

RESEARCH ARTICLE

Evaluation of salicylic acid fatty ester prodrugs for UV protection

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Abstract

The purpose of this study was to investigate the physicochemical properties and *in vitro* evaluation of fatty ester prodrugs of salicylic acid for ultraviolet (UV) protection. The physicochemical properties such as lipophilicity, chemical stability and enzymatic hydrolysis were investigated with the following fatty ester prodrugs of salicylic acid: octanoyl (C8SA), nonanoyl (C9SA), decanoyl (C10SA), lauroyl (C12SA), myristoyl (C14SA) and palmitoyl oxysalicylate (C16SA). Furthermore, their skin permeation and accumulation were evaluated using a combination of common permeation enhancing techniques such as the use of a lipophilic receptor solution, removal of stratum corneum and delipidization of skin. Their k' values were proportional to the degree of carbon-carbon saturation in the side chain. All these fatty esters were highly stable in 2-propanol, acetonitrile and glycerin, but unstable in methanol and ethanol. They were relatively unstable in liver and skin homogenates. In particular, C16SA was mostly hydrolyzed to its parent compound in hairless mouse liver and skin homogenates, suggesting that it might be converted to salicylic acid after its topical administration. In the skin permeation and accumulation study, C16SA showed the poorest permeation in all skins, suggesting that it could not be permeated in the skin. Furthermore, C14SA and C16SA were less accumulated in delipidized skin compared with normal skin or stripped skin, suggesting that these esters had relatively strong affinities for lipids compared with the other prodrugs in the skin. C16SA showed significantly higher dermal accumulation in all skins compared with its parent salicylic acid. Thus, the palmitoyl oxysalicylate (C16SA) might be a potential candidate for UV protection due to its absence of skin permeation, smaller uptake in the lipid phase and relatively lower skin accumulation.

Keywords: Salicylic acid fatty ester prodrugs, UV protection agent, skin permeation, skin accumulation

Introduction

Topical medications for local therapeutics would be expected to avoid systemic absorption and penetrate substantially into deeper tissues as much as possible. Unfortunately, most of the disorders requiring such medication are found in the area of high permeation flux¹. There have been many trials to develop a suitable method for minimizing the systemic absorption and adverse effects of topical medications. More recently, there has been interest in permeation retardants that may be used in topical formulations to prevent the permeation of active ingredients². However, not all of these permeation

retardants have yet been proven to be commercially viable and pharmaceutically suitable and in many cases retardation has been achieved by compromising systemic and local toxicities of the retardants³. Therefore, it would be advantageous if the systemic absorption of topical agents could be minimized irrespective of the sites or altered skin barrier function without the co-application of other chemical retardants.

Although many fatty alcohols/acids/esters have been shown to act as penetration enhancers for a variety of drugs, their enhancing efficiency has been found to have a parabolic relationship with their lipophilicity⁴⁻⁹.

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In addition to their permeation enhancing effect, the self-permeability of highly lipophilic molecules has been reported to be low due to their accumulation in the stratum corneum (SC) resulting from low aqueous solubility^{10,11}. Such compounds may sometimes be ineffective retardants for other molecules. Their self-permeation could eventually increase because of their enzymatic degradation into the parent drug and free enhancer molecule (fatty alcohols/acids/esters). Thus, their lipophilicity and enzymatic stability in skin would be an important factor for their permeation retardant effect.

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^{12–14}. Similarly, parabolic relationships were found between skin permeation rate and lipophilicity, indicating a possibility for designing suitably lipophilic fatty esters that would not be subject to systemic wash-out. However, they were enzymatically unstable, which precluded their use as local topical agents. Therefore, lipophilicity and stability have been reported to be improved by synthesizing fatty ester derivatives¹⁵. If suitably lipophilic and esterase-stable fatty ester derivatives could be synthesized, their skin permeation might be low due to their lipophilicity and low aqueous solubility and the application of exogenous esters might result in a more efficient barrier function^{16–20}.

Salicylic acid is widely used in dermatological preparations and is most commonly included as a UV-B (290–320 nm) filter²¹. The excellent skin penetration properties have been documented in numerous rat and human studies^{22,23}. Only a few reports have dealt with the relationship between the structural features of a series of salicylic compounds and percutaneous absorption. Wurster and Kramer²⁴ applied salicylate esters topically to humans and showed that excretion rates depended on lipophilicity. This relationship was later confirmed by Yano

et al.²⁵, who studied *in vivo* percutaneous absorption in humans and found a parabolic relationship between the amount absorbed and lipophilicity of a series of salicylic compounds.

In this study to select a UV protection agent for improved dermal accumulation of salicylic acid among salicylic acid fatty ester prodrugs such as octanoyl (C8SA), nonanoyl (C9SA), decanoyl (C10SA), lauroyl (C12SA), myristoyl (C14SA) and palmitoyl oxysalicylate (C16SA), their stability in organic solvents, skin and liver homogenates was investigated. The skin penetration and accumulation of the salicylic acid fatty ester prodrugs were evaluated using a hairless rat model.

Materials and methods

Materials

The salicylic acid fatty ester prodrugs (Figure 1) octanoyl oxysalicylate (C8SA), nonanoyl oxysalicylate (C9SA), decanoyl oxysalicylate (C10SA), lauroyl oxysalicylate (C12SA), myristoyl oxysalicylate (C14SA) and palmitoyl oxysalicylate (C16SA) were kindly supplied by Medical Chemistry Laboratory at Dongseo University (Busan, South Korea). Methanol, ethanol, 2-propanol, acetonitrile, glycerin and polyethylene glycol 400 were purchased from Duksan Chemical Co. (Ansan, South Korea). All other reagents were of analytical grade and used without further purification.

Determination of lipophilic index

The capacity factor (k') was determined according to our earlier described method¹⁴. The k' values of salicylic acid fatty ester prodrugs were determined isocratically using high-performance liquid chromatography (HPLC) equipped with a Hitachi diode array detector (L-7450) at 225 nm and D-7000 HSM program. HPLC separation was conducted with 50 μ l injection volume on a C8 column (Inertsil HPLC column C8-3, particle size 5 μ m,

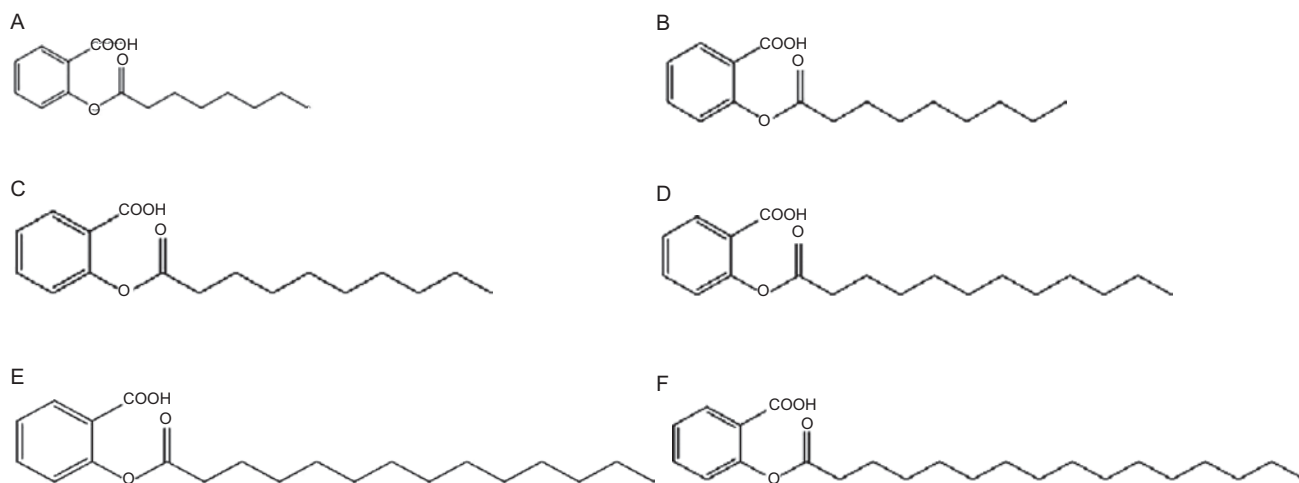


Figure 1. Structures of fatty ester prodrugs of salicylic acid: (A) octanoyl oxysalicylate (C8SA), (B) nonanoyl oxysalicylate (C9SA), (C) decanoyl oxysalicylate (C10SA), (D) lauroyl oxysalicylate (C12SA), (E) myristoyl oxysalicylate (C14SA) and (F) palmitoyl oxysalicylate (C16SA).

4.6 × 150 mm). The mobile phase consisted of methanol, acetonitrile and distilled water (4:4:2 volume ratio) followed by adjustment to pH 4.4 with 0.1% acetic acid. The eluent was monitored at 225 nm with a flow rate of 1.0 ml/min. Retention times of each compound were measured and k' value was calculated from the following equation:

$$k' = \frac{(t_i - t_0)}{t_0}$$

where t_0 is the retention time of the mobile phase and t_i is the retention time of each compound¹³.

Furthermore, the partition coefficient ($\log P$) of salicylic acid fatty esters was calculated by ChemBioDraw (Ultra 11.0; CambridgeSoft Co., Cambridge, MA).

Chemical stability in organic solvents

The chemical stability of salicylic acid fatty ester prodrugs was determined in the following organic solvents: methanol, ethanol, 2-propanol, acetonitrile, glycerin and polyethylene glycol 400¹². An excess of salicylic acid fatty esters (100 µg) was added to 1 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) and filtered through a membrane filter (0.45 µm). The concentration of remaining esters was then analyzed by HPLC as described above. Considering the initial concentration of each ester as 100%, the remaining percentage of ester in each solution was determined as the function of time.

Enzymatic hydrolysis

All animal care and 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 Toxicology²⁶. Skin and liver homogenates were prepared by homogenizing freshly collected hairless mouse skin or livers with phosphate buffer solution (pH 7.4) to 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. Although the amounts of proteins in the homogenates were important in enzymatic hydrolysis, they were not measured in order to focus the enzymatic hydrolysis of prodrugs in the homogenates. One milliliter of ester solutions in 2-propanol (100 µg/ml) were mixed with 4 ml homogenates and shaken at 37°C. At predetermined time intervals, 0.25 ml of aliquots were sampled and mixed with 0.05 ml 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 supernatants were mixed with 0.5 ml acetonitrile again, vortexed and centrifuged at 10,000 g for 5 min. The clear supernatants were analyzed by HPLC as described above. Considering the initial concentration of each ester as 100% and that of salicylic acid as 0%, their remaining percentage in each solution were determined as a

function of time. Gentamycin (0.01% w/v) was used in all media to prevent the bacterial degradation of esters and homogenates¹².

Preparation of SC-stripped and delipidized skins

SC-stripped skins were obtained by stripping the intact skins with adhesive tape 30 times²⁰. Delipidized skins were prepared from full-thickness skin using methylene chloride as an extracting solvent according to the method described by Kuo et al.²⁷ A piece of hairless mouse skin was mounted on a diffusion cell with the SC side facing the donor compartment to extract lipids, whereas phosphate-buffered saline (pH 7.4) containing 0.01% (w/v) gentamycin was placed in 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 a more efficient lipid extraction.

Skin permeation of salicylic acid fatty ester prodrugs

Freshly excised hairless rat skin was mounted in open modified Franz-type diffusion cells with an available diffusion area of 2.01 cm² and a receptor volume of approximately 13 ml (12.4–13.2 ml). The diffusion cells were placed in a thermostated water bath with a constant temperature of 37°C at the skin surface. The receptor compartments contained a mixture of glycerin and phosphate-buffered saline (pH 7.4) (50:50, 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 fatty ester prodrug was applied to the SC side of the membrane. Saturation was considered to be achieved by sonicating an excess of materials in 2-propanol at 37°C^{14,15}. At appropriate time intervals, the receptor fluid (200 µl) was withdrawn and replaced by fresh and thermostated receptor fluid. The permeation was followed for 24 h, a time period which had previously been shown to be sufficient¹². It was ensured that sink conditions were present and that the sample concentrations were well above the limit of quantitation of the analytical methods. The experiments were carried out in triplicate. The samples were kept at 4°C for a maximum of 2 days until HPLC analysis could be performed.

Skin accumulation of salicylic acid fatty ester prodrugs

Accumulation of the salicylic acid fatty ester prodrugs in the skin at the end of the permeation study was determined by cutting away the effective permeation areas of the skin samples and then washing them with distilled water to remove any surface adhered materials²⁸. Skin permeation area samples were then minced into small pieces and homogenized with four-fold distilled water for 15 min in an ice bath. Each sample (0.45 ml) was mixed with 0.05 ml internal standard solution (valsartan 1 mg/ml) and 0.5 ml acetonitrile. Then 0.1 ml of clear supernatant, which was obtained after vortexing and centrifugation for 10 min at 10,000 g, was mixed again

with 0.9 ml acetonitrile and centrifuged for 10 min at 10,000g. The supernatant was filtered through a syringe filter (0.45 μm) and analyzed by HPLC for assay of salicylic acid fatty esters.

Results and discussion

Lipophilicity

Lipophilicity is very important for dermal permeation because the SC, the major barrier to drug permeation, is lipid in nature and generally favors permeation by lipophilic drugs¹⁴. The molecular weights of the salicylic acid fatty ester prodrugs (Figure 1) ranged from 264 to 377 g/mol (Table 1). Their lipophilicity was determined in terms of capacity factors (k') and partition coefficient ($\log P$) calculated from the difference of their retention times and by ChemBioDraw, respectively. Their k' values were proportional to their $\log P$, suggesting that their k'

value is consistent with their lipophilicity¹². The k' values ranged between 1.82 (octanoyl oxysalicylate) and 14.64 (palmitoyl oxysalicylate) (Table 1). Moreover, the homologous series of esters showed k' values proportional to the number of carbon atoms present (C-C) in the side chain. Based on the capacity factor, the lipophilicity of salicylic acid fatty esters was in the order of octanoyl oxysalicylate (C8SA) < nonanoyl oxysalicylate (C9SA) < decanoyl oxysalicylate (C10SA) < lauroyl oxysalicylate (C12SA) < myristoyl oxysalicylate (C14SA) < palmitoyl oxysalicylate (C16SA).

Chemical stability

The chemical hydrolysis of these fatty ester prodrugs was determined by their stability in the organic solvents methanol, ethanol, 2-propanol, acetonitrile, glycerin and polyethylene glycol 400 (Figures 2 and 3). All of the fatty esters were highly stable in 2-propanol, acetonitrile

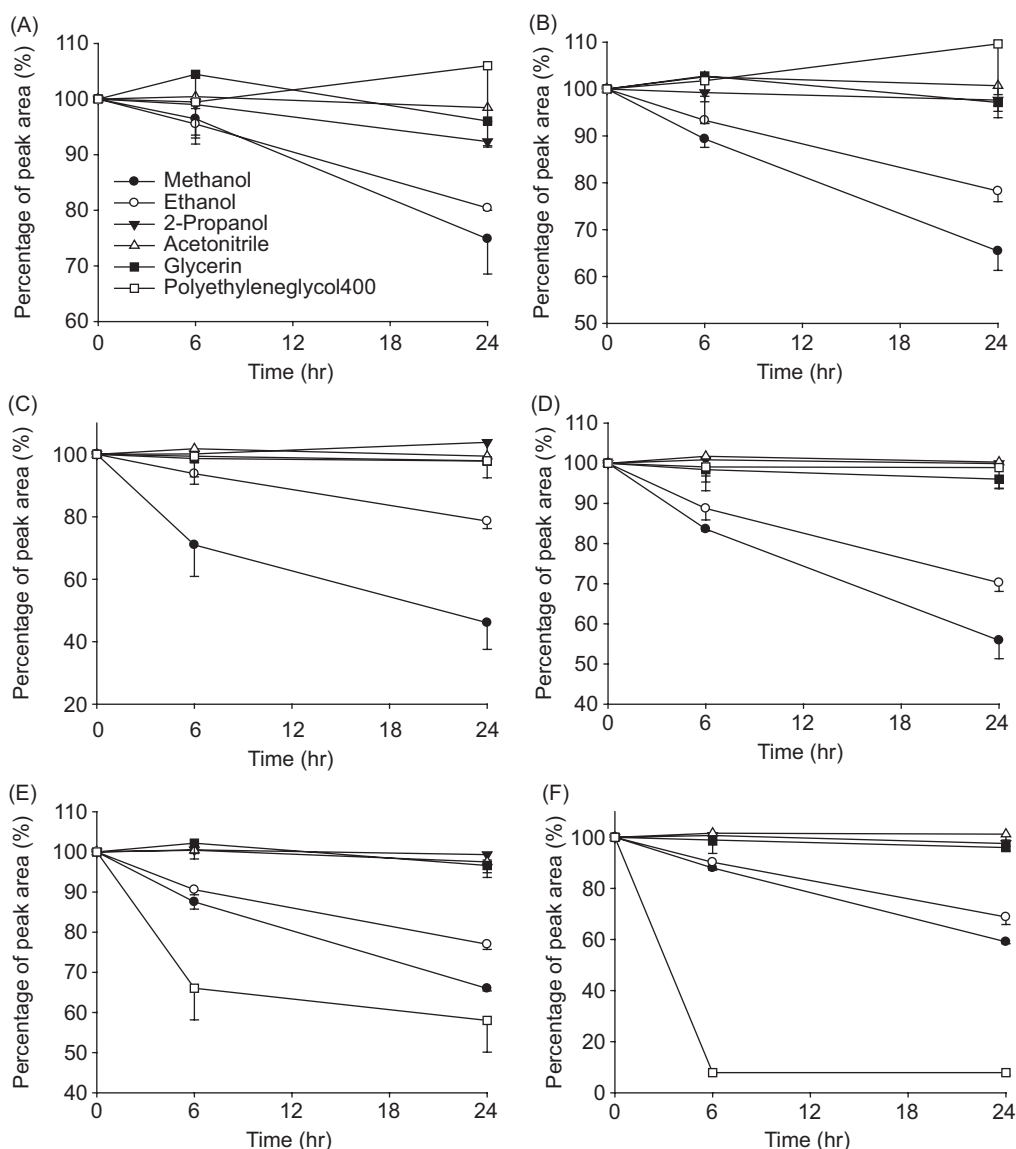


Figure 2. Chemical stability of fatty ester prodrugs in various organic solvents: (A) C8SA, (B) C9SA, (C) C10SA, (D) C12SA, (E) C14SA and (F) C16SA. Each value represents the mean \pm SD ($n=3$).

and glycerin. They showed the lowest levels of 40–70% against the initial concentration in methanol and ethanol at 24 h (Figure 3), indicating that they were unstable in these solvents. C8SA, C9SA, C10SA and C12SA were highly stable in polyethylene glycol 400 but C14SA and C16SA were unstable in this solvent. Furthermore, C14SA was less stable in this solvent than C16SA (58% vs. 8% at 24 h). Thus, the salicylic acid fatty ester prodrugs with smaller molecular weights were more stable in organic solvents than those with larger molecular weights.

Enzymatic hydrolysis

Enzymatic hydrolysis profiles of fatty ester prodrugs are illustrated in Figure 4. All prodrugs were well hydrolyzed in both hairless mouse skin and liver homogenates. In 6 h, all prodrugs were entirely hydrolyzed in hairless mouse liver homogenates (Figure 4A) suggesting that they were very unstable in these homogenates. After 4 days, all prodrugs were entirely hydrolyzed in hairless mouse skin homogenates. Moreover, even if there were no significant differences, the fatty ester prodrugs with long carbon chains were more hydrolyzed in skin homogenates (Figure 4B). The rate of ester hydrolysis was proportional to the lipophilicity and K value of the compound. In this study, the salicylic acid fatty ester prodrugs were readily hydrolyzed to the parent compounds in hairless mouse liver and skin homogenates. Thus, especially C14 and C16 with long carbon chains may be converted to salicylic acid after their topical administration and may have a potential use as UV protective agents in pharmaceutical formulations^{13,14}.

Skin permeation of salicylic acid fatty ester prodrugs

In the *in vitro* permeation studies, the intact SC-stripped and delipidized hairless mouse skins were used in Franz-type diffusion cells to examine the permeation characteristics of salicylic acid fatty ester prodrugs. The prodrug-containing suspensions were applied as

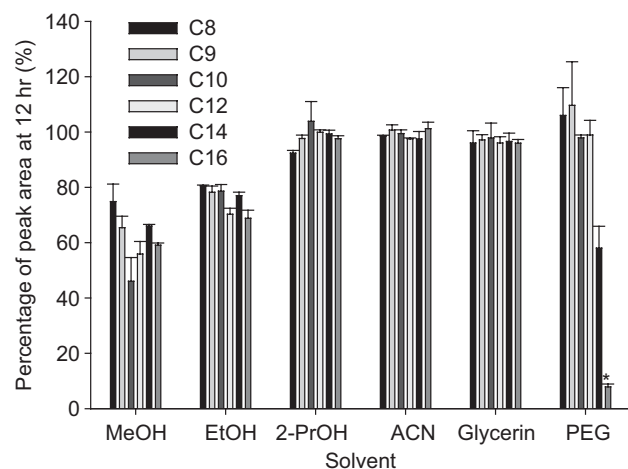


Figure 3. Chemical stability of fatty ester prodrugs in various organic solvents at 24 h. Each value represents the mean \pm SD ($n=3$). * $P<0.05$ compared with other solvents.

saturated solutions to maintain constant diffusion and maximum flux. Before the permeation study, the stability of fatty ester prodrugs were investigated in a receptor medium, resulting in the good stability of all prodrugs for 3 days ($96.8 \pm 3.5\%$). After application of prodrugs, the metabolite parent compound was found in the receptor fluid rather than the intact salicylic acid fatty ester prodrugs. This was in good correlation with a previously reported study²⁹.

In the intact skins, the highest permeation was observed for C12SA followed by C10SA (Figure 5A). All other esters showed poor permeation profiles compared with C10SA and C12SA ($P<0.05$). Our results suggest that C10SA and C12SA were well permeated throughout the skin. Among the prodrugs tested, C14 and C16 showed

Table 1. Molecular weight, capacity factor (k') and log P value of fatty ester prodrugs of salicylic acid.

Compounds	MW	k'	Log P
Octanoyl oxysalicylate (C8SA)	264.35	1.82	3.92
Nonanoyl oxysalicylate (C9SA)	278.38	2.12	4.33
Decanoyl oxysalicylate (C10SA)	292.41	2.83	4.75
Lauroyl oxysalicylate (C12SA)	320.47	5.15	5.59
Myristoyl oxysalicylate (C14SA)	348.53	8.26	6.42
Palmitoyl oxysalicylate (C16SA)	376.59	14.64	7.26

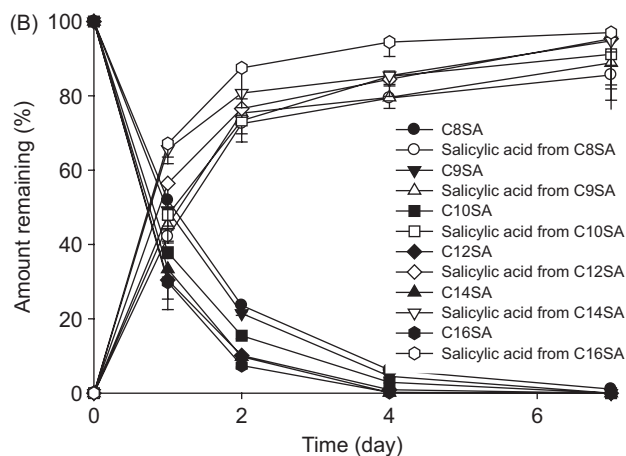
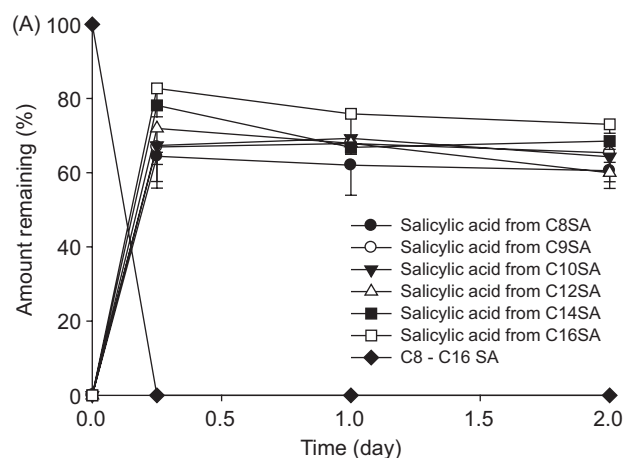


Figure 4. Hydrolysis of fatty ester prodrugs in hairless mouse liver (A) and skin (B) homogenates. Each value represents the mean \pm SD ($n=3$).

the lowest permeations of salicylic acid, even if no significant differences were found. Thus, C14SA and C16SA could hardly permeate through the skin and/or might be degraded by skin esterase.

The prodrugs C8SA and C9SA showed the significantly highest permeations in SC-stripped skin (Figure 5B) and delipidized skin (Figure 5C). Similarly, C14 and C16 showed the significantly lowest permeations of salicylic acid. Stripping and delipidization had both positive and negative impacts on the permeation profiles of parent salicylic acid from the applied esters. Except for C16SA, all esters showed significantly higher permeation of salicylic

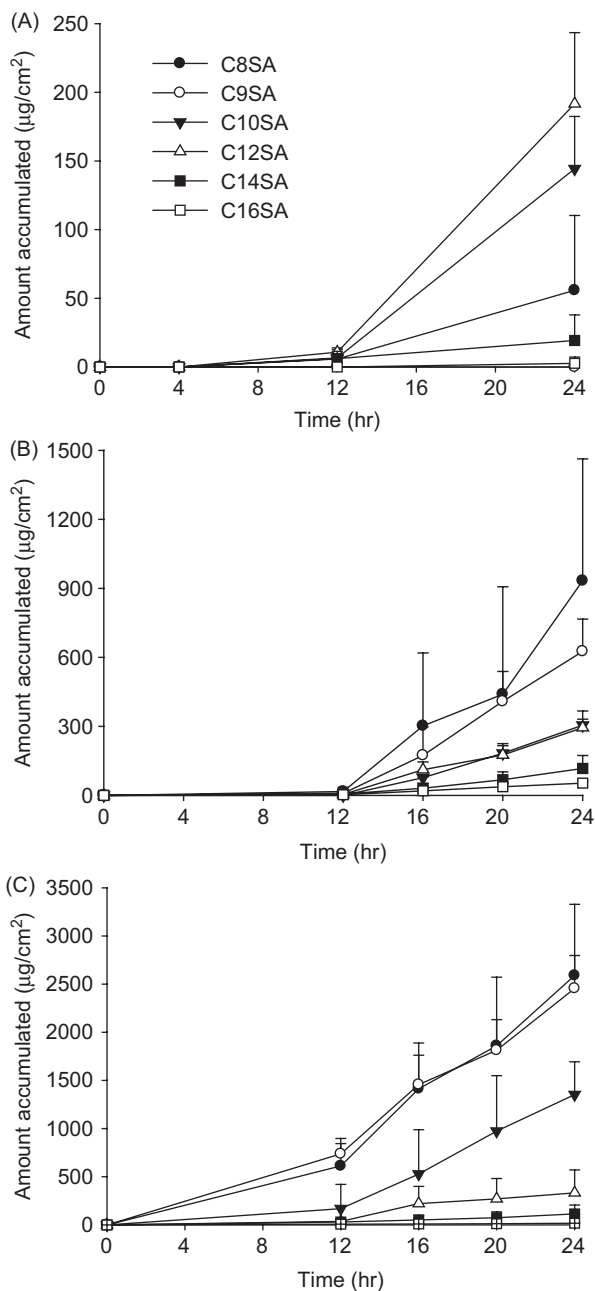


Figure 5. Skin permeation amounts of fatty ester prodrugs on intact (A), stratum corneum-stripped (B) and delipidized (C) hairless mouse skin. All compounds were detected but as parent drug forms rather than as fatty ester prodrugs. Each value represents the mean \pm SD ($n=3$).

acid in SC-stripped and delipidized skins compared with intact skins ($P<0.05$). C16SA had similar permeation of salicylic acid in all skins. In particular, C16SA showed the poorest permeation in all skins compared with the other prodrugs (Figure 5).

As shown in Figure 6, there were no correlations between permeated amounts of salicylic acid and $\log P$ in intact skin. However, in SC-stripped and delipidized skins, the higher the $\log P$, the lesser the permeated amounts of salicylic acid. The permeated amount of salicylic acid from these fatty ester prodrugs was highest in delipidized skin followed by stripped and intact skin. Their relatively higher lipophilicity might have been responsible for their low permeability into the receptor solution through intact skin. Exogenous bulk lipids provided by ester side chains might have contributed to an increment in the total lipid proportion^{16-19,30}. As the ester side chains had a structural similarity with the indigenous fatty acids in skin, they might have allied with their naturally occurring counterparts in the lipid phase. This might have contributed to an increment in the total lipid content, and lead to favorable molecular arrangements and interactions to create order and hence chain rigidity rather than disorder of the skin lipid layer and consequently a more effective barrier^{31,32}.

From the results of these permeation studies, C16SA could be a potential candidate for UV protection because it showed similar permeation of salicylic acid in all skins and the poorest permeation level in all skins compared with the other prodrugs.

Skin accumulation of salicylic acid fatty ester prodrugs

Among the prodrugs tested, C10SA and C12SA showed the highest accumulation in intact skin compared with the other prodrugs (Figure 7). Furthermore, C10SA and C12SA prodrugs showed significantly higher accumulations in intact skin than in SC-stripped skin and delipidized skin ($P<0.05$). These results could be explained by the affinity of these prodrugs for the SC. The higher accumulations in SC-stripped skin than in delipidized skin were contributed by C10SA and C12SA, which

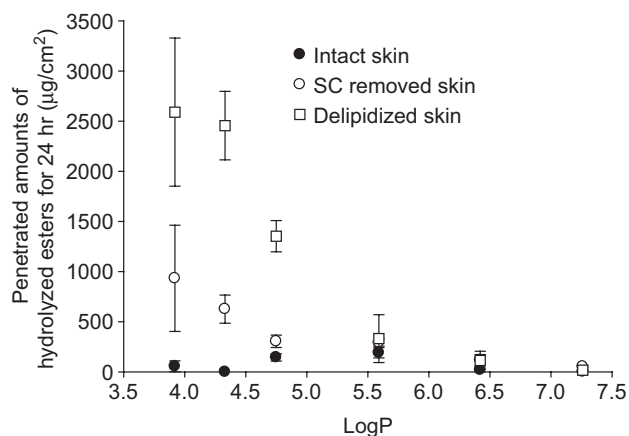


Figure 6. Correlation of skin permeation of fatty ester prodrugs in 24 hr and $\log P$.

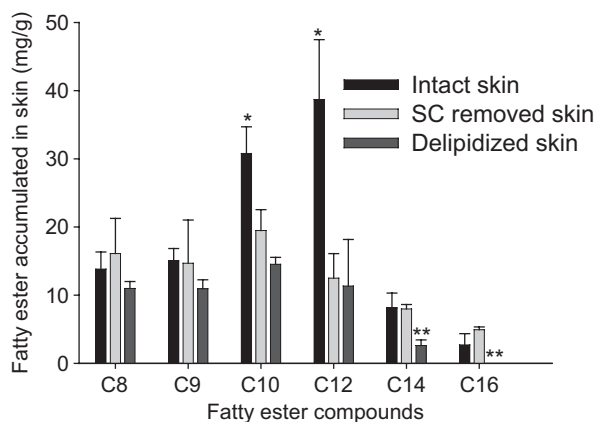


Figure 7. Skin accumulation amounts of fatty ester prodrugs. Each value represents the mean \pm SD ($n=3$). For comparison between two groups: * $P<0.05$, accumulation profiles in intact skin vs. accumulation profiles of SC-stripped skin and delipidized skin. ** $P<0.05$, accumulation profiles in delipidized skin vs. accumulation profiles of intact skin and SC-stripped skin.

shows their greater affinity for SC lipids than for dermal lipids.

On the other hand, C14SA and C16SA showed relatively lower accumulations in intact skin than the other prodrugs did. Similarly, C14SA and C16SA had relatively lower accumulations in SC-stripped skin and delipidized skin compared with the other prodrugs, resulting from their poorer affinity to skin (Figure 7). Moreover, the accumulations of C14SA and C16SA were significantly lower in delipidized skin compared with intact skin and SC-stripped skin ($P<0.05$).

These skin accumulations of salicylic ester prodrugs showed a parabolic relationship with their log P values (Figure 8). In the case of compounds other than C14SA and C16SA, the accumulations of salicylic ester prodrugs were increased in all skins as the lipophilicity increased. C14SA and C16SA, both with highly lipophilic molecules, showed lower accumulations than other prodrugs due to their low aqueous solubility^{10,11}.

For drugs that permeate mainly through the lipid bilayer region (intercellular pathway), similar permeability coefficients should be observed for those transported through the SC-stripped skin and delipidized skin. In the case of C14SA and C16SA, there was no significant difference ($P>0.05$) in ester accumulation in SC-stripped and delipidized skins, suggesting that the predominant route for their passive penetration of the SC layer might be the intercellular pathway¹². Lower accumulations of C14SA and C16SA compounds in delipidized skin compared with intact skin or stripped skin suggested that these esters had relatively strong affinities for lipids compared with other components in the skin. They were not highly taken up in both lipid and proteinaceous phases. Their smaller uptake in the lipid phase had no positive effects in their skin permeation because of their strong affinity for skin lipids and other skin components^{32,33}. Thus, C14SA and C16SA could be used as UV protective agents because they showed lower accumulations and smaller

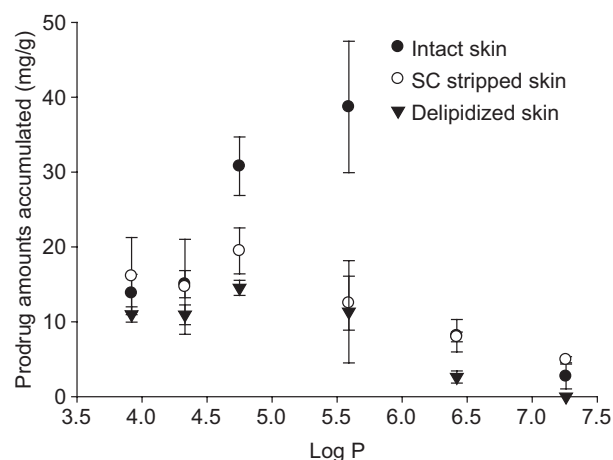


Figure 8. Correlation of skin accumulation of fatty ester prodrugs and log P .

uptakes in the lipid phase than the other prodrugs due to their low aqueous solubility.

Conclusions

In conclusion, the palmitoyl oxysalicylate (C16SA) was readily hydrolyzed to its parent compound in hairless mouse liver and skin homogenates, suggesting that it might be converted to salicylic acid after its topical administration. Furthermore, it showed the lowest permeation of salicylic acid in all skins and a lower accumulation and smaller uptake in the lipid phase than compared with the other prodrugs. Thus, the palmitoyl oxysalicylate (C16SA) could be a potential candidate for UV protection.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article. This work was supported by Korea Research Foundation Grant (KRF-2004-042-E00172).

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