The relatively higher lipophilicity might have been responsible for the low permeability of these compounds into the receptor solution, resulting in low permeability of salicylic acid into receptor solution through intact skin. Exogenous bulk lipid provided by the amide side chains might have contributed to an increment in the total lipid proportion (Feingold et al., 1990; Holleran et al., 1993; Man et al., 1993). As the amide side chains have structural similarity to the natural fatty acids in skin, they might have allied with their naturally occurring counterparts in the lipid phase, contributing to an increment in total lipid content. Alternatively, this may have led to favourable molecular arrangements and interactions to create order rather than disorder in the skin lipid layer and consequently making it a more effective barrier (Feingold et al., 1990; Holleran et al., 1993; Tsai et al., 1996). However, in the case of SC-stripped and delipidised skins, the permeation rates of these amide prodrugs were also very low as mentioned above. This might be explained by the strong affinity for both dermal lipids and SC lipids. Moreover, low aqueous solubility could be another possible reason for the low permeation of these drugs. It has also been reported that not only optimum lipophilicity, but also adequate aqueous solubility are the main determinants for drug permeation through skin (Kerr et al., 1998; Taylor and Sloan, 1998; Rautio et al., 2000). Enhanced

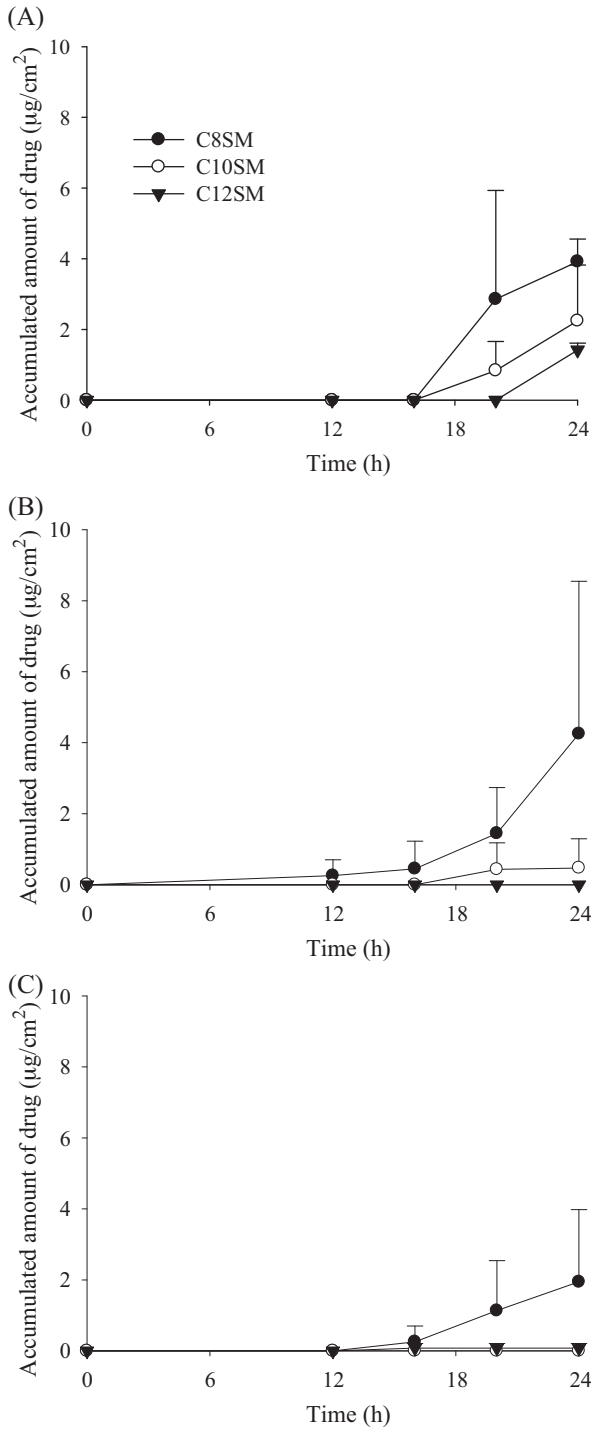

Fig. 4. Degree of skin permeation of amide prodrugs on intact (A), stratum corneum-stripped (B) and delipidised (C) hairless mouse skin. All compounds were detected not as amide prodrugs but as the parent drug form. Each value represents the mean ± S.D. ($n = 3$).

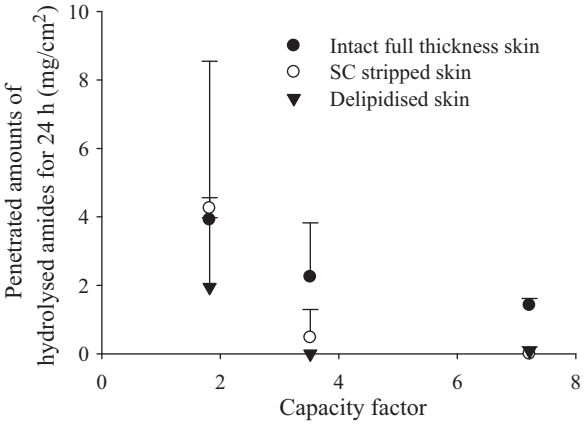

Fig. 5. Correlation of skin permeation of amide prodrugs in 24 h and capacity factor.

lipophilicity might increase partitioning into the stratum corneum, forming a reservoir, but subsequent transport into the aqueous milieu beneath may be limited by both the prodrugs aqueous solubility and the ability of epidermal enzymes to convert the prodrug into a more polar metabolite.

From these permeation studies, C12SM could be a potential candidate for UV protection because it showed the poorest permeation of salicylic acid in all skin preparations compared to the other prodrugs.

3.5. Skin accumulation

In general, as shown in Fig. 6, the accumulated amounts of the parent salicylic acid released via enzymatic hydrolysis of the amide prodrugs were large and comparable in delipidised skin and intact skin, both of which gave significantly higher accumulation than in the SC-stripped skin, irrespective of the number of carbon atoms in the side chain. These data indicated that all three amide prodrugs had very strong affinity for lipids compared to other components in the skin. Judged by the relatively lower accumulation in the SC-stripped skin in comparison with that in the delipidised skin, the amide prodrugs seemed to have a stronger affinity for SC lipids than dermal lipids, suggesting that SC was a major barrier to permeation and its impermeability into the receptor solution.

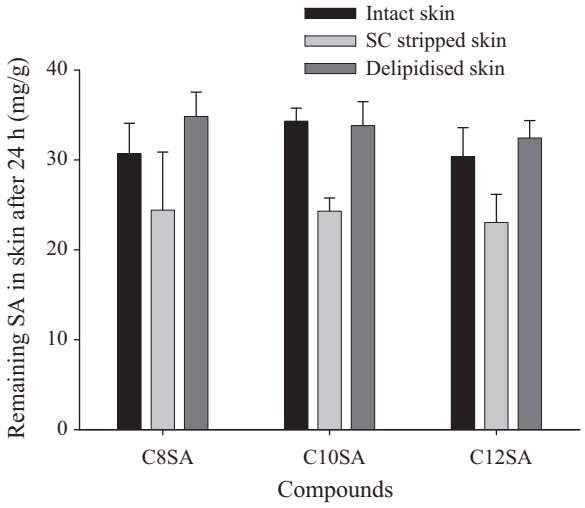

Fig. 6. Degree of skin accumulation of salicylic acid produced by the hydrolysis of prodrugs. Each value represents the mean ± S.D. ($n = 3$).

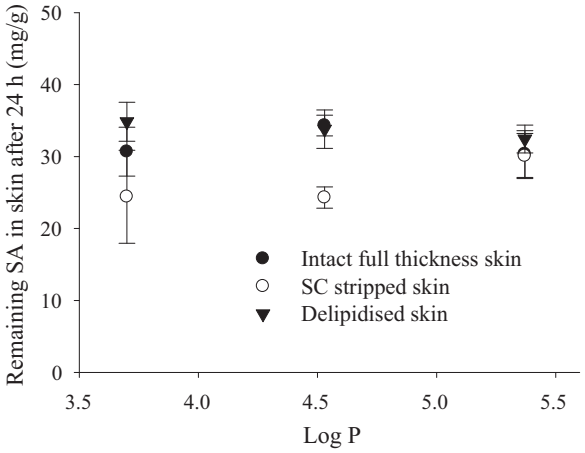

Fig. 7. Correlation of skin accumulation of salicylic acid and log P value of amide prodrugs.

The difference between the SC-stripped skin and delipidised skin also suggested different permeation mechanisms of the amide prodrugs compared to that of the ester prodrug. As reported by Lee et al. (1993) for drugs, which permeate mainly through the lipid bilayer region (intercellular pathway), similar permeability coefficients should be observed for those transported through the SC-stripped skin and delipidised skin. In our present study, there was a statistically significant difference ($p < 0.05$) in accumulation in SC-stripped and delipidised skins, suggesting that the predominant route for their passive penetration of the SC layer might be the transcellular pathway instead of the intercellular pathway.

In the study on ketorolac fatty ester prodrugs, it was reported that drug accumulation in skin has an inversely relationship with its lipophilicity (Bhandari et al., 2007). However, in this study, it was interesting to note that skin accumulation of the amide prodrugs did not increase in proportion to increased lipophilicity (Fig. 7). Instead, the amount of accumulated drug in the skin only varied within a narrow range (24.4–34.3 mg/g) as a result of prodrug log P variation (Fig. 7). This discrepancy was reasonably thought to be due to the difference in the nature of prodrug used in each study. This could be explained by the lower aqueous solubility of the amide prodrugs compared to the ester prodrug, which possibly limited drug accumulation (Goodman and Barry, 1989; Im et al., 2011; Williams and Barry, 1992). As mentioned above, only when a compound had suitable both aqueous solubility and lipid solubility, it could be transferred into stratum corneum, which was hydrophobic in nature and subsequently to be transferred to the underlying aqueous tissues. Relatively lower aqueous solubility limits the permeation of a prodrug through the skin, resulting in significant accumulation of the prodrug in the stratum corneum, where the amide prodrug can be hydrolysed into the parent compound salicylic acid.

4. Conclusion

In conclusion, after application as a sunscreen, N-dodecyl salicylamide (C12SM) was readily hydrolysed into its parent compound salicylic acid both in the hairless mouse liver and skin homogenates, suggesting that it might be converted to salicylic acid. Furthermore, this compound showed the lowest permeation of salicylic acid in all skin preparations compared to the other prodrugs. It had a high affinity for the stratum corneum and its accumulation was restricted to the uppermost layer. Thus, this amide prodrug

could be a safer topical sunscreen agent with minimum potential for systemic absorption.

Acknowledgements

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