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TRANSDERMAL DELIVERY OF RISEDRONATE USING CHEMICAL ENHANCERS FOR IMPROVED SKIN PENETRATION

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ABSTRACT

Background: Risedronate sodium (RIS) is effective for bone diseases but has low bioavailability and severe side effects. This study investigates the use of hydrophilic enhancers to improve the efficiency of RIS's transdermal delivery. **Methods:** This study involved preparing topical samples of RIS with various enhancers, including ethanol (EtOH), dimethyl sulfoxide (DMSO), Dimethylene glycol monomethyl ether (DGME), and propylene glycol (PG). In vitro permeation tests were conducted using hairless mouse skin in Franz diffusion cells, and skin irritation tests were performed on mice. **Results:** The cumulative amount of RIS after 24 hours significantly increased with penetration enhancers: 6.02 µg/cm² (RIS alone), 90.22 µg/cm² (20% DGME), 67.31 µg/cm² (20% PG), 266.31 µg/cm² (20% DMSO), and 784.52 µg/cm² (20% EtOH). EtOH showed a dose-dependent increase, with 1,302.76 µg/cm² at 50% concentration. Further experiments using DMSO and EtOH at concentrations of 5% and 10% identified the optimal permeation enhancement as follows: 201.36 ± 31.6 µg/cm² (5% DMSO), 183.03 ± 31.6 µg/cm² (10% DMSO), 261.71 ± 164.93 µg/cm² (5% EtOH), 569.21 ± 197.67 µg/cm² (10% EtOH). **Discussion:** EtOH and DMSO significantly enhanced RIS penetration by modifying the skin's structure. The study suggests that adjusting the concentration of these enhancers can control the penetration profile, offering a promising alternative to oral delivery. **Conclusions:** This study demonstrated that chemical enhancers significantly improved the skin penetration of RIS. The transdermal delivery of RIS can help reduce the side effects of oral delivery of the drug and thus improve patients' compliance.

INTRODUCTION

Risedronate sodium (1-hydroxy-2[3-pyridinyl] ethylidene bisphosphonic acid monosodium salt, RIS) has been approved for treating various metabolic bone diseases such as osteoporosis, Paget's disease [1,2]. RIS inhibits bone resorption by binding to hydroxyapatite and deactivating the enzyme farnesyl pyrophosphate synthase (FPPS), subsequently preventing osteoclast-mediated bone resorption [3]. Despite its potent therapeutic effects, RIS has low bioavailability and requires inconvenient administration protocols, including maintaining an upright position and avoiding food intake for several hours after dosing [4]. Furthermore, adverse effects include esophagitis, acid reflux, and atypical fractures. Long-term use can lead to bisphosphonate-related osteonecrosis of the jaw (BRONJ) [5,6]. Long-term use of bisphosphonates can result in severe adverse effects, including bisphosphonate-

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related osteonecrosis of the jaw (BRONJ). This disorder has been reported during tooth extraction and also after long-term usage of bisphosphonates [7]. Considering the poor compliance and adverse effects, an alternative administration route for RIS should be developed to eliminate the problems associated with oral administration. Transdermal drug delivery has been considered a suitable alternative to oral delivery of drugs and hypodermic injections [8], potentially reducing gastrointestinal issues and enhancing bioavailability [9,10]. The skin barrier, specifically the stratum corneum (SC), is a highly effective barrier that restricts the passage of drugs to small, moderately lipophilic molecules [11,12]. RIS has a small molecular weight, but due to the phosphate group, it is highly ionized in aqueous solutions, making it highly soluble in water. On the other hand, it has low affinity for lipids. The partition coefficient of RIS has not been thoroughly investigated due to its low solubility in octanol [13]. Due to their structural characteristics, RIS and other bisphosphonate drugs have limited suitability for topical administration. The limited permeability of RIS is a primary factor contributing to the scarcity of research on its transdermal delivery. Numerous studies have explored transdermal delivery strategies for RIS, including nanocarrier-based drug delivery systems and microneedle technologies [14, 15]. However, commercialization remains unrealized, likely due to manufacturing complexities and a lack of long-term stability verification for these approaches. In general, the initial strategies to enhance drug permeability through the skin involved the use of chemical agents called penetration enhancers. These substances increase drug permeation by altering the skin's barrier properties [16,17]. Despite extensive research on RIS delivery systems, prior studies have not systematically investigated their skin penetration profile using traditional chemical enhancers. Therefore, this study addresses this gap by investigating clinically available hydrophilic, water-soluble penetration enhancers such as EtOH, DMSO, PG, DGME, and NMP, which facilitate drug permeation by modulating the skin's barrier properties [16,17]. The efficacy of these agents was validated through in vitro skin penetration tests using hairless mouse models.

MATERIALS AND METHODS

Materials

RIS was sourced from Langfang Shinya Chemicals Co. Ltd. (Hong Kong, China). Diethylene glycol monomethyl ether (DGME) and propylene glycol (PG) were obtained from Daejung Chemicals Co. Ltd. (Seoul, Korea). Dimethyl sulfoxide (DMSO), *N*-methyl-2-pyrrolidone (NMP), and ethanol (EtOH) were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA), and High-performance liquid chromatography (HPLC)-grade solvents were purchased from J.T. Baker (Mallinckrodt Baker Inc., Phillipsburg, NJ, USA). Distilled water was used in all experiments.

Preparation of topical samples

All the chemical enhancers used in this study were water-soluble. First, RIS was dissolved in distilled water, and its pH was adjusted to 7.4, 6.0, and 4.0 using diluted sodium hydroxide and hydrochloric acid solutions. Subsequently, the chemical enhancers were added to the primary solution containing RIS and incubated for 1 day at room temperature before conducting the *in vitro* penetration test. The concentration of RIS in the aqueous solution containing different enhancers was 2% (w/w).

In vitro permeation test

Hairless mice were obtained from Orient Bio Inc. (Seoungnam, Gyeonggi, Korea). The animals were euthanized, and their full-thickness skins were harvested. Subcutaneous fat and subdermal tissues were meticulously removed. The skin samples were washed with phosphate-buffered saline (PBS) & stored at -20°C for up to four weeks before use. Before conducting the in vitro penetration tests, we inspected the hairless mouse skin for any perforations by placing it on a Franz diffusion cell and using a flashlight. The tests were performed using a Franz diffusion cell with an area of 2.01 cm^2 and a volume of 10 mL. Normal saline (0.9% sodium chloride solution) served as the medium for the receptor. The prepared sample containing RIS (200 µg) was applied to the skin. Subsequently, 0.5-mL aliquots were collected from the receptor part of the diffusion cells at specified intervals of 2, 4, 8, 12, and 24 hours, and the cells were refreshed with fresh medium. All experiments were performed at 37 ± 1°C and stirred at 200 rpm by using magnetic stirring bars for 24 h.

Skin irritation test

The hairless mice used in the skin irritation tests were also purchased from Orient Bio Inc. Samples containing RIS and enhancers were applied to the dorsal surface of the mice once daily for 3 days. The total amount of sample used was 100 µg, containing 2 µg of RIS. To assess the degree of irritation, all mice were photographed at 0, 24, 48, and 72 hours. The reactions were categorized as negative, mild (characterized by erythema

alone), moderate (involving erythema accompanied by edema), or severe (exhibiting erythema, edema, and vesiculation). All animal experiments were conducted in compliance with the guidelines set by the Seoul National University Institutional Animal Care and Use Committee (SNU-160805-11).

HPLC analysis

The amount of RIS was analyzed using high-performance liquid chromatography (HPLC). The HPLC system employed was an Agilent 1100, equipped with a pump and a detector set to wavelengths of 262 nm for excitation and 360 nm for emission. Chromatographic separation was facilitated by a Waters C18 column (150 mm × 4.6 mm, 5 µm) using a mobile phase composed of an aqueous solution containing 5 mM sodium pyrophosphate and 5 mM tetra-n-butyl ammonium hydrogen bromide at a pH of 7.0, mixed with acetonitrile in a ratio of 93:7. The sample was analyzed at a flow rate of 1 mL/min, with a total analysis time of 30 minutes.

Data analysis

Steady-state flux (J_s; µg/cm^2/h), lag time (T_L), and permeability coefficient (P; cm/h) are defined by the following equations:

$$Js = dQr/Adt \qquad P = Js/Cs$$

dQr: *the change in quantity of the drug;* ***A:*** *the effective diffusion area;* ***dt:*** *the change in time;* ***Cs:*** *the solubility of the donor solutions (initial drug concentration in their donor phase)*

All quantitative experiments were conducted at least three times. Statistical analysis was performed using the GraphPad Prism 5 (GraphPad Software Inc.). All results are indicated as the mean and Standard deviation.

RESULTS

The enhancing effect of various penetration enhancers

We investigated the effect of chemical enhancers, including EtOH, DGME, DMSO, NMP, and PG, on hairless mouse skin. For preliminary investigations, samples were prepared with a concentration of 2% RIS and 20% chemical enhancers. The structures of the permeation enhancers are illustrated in Figure 1. Initially, *in vitro* permeation tests were conducted for RIS with 20% enhancers to evaluate the enhancement effects of these compounds. The permeation parameters of RIS with 20% penetration enhancers are presented in Figure 2. After 24 hours, the cumulative amount of RIS was determined as follows: 6.02± 4.51 µg/cm^2 (RIS only), 90.22 ± 24.99 µg/cm^2 (RIS in 20% DGME), 67.31 ± 45.36 µg/cm^2 (RIS in 20% PG), 266.31 ± 47.80 µg/cm^2 (RIS in 20% DMSO), and 784.52 ± 41.66 µg/cm^2 (RIS in 20% EtOH). The permeation profiles of RIS with 20% DMSO and RIS with 20% EtOH were higher than those of RIS with other enhancers.

In vitro penetration tests using various concentrations of enhancers

We performed additional permeation tests on RIS with varying concentrations of DMSO and EtOH to determine the optimal concentration of these two enhancers. Figure 3 and Table 1 describe the results of the *in vitro* permeation tests. Based on the results of the preliminary *in vitro* tests using 20% enhancers, we concluded that DMSO and EtOH are appropriate enhancers for RIS. The amounts of RIS penetrated using 5% and 10% DMSO were found to be 201.36 ± 31.6 µg/cm² & 183.03 ± 31.6 µg/cm², respectively, after 24 hours; these amounts were lower than those of RIS penetrated by the cells when RIS was used with 20% DMSO.

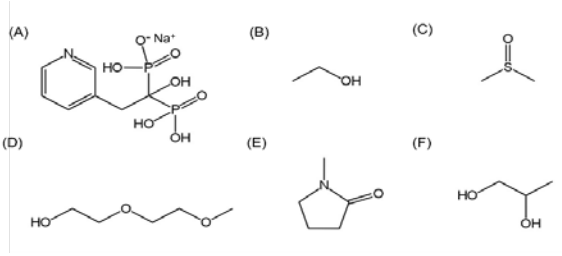

Figure 1: The chemical structures of RIS (A) and EtOH (B), DMSO (C), DGME (D), NMP (E), and PG (F)

Generally, when DMSO is administered as a penetration enhancer, it must be administered at a concentration of 60% to exhibit its enhancing effects [18]. We also evaluated the penetration of RIS in combination with 50% DMSO; however, 50% DMSO was not suitable for the sufficient dissolution of RIS, and thus, it formed precipitates.

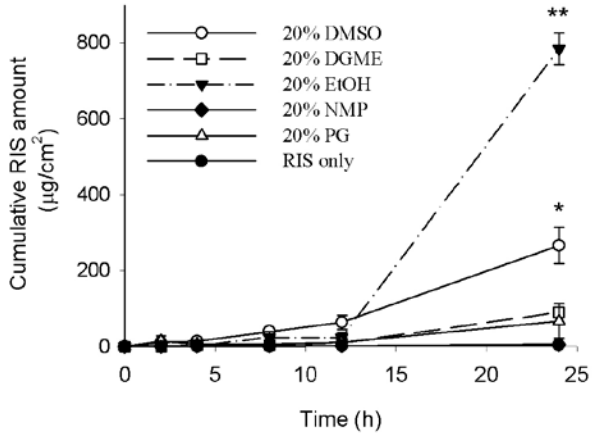

Figure 2: In vitro skin penetration test of RIS in combination with 20% of the enhancers such as EtOH, NMP, DGME, PG,

and DMSO. All the samples were prepared with 2% RIS, and the pH was adjusted to 7.4. Normal saline was used as the medium for the receptor. Results are presented as the mean and the standard deviation (n=3). (*: p < 0.05, **: p < 0.005). Significance was analyzed by comparison with the RIS-only group.

In addition to DMSO, 20% EtOH showed a high enhancing effect on the penetration of RIS. We performed the penetration tests at the concentrations of 5%, 10%, 20%, and 50% of the enhancers. Unlike 50% DMSO, 50% EtOH was an appropriate co-solvent that could sufficiently dissolve RIS. The *in vitro* permeation profiles are shown in Figure 3, which shows that the cumulative concentrations of RIS penetrating into the cells at different concentrations of EtOH after 24 h were 261.71 ± 164.93 μg/cm^2 (5% EtOH), 569.21 ± 197.67 μg/cm^2 (10% EtOH), 784.52 ± 41.66 μg/cm^2 (20% EtOH), and 1,302.76 ± 82.36 μg/cm^2 (50% EtOH). These results showed that RIS with 50% EtOH induced the highest penetration of RIS. Lag times were similar for all EtOH concentrations, except for 50% EtOH, which exhibited a short lag time of 4.08 ± 2.83 min.

In vitro penetration tests under different pH conditions

The results of the skin penetration test are presented in Fig. 4. The observed amounts of RIS after 24 h were 9.69 ± 3.51 μg/cm^2 (with 20% DMSO) and 25.17 ± 9.54 μg/cm^2 (with 20% EtOH) at pH 6.0, and it was 6.23 ± 0.30 μg/cm^2 (with 20% DMSO) and 9.30 ± 1.58 μg/cm^2 (with 20% EtOH) at pH 4.0. These results show that the amount of RIS penetrating the cells was lower at pH 6.0 and 4.0 than at pH 7.4. Ionization of RIS decreases at low pH because of the deprotonation of the phosphonate groups of RIS; this, in turn, explains the decreased flux.

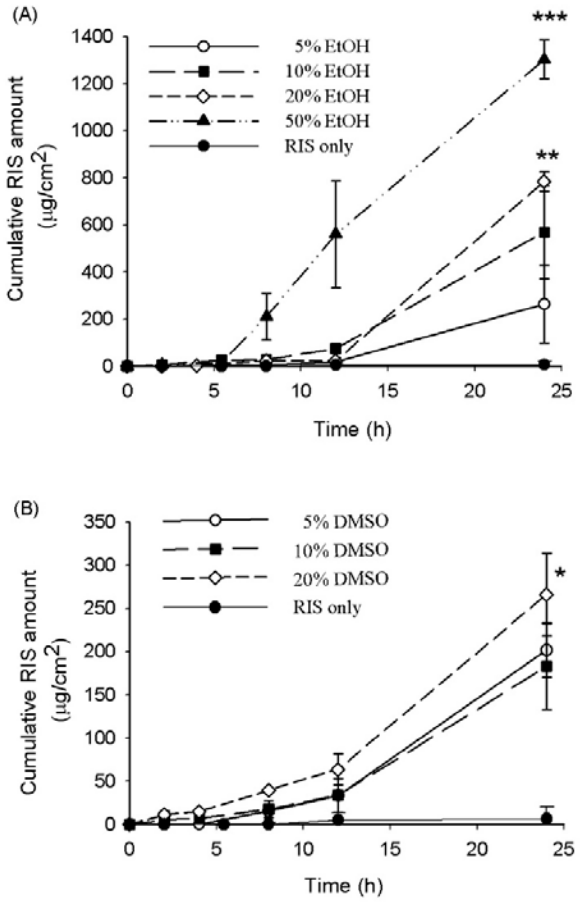

Figure 3. In vitro skin penetration test of RIS with EtOH (A) and DMSO (B) at various concentrations (5%, 10%, 20%, and 50%). All the samples were prepared with 2% RIS, and the pH was adjusted to 7.4. Normal saline was used as the medium for the receptor. Results are presented as the mean and the standard deviation (n=3). (*: p < 0.05; **: p < 0.005, ***: p < 0.001, Significance was analyzed by comparison with RIS only group)

Table 1: Permeation parameters of 2% RIS across excised hairless mouse skin in the presence of chemical enhancers. Q_{12} (μg/cm²): the cumulative amount of the drug permeated for 12 hours.

	Flux (μg/cm²·h)	Lag Time (h)	Q_{12} (μg/cm²)	P × 10³ (cm/h)
20% DGME	5.8 ± 1.21	8.7 ± 2.20	9.3 ± 2.05	0.2 ± 0.03
20% NMP	0.3 ± 0.10	7.9 ± 2.44	2.4 ± 3.90	0.0 ± 0.00
20% PG	4.0 ± 2.69	7.6 ± 0.04	11.5 ± 7.94	0.1 ± 0.01
5% DMSO	12.2 ± 2.28	7.8 ± 0.48	3.6 ± 2.30	0.3 ± 0.06
10% DMSO	10.8 ± 2.53	7.6 ± 0.92	8.4 ± 3.81	0.3 ± 0.07
20% DMSO	14.8 ± 2.77	6.3 ± 0.04	63.7 ± 18.07	0.4 ± 0.07
5% EtOH	17.0 ± 0.81	9.0 ± 0.16	18.8 ± 9.92	0.4 ± 0.28
10% EtOH	36.0 ± 13.30	8.3 ± 0.55	75.0 ± 0.91	0.9 ± 0.35
20% EtOH	51.5 ± 2.84	9.3 ± 0.04	12.9 ± 13.86	1.3 ± 0.07
50% EtOH	66.8 ± 8.59	4.1 ± 2.83	560.9 ± 227.96	1.7 ± 0.22

Skin irritation test

Following the application of RIS with various enhancers, a skin irritation test was conducted. Despite RIS with 50% EtOH demonstrating the highest permeation rate, its use in formulations was ruled out due to its potential to irritate [16,19]. Consequently, we selected RIS with 20% DMSO and 20% EtOH for further evaluation in the skin irritation test. The application of 2% RIS with 20% DMSO and 20% EtOH resulted in no observable irritation (Figure 5). Inspection of the application site indicated that the skin appeared clear and unaffected.

DISCUSSION

The purpose of this study is to investigate the feasibility of developing a transdermal delivery agent for RIS, an osteoporosis treatment, utilizing already proven enhancers. Although various enhancers were available, we chose the water-soluble and commonly used enhancers because RIS is water-soluble and has poor lipophilicity. EtOH and DMSO are the most widely studied penetration-enhancing molecules [16,18]. EtOH enhances permeability through a dual-action structural modification, expanding inter-lipid chain spacing in the SC by 0.3 nm to create transient diffusion channels. It denatures keratin proteins, thereby reducing barrier resistance [20]. Additionally, PG, DGME, and NMP are also widely used as penetration enhancers for transdermal delivery [21-23]. Propylene glycol (PG) exhibits a similar mechanism to Ethanol, targeting the polar head regions of SC lipids to disrupt hydrogen bonding networks [24]. This induces lipid bilayer disorder and enhances drug solubility at aqueous-lipid interfaces. DGME destabilizes the lipid-protein interactions while acting as a biphasic cosolvent. Its amphiphilic nature optimizes drug solubility across hydrophilic and hydrophobic SC domains, achieving peak efficacy at 24% concentration [25]. NMP synergizes lipid fluidization with molecular complexation. At 10% concentration, it forms supramolecular drug complexes via hydrogen bonding and π-π interactions, enhancing SC partitioning while minimizing irritation risks-a critical advantage over higher concentrations (>15%) associated with protein denaturation [26].

Although the optimal concentration of each enhancer may vary, experiments were conducted using a 20% concentration for all enhancers to allow for relative comparison. RIS with DGME & PG also exhibited enhanced effects; however, the enhancing effects of DGME and PG were lower than those of DMSO & EtOH. These results showed that RIS may be developed for transdermal use by using various chemical enhancers.

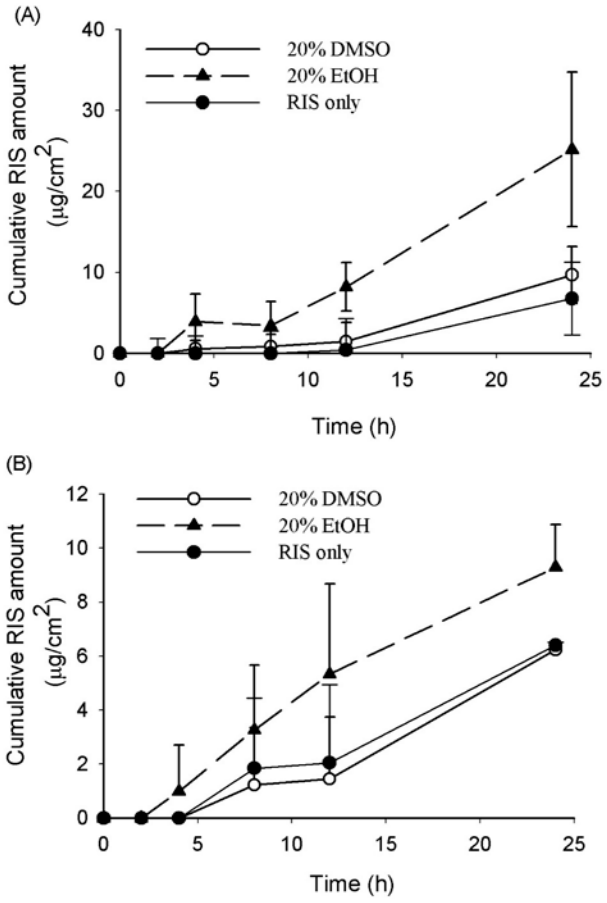

Figure 4: In vitro skin penetration test of RIS alone and RIS with 20% ethanol and 20% DMSO at pH 6.0 (A) and 4.0 (B). All the samples were prepared with 2% RIS. Normal saline was used as the medium. Results are presented as the mean and the standard deviation (n=3).

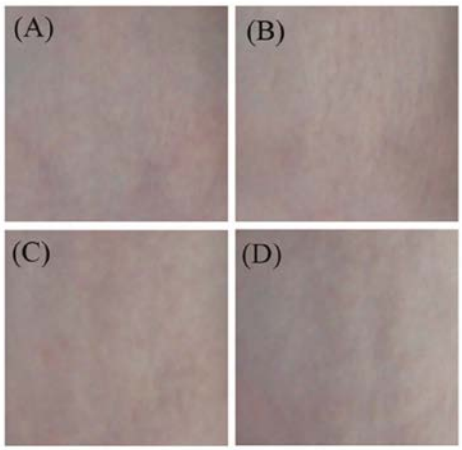

Figure 5: Skin irritation test performed on the dorsal surface of the hairless mice. The images were acquired 4 days after the dorsal surface of the skin was treated with 20% EtOH (A), 20% DMSO (B), RIS in 20% EtOH (C), and RIS in 20% DMSO (D). The samples were administered every day for 3 days.

DMSO is well recognized for its ability to enhance the permeation of numerous drugs and has been extensively utilized across various fields of pharmaceutical science. DMSO functions by modifying the keratin in the SC and other proteins involved in the structural organization of the skin, thereby facilitating drug penetration into the skin layers [27]. DMSO preferentially fluidizes the SC lipid bilayers, disrupting their ordered architecture to enhance drug partitioning, which is maximized at a concentration of 5% [28]. Although the degree of penetration enhancement by DMSO was less pronounced compared to EtOH, it was notable that only a 5% concentration of DMSO was sufficient to induce enhanced permeation of RIS through the hairless mouse skin, unlike other enhancers.

RIS is a negatively charged molecule that easily reacts with metal ions, which prevents its penetration into the SC. Thus, the pH of the vehicle is a crucial factor influencing the permeation of RIS. RIS has 5 pKa values of 1.6, 2.2, 5.9, 7.1, and 11.7. Theoretically, four hydrogen atoms of the phosphonate group are dissociated and form a negative charge at physiological pH. To investigate the effect of pH, we analyzed the permeability of RIS at pH 6.0 and 4.0. Severe pH conditions, such as those with a pH below 4.0 (highly acidic) and above 8.0 (highly alkaline), are not favorable for pharmaceutical products, particularly those intended for long-term use or use by the elderly. Several studies have indicated that the maintenance of the SC is pH-dependent and well-tolerated within a range of 4 to 6 and at least 7.4 [29,30]. EtOH is widely used in the preparation of topical formulations of drugs and cosmetics. Similar to water, EtOH permeates rapidly through the human skin [31]. EtOH enhances permeability through dual-action structural modifications: It expands inter-lipid chain spacing in the SC by 0.3 nm, creating transient diffusion channels and denaturing keratin proteins, thereby reducing barrier resistance and facilitating the drug's entry into the SC [20].

In essence, EtOH modifies the drug's solubility in the vehicle and enhances its partitioning into the SC [32]. Furthermore, the rapid flux of EtOH leads to its depletion from the vehicle, which subsequently increases the concentration of RIS. Thus, higher concentrations of RIS lead to a supersaturated state, which modifies the thermodynamic activity [33,34]. Due to these reasons, RIS with EtOH exhibits a high penetration and release profile. The highest penetration amount of RIS in 50% EtOH explained the synergistic effect of the drag effect, thermodynamic effect, and skin fluidity due to the altered structure of the SC. Therefore, it is possible to control the penetration profile by adjusting the concentration of EtOH. RIS is more soluble in water under alkaline conditions because of ionization. Generally, the penetration of RIS across the SC decreases when the degree of ionization increases. However, the more RIS was ionized, the more easily it dissolved in aqueous solutions. For example, the flux of 5-fluorouracil was increased at a higher pH, which was related to ionization and solubility in the vehicles [35]. Therefore, it can be concluded that the enhancing effects of DMSO and EtOH can be maximized at pH 7.4 among these conditions. In this study, the skin irritation experiment also did not show any signs of irritation. Additionally, it has been reported that when RIS and alendronate were exposed to an open skin test involving friction and puncture on human skin, no signs of irritation or other adverse reactions were observed. Although the experiments and results are not sufficient to develop a transdermal system for RIS, the possibility of using RIS to deliver drugs via an alternative, easy, and convenient route appears promising.

CONCLUSION

This study showed that various enhancers could significantly influence the skin penetration of RIS. RIS showed the best penetration profile through the hairless mouse skin with 50% EtOH. RIS with 20% EtOH and 20% DMSO also caused enhanced flux of RIS, and they could be potential candidates for the topical delivery of RIS. Based on these results, we concluded that RIS penetrates sufficiently when delivered with chemical enhancers. Furthermore, no skin irritation was observed when RIS was used with these enhancers. The enhanced skin penetration of RIS can help reduce the side effects associated with oral drug delivery and thus improve patient compliance.

FINANCIAL ASSISTANCE

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTION

So Hee Nam collected the data, performed experiments, and drafted the manuscript. So Hee Nam made all contributions to this article.

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