

## Effect of Enhancers on *in vitro* and *in vivo* Skin Permeation and Deposition of S-Methyl-L-Methionine

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### Abstract

S-methyl-L-methionine (SMM), also known as vitamin U, is commercially available as skin care cosmetic products for its wound healing and photoprotective effects. However, the low skin permeation expected of SMM due to its hydrophilic nature with a log P value of -3.3, has not been thoroughly addressed. The purpose of this study thus was to evaluate the effect of skin permeation enhancers on the skin permeation/deposition of SMM. Among the enhancers tested for the *in vitro* skin permeation and deposition of SMM, oleic acid showed the most significant enhancing effect. Moreover, the combination of oleic acid and ethanol further enhanced *in vitro* permeation and deposition of SMM through hairless mouse skin. Furthermore, the combination of oleic acid and ethanol significantly increased the *in vivo* deposition of SMM in the epidermis/dermis for 12 hr, which was high enough to exert a therapeutic effect. Therefore, based on the *in vitro* and *in vivo* studies, the combination of oleic acid and ethanol was shown to be effective in improving the topical skin delivery of SMM, which may be applied in the cosmetic production process for SMM.

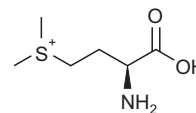
**Key Words:** S-methyl-L-methionine, Vitamin U, Skin permeation, Deposition, Enhancers

### INTRODUCTION

S-methyl-L-methionine (SMM), also known as vitamin U, is a functional biomolecule having cationic structure with  $\alpha$ -amino acid end group (Fig. 1). Natural sources of SMM are mainly raw cabbage and green vegetables such as broccoli and celery. Its molecular weight is 164.25 g/mol with a log *p*-value of -3.3, which indicates that it is very hydrophilic (Patel and Prajapati, 2012).

Diverse pharmacological efficacy of SMM has been reported. Oral administration of SMM reduced the liver damage induced by valproic acid in rats (Sokmen *et al.*, 2012). In experimental nephrotic hyperlipidemia, SMM ameliorated cholesterol and phospholipid levels in blood (Seri *et al.*, 1979). Moreover, SMM showed protective effect not only on rat gastro-intestinal mucosa (Ichikawa *et al.*, 2009) but also on oesophagogastric ulcers in pig studies (Kopinski *et al.*, 2007).

Studies on the effect of topical skin administration of SMM have begun recently. The topical administration of SMM en-



**Fig. 1.** Chemical structure of S-methyl-L-methionine (SMM, vitamin U).

hanced the repair of skin damage by activation of fibroblasts, thereby showing a wound healing effect (Kim *et al.*, 2010). SMM also showed a photoprotective effect by reducing UV-induced skin damage (Kim *et al.*, 2015). Based on these results, SMM was developed as a wound healing agent and skin care cosmetic product. However, there has been no systematic study reported up to date on the skin permeation/deposition of SMM even though the skin permeation of SMM is expected to be low due to its high hydrophilicity. Thus, this study has been initiated with the assumption that the use of skin permeation

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enhancer could improve the skin permeation and/or deposition of SMM, thereby further increasing its therapeutic efficacy. Various skin permeation enhancers with different mechanism of action have been reported (Rachakonda *et al.*, 2008). However, only a few them could significantly enhance the skin permeation of hydrophilic compounds (Karande *et al.*, 2004), and skin irritation has always been a critical issue (Lashmar *et al.*, 1989). Thus, the objective of this study was to optimize and evaluate the skin permeation enhancer on the *in vitro* skin permeation and deposition of SMM in hairless mouse model. Also, the synergistic effect of skin permeation enhancers on *in vitro* and *in vivo* skin deposition of SMM was systematically investigated.

## MATERIALS AND METHODS

### Materials

SMM was purchased from Sigma Chemical Co (St. Louis, MO, USA). Propylene glycol (PG) was purchased from Samchun Chemical Co., Ltd (Pyeongtaek, Korea). Isopropyl myristate (IPM), oleic acid, and limonene were purchased from Sigma Chemical Co. HPLC grade methanol, ethanol and acetonitrile were purchased from Thermo Fisher Scientific Co (Pittsburgh, PA, USA).

### Animals

For *in vitro* and *in vivo* evaluation, male hairless mice (18–20 g) were obtained from Orient Bio Inc (Sungnam, Korea). They had free access to water and food before the studies. Experimental protocols for the animals (SNU-111007-4-2) used in this study were reviewed by the Animal Care and Use Committee of the College of Pharmacy, Seoul National University (Seoul, Korea) and were in accordance with the National Institutes of Health's Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publication Number 85-23, revised 1985).

### *In vitro* skin permeation and deposition studies

Evaluation of *in vitro* permeation and deposition of SMM through hairless mouse skin was carried out by using Keshary-Chien diffusion cells at 32°C, which have 1.77 cm<sup>2</sup> of the surface area for diffusion. After sacrificing the hairless mice by cervical dislocation, the dorsal skin was cut to about 3 cm x 3 cm size and the subcutaneous fat was removed. Then, they were fixed between the donor and receptor cells, laying the stratum corneum toward the donor cells. The donor cells contained 2% (w/v) or 5% (w/v) SMM in the 50:50 (v/v) mixture (1.0 mL) of PG and double distilled water (DDW) with or without permeation enhancers, and were covered with parafilm to prevent evaporation. The receptor cells were filled with DDW (13.0 mL) and continuously stirred by magnetic bar. After applying SMM solution on the donor cells, 0.5 mL of the receptor solution was collected at 3, 6, 9, and 12 hr and added immediately with an equal volume of fresh media. SMM in the receptor solutions was analyzed using LC-MS/MS.

For *in vitro* skin deposition studies, the skin was removed from the diffusion cells at 6 and 12 hr after applying SMM solution and washed out with methanol. In order to determine the amount of SMM in stratum corneum by tape stripping method, cellophane adhesive tape (CuDerm Cooperation, Dallas, TX, USA) was applied three times on the stratum corneum of the

skin. Then, the tapes of each time samples were separately collected into the 15 mL tube and added with mobile phase (3.0 mL). SMM was extracted from the tapes by shaking the tube for 3 hr, followed by centrifugation for 5.0 min at 16,100×g. The skin samples after removing the stratum corneum (i.e., epidermis and dermis) were chopped and collected into the 15 mL tube. After adding mobile phase (3.0 mL), they were homogenized using ULTRA-TURAX® T25basic (IKA, Staufen, Germany), followed by centrifugation for 1.5 min at 3,660×g. The amount of SMM in the stratum corneum and epidermis/dermis at 6 and 12 hr was analyzed using LC-MS/MS.

### *In vivo* skin deposition study

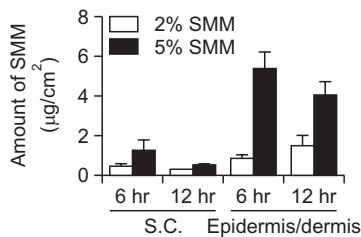
Hairless mice were lightly anesthetized with ether and each mouse was fixed laying the dorsal skin upward. A specially designed cylinder-type chamber which has a diffusion area of 2.14 cm<sup>2</sup> was put on the dorsal skin of mice and fixed with surgical glue (Vet bond®, 3M Co., St. Paul, MN, USA) (Valiveti *et al.*, 2004; Jung *et al.*, 2013). Aliquot of PG solution containing 2% (w/v) of SMM with or without enhancer [i.e., 2% (w/v) oleic acid with or without 10% (w/v) ethanol] was applied into the chamber for topical administration of SMM at 80 mg/kg dose. The hairless mice were sacrificed by cervical dislocation at 6 and 12 hr, and then the skin samples were prepared as described in *in vitro* skin deposition study to determine the amount of SMM.

### LC-MS/MS analysis of SMM

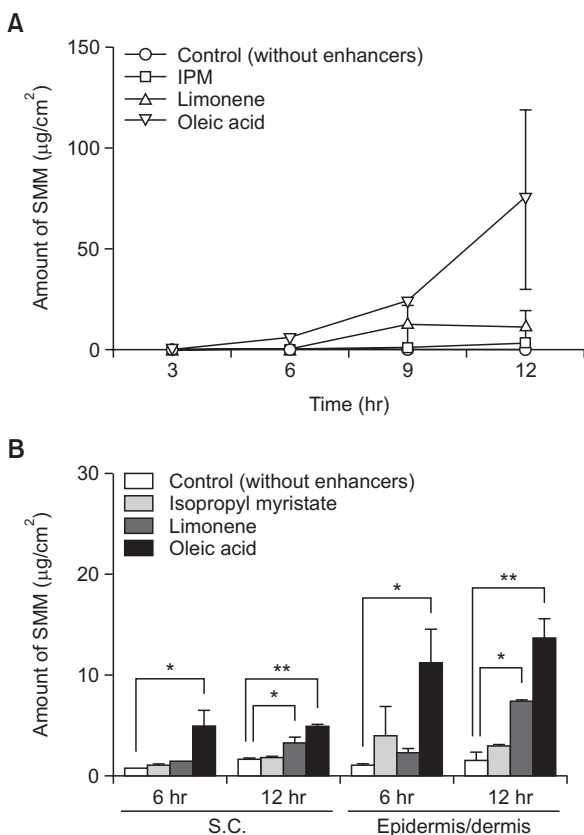
SMM was analyzed by using LC-MS/MS following the method in the literature with slight modification (Scherb *et al.*, 2009). Samples were transferred to the mass vials and a 3 µL aliquot was injected into Synergi Max-RP column (75 mm×4.6 mm, 4 µm; Phenomenex, Torrance, CA, USA). The mobile phase was 73% acetonitrile and 27% DDW containing 0.2% formic acid at the flow rate of 0.5 mL/min. The column eluents were detected with an Agilent LC-MS/MS system equipped with an Agilent Technologies 1260 Infinity HPLC system (Santa Clara, CA, USA) and Agilent Technologies 6430 Triple Quad LC-MS system. Optimization was conducted in the multiple reaction monitoring (MRM) mode with positive electrospray ionization (ESI). The gas temperature, gas flow, nebulizer pressure, and capillary voltage were 300°C, 11 L/min, 15 psi, and 4500 V, respectively. The m/z value of precursor to product ion, fragment voltage, collision energy, and cell accelerator voltage were 163.9 to 102.0 (Fig. S1), 73 V, 7eV, and 1 V, respectively. The data were processed using the MassHunter Workstation Software Quantitative Analysis (vB.05.00; Agilent Technologies). The retention time of SMM was 1.1 minutes (Fig. S2a). The mean correlation coefficient (R<sup>2</sup>) for the calibration curve was over 0.999 (Fig. S2b). The lower limit of quantification (LLOQ) of SMM was 20 ng/mL. Precision and accuracy values were within acceptable ranges (Table S1).

### Statistical analysis

All experiments were carried out at least three times (n≥3). A p-value less than 0.05 was considered to be statistically significant using one-way ANOVA. Data were shown as mean ± standard deviation.



**Fig. 2.** *In vitro* skin deposition of SMM at 6 hr and 12 hr in the stratum corneum (SC) and the epidermis/dermis of hairless mouse skin after applying 2% (w/v) or 5% (w/v) of SMM in the 50:50 (v/v) mixture of PG and DDW (Mean ± SD, n=3).



**Fig. 3.** Effect of absorption enhancers (5%, w/v) on (A) the *in vitro* skin permeation profiles of SMM and (B) the skin deposition of SMM at 6 hr and 12 hr in the stratum corneum (SC) and the epidermis/dermis of hairless mouse skin. SMM was applied at 2% (w/v) in the 50:50 (v/v) mixture of PG and DDW (Mean ± SD, n=3) (B). \* $p < 0.05$ , \*\* $p < 0.01$ .

**RESULTS**

**Effect of SMM concentration on *in vitro* skin permeation and deposition**

The *in vitro* skin permeation study showed that the amount of SMM in the receptor solution after 12 hr of applying up to 20% (w/v) of SMM was below the detection limit (data not shown). However, the skin deposition of SMM after applying 2% (w/v) or 5% (w/v) of SMM, which were selected based on the efficacy studies in the literature (Kim *et al.*, 2010, 2015),

**Table 1.** Effect of absorption enhancers (5%, w/v) on the *in vitro* skin permeation parameters of SMM dissolved in the 50:50 (v/v) mixture of PG and DDW at 2% (w/v)

Enhancer	Flux (µg/hr/cm²)	Permeability coefficient (×10 <sup>-4</sup> cm/hr)	Lag time (hr)
Without enhancers	ND	ND	ND
Isopropyl myristate	0.35 ± 0.36	0.18 ± 0.18	3.46 ± 0.66
Limonene	1.38 ± 0.82	0.69 ± 0.41	1.51 ± 0.43
Oleic acid	7.98 ± 4.43**	3.99 ± 2.21**	4.11 ± 0.25

ND: below the detection limit.  
 \*Significantly different from the IPM group ( $p < 0.05$ ).  
 \*\*Significantly different from the Limonene group ( $p < 0.05$ ).

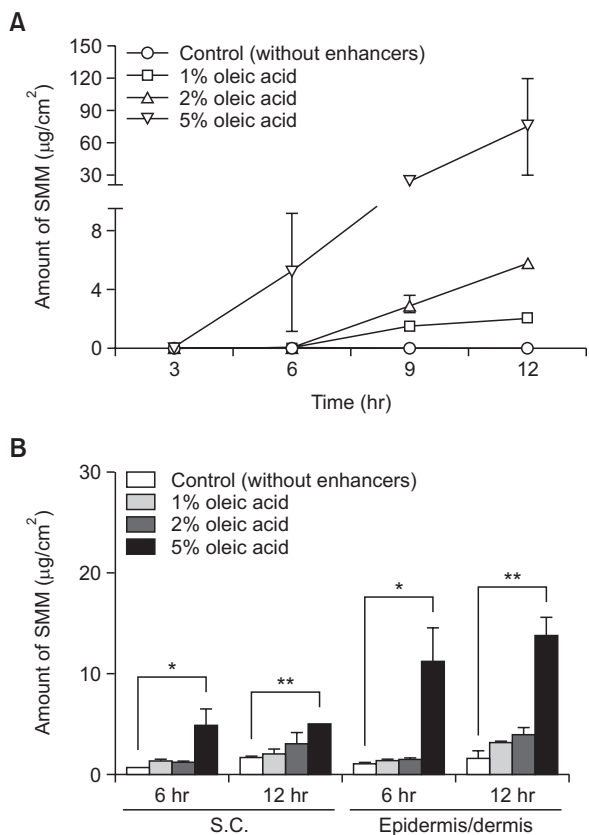
was detectable by LC-MS/MS analysis. As shown in Fig. 2, application of 5% (w/v) SMM resulted in higher deposition at both 6 hr and 12 hr than those of 2% (w/v). It is also interesting to note that the deposition of SMM in the epidermis/dermis was always higher than that of the stratum corneum, regardless of application time and SMM concentration. Since application of 2% (w/v) SMM was high enough to determine its skin deposition, further studies to investigate the effect of permeation enhancer was conducted at this concentration.

**Effect of enhancers on *in vitro* skin permeation and deposition of SMM**

To investigate the effect of enhancers on the *in vitro* skin permeation and deposition of SMM (2%, w/v), three different enhancers were added at 5% (w/v) in the donor solution. Fig. 3A shows the *in vitro* skin permeation profiles of SMM, and its permeation parameters are summarized in Table 1. Skin permeation of SMM without enhancer was negligible, and was below the detection limit. However, the addition of enhancers increased the flux of SMM compared with the control group (without enhancer). The most effective enhancer among tested was oleic acid, which significantly increased the flux of SMM up to 7.98 ± 4.43 µg/hr/cm². Fig. 3B shows the effect of enhancer on the *in vitro* skin deposition of SMM at 6 hr and 12 hr. Oleic acid significantly increased the amount of SMM in both the stratum corneum and epidermis/dermis at 6 h and 12 hr, which is consistent with the results of the *in vitro* skin permeation study (Fig. 3A, Table 1).

**Effect of oleic acid concentration on *in vitro* skin permeation and deposition of SMM**

Fig. 4A shows the effect of oleic acid concentration on the *in vitro* skin permeation of SMM, and Table 2 summarizes the permeation parameters. Although oleic acid increased the skin permeation of SMM in a dose-dependent manner, significant increase of the SMM flux was observed in the 5% oleic acid group only (Table 2). Moreover, skin deposition study also consistently show that the amount of SMM in the stratum corneum and epidermis/dermis significantly increased with the addition of 5% oleic acid only (Fig. 4B). The amount of SMM in the epidermis/dermis at 12 hr was 9.43 times higher in 5% oleic acid group compared with the control group (without enhancer). Although oleic acid was the most effective skin permeation enhancer for SMM among tested, it is known to cause skin irritation at higher than 5% concentration (Songkro,



**Fig. 4.** Effect of oleic acid concentration on (A) the *in vitro* skin permeation profiles of SMM and (B) the skin deposition of SMM at 6 hr and 12 hr in the stratum corneum (SC) and the epidermis/dermis of hairless mouse skin. SMM was applied at 2% (w/v) in the 50:50 (v/v) mixture of PG and DDW (Mean  $\pm$  SD, n=3) (B). \* $p$ <0.05, \*\* $p$ <0.01.

**Table 2.** Effect of oleic acid concentration on the *in vitro* skin permeation parameters of SMM dissolved in the 50:50 (v/v) mixture of PG and DDW at 2% (w/v)

Enhancer	Flux ( $\mu\text{g/hr/cm}^2$ )	Permeability coefficient ( $\times 10^{-4}$ cm/hr)	Lag time (hr)
Without enhancers	ND	ND	ND
1% oleic acid	0.25 $\pm$ 0.02	0.13 $\pm$ 0.01	4.00 $\pm$ 0.03
2% oleic acid	0.68 $\pm$ 0.05	0.34 $\pm$ 0.03	4.29 $\pm$ 0.11
5% oleic acid	7.98 $\pm$ 4.43*#	3.99 $\pm$ 2.21**	4.11 $\pm$ 0.25

ND: below the detection limit.

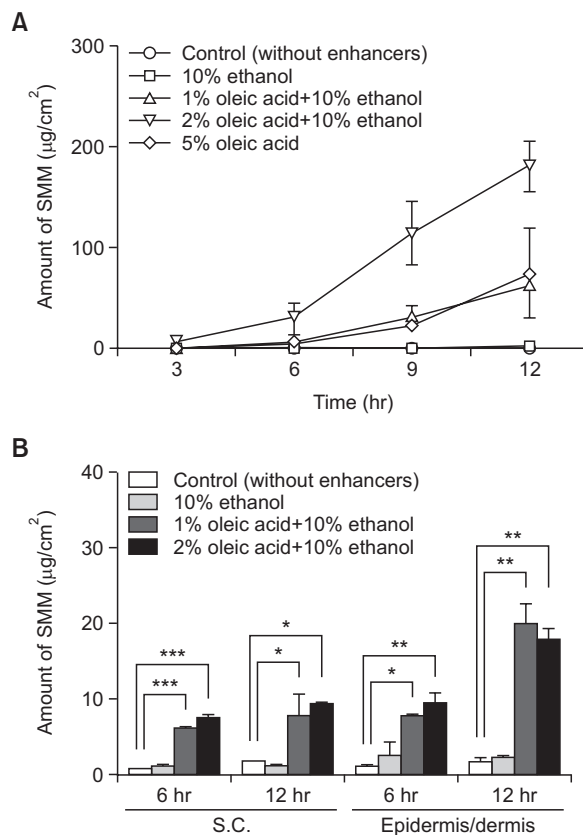
\*Significantly different from the 1% oleic acid group ( $p$ <0.05).

#Significantly different from the 2% oleic acid group ( $p$ <0.05).

2009). Thus, further study on the synergistic skin permeation-enhancing effect of ethanol and oleic acid was investigated to minimize the use of oleic acid.

#### Synergistic effect of oleic acid and ethanol on *in vitro* permeation and deposition of SMM

*In vitro* skin permeation profiles of SMM when 10% ethanol was added alone or in combination with oleic acid are shown



**Fig. 5.** Synergistic effect of oleic acid and ethanol on (A) the *in vitro* skin permeation profiles of SMM and (B) the skin deposition of SMM at 6 hr and 12 hr in the stratum corneum (SC) and the epidermis/dermis of hairless mouse skin. SMM was applied at 2% (w/v) in the 50:50 (v/v) mixture of PG and DDW (Mean  $\pm$  SD, n=3) (B). \* $p$ <0.05, \*\* $p$ <0.01, \*\*\* $p$ <0.001.

in Fig. 5A. Synergistic effect of ethanol and oleic acid on the permeation parameters is summarized in Table 3. Addition of 10% ethanol (without oleic acid) showed a permeation-enhancing effect, and increased the flux of SMM up to 0.27  $\mu\text{g/hr/cm}^2$ , which is comparable to the effect of 2% oleic acid (Table 2). Moreover, when 10% ethanol was added with 1% oleic acid, the flux of SMM (7.11  $\mu\text{g/hr/cm}^2$ ) was comparably enhanced to that with 5% oleic acid (7.98  $\mu\text{g/hr/cm}^2$ , Table 1). When 2% oleic acid was added with 10% ethanol, the flux of SMM synergistically increased up to 19.83  $\mu\text{g/hr/cm}^2$  (Table 2). The lag time also showed decreasing tendency when ethanol and oleic acid were added in combination. The lag time of permeation decreased to 3.22 hr when 2% oleic acid was added with 10% ethanol (Table 3), while that of 2% oleic acid alone was 4.29 hr (Table 2). Fig. 5B shows that the *in vitro* skin deposition of SMM synergistically increased when oleic acid was added with 10% ethanol. The amount of SMM in epidermis/dermis at 12 hr increased 12.3 times when 2% oleic acid and 10% ethanol was added in combination, compared to the control group (without enhancer).

#### *In vivo* skin deposition of SMM in hairless mice

The effect of permeation enhancer on the *in vivo* skin deposition of SMM was consistent with that of the *in vitro* result, as shown in Fig. 6. The addition of 2% oleic acid increased

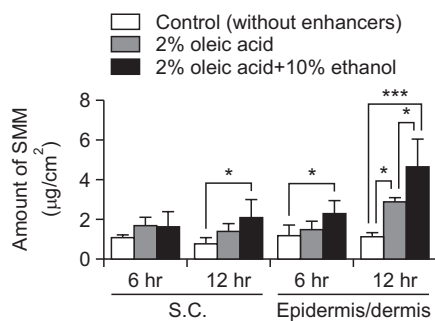
**Table 3.** Synergistic effect of oleic acid and ethanol on the *in vitro* skin permeation parameters of SMM dissolved in the 50:50 (v/v) mixture of PG and DDW at 2% (w/v)

Enhancer	Flux (μg/hr/cm <sup>2</sup> )	Permeability coefficient (×10 <sup>-4</sup> cm/hr)	Lag time (hr)
Without enhancers	ND	ND	ND
10% ethanol	0.27 ± 0.34	0.14 ± 0.17	-
10% ethanol+ 1% oleic acid	7.11 ± 0.84*	3.56 ± 0.42*	3.85 ± 0.29
10% ethanol+ 2% oleic acid	19.83 ± 3.33**	9.91 ± 1.67**	3.22 ± 0.07

ND: below the detection limit.

\*Significantly different from the 1% oleic acid group (*p*<0.05).

\*\*Significantly different from the 10% ethanol+1% oleic acid group (*p*<0.05).



**Fig. 6.** Effect of oleic acid and ethanol on the *in vivo* skin deposition of SMM at 6 hr and 12 hr in the stratum corneum (SC) and the epidermis/dermis of hairless mouse skin after applying 2% (w/v) of SMM in the 50:50 (v/v) mixture of PG and DDW (Mean ± SD, n=3). \**p*<0.05, \*\*\**p*<0.001.

the deposition of SMM in the epidermis/dermis at 6 hr and 12 hr, compared to the control (without enhancer). Moreover, 2% oleic acid and 10% ethanol synergistically increased the deposition of SMM up to 4.65 μg/cm<sup>2</sup> at 12 hr in epidermis/dermis, which was 4.2 times higher than that of the control.

## DISCUSSION

The target site of SMM for wound healing and photoprotective effect is the epidermis/dermis of the skin which includes the fibroblast and keratinocyte (Barry, 2001; Benson, 2005). However, the stratum corneum on the outermost layer of the skin works as an absorption barrier, and thus would be the main hurdle for the topical skin delivery of SMM. Since intercellular micro-route of stratum corneum is lipophilic in nature, the molecule with optimum lipophilicity is a prerequisite for permeation. However, SMM is a hydrophilic compound with a log *p*-value of -3.3, which leads to the expectation that the skin permeation of SMM would be poor (Patel and Prajapati, 2012).

Since topical skin delivery of SMM has not been previously reported, the goal of this study was therefore to first of all evaluate the skin permeation and deposition of SMM, after which

to investigate the effect of enhancers. Fortunately, the skin deposition of SMM in epidermis/dermis was detectable by LC-MS/MS analysis (Fig. 2), although the *in vitro* skin permeation without enhancer was not (Table 1). However, the amount of SMM from the *in vivo* skin deposition study was less than that from the *in vitro* study (Fig. 5B vs. Fig. 6), which also should be considered when conducting *in vitro* study to estimate *in vivo* skin deposition.

Due to the difficulties in obtaining human skin and its variability of lipid content, animal models, particularly hairless mouse whose hair follicles are underdeveloped and less dense (Lauer *et al.*, 1995), have been generally used as an alternative to the human skin (Bond and Barry, 1988). In many studies, the correlation of permeation characteristics between hairless mouse and human skins has been reported for many drugs, which is due to the similarity of skin lipid composition between two skins (Durrheim *et al.*, 1980; Jung and Maibach, 2015; Jung *et al.*, 2015). It is well known that the major skin permeation route is transepidermal pathway, and transappendageal pathway (including hair follicles and sweat gland) contributes less than 0.1% of the total permeation amount (Bond and Barry, 1988). Moreover, it was reported that *in vivo* absorption of ionic solutions through hairy skin and hairless skin was not significantly different (Wahlberg, 1968). Based on these previous literatures, we believe that hairless mouse is a suitable model, and hair follicles in hairless mouse do not affect the *in vitro* and *in vivo* skin permeation of SMM.

In order to further increase the skin permeation and deposition in topical delivery, formulation with skin permeation enhancers is a simple and useful method (Songkro, 2009; Marwah *et al.*, 2016). Results of this study showed that oleic acid, alone and in combination with ethanol, is a useful enhancer for SMM among tested, and significantly increases both *in vitro* skin permeation and deposition of SMM. Oleic acid is one of the most potent skin permeation enhancers and has a different mechanism on enhancing skin permeability from its cis unsaturated double bonds structure (Trommer and Neubert, 2006). Due to its structure, oleic acid is known to increase the fluidity of the stratum corneum and form separate phases within the bilayer lipids in stratum corneum, thereby forming a permeable interfacial defect to increase permeation of hydrophilic molecules through the skin (Koyama *et al.*, 1994; Trommer and Neubert, 2006; Songkro, 2009). However, addition of more than 5% oleic acid causes skin irritation (Sintov *et al.*, 1998; Songkro, 2009), and thus less than 2% is recommended for topical skin delivery formulations (Sintov *et al.*, 1998). Diverse skin permeation-enhancing mechanisms of ethanol have been reported, which include lipid extraction effect (Lachenmeier, 2008). High concentration of ethanol also could cause skin irritation, but up to 10% ethanol in formulation is commonly used without adverse effects (Lachenmeier, 2008). Moreover, many studies already reported that the combination of oleic acid and ethanol synergistically enhance the skin permeation and deposition in skin delivery (Koyama *et al.*, 1994; Kim and Chien, 1995; Jung *et al.*, 2013). Consistent with the previous reports, 1% oleic acid in combination with 10% ethanol showed comparable *in vitro* skin permeation of SMM to that of 5% oleic acid (7.11 μg/hr/cm<sup>2</sup> vs. 7.98 μg/hr/cm<sup>2</sup>, Table 1, 2). Moreover, 2% oleic acid in combination with 10% ethanol further increased the flux of SMM up to 19.83 μg/hr/cm<sup>2</sup> (Table 2). It is also notable that the *in vitro* skin deposition of SMM synergistically increased by combining oleic

acid and ethanol. For example, *in vitro* deposition of SMM in epidermis/dermis at 12 hr was 3.77  $\mu\text{g}/\text{cm}^2$  with 2% oleic acid (Fig. 4B), while that was 17.80  $\mu\text{g}/\text{cm}^2$  when 10% ethanol was added in combination with oleic acid (Fig. 5B). In the *in vivo* skin deposition study, 2% oleic acid in combination with 10% ethanol resulted in 4.65  $\mu\text{g}/\text{cm}^2$  SMM at 12 hr in epidermis/dermis (Fig. 6).

The concentration of SMM in its target epidermis/dermis is closely related to its efficacy for skin care cosmetic products. In the *in vivo* skin deposition study, the amount of SMM in epidermis/dermis at 12 hr without permeation enhancer (1.10  $\mu\text{g}/\text{cm}^2$ , Fig. 6) was converted to its SMM concentration, assuming that the thickness of epidermis/dermis of hairless mouse is about 300  $\mu\text{m}$  (Calabro *et al.*, 2011) and SMM is homogeneously distributed. The calculated concentration of SMM in the 2.14  $\text{cm}^2$  surface area of epidermis/dermis was about 223  $\mu\text{M}$ . The skin permeability of human skin is known to be at least two times lower than that of hairless mouse skin (Jung *et al.*, 2015; Seo *et al.*, 2016), and SMM showed wound healing and photoprotective effect in human dermal fibroblast at higher than 100  $\mu\text{M}$  concentration (Kim *et al.*, 2010, 2015). Thus, it was necessary to further increase the skin deposition of SMM in epidermis/dermis. The amount of SMM in epidermis/dermis at 12 hr by the addition of 2% oleic acid and 10% ethanol was 4.65  $\mu\text{g}/\text{cm}^2$ , which is equivalent to about 943  $\mu\text{M}$ .

Thus, it can be concluded that the therapeutically significant concentration of *in vivo* skin deposition of SMM can be achieved when 2% oleic acid in combination with 10% ethanol was used as permeation enhancer. Further studies are underway in this laboratory to develop gel-type topical formulations of SMM.

## CONFLICT OF INTEREST

The authors have declared no conflict of interest.

## ACKNOWLEDGMENTS

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## REFERENCES

- Barry, B. W. (2001) Novel mechanisms and devices to enable successful transdermal drug delivery. *Eur. J. Pharm. Sci.* **14**, 101-114.
- Benson, H. A. (2005) Transdermal drug delivery: penetration enhancement techniques. *Curr. Drug Deliv.* **2**, 23-33.
- Bond, J. R. and Barry, B. W. (1988) Limitation of hairless mouse skin as a model for *in vitro* permeation studies through human skin: hydration damage. *J. Invest. Dermatol.* **90**, 486-489.
- Calabro, K., Curtis, A., Calarneau, J. R., Krucker, T. and Bigio, I. (2011) Gender variation in the optical properties of skin in murine animal models. *J. Biomed. Opt.* **16**, 011008.
- Durrheim, H., Flynn, G. L., Higuchi, W. I. and Behl, C. R. (1980) Permeation of hairless mouse skin: 1. Experimental methods and comparison with human epidermal permeation by alkanols. *J. Pharm. Sci.* **69**, 781-786.
- Ichikawa, T., Ito, Y., Saegusa, Y., Iwai, T., Goso, Y., Ikezawa, T. and Ishihara, K. (2009) Effects of combination treatment with famotidine and methylmethionine sulfonium chloride on the mucus barrier of rat gastric mucosa. *J. Gastroenterol. Hepatol.* **24**, 488-492.
- Jung, E. C. and Maibach, H. I. (2015) Animal models for percutaneous absorption. *J. Appl. Toxicol.* **35**, 1-10.
- Jung, E. J., Kang, Y. P., Yoon, I. S., Kim, J. S., Kwon, S. W., Chung, S. J., Shim, C. K. and Kim, D. D. (2013) Effect of permeation enhancers on transdermal delivery of fluoxetine: *in vitro* and *in vivo* evaluation. *Int. J. Pharm.* **456**, 362-369.
- Jung, E. J., Lee, E. Y., Choi, H. K., Ban, S. J., Choi, S. H., Kim, J. S., Yoon, I. S. and Kim, D. D. (2015) Development of drug-in adhesive patch formulation for transdermal delivery of fluoxetine: *in vitro* and *in vivo* evaluation. *Int. J. Pharm.* **487**, 49-55.
- Karande, P., Jain, A. and Mitragotri, S. (2004) Discovery of transdermal penetration enhancers by high-throughput screening. *Nat. Biotechnol.* **22**, 192-197.
- Kim, D. D. and Chien, Y. W. (1995) Transdermal delivery of zalcitabine: *in vitro* skin permeation study. *AIDS* **9**, 1331-1336.
- Kim, W. S., Seo, H. M., Kim, W. K., Choi, J. S., Kim, I. Y. and Sung, J. H. (2015) The photoprotective effect of s-methylmethionine sulfonium in skin. *Int. J. Mol. Sci.* **16**, 17088-17100.
- Kim, W. S., Yang, Y. J., Min, H. G., Song, M. G., Lee, J. S., Park, K. Y., Kim, J. J., Sung, J. H., Choi, J. S. and Cha, J. H. (2010) Accelerated wound healing by s-methylmethionine sulfonium: evidence of dermal fibroblast activation via the ERK 1/2 pathway. *Pharmacology* **85**, 68-76.
- Kopinski, J. S., Fogarty, R. and McVeigh, J. (2007) Effect of s-methylmethionine sulphonium chloride on oesophagogastric ulcers in pigs. *Aust. Vet. J.* **85**, 362-367.
- Koyama, Y., Bando, H., Yamashita, F., Takakura, Y., Sezaki, H. and Hashida, M. (1994) Comparative analysis of percutaneous absorption enhancement by d-limonene and oleic acid based on a skin diffusion model. *Pharm. Res.* **11**, 377-383.
- Lachenmeier, D. W. (2008) Safety evaluation of topical applications of ethanol on the skin and inside the oral cavity. *J. Occup. Med. Toxicol.* **3**, 26.
- Lashmar, U. T., Hadgraft, J. and Thomas, N. (1989) Topical application of penetration enhancers to the skin of nude mice: a histopathological study. *J. Pharm. Pharmacol.* **41**, 118-122.
- Lauer, A. C., Lieb, L. M., Ramachandran, C., Flynn, G. L. and Weiner, N. D. (1995) Transfollicular drug delivery. *Pharm. Res.* **12**, 179-186.
- Marwah, H., Garg, T., Goyal, A. K. and Rath, G. (2016) Permeation enhancer strategies in transdermal drug delivery. *Drug Deliv.* **23**, 564-578.
- Patel, A. D. and Prajapati, N. K. (2012) Review on biochemical importance of vitamin-u. *J. Chem. Pharm. Res.* **4**, 209-215.
- Rachakonda, V. K., Yerramsetty, K. M., Madihally, S. V., Robinson, R. L., Jr. and Gasem, K. A. (2008) Screening of chemical penetration enhancers for transdermal drug delivery using electrical resistance of skin. *Pharm. Res.* **25**, 2697-2704.
- Scherb, J., Kreissl, J., Haupt, S. and Schieberle, P. (2009) Quantitation of s-methylmethionine in raw vegetables and green malt by a stable isotope dilution assay using LC-MS/MS: comparison with dimethyl sulfide formation after heat treatment. *J. Agric. Food Chem.* **57**, 9091-9096.
- Seo, J. E., Kim, S. and Kim, B. H. (2016) *In vitro* skin absorption tests of three types of parabens using Franz diffusion cell. *J. Expo. Sci. Environ. Epidemiol.* [Epub ahead of print].
- Seri, K., Amemiya, K., Sugimoto, H. and Kato, T. (1979) Effects of s-methylmethionine (vitamin u) on experimental nephrotic hyperlipidemia. *Arzneimittelforschung* **29**, 1517-1520.
- Sintov, A., Ze'evi, A., Uzan, R. and Nyska, A. (1999) Influence of pharmaceutical gel vehicles containing oleic acid/sodium oleate combinations on hairless mouse skin, a histological evaluation. *Eur. J. Pharm. Biopharm.* **47**, 299-303.
- Sokmen, B. B., Tunali, S. and Yanadaq, R. (2012) Effect of vitamin u (s-methyl methionine sulphonium chloride) on valproic acid induced liver injury in rats. *Food Chem. Toxicol.* **50**, 3562-3566.
- Songkro, S. (2009) An overview of skin penetration enhancers: penetration enhancing activity, skin irritation potential and mechanism of action. *Songklanakarinn J. Sci. Technol.* **31**, 299-321.
- Trommer, H. and Neubert, R. H. (2006) Overcoming the stratum corneum: the modulation of skin penetration. *Skin Pharmacol. Physiol.*

ol. **19**, 106-121.

Valiveti, S., Hammell, D. C., Earles, D. C. and Stinchcomb, A. L. (2004) Transdermal delivery of the synthetic cannabinoid WIN 55,212-2: *in vitro* *in vivo* correlation. *Pharm. Res.* **21**, 1137-1145.

Wahlberg, J. E. (1968) Transepidermal or transfollicular absorption? *In vivo* and *in vitro* studies in hairy and non-hairy guinea pig skin with sodium ( $^{22}\text{Na}$ ) and mercuric ( $^{203}\text{Hg}$ ) chlorides. *Acta Derm. Venereol.* **48**, 336-344.