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The effects of fatty acids in propylene glycol on the percutaneous absorption of alendronate across the excised hairless mouse skin

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Abstract

The effects of fatty acids at various concentrations in propylene glycol (PG) on the *in vitro* permeation of alendronate from solution formulations and formulated pressure-sensitive adhesive (PSA) transdermal delivery systems through excised hairless mouse skin were investigated. Caprylic acid, capric acid, lauric acid, oleic acid and linoleic acid at concentrations of 3, 6 and 10% were employed as a fatty acid. The highest maximum permeation flux was obtained with 3% capric acid in PG followed by 6% capric acid and 3% oleic acid from solution formulations; the enhancement factor by the addition of 3% capric acid to PG was 20.5 compared to PG alone. On the contrary, from PSA transdermal delivery systems, the highest enhancement factor of 2.9 was attained with 6% caprylic acid in PG compared to PG alone. The maximum permeation flux and lag time from PSA transdermal delivery systems by the addition of 6% caprylic acid to PG were $195.68 \pm 26.6\,ng/cm^2/h$ and $0.6 \pm 0.3\,h$ whereas PG without fatty acids showed $67.3 \pm 5.8\,ng/cm^2/h$ and $0.5 \pm 0.4\,h$, respectively. The PSA transdermal delivery systems initially provided very high permeation rate followed by a gradual decrease regardless of the fatty acids. The highest release rate was also obtained with the formulation containing 6% caprylic acid in PG although release rates were not matched with permeation rates perfectly. In conclusion, for effective transdermal delivery system of alendronate, 6% caprylic acid in PG could be employed.

Keywords: Alendronate; Percutaneous absorption; Propylene glycol; Fatty acids

1. Introduction

Alendronate sodium (sodium 4-amino-1-hydroxybutylidene-1,1-bisphosphonate trihydrate) is a potent inhibitor of bone resorption mediated by osteoclast (Schenk et al., 1986; Sato and Grasser, 1990; Beek et al., 2003; Gur et al., 2005). It has been used for the treatment of a variety of bone diseases including osteoporosis, Paget's disease and metastatic bone disease (Hosking et al., 1998; Russell and Rogers, 1999).

Even though alendronate is considered as the drug of choice for the treatment of osteoporosis, its use has been limited due to the complexity of administration. Alendronate has very low bioavailability less than 1%, and the absorption is reduced by co-administered drugs or foods by 60% (Ptáček et al., 2002; Lacy et al., 2007), therefore, it is recommended that the drug be administered at least 30 min before taking other drugs or foods.

The main adverse drug effects are gastrointestinal troubles including esophagitis, abdominal pain and acid reflux. To prevent these adverse drug events, patients should sit up or walk for more than 30 min after taking the drug (Lacy et al., 2007). Considering most patients with osteoporosis are elderly people, many patients have difficulties with following the administration procedure.

Since the transdermal delivery reduces the gastroinstestinal problem, it has been recognized as an alternative route to oral delivery (Ansel et al., 1995; Gwak and Chun, 2002; Cho and Gwak, 2004). In addition, the advantages of the transdermal delivery are as follows: avoidance of metabolism by oral administration, and constant maintenance of plasma drug concentration.

The stratum corneum is known to have an excellent barrier property against skin penetration. Four mechanisms have been suggested to overcome this problem. One possible mechanism

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is the reduction of skin resistance as a permeability barrier by disruption of tightly packed lipid regions of stratum corneum, which consequently increases penetration through the intercellular lipid matrix (Aungst et al., 1990). Another mechanism is increased skin/vehicle partitioning of the drug. Green et al. (1988) reported that the permeation enhancement of naphazoline by fatty acids could be caused by ion pair formation between drug and fatty acids, resulting in the increase of partitioning into the stratum corneum. A third likely mechanism of skin permeation enhancement is increased solvent transport into or across the skin. The results of increased solvent penetration may include increased drug solubility in the skin and increased skin penetration of the drug if the drug has a high affinity for the solvent (Yamada et al., 1987). The remaining proposed mechanism is increased drug solubility in the vehicle. Generally, acidic enhancers have been used to increase the solubility of basic drugs, and vice versa (Aungst et al., 1990).

The objective of this paper was to examine the feasibility of developing alendronate transdermal delivery systems by investigating the effects of fatty acids in PG on the percutaneous absorption of alendronate using the hairless mouse skin.

2. Materials and methods

2.1. Materials

Alendronate sodium trihydrate and pamidronate sodium (internal standard, IS) were kindly supplied by Whanin Pharmaceutical Company and Hallim Pharmaceutical Company, respectively. Propylene glycol monolaurate (PGML, Lauroglycol® 90), propylene glycol monocaprylate (PGMC, Capryol® 90) and diethylene glycol monoethyl ether (DGME, Transcutol® P) were obtained from Gattefossé (Gennevilliers Cedex, France). Ethanol, propylene glycol (PG), isopropyl alcohol (IPA), calcium chloride, sodium dihydrogen phosphate, sodium hydroxide, hydrochloric acid, potassium phosphate, sodium acetate, acetic acid, sodium citrate and citric acid (Duksan Pure Chemical Co., Ansan, Korea) were used. Isopropyl myristate (IPM), caprylic acid, capric acid, lauric acid, oleic acid, linoleic acid and 9-fluorenyl methylchloroformate (FMOC) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The diethylamine (DEA) solid-phase extraction (SPE) cartridges were used (Strata, Phenomenex, USA). Acetonitrile and methanol used were of HPLC grade. Acrylic pressure-sensitive adhesive (PSA) solutions in organic solvents which were Duro-Tak® 87–2196 (copolymer: acrylate-vinylacetate, functional group: –COOH, 45% solution of self-crosslinking acrylic copolymer, 3000 cps, solubility parameter 16), Duro-Tak® 87–2100 (copolymer: acrylate, functional group: –COOH, 51.5% solution of self-crosslinking acrylic copolymer, 8500 cps, solubility parameter 16) and Duro-Tak® 87–2510 (copolymer: acrylate, functional group: –OH, 40.5% solution of non-crosslinking acrylic copolymer, 4500 cps, solubility parameter 16) were obtained from National Starch and Chemical Company (Bridgewater, NJ, USA). Other reagents were of analytical grade.

2.2. Animals

Male hairless mice aged 6–8 weeks were purchased from Samtako Bio Korea Co., Ltd. (Osan, Korea). Animals were treated humanely.

2.3. Analysis

Samples were analyzed by high-performance liquid chromatography (HPLC). The HPLC system consisted of a pump (L-7100, Hitachi, Japan), with a detector (L-7480, Hitachi, Japan) set at 260 and 310 nm for excitation and emission, respectively. An ODS column (Capcell Pack C18, Shiseido, Japan) equipped with a C18 Radial Pak insert was used. A simple gradient method was used in which the starting mobile phase was composed of solution A and solution B at the ratio of 28:72 (v/v) and delivered at a flow rate of 1.5 ml/min; solution A and solution B were made up of acetonitrile and methanol (1:1), and 25 mM citric acid buffer and 25 mM sodium phosphate buffer (1:1), respectively. The starting mobile phase was changed to methanol, solution A and solution B (20:28:52) at 13 min and hold at this composition for 13 min. Then the original mobile phase was again pumped to restore starting conditions. The total run time was 30 min.

2.4. Preparation of the samples

Samples from release, permeation, solubility and partition coefficient studies were analyzed using the method by Ptáček et al. (2002). This method involved corprecipitation with calcium phosphate, separation on DEA SPE cartridge and derivatization with FMOC in citrate buffer (pH 11.9). Calibration curves were constructed by plotting the peak area ratios of alendronate to IS versus the concentrations of alendronate in solution.

2.5. Solubility determination

An excess amount of alendronate sodium was added to the various pure solvents or co-solvents, and shaken at 37 °C for more than 48 h. The solutions were then centrifuged at 7500 rpm for 5 min, and the supernatant was assayed by HPLC after appropriate dilution.

2.6. Partition coefficient determination

Lipophilic and hydrophilic phases were saturated with each other before the experiment. The compositions of lipophilic/hydrophilic phase were *n*-octanol/water and toluene/PG with or without fatty acids. Alendronate solution (500 μg/ml) was prepared with hydrophilic phase saturated with lipophilic phase. One milliliter of this solution was then transferred to 10 ml centrifuge tube containing 1 ml of lipophilic phase saturated with hydrophilic phase. The tube was vortexed for 30 min and centrifuged at 3000 *g* for 5 min, and the drug concentrations in both phases were determined by HPLC.

2.7. Preparation of alendronate transdermal delivery systems

Ninety milligram of alendronate sodium (alendronate 53.8 mg) was dissolved in 2 ml of PG with or without fatty acids, and then mixed with 5 g of three kinds of acrylic adhesive solutions: Duro-Tak® 87–2196, Duro-Tak® 87–2510, and Duro-Tak® 87–2100. The fatty acids used were 3, 6 and 10% caprylic acid, capric acid, lauric acid, oleic acid and linoleic acid. Alendronate PSA transdermal delivery systems were prepared by casting the above solutions on a polyester release liner coated with silicone (Gelroflex ALU-PET 100 μ-2S DR, 3M, USA) using a casting knife. The area of the cast solutions was 10 cm × 15 cm per 7 g solution. They were set at room temperature for 10 min to evaporate the solvents, and then dried for 20 min in an oven set at 37 °C. The dried film was transferred onto a backing film (Scotchpak 1109, 3M, USA).

2.8. Procedure for skin permeation from solution formulations and PSA transdermal delivery systems

After sacrificing with ether, the dorsal skin of each hairless mouse was excised. One milliliter of alendronate solution formulation (2 mg/ml) or PSA transdermal delivery systems of an appropriate size were applied to the epidermal side of the skin, and mounted on a Franz Cell permeation system (Diffusion Cell Drive System, Labfine, Korea); the dermal side was in contact with the receptor compartment. The theoretical amount of drug loaded to the epidermal side from PSA transdermal delivery systems was 1.06 mg. Receptor compartment cells were filled with pH 7.4 potassium phosphate buffer and the media were stirred by a Teflon-coated magnetic bar to keep them well mixed. The permeation media were maintained at 37 °C. The surface area of the receiver cell opening was 1.766 cm^2, and the cell volume was 12 ml. At predetermined time intervals, 1 ml of receptor solutions was withdrawn, and the amount of alendronate permeated was determined by HPLC.

2.9. Procedure for alendronate release from PSA transdermal delivery systems

Alendronate transdermal delivery systems prepared were mounted on a Franz Cell permeation system; the drug loaded-layer was in contact with the receptor compartment. Receptor compartment cells were filled with pH 7.4 potassium phosphate buffer and the media were stirred by a Teflon-coated magnetic bar to keep them well mixed. The released media were maintained at 37 °C. At predetermined time intervals, 1 ml of receptor solutions were withdrawn, and the amount of alendronate released from various PSA transdermal systems was determined by HPLC.

2.10. Statistical analysis

All the values are expressed as the mean ± S.D. The permeation flux of the formulated transdermal delivery systems was compared with Kruskal Wallis test, which was followed by a posterior test with an unpaired *t*-test using the Bonferroni correction. A *P*-value of less than 0.05 was considered significant.

3. Results and discussion

3.1. Effects of fatty acids in PG on the permeation of alendronate from solution formulations

PG was selected as a vehicle due to its relatively high solubility and physicochemical characteristics which is well mixed with PSA. We employed a lot of vehicles to examine their solubilities. The overall solubilities of alendronate in pure vehicles used were fairly poor, which were below 2 μg/ml except for PG. The solubility of alendronate ($n = 3$) in PG, PGML, PGMC, oleyl alcohol, DGME, IPM, IPA and ethanol was found to be 2110.3 ± 432.6, 15.1 ± 2.6, 19.4 ± 3.7, 3.8 ± 0.7, 1.5 ± 0.4, 1.0 ± 0.1, 0.4 ± 0.1 and 2.2 ± 0.05 ng/ml, respectively. Partition coeffcient$_{n\text{-octacol/water}}$ was calculated to be 0.013 ± 0.0007, which was very low.

PG is known to have relatively low skin cell toxicity (Ponec et al., 1989, 1990; Kadir et al., 1993), and has been widely used for formulation of transdermal delivery systems. It was suggested that the probable mechanism of PG is solvating alpha keratin and occupying hydrogen-bonding sites, thus reducing drug/tissue binding (Barry, 1987). On the contrary, fatty acids are known to be enhancers with lipophilic properties, and many studies have shown that the skin permeability enhancing effects of fatty acids are greatest with PG vehicles (Cooper, 1984; Cooper et al., 1985; Aungst et al., 1986; Yamada et al., 1987).

We investigated the effects of various fatty acids on the alendronate permeation. Five fatty acids at concentrations of 3, 6 and 10% were compared: three were saturated fatty acids-caprylic acid (C_8), capric acid (C_{10}) and lauric acid (C_{12}); and two were unsaturated fatty acids-oleic acid (C_{18} with one double bond) and linoleic acid (C_{18} with two double bonds). Most of the studies on fatty acid penetration enhancers have focused mainly on oleic acid, and to a lesser extent, linoleic acid. Both oleic acid and linoleic acid are unsaturated fatty acids with a *cis*-configuration. Accordingly, it has been proposed that the effects of these compounds are associated with the linked structure owing to the *cis* double bond (Barry, 1987; Cooper, 1984; Golden et al., 1987). Among saturated, straight-chain fatty acids, capric acid and lauric acid were previously shown to be the most effective enhancers for naloxone permeation (Aungst et al., 1986).

The maximum permeation flux (0.17 ± 0.07 μg/cm^2/h) of alendronate in PG was very low and lag time (0.9 ± 0.3 h) was short. With the addition of fatty acids, the permeation rates increased markedly up to 20.5 times as depicted in Fig. 1. The highest maximum flux was obtained with the addition of 3% capric acid, which was 3.52 ± 0.92 μg/cm^2/h, followed by 6% capric acid (3.43 ± 0.005 μg/cm^2/h), 3% oleic acid (2.71 ± 1.27 μg/cm^2/h) and 3% caprylic acid (2.31 ± 0.58 μg/cm^2/h). The longest lag time (8.4 ± 1.1 h) was found in the formulation of 6% caprylic acid in PG, and the formulation containing 3% capric acid in PG showed the lag time of 5.1 ± 0.7 h. There was no correlation between fatty acid concentration and maximum permeation flux or lag time except

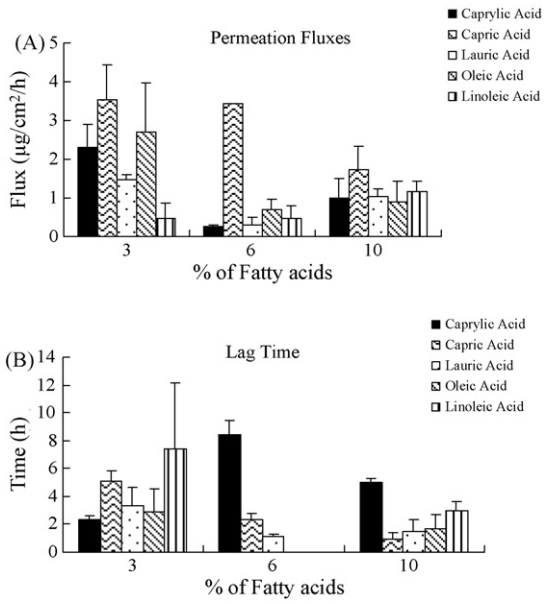

Fig. 1. The permeation flux (A) and lag time (B) of alendronate from solution formulations containing various concentrations of fatty acids in propylene glycol ($n = 3$).

for capric acid. Capric acid showed higher permeation rate with lower fatty acid concentration.

We investigated the mechanism of enhancing effects by capric acids. Base on the equation of $J_s = DKC/h$ (Barry, 1983), the permeation flux could be enhanced by the increase of diffusion coefficient (D), partition coefficient (K) and/or the constant drug concentration dissolved in a formulation (C), assuming that the skin thickness (h) is constant. In this study, saturated drug concentration of 2 mg/ml was used, in which drug was saturated in all vehicles used, resulting in the maximized and constant thermodynamic activity. K was obtained by measuring organic solvent/vehicle partition coefficients. Increases in permeation rates not accounted for by increases in K were assumed to involve increased skin diffusivity indicating barrier disruption (Aungst et al., 1990). Even though n-octanol is known to have a similar polarity to that of the lipids of skin (Raykar et al., 1988), toluene was used as the lipophilic phase for partition coefficients of alendronate from PG with or without fatty acids because n-octanol was miscible with PG.

The results revealed that the enhancing effects by the addition of capric acids were not attributable to increased K. The addition of capric acids rather decreased the K values regardless of the concentrations. The partition coefficients were calculated to be 9.7 ± 0.9, 1.1 ± 0.2, 1.9 ± 0.4 and 2.0 ± 0.1 for PG only, PG with 3, 6 and 10% capric acid, respectively. It was, therefore, thought that the enhancing effect by capric acid was mainly due to increased skin diffusivity by barrier disruption.

The enhancing effect by the addition of fatty acids to PG has been widely studied, and the binary system was considered to disorganize the multilaminate hydrophilic–lipophilic layers located intercellularly in the stratum corneum, consequently promoting percutaneous absorption of drugs (Nomura et al., 1990). Specially, using differential scanning calorimetry and infrared spectroscopy, it has been suggested that fatty acids can change the physicochemical properties of skin in which they disrupt the packed structure of the intracellular lipids of the stratum corneum (Golden et al., 1987).

3.2. Effects of fatty acids in PG on the permeation of alendronate from pressure-sensitive adhesive transdermal delivery systems

To evaluate the effects of PSAs, three kinds of acrylic adhesives were employed; Duro-Tak® 87–2196, 87–2100 and 87–2510. Ninety milligrams of alendronate sodium (alendronate 53.8 mg) was dissolved in 2 ml of PG and mixed with 5 g of PSA. Among PSAs used, Duro-Tak® 87–2100 and 87–2510 were not mixed with the vehicle, and phase-separation occurred. Therefore, Duro-Tak ® 87–2196 was chosen as the PSA for the fabrication of alendronate PSA transdermal delivery systems.

The permeation rate and lag time of alendronate from the formulated PSA transdermal systems including PG with/without fatty acids are summarized in Table 1. Unlike solution formulations, the addition of fatty acids failed to increase the permeation flux dramatically, and some formulations rather decreased the permeation flux. The overall permeation fluxes of alendronate from PSA transdermal systems were quite lower than those from solution formulations. The difference was possibly thought to be the solubility or diffusivity change by mixing with PSA.

The highest enhancement factor of 2.9 was attained with the addition of 6% caprylic acid compared to PG alone. Like the enhancing effects by capric acid in the solution formulations, the enhancing effect by the addition of caprylic acid to PG in PSA transdermal delivery systems was attributable to the increased diffusivity because the partition coefficients rather decreased by the addition of caprylic acid; the partition coefficients (PG/toluene) were 9.7 ± 0.9, 4.1 ± 1.3, 3.8 ± 0.5 and

Table 1
Permeation flux (J_s)and lag time (T_L) of alendronate from formulated transdermal delivery systems containing propylene glycol (PG) in the presence and absence of fatty acids at various concentrations

Vehicles	J_s (ng/cm²/h)	T_L (h)
PG	67.25 ± 5.77	0.5 ± 0.4
Caprylic acid (3%)	86.46 ± 1.34*	3.2 ± 0.03
Caprylic acid (6%)	195.68 ± 26.6*	0.6 ± 0.3
Caprylic acid (10%)	55.05 ± 6.80	0.2 ± 0.1
Capric acid (3%)	74.44 ± 11.47	1.3 ± 0.5
Capric acid (6%)	30.07 ± 23.25	1.2 ± 0.9
Capric acid (10%)	22.18 ± 8.42*	0.9 ± 0.5
Lauric acid (3%)	62.51 ± 3.47	NA
Lauric acid (6%)	32.75 ± 2.54*	NA
Lauric acid (10%)	51.61 ± 4.04	NA
Oleic acid (3%)	46.28 ± 1.89*	0.04 ± 0.04
Oleic acid (6%)	128.22 ± 10.28*	0.4 ± 0.3
Oleic acid (10%)	142.63 ± 2.75*	0.5 ± 0.3
Linoleic acid (3%)	41.06 ± 4.17*	0.1 ± 0.1
Linoleic acid (6%)	74.29 ± 4.17	0.3 ± 0.1
Linoleic acid (10%)	79.78 ± 8.14	0.1 ± 0.1

Data were expressed as the mean ± S.D. ($n = 3$). There was statistical difference between J_s ($P < 0.05$). NA, not available.
* $P < 0.05$ compared to PG.

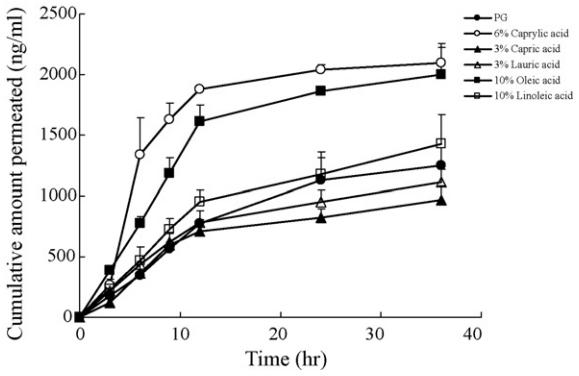

Fig. 2. Effect of fatty acids at various concentrations in propylene glycol on the permeation of alendronate from formulated transdermal delivery systems ($n = 3$).

3.7 ± 0.8 for 0, 3, 6 and 10% caprylic acid-containing PG, respectively. There was no correlation between solution formulation and PSA transdermal delivery systems with respect to either permeation flux or lag time.

Fig. 2 shows the cumulative amount of alendronate permeated across the hairless mouse skin from PSA transdermal delivery systems containing PG only and five fatty acids at the representative concentration in PG which showed the maximum highest flux among the concentrations of 3, 6 and 10%. The PSA transdermal delivery systems initially provided very high permeation rate followed by a gradual decrease regardless of the fatty acids. The decrease in permeation rate with time was attributable to the rapid reduction by rapid drop in alendronate concentration in the donor compartment. The graph in the initial phase in most formulations showed 1st order straight line, indicating that the permeation occurred very rapidly after administration. The lag time was shorter than 1 h except for 3% caprylic acid and 3 and 6% capric acid (Table 1).

In the case of unsaturated fatty acids such as oleic acid and linoleic acid, the maximum permeation flux showed fatty acid concentration-dependent manner even though 6 and 10% did not give significant difference.

Fig. 3 shows the release profile from PSA transdermal delivery systems. Release rates were not matched with permeation rates perfectly; oleic acid (3%) and capric acid (10%) showed very high release rate even though permeation rate was very low. However, 6% caprylic acid in PG showed the highest rates with respect to both release and permeation rate among vehicles employed in this study. It was found that the releases from the formulated PSA transdermal delivery systems were proportional to the square root of time, consistent with the matrix-controlled diffusion model ($Q' = k't^{1/2}$, Q': amount released, k': release rate constant) (Chien and Lambert, 1974). The release rate constants were calculated to be 12.3 ± 0.2, 14.1 ± 0.2, 0.9 ± 0.08, 8.8 ± 0.6 and 11.3 ± 0.6 μg/cm^2/h$^{1/2}$ in the formulations of 3% caprylic acid, 6% caprylic acid, 6% capric acid, 10% capric aid and 3% oleic acid in PG, respectively.

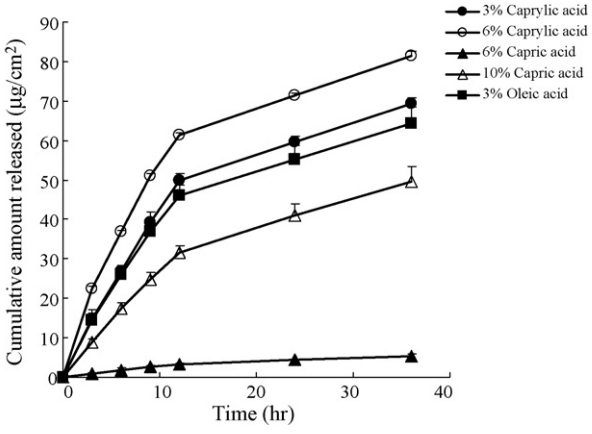

Fig. 3. Effect of fatty acids at various concentrations in propylene glycol on the release of alendronate from formulated transdermal delivery systems ($n = 3$).

This study was to examine the feasibility of developing alendronate transdermal delivery system. Among vehicles used in this study, 6% caprylic acid in PG showed the most excellent permeation profile; the permeation flux of the formulation containing 6% caprylic acid in PG with theoretical drug dose of 358.7 μg/cm^2 was 195 ng/cm^2/h. If the patch is formulated at the size of 10 cm × 10 cm, the drug amount permeated per hour will be 19.5 μg with a drug dose of 35.9 mg, resulting in the total permeated amount of 468 and 936 μg for 1 and 2 day application, respectively, which is 0.67–1.34% of 70 mg oral dose. It should be cautious, however, to conclude that the formulation could be used in human because it is known that hairless mouse skin is increased in permeability compared to human skin (Bond and Barry, 1988) Nevertheless, this study was considered to provide some preliminary data to develop alendronate transdermal delivery systems.

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References

Ansel, H.C., Popovich, N.G., Allen, L.V., 1995. Pharmaceutical Dosage Forms and Drug Delivery Systems. Williams & Wilkins, Malvern, pp. 357–372.

Aungst, B.J., Blake, J.A., Hussain, M.A., 1990. Contributions of drug solubilization, partitioning, barrier disruption, and solvent permeation to the enhancement of skin permeation of various compounds with fatty acids and amines. Pharm. Res. 7, 712–718.

Aungst, B.J., Rogers, N.J., Shefter, E., 1986. Enhancement of naloxone penetration through human skin in vitro using fatty acids, fatty alcohols, surfactants, sulfoxides and amides. Int. J. Pharm. 33, 225–234.

Barry, B.W., 1983. Dermatological Formulations. Marcel Dekker, New York, pp. 49–94.

Barry, B.W., 1987. Mode of action of penetration enhancers in human skin. J. Control. Res. 6, 85–97.

Bond, J.R., Barry, B.W., 1988. Hairless mouse skin is limited as a model for assessing the effects of penetration enhancers in human skin. J. Invest. Dermatol. 90, 810–813.

Beek, E.R., Cohen, L.H., Leroy, I.M., Ebetino, F.H., Löwik, C.W.G.M., Papapoulos, S.E., 2003. Differentiating the mechanisms of antiresorptive action of nitrogen containing bisphosphonate. Bone 33, 805–811.

Chien, Y.W., Lambert, H.J., 1974. Controlled drug release from polymeric delivery devices II: differentiation between partition-controlled and matrix-controlled drug release mechanism. J. Pharm. Sci. 63, 515–519.

Cho, Y.A., Gwak, H.S., 2004. Transdermal delivery of ketorolac tromethamine: effects of vehicles and penetration enhancers. Drug Dev. Ind. Pharm. 30, 557–564.

Cooper, E.R., 1984. Increased skin permeability for lipophilic molecules. J. Pharm. Sci. 73, 1153–1156.

Cooper, E.R., Merritt, E.W., Smith, R.L., 1985. Effect of fatty acids and alcohols on the penetration of acyclovir across human skin in vitro. J. Pharm. Sci. 74, 688–689.

Golden, G.M., McKie, J.E., Potts, R.O., 1987. Role of stratum corneum lipid fluidity in transdermal drug flux. J. Pharm. Sci. 76, 25–28.

Green, P.G., Guy, R.H., Hadgraft, J., 1988. In vitro and in vivo enhancement of skin permeation with oleic acid and lauric acids. Int. J. Pharm. 48, 103–111.

Gur, A., Colpan, L., Cevik, R., Nas, K., Sarac, A.J., 2005. Comparison of zinc excretion and biochemical markers of bone remodeling in the assessment of the effects of alendronate and calcitonin on bone in postmenopausal osteoporosis. Clin. Biochem. 38, 66–72.

Gwak, H.S., Chun, I.K., 2002. Effect of vehicles and penetration enhancers on the in vitro percutaneous absorption of tenoxicam through hairless mouse skin. Int. J. Pharm. 236, 57–64.

Hosking, D., Chilvers, C., Christiansen, C., Ravn, P., Wasnich, R., Ross, P., McClung, M., Balske, A., Thompson, D., Daley, M., Yates, A.J., 1998. Prevention of bone loss with alendronate in postmenopausal women under 60 years of age. Early Postmenopausal Intervention Cohort Study Group. N. Engl. J. Med. 338, 485–492.

Kadir, R., Ponec, M., Tiemessen, H.L.G.M., Junginger, H.E., Boddé, H.E., 1993. Oleyl surfactants as skin penetration enhancers: effects on human stratum corneum permeability and in vitro toxicity to cultured human skin cells. In: Walters, K.A., Hadgraft, J. (Eds.), Pharmaceutical Skin Penetration Enhancement, vol. 59. Marcel Dekker, Inc., New York, pp. 215–228.

Lacy, C.F., Amstrong, L.L., Goldman, M.P., Lance, L.L., 2007. Drug Information Handbook. Lexi-comp, Hudson, pp. 63–64.

Nomura, H., Fusao, K., Sugimoto, Y., Miyashita, Y., Dohi, M., Kato, Y., 1990. Percutaneous absorption of indomethacin from mixtures of fatty alcohol and propylene glycol (FAPG base) through rat skin: effects of oleic acid added to FAPG base. Chem. Pharm. Bull. 38, 1421–1424.

Ponec, M., Haverkort, M., Soei, Y.L., Kempenaar, J., Boddé, H.E., 1990. Use of human keratinocyte and fibroblast cultures for toxicity studies of topically applied compounds. J. Pharm. Sci. 79, 312–316.

Ponec, M., Haverkort, M., Soei, Y.L., Kempenaar, J., Brussee, H., Boddé, H.E., 1989. Toxicity screening of *n*-alkylazacycloheptan-2-one derivatives in cultured human skin cells: structure–toxicity relationships. J. Pharm. Sci. 78, 738–741.

Ptáček, P., Klima, J., Macek, J., 2002. Determination of alendronate in human urine as 9-fluorenylmethyl derivative by high-performance liquid chromatography. J. Chromatogr. B 767, 111–116.

Raykar, P.V., Fung, M.C., Anderson, B.D., 1988. The role of protein and lipid domains in the uptake of solutes by human stratum corneum. Pharm. Res. 5, 140–150.

Russell, R., Rogers, M., 1999. Bisphosphonates: from laboratory to the clinic and back again. Bone 25, 97–106.

Sato, M., Grasser, W., 1990. Effect of bisphosphonates on isolated rat osteoclasts as examined by reflected light microscopy. J. Bone Miner. Res. 5, 31–40.

Schenk, R., Eggi, D.P., Fleisch, D.P., Rosini, S., 1986. Quantitative morphometric evaluation of the inhibitory activity of new amino-bisphosphonates on bone resorption in the rat. Calcif. Tissue Int. 38, 342–349.

Yamada, M., Uda, Y., Tanigawara, Y., 1987. Mechanism of enhancement of percutaneous absorption of molsidomine by oleic acid. Chem. Pharm. Bull. 35, 3399–3406.