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Ion pairs of risedronate for transdermal delivery and enhanced permeation rate on hairless mouse skin

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ABSTRACT

Ion-paired solutions of risedronate (RIS) with L-arginine (ARG), L-lysine (LYS), and diethylenetriamine (DETA) were tested *in vitro* for their potential to enhance the penetration of RIS across the skin of hairless mouse. The xylene solubilities of RIS paired with ARG, LYS, and DETA in molar ratios of 1:2, 1:2, and 1:1 were 8.9%, 12.0%, and 2.1%, respectively, in comparison with the solubility in deionized water, but non-ion-paired RIS was not detected in xylene. *In vitro* permeation tests were performed on the skin of hairless mice, and the results indicated that ion-paired RIS could penetrate mice skin about 36 times more effectively than RIS alone. The cumulative amount of ion paired RIS after 24 h resulted in $475.18 \pm 94.19\ \mu g/cm^2$ and $511.21 \pm 106.52\ \mu g/cm^2$ at molar ratio of 1:2 and 1:1. The cumulative amount of RIS alone was as low as $14.13 \pm 5.49\ \mu g/cm^2$ in 24 h. The hairless mice showed no skin irritation after a single administration of RIS alone and ion-paired RIS (1:2 molar ratio with ARG, and 1:1 molar ratio with DETA). In this study, we found that RIS can be delivered transdermally, and the ion-paired system in an aqueous solution showed an enhanced flux through the skin barrier.

1. Introduction

Risedronate (RIS) is a well-known inhibitor of osteoclastic activity and is widely used in the clinical treatment of various system metabolic bone diseases, mainly osteopenia and osteoporosis. In both animals and humans, RIS inhibits bone resorption and thereby slows down the process of bone loss and also maintains bone mass, bone microstructure, and strength in the relevant anatomical sites such as the femur and vertebra. RIS works by blocking the action of the enzyme farnesyl pyrophosphate synthase (FPPS) (Assael, 2009; Ruggiero and Drew, 2007). This reduces osteoclastic bone resorption via accumulation of unprenylated small GTPases within the osteoclast. However, RIS and other bisphosphonates (BPs), which have been chosen for the treatment of osteoporosis, have a number of limitations in administration. RIS has a bioavailability of less than 0.5%, and its absorption rate is reduced by the co-administration of other drugs or food (Mitchell et al., 1999). RIS can also cause esophagitis, abdominal pain, acid reflux, acute phase reactions, bone, muscle and joint pain, and atypical fragility fractures (Abrahamsen, 2010; Solomon et al., 2009). Therefore, patients treated with RIS experience various types of adverse effects. The most serious adverse effect of RIS is bisphosphonate-related osteonecrosis of the jaw (BRONJ), which results from the ingestion of the drug. This disorder was reported during tooth extraction and also after long-term usage of BPs (Brooks et al., 2007; Ruggiero et al., 2004). Thus, it is necessary to develop an alternate administration route to eliminate the problems caused by oral administration of RIS.

Transdermal drug delivery is one of the alternative routes to prevent or reduce the adverse effects of RIS and increase its bioavailability. Because of the lower incidence of gastrointestinal problems associated with this route of administration, it is more advantageous than oral delivery and hypodermic injection (Brown et al., 2006; Prausnitz and Langer, 2008; Ramachandran and Fleisher, 2000). Thus, transdermal delivery of RIS has solved or reduced many problems associated with the other modes of delivery.

RIS is soluble in water, but practically insoluble in almost all types of organic solutions. Moreover, RIS is a highly ionized and very acidic molecule. Therefore, it may have a low permeation flux in transdermal administration. To date, there has been little research on the transdermal delivery of RIS because of its poor lipophilicity and the skin barrier. The skin barrier, stratum corneum (SC), is a very strong barrier system (Forslind, 1994). It has an excellent barrier function that allows only small, moderately lipophilic, and highly potent molecules to be administered via the transdermal route (Naik et al., 2000).

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One approach to overcome this limitation is to create ion pairs by combining an oppositely charged species with a charged permeant. In theory, this gives rise to a neutralized complex, which partitions into the membrane and may dissociate to liberate the charged species (Neubert, 1989; Valenta et al., 2000). The formation of ion pairs can increase the penetration of drugs through the skin by decreasing the charge and increasing the hydrophobicity (Barry, 2001; Wang et al., 2008). Therefore, we considered an ion-pairing system to enhance the flux of RIS through the skin. Pairing with a counter ion reduced the degree of charge of RIS and increased both hydrophobicity and lipophilicity.

The objective of this study was to investigate the possibility of transdermal delivery of RIS and to verify the enhancing effect of the RIS ion-pair system by performing *in vitro* penetration tests on the skin of hairless mice.

2. Materials and methods

2.1. Materials

RIS (risedronate monosodium) was purchased from Langfang Shinya Chemicals Co., Ltd. (Hongkong, China). ARG, LYS, DETA, PEG, xylene, propylene carbonate, and 9-diethylamino-5Hbenzo[*a*]phenoxazine-5-one (Nile red dye) were purchased from Sigma Chemicals Co. Ltd. (St. Louis, MO, USA). High performance liquid chromatography (HPLC)-grade solvents were purchased from J.T. Baker (Mallinckrodt Baker, Inc., Phillipsburg, NJ). Distilled water (DW) was used throughout the experiment.

2.2. Formation of ion pairs

RIS was dissolved in DW at a concentration of 80 mg/mL. ARG, LYS, and DETA were also dissolved in DW at molar ratios of 1:1, 1:2, and 1:4 with the 80 mg/mL solution of RIS as a reference. All the samples were adjusted to pH 7.4 by using a solution of diluted sodium hydroxide. Thereafter, equal volumes of a solution of RIS and counter ions were mixed, slightly stirred, and incubated overnight at room temperature. Then, the samples were mixed with 20% (w/w) PEG in a 1:1 ratio for the *in vitro* permeation test. The final aqueous solution contained 2% RIS (w/w) and equal amounts of the counter ion in 10% (w/w) PEG solution. Ion pairs of RIS with counter ions were represented to RIS_ARG (DETA or LYS) 1 equiv., RIS_ARG (DETA or LYS) 2 equiv. and RIS_ARG (DETA or LYS) 4 equiv. in molar ratio of 1:1, 1:2, and 1:4. For the skin-irritation tests, samples were prepared in 1% xhanthan gum to enhance their adhesiveness and viscosity.

2.3. Partitioning test of ion-paired risedronate

The partitioning of ion-paired RIS in the organic phase was determined in propylene carbonate/water and xylene/water systems. All the aqueous samples were prepared at pH 7.4. Initially, DW containing the counter ions was prepared at an appropriate molar ratio of RIS and then added to an appropriate organic solution at a 1:2 volume ratio. Then, the solutions were vigorously stirred for 24 h to saturate the 2 phases. DW containing the drug was then added to the primary solution such that the aqueous and organic phases had the same volume ratio and stirred at 200 rpm at 30 °C for 48 h until equilibrium was achieved. Subsequently, the final solution was centrifuged at 3000 rpm for 15 min, and the phases of the solution were separated to investigate the concentration of the drug in the organic and aqueous phases. The concentration of RIS was investigated by HPLC after appropriate dilutions with elution buffer. The experiments were performed in triplicate. All the data were expressed in terms of the relative amount of RIS in the organic phase (%).

$$\text{Relative amount of RIS in organic phase (\%)} = \frac{\text{total amount of TIS in xylene (or PC)}}{\text{total amount of RIS in DW}} \times 100$$

2.4. HPLC analysis

RIS was analyzed by HPLC. The HPLC system (1100, Agilent, USA) consisted of a pump with a detector set at 262 nm and 360 nm for excitation and emission. Chromatographic separation was achieved on Waters symmetry C18, 150 mm × 4.6 mm, 5 μm column by using a mobile phase containing a mixture of aqueous 5 mM sodium pyrophosphate and 5 mM tetra-*n*-butyl ammonium hydrogen bromide at pH 7.0, and acetonitrile in the ratio of 93:7. The sample was delivered at a flow rate of 1 mL/min, and the total run time was 30 min.

2.5. In vitro skin permeation test

Hairless mice were purchased from Orient Bio, Inc. (Seoungnam, Gyeonggi, Korea). The mice were killed and full-thickness skins were obtained. The subcutaneous fat and subdermal tissues were removed carefully. The skin was washed with PBS and stored at −20 °C up to a maximum of 4 weeks before use. *In vitro* release tests were performed using a Franz diffusion cell with a diffusion area of 2.01 cm^2 and receiver volume of 10 mL. Normal saline (0.9% sodium chloride) was used as the receptor medium. The sample (200 mg) was added onto the skin in the donor compartment. Then, 0.5-mL aliquots were collected from the receptor side of the diffusion cells at designated intervals of 2, 4, 8, 12, and 24 h and replaced with the fresh media. The receptor medium was maintained at 37 ± 1 °C and stirred at 200 rpm using magnetic stirring bars for 24 h. The animal study was approved by the animal care committee of Seoul National University.

2.6. Skin barrier integrity

The SC barrier integrity was performed through Nile red staining and water contents of skin specimens. After *in vitro* penetration test, hairless mice skin were obtained, washed slightly with PBS and prepared to frozen sections. Briefly, 10 μm thick cryostat sections were stained with Nile red solution (2.5 μg/mL in 75% glycerin) for 10 min. Specimens of the upper most skin were examined using fluorescence microscope because Nile red interacts with lipids such as phospholipids, cholesterol and triglycerides (Greenspan and Gutman, 1993). Next, water contents of skin after *in vitro* penetration test were evaluated by freeze drying, weighing and calculation. Before freeze drying, we eliminated the water remained in the skin surface sufficiently. All mice skins used *in vitro* were compared with both hairless mice skin prepared right after sacrifice and frozen hairless mice skin.

2.7. Skin irritation test

Hairless mice for skin irritation tests were purchased from Orient Bio, Inc. (Seoungnam, Gyeonggi, Korea). RIS was applied on the backside of the mice, and the skin response to RIS treatment was visually assessed. The samples were prepared using 2% RIS with DETA and ARG in molar ratios of 1:1 and 1:2 in 1% xhanthan for enhanced adhesiveness. The samples were applied for an hour on the backside of mice and carefully removed with gauze after 1 h. All the mice were photographed to validate the degree of irritation at 0, 24, 48, and 72 h. Reactions were graded as negative,

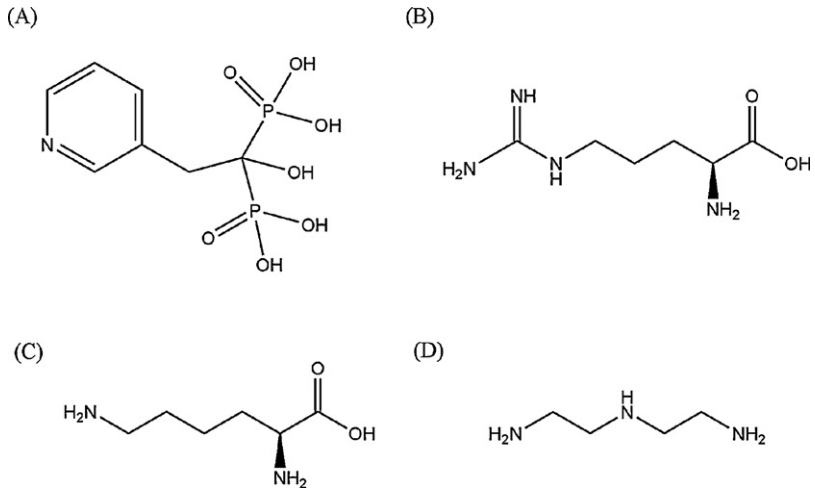

Fig. 1. The chemical structures of RIS (A), ARG (B), LYS (C), and DETA (D).

mild (erythema alone), moderate (erythema with edema), or severe (erythema, edema, and vesiculation).

2.8. Statistical analysis

We performed all the quantitative experiments at least in triplicate. The data were presented as mean values ± standard deviation of the mean. We assessed the statistical significance of the results by the two-tailed Student's t test. $p \leq 0.05$ was considered to indicate significant differences.

3. Results and discussion

3.1. Partitioning of ion-paired risedronate in organic phase

RIS was paired with ARG, LYS, and DETA, which have cationic charges under physiological conditions. All chemical structures of RIS and counter ions were represented in Fig. 1. These counter ions were reported to increase the penetration rate by the ion pair formation through the skin of hairless mice (Fini et al., 1999; Ruland and Kreuter, 1992). Partition coefficient is an important factor that determines the possibility of topical drug delivery (Banerjee et al., 1980; Barry, 1987). Previous studies have reported that ion-pairing with counter ions enhances the lipophilicity of a molecule (Takacs-Novak and Szasz, 1999). Generally, lipophilicity is determined by performing a partition coefficient test in octanol/water system. However, when we performed this test, we were unable to detect RIS in octanol (data not shown). Therefore, we chose to perform this test in xylene/water and propylene carbonate/water. Xylene has been used in an alternative lipophilic phase instead of octanol. Propylene carbonate is a well-known solvent that has the ability to enhance the flux of some molecules for topical delivery (Schroeder et al., 2007). The partitioning results of RIS in the organic phase are shown in Fig. 2. RIS was paired with ARG, LYS, and DETA at molar ratios of 1:1, 1:2, and 1:4, respectively. All the counter ions had the ability to solubilize RIS in the organic phase at different degrees. In the case of ARG, the solubility of ion pairs increased at molar ratios of 1:1 and 1:2, but ion pairing at a molar ratio of 1:4 resulted in a lower solubility than that observed in ion pairing at a molar ratio of 1:2 in all solvents. This is because the carboxylic acid group of ARG has an anionic charge at pH 7.4, and this interrupted the formation of an ion pair leading to a lower solubility. Ion pairing with DETA, however, showed increased solubility in both the phases. Although the results were different in each organic solvent, all ion-paired groups could dissociate in the organic phase.

3.2. Skin permeation of ion paired risedronate

We investigated the effect of ion pairing using hairless mice skin. For many years, hairless mouse skin has been used in experiments to investigate the flux through the skin layer in dermatology (Benavides et al., 2009). Hairless mouse skin is easily permeable than human skin, which is reliable for examining the skin transport

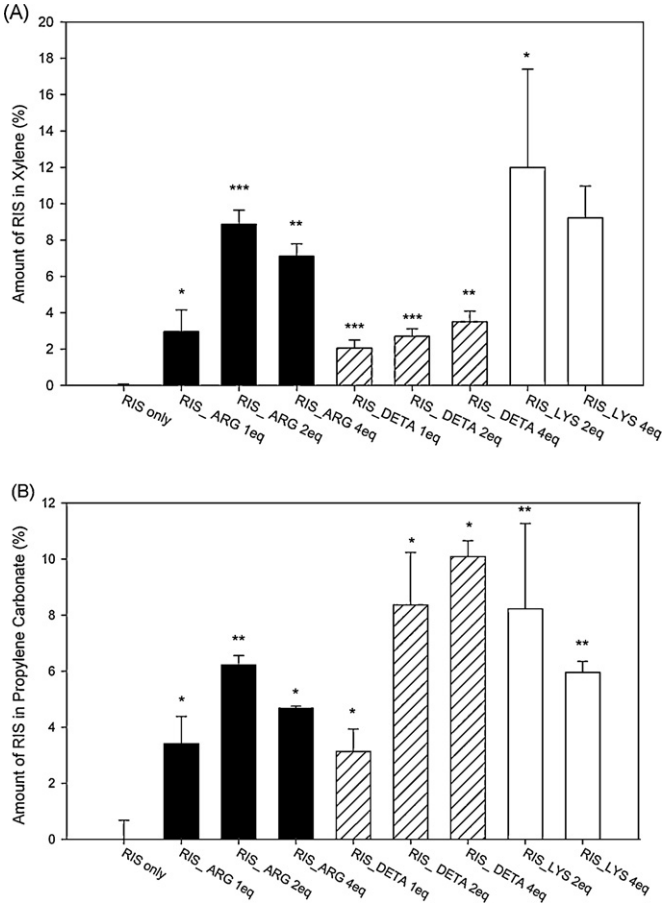

Fig. 2. Partitioning test of ion-paired RIS in xylene (A) and propylene carbonate (B); the concentration of RIS in the total solution (the mixture of organic phase and aqueous phase) was 2% (w/w). All the data were expressed in terms of the relative amount of RIS in the organic phase over the aqueous phase ($^{*}p \leq 0.05$, $^{**}p \leq 0.005$, $^{***}p \leq 0.0005$).

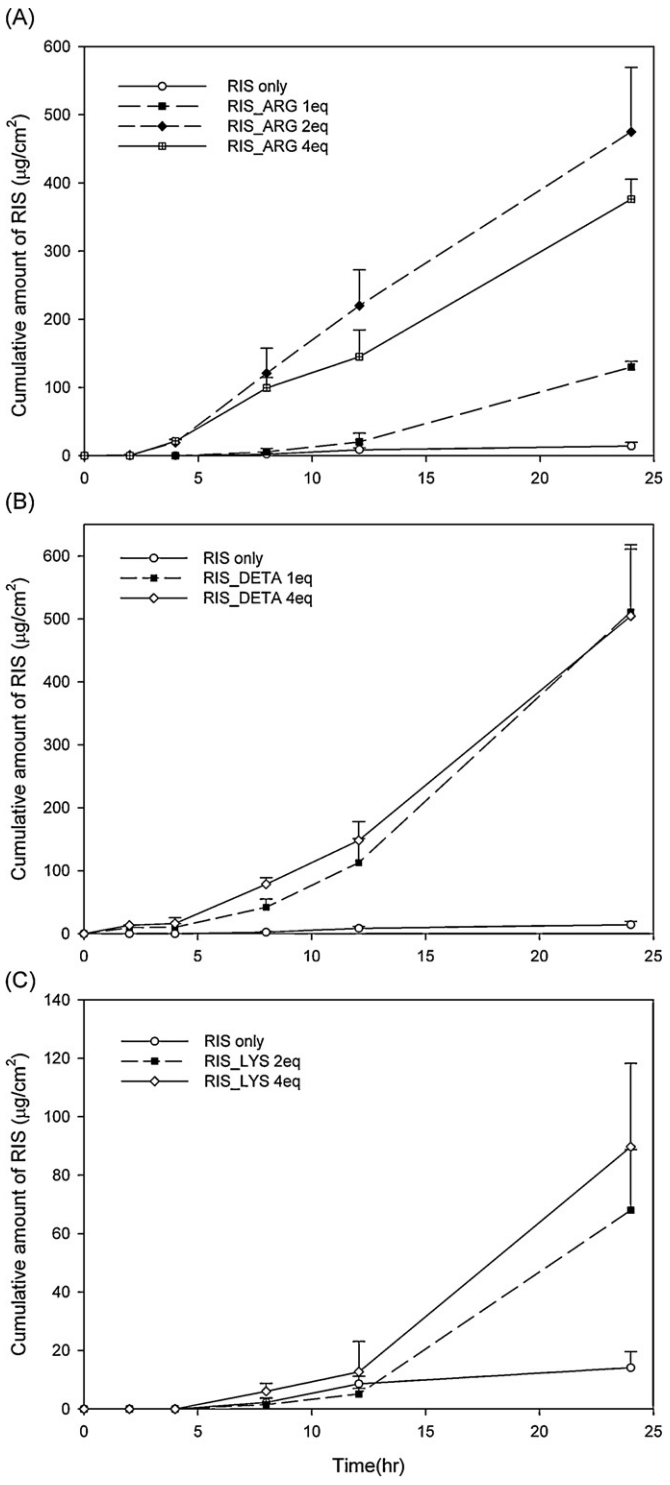

Fig. 3. *In vitro* skin penetration test of RIS only, RIS with ARG (A), RIS with DETA (B), and RIS with LYS (C). Samples were prepared in deionized water containing 10% PEG (Mw, 400) with 2% RIS adjusted at pH 7.4. Normal saline was used as the receptor medium.

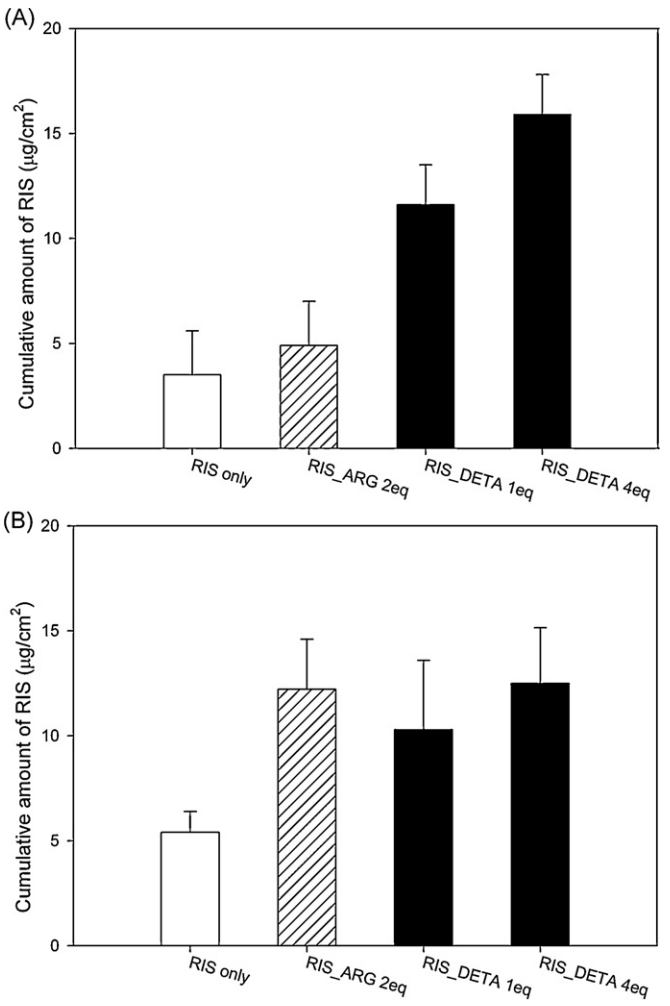

Fig. 4. *In vitro* skin penetration test; cumulative amount of RIS and ion-paired RIS of low concentration for 24 h. Concentration of RIS in the samples were 0.5% (A) and 1% (B).

of drug during initial development of a new drug or system (Kao et al., 1988). We chose hairless mouse skin for reasons such as that it is easily acceptable model with limited variability among individuals, and similar hair follicle density to human skin (Bronaugh et al., 1982).

The penetration profiles are shown in Fig. 3. The cumulative amount of RIS alone (2%, w/w) on the skin of hairless mice was as low as $14.13 \pm 5.49\ \mu g/cm^2$ in 24 h. The total amounts of RIS–ARG that penetrated through the mice skin were $129.93 \pm 8.43\ \mu g/cm^2$ and $475.18 \pm 94.19\ \mu g/cm^2$ when the molar ratios were 1:1 and 1:2, but the total amount of RIS–ARG that penetrated through mice skin was $376.14 \pm 29.20\ \mu g/cm^2$ when the molar ratio was 1:4. The inhibitory effect of excess ARG at molar ratios greater than 1:2 correlated with the results of the solubility test. Ion pairing with LYS at a ratio of 1:2 allowed penetration to $68.01 \pm 20.71\ \mu g/cm^2$, which was lower than the penetration obtained at any molar ratio of ARG. The flux of the RIS–LYS system showed no significant difference at molar ratios of 1:2 and 1:4 because the first amine group of LYS had a lower capacity for ion pairing with the phosphate group of RIS than the guanidyl group of ARG. In the case of DETA, the ion-paired RIS showed penetration rate of $511.21 \pm 106.52\ \mu g/cm^2$ and $504.73 \pm 103.32\ \mu g/cm^2$ at molar ratios of 1:1 and 1:2, respectively. In the case of the RIS–DETA ion pair, no changes in the penetration rate (increase or decrease) were observed with an increase of DETA. DETA at molar ratio of 1:1 is sufficient for enhancing the penetration rate of RIS. Unlike ARG, DETA has only amine groups and a cationic charge in an aqueous solution, and the primary amine is hydrogenated at physiological condition. Therefore, in this ion pair, molar ratios greater than 1:1 have no effect on the penetration of ion-paired RIS through the skin.

A permeant must partition into the membrane in order to cross the SC. This process of partitioning could be a rate-limiting step in the permeation process (Potts and Guy, 1992). Hydrophilic and

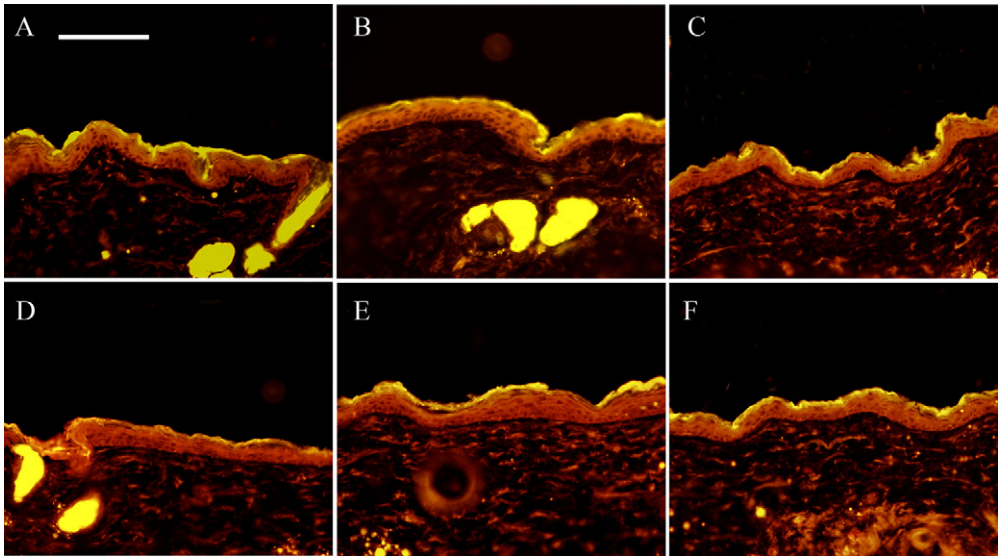

Fig. 5. Fluorescence microscopic examination with Nile red staining for lipids. Images represented skin prepared after sacrifice (A), frozen skin after sacrifice (B), 10% PEG treated skin (C), RIS only treated skin (D), RIS_ARG 2 equiv. treated skin (E) and RIS_DETA 1 equiv. treated skin (F). (C)–(F) were skin specimens used in *in vitro* penetration test. Scale bar = 50 μm.

ionized molecules are likely to permeate poorly via the intracellular route (Lauer et al., 1995), but they could penetrate the membrane through the shunt route (Barry, 2002). However, the amounts of these permeants may be somewhat low if the species were unionized and passed mainly through the lipoidal intercellular route. In theory, an ion-pairing complex is formed between oppositely charged molecules, and this bond leads to a decrease in the total charge, thus allowing the complex to partition into the membrane (Megwa et al., 2000). Thus, the ion-pairing system can allow RIS to partition in the organic phase. The balance between hydrophilicity and lipophilicity of drugs is very important for the topical delivery system. Drug flux is the product of the permeability coefficient and the effective drug concentration in the vehicle (Michaels et al., 1975). We paired 2% RIS with counter ions to enhance the liphophilicity of RIS, which in turn enhanced the permeability of the drug. Flurbiprofen flux has been reported to improve through the formation of an ion pair (Ma et al., 2010). In that study, amine derivatives were used as counter ions. An ion-paired solution of methotrexate in ARG/water/propyleneglycol was evaluated for enhanced permeation across the rabbit nasal mucosa (Ivaturi and Kim, 2009). Ion pairs of glipizide with amine derivatives also showed enhanced permeability through the abdominal skin of rats (Tan et al., 2009). In another study, transdermal delivery of adefovir, an acyclic nucleoside phosphonate, was represented to have enhanced flux through the porcine skin with 6-dimethylaminohexanoic acid dodecyl ester (DDAK) by the formation of an ion pair (Novotny et al., 2009; Vávrová et al., 2008).

However, Bjerrum's equation, which describes a critical separation distance for the formation of an ion pair, highlights the importance of the dielectric constants: a solvent with high dielectric constant such as water ($\varepsilon = 78.5$) is unfavorable for ion-pair formation (Prue, 1969). Although PEG has a low dielectric constant, a donor vehicle consisting of 90% water and only 10% PEG still had a high dielectric constant. Unfortunately, RIS cannot be completely solubilized in organic solutions. There are few hydrophobic or lipophilic solvents that can dissolve RIS sufficiently. That is why we chose water as the major vehicle. Although 10% PEG solution was considered as an undesirable system for ion pairing in theory, the results of *in vitro* penetration test showed the enhancing effect of counter ions in aqueous solution in case of RIS. We performed NMR analysis of RIS paired with ARG in D_2O at pH 7.4 and examined the peak broadening of the guanidyl group of ARG. However, we could not observe the chemical shift of RIS or ARG (data now shown). In this result, we concluded that the combination of RIS and counter ions was not sufficiently stable, but the 2 components actively interacted with each other. The results of the solubility and penetration tests support this hypothesis.

Additionally, we assessed the thermodynamic activity of vehicle using 20% and 40% PEG. Permeation of the solvent through the skin could alter the thermodynamic activity of drug in the vehicle which would be a driving force for diffusion (Wotton et al., 1985). RIS did not dissolve in 40% PEG solution sufficiently. Therefore, we performed the experiments using donor samples of RIS only, RIS_ARG 2 equiv. and RIS_DETA 1 equiv. in 20% PEG solution. All samples did not show the enhanced penetration rate compared with the samples using 10% PEG solution. The total amounts of RIS for 24 h were recorded 6.94 ± 2.98 μg/cm^2 without counter ion, 10.49 ± 3.45 μg/cm^2 with 1:2 molar ratio of ARG and 29.52 ± 5.23 μg/cm^2 with an 1:1 molar ratio of DETA. These results indicate that PEG had no enhancing effect as a vehicle even though it has higher concentration. Furthermore, the flux of ion paired RIS was decreased in 20% PEG, which may be explained by different solubilities of RIS, ARG, and DETA in PEG solution and changes of ion pair stability. In case of binary vehicles, optimization of vehicle composition is an important factor for transdermal delivery (Pardo et al., 1990). RIS is soluble in water and little soluble in PEG. In our result, 10% PEG was an adequate vehicle for ion paired RIS.

We also identified an appropriate drug concentration to allow effective ion pairing with counter ions. An appropriate concentration of RIS and counter ions is required because ion pair formation is slightly unstable in an aqueous solution. Fig. 4 shows that the ion pairing effect declined heavily at RIS concentrations less than 2%, which also explained the unstable ion-pairing system in water. Although ion-pair formations containing RIS are considered slightly unstable, they had a remarkable enhancing effect on RIS penetration into hairless mouse skin. In further studies, we plan to develop a stable vehicle for the ion pairing of RIS and counter ions.

3.3. Skin integrity

We investigated whether the donor samples or the process of *in vitro* penetration test affected the skin barrier by changing

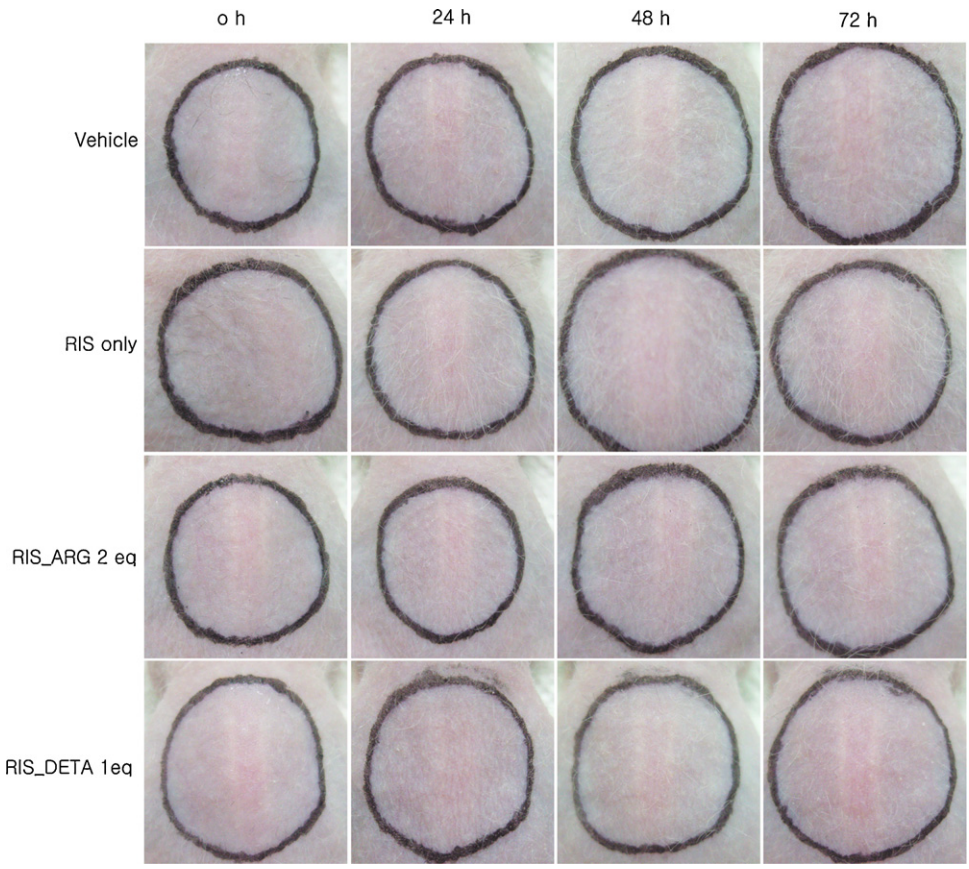

Fig. 6. Skin irritation test performed on the backside of hairless mice; photographs of backside of the skin treated with RIS (2%, w/w) alone, RIS–ARG in a molar ratio of 1:2, RIS–DETA in a molar ratio of 1:2, and vehicle (10% PEG, 1% xhantan gum) at 24 h, 48 h, and 72 h after single spreading and wiping.

lipid contents of the SC. Barrier function of the SC was evaluated by assessing the intercellular and epidermal lipids using Nile red staining. We observed similar intense yellow fluorescence on the uppermost area of skin, indicating no significant changes in the amount of epidermal lipids (Fig. 5). Next, we determined the water contents in hairless mice skin. The water contents of skin were $69.52 \pm 1.28\%$ in skin after sacrifice, $71.00 \pm 2.17\%$ in frozen skin, $74.05 \pm 3.11\%$ in skin treated with vehicle, $71.64 \pm 2.78\%$ in skin treated RIS only, $74.25 \pm 3.08\%$ in skin treated with RIS_ARG 2 equiv., and $70.49 \pm 4.59\%$ in skin treated RIS_DETA 1 equiv. All skin specimens used for *in vitro* penetration test contained similar or little higher water contents compared with the fresh skin. Therefore, we concluded that the enhanced skin penetration rate of RIS was not due to physical damage during skin preparation or *in vitro* test but due to the enhancing effect of ion pairs.

3.4. Skin irritation

We performed the skin irritation test after a single application of ion-paired RIS on the backside of a hairless mouse, as shown in Fig. 6. We observed that application of 2% RIS in 10% PEG and 1% xhantan gum solution with counter ions such as ARG and DETA did not cause any irritation. Photographs of the application site showed a clear and normal skin condition 24, 48, and 72 h after spreading. When both RIS and alendronate (ALN) were put through the open skin test on rubbed and pricked human skin, application of ALN resulted in a skin reaction, while no reaction was observed in the case of RIS (Brinkmeier et al., 2007). It was reported that an ALN patch induced skin irritation, while incorporation of butylhydroxytoluene to the ALN patch suppressed the alendronate-induced skin damage (Kusamori et al., 2010). This result was in accordance with our findings that RIS did not cause any skin irritation and supported the conformity of RIS for topical delivery candidacy.

4. Conclusions

The results of this study showed that the RIS flux through hairless mouse skin after administration of ion pairs was 36 times more than that after administration of RIS alone. Counter ions of RIS, such as ARG, LYS, and DETA, were effective amine materials that increased the permeation of RIS in an aqueous solution containing only 10% PEG. The partitioning ability of an ion pair was confirmed by the solubility test, and the enhanced partitioning ability resulted in enhanced solubility of ion-paired RIS in xylene and propylene carbonate. Considering the low daily dose of RIS, the pharmaceutical effect, and the minimum skin irritation, the ion-paired RIS is a promising method to deliver RIS through the transdermal route and can serve as an alternative to the oral route.

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References

Abrahamsen, B., 2010. Adverse effects of bisphosphonates. Calcif. Tissue Int. 86, 421–435.
Assael, L.A., 2009. Oral bisphosphonates as a cause of bisphosphonate-related osteonecrosis of the jaws: clinical findings, assessment of risks, and preventive strategies. J. Oral Maxillofac. Surg. 67, 35–43.

Banerjee, S., Yalkowsky, S.H., Valvani, C., 1980. Water solubility and octanol/water partition coefficients of organics. Limitations of the solubility-partition coefficient correlation. Environ. Sci. Technol. 14, 1227–1229.
Barry, B., 1987. Mode of action of penetration enhancers in human skin. J. Control. Release 6, 85–97.
Barry, B., 2001. Novel mechanisms and devices to enable successful transdermal drug delivery. Eur. J. Pharm. Sci. 14, 101–114.
Barry, B., 2002. Drug delivery routes in skin: a novel approach. Adv. Drug Deliv. Rev. 54, S31–S40.
Benavides, F., Oberyszyn, T.M., VanBuskirk, A.M., Reeve, V.E., Kusewitt, D.F., 2009. The hairless mouse in skin research. J. Dermatol. Sci. 53, 10–18.
Brinkmeier, T., Kügler, K., Lepoittevin, J.-P., Frosch, P.J., 2007. Adverse cutaneous drug reaction to alendronate. Contact Dermat. 57, 123–125.
Bronaugh, R.L., Stewart, R.F., Congdon, E.R., 1982. Methods for in vitro percutaneous absorption studies. II. Animal models for human skin. Toxicol. Appl. Pharmacol. 62, 481–488.
Brooks, J.K., Gilson, A.J., Sindler, A.J., Ashman, S.G., Schwartz, K.G., Nikitakis, N.G., 2007. Osteonecrosis of the jaws associated with use of risedronate: report of 2 new cases. Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod. 103, 780–786.
Brown, M.B., Martin, G.P., Jones, S.A., Akomeah, F.K., 2006. Dermal and transdermal drug delivery systems: current and future prospects. Drug Deliv. 13, 175–187.
Fini, A., Fazio, G., Gonzalez-Rodriguez, M., Cavallari, C., Passerini, N., Rodriguez, L., 1999. Formation of ion-pairs in aqueous solutions of diclofenac salts. Int. J. Pharm. 187, 163–173.
Forslind, B., 1994. A domain mosaic model of the skin barrier. Acta Derm. Venereol. 74, 1.
Greenspan, P., Gutman, R.L., 1993. Detection by nile red of agarose gel electrophoresed native and modified low density lipoprotein. Electrophoresis 14, 65–68.
Ivaturi, V.D., Kim, S.K., 2009. Enhanced permeation of methotrexate in vitro by ion pair formation with L-arginine. J. Pharm. Sci. 98, 3633–3639.
Kao, J., Hall, J., Helman, G., 1988. In vitro percutaneous absorption in mouse skin: influence of skin appendages. Toxicol. Appl. Pharmacol. 94, 93–103.
Kusamori, K., Katsumi, H., Abe, M., Ueda, A., Sakai, R., Hayashi, R., Hirai, Y., Quan, Y.-S., Kamiyama, F., Sakane, T., Yamamoto, A., 2010. Development of a novel transdermal patch of alendronate, a nitrogen-containing bisphosphonate, for the treatment of osteoporosis. J. Bone Miner. Res. 25, 2582–2591.
Lauer, A.C., Lieb, L.M., Ramachandran, C., Flynn, G.L., Weiner, N.D., 1995. Transfollicular drug delivery. Pharm. Res. 12, 179–186.
Ma, X., Fang, L., Guo, J., Zhao, N., He, Z., 2010. Effect of counter ions and penetration enhancers on the skin permeation of flurbiprofen. J. Pharm. Sci. 99, 1826–1837.
Megwa, S.A., Cross, S.E., Benson, H.A.E., Roberts, M.S., 2000. Ion pair formation as a strategy to enhance topical delivery of salicylic acid. J. Pharm. Pharmacol. 52, 919–928.
Michaels, A., Chandrasekaran, S., Shaw, J., 1975. Drug permeation through human skin: theory and in vitro experimental measurement. AIChE J. 21, 985–996.
Mitchell, D.Y., Heise, M.A., Pallone, K.A., Clay, M.E., Nesbitt, J.D., Russell, D.A., Melson, C.W., 1999. The effect of dosing regimen on the pharmacokinetics of risedronate. Br. J. Clin. Pharmacol. 48, 536–542.
Naik, A., Kalia, Y.N., Guy, R.H., 2000. Transdermal drug delivery: overcoming the skin's barrier function. Pharmaceut. Sci. Technol. Today 3, 318–326.
Neubert, R., 1989. Ion pair transport across membranes. Pharm. Res. 6, 743–747.
Novotny, J., Kova ikova, P., Novotny, M., Jan ova, B., Hrabalek, A., Vavrova, K., 2009. Dimethylamino acid esters as biodegradable and reversible transdermal permeation enhancers: effects of linking chain length, chirality and polyfluorination. Pharm. Res. 26, 811–821.
Pardo, A., Shiri, Y., Cohen, S., 1990. Percutaneous absorption of physostigmine: optimization of delivery from a binary solvent by thermodynamic control. J. Pharm. Sci. 79, 573–578.
Potts, R.O., Guy, R.H., 1992. Predicting skin permeability. Pharm. Res. 9, 663–669.
Prausnitz, M.R., Langer, R., 2008. Transdermal drug delivery. Nat. Biotechnol. 26, 1261–1268.
Prue, J., 1969. Ion pairs and complexes: free energies, enthalpies, and entropies. J. Chem. Educ. 46, 12.
Ramachandran, C., Fleisher, D., 2000. Transdermal delivery of drugs for the treatment of bone diseases. Adv. Drug Deliv. Rev. 42, 197–223.
Ruggiero, S., Drew, S., 2007. Osteonecrosis of the jaws and bisphosphonate therapy. J. Dent. Res. 86, 1013.
Ruggiero, S.L., Mehrotra, B., Rosenberg, T.J., Engroff, S.L., 2004. Osteonecrosis of the jaws associated with the use of bisphosphonates: a review of 63 cases. J. Oral Maxillofac. Surg. 62, 527–534.
Ruland, A., Kreuter, J., 1992. Influence of various penetration enhancers on the in vitro permeation of amino acids across hairless mouse skin. Int. J. Pharm. 85, 7–17.
Schroeder, I.Z., Franke, P., Schaefer, U.F., Lehr, C.M., 2007. Delivery of ethinylestradiol from film forming polymeric solutions across human epidermis in vitro and in vivo in pigs. J. Control. Release 118, 196–203.
Solomon, D.H., Rekedal, L., Cadarette, S.M., 2009. Osteoporosis treatments and adverse events. Curr. Opin. Rheumatol. 21, 363.
Takacs-Novak, K., Szasz, G., 1999. Ion-pair partition of quaternary ammonium drugs: the influence of counter ions of different lipophilicity, size, and flexibility. Pharm. Res. 16, 1633–1638.
Tan, Z., Zhang, J., Wu, J., Fang, L., He, Z., 2009. The Enhancing Effect of Ion-pairing on the skin permeation of glipizide. AAPS PharmSciTech 10, 967–976.
Vávrová, K., Lorencová, K., Klimentová, J., Novotný, J., Holý, A., Hrabálek, N.A., 2008. Transdermal and dermal delivery of adefovir: effects of pH and permeation enhancers. Eur. J. Pharm. Biopharm. 69, 597–604.
Valenta, C., Siman, U., Kratzel, M., Hadgraft, J., 2000. The dermal delivery of lignocaine: influence of ion pairing. Int. J. Pharm. 197, 77–85.
Wang, M., Fang, L., Ren, C., Li, T., 2008. Effect of ion pairing and enhancers on scutellarin skin permeability. J. Pharm. Pharmacol. 60, 429–435.
Wotton, P.K., Møllgaard, B., Hadgraft, J., Hoelgaard, A., 1985. Vehicle effect on topical drug delivery. III. Effect of azone on the cutaneous permeation of metronidazole and propylene glycol. Int. J. Pharm. 24, 19–26.