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ORIGINAL ARTICLE

Effects of amines on percutaneous absorption of alendronate

Jiae Whang, and Hyesun Gwak

College of Pharmacy and Division of Life and Pharmaceutical Sciences, Ewha Womans University, 11-1 Daehyun-Dong Seodaemun-Gu, Seoul 120-750, Korea

Abstract

Objective: The aim of this study was to examine the effects of amines on the permeation of alendronate using solution formulations and pressure-sensitive adhesive (PSA) transdermal delivery systems (TDS).

Materials and methods: Monoethanolamine (MEA), diethanolamine (DEA), triethanolamine (TEA), diethylamine (DEYA), and triethylamine (TEYA) at concentrations of 3, 6, and 10% were added to propylene glycol (PG) containing 6% caprylic acid. *In vitro* and *in vivo* experiments were conducted using alendronate solution and PSA TDS formulations.

Results: When using saturated solution formulations, 3% TEA and 10% DEYA showed high permeation rates of 8.20 ± 0.80 and 7.87 ± 0.18 μg/cm^2/h, respectively. The maximum permeation flux of 1.79 ± 0.28 μg/cm^2/h from 1 mg/ml solution was obtained with the addition of 10% DEYA followed by the addition of 10% TEYA (1.72 ± 0.72 μg/cm^2/h). The highest enhancement factor of 1.86 was obtained with alendronate PSA TDS containing 10% MEA compared with no amine. In the *in vivo* study, the amount remaining to be excreted (ARE) at time 0 (Ae_∞) and ARE at time *t* [Ae(*t*)] differed between TDS and oral delivery significantly ($P < 0.01$). The TDS containing 10% MEA showed the highest Ae_∞ (19.5 ± 6.93 μg), which was 2.7- and 2.2-fold, compared with oral and no amine administration, respectively.

Conclusion: Based on the results, TDS with 10% MEA in PG containing 6% caprylic acid could be a good candidate for the alendronate TDS.

Keywords: Alendronate; amines; permeation flux; pharmacokinetics; transdermal delivery system

Introduction

Alendronate sodium (sodium [4-amino-1-hydroxy-1-(hydroxy-oxido-phosphoryl)-butyl] phosphonic acid trihydrate) is a potent bisphosphophate that selectively inhibits osteoclast-mediated bone resorption (van Beek et al., 2003; Gur et al., 2005), increases bone mineral density (BMD) (Boivin et al., 2000) and reduces the incidence of vertebral, hip, and other fractures (Black et al., 2000; Hochberg et al., 2002). It has been used to treat and prevent osteoporosis and to treat Paget's disease and bone metastases (Rodan and Martin, 2000; Cranney et al., 2002).

As with all potent bisphosphonates, the systemic bioavailability of alendronate after oral dosing is low due to its very poor lipophilicity (Porras et al., 1999). Intake together with meals and beverages other than water further reduces the bioavailability (Gertz et al., 1993). The absorbed drug rapidly partitions, and the drug is either taken up by bone tissue or excreted unchanged by the kidney (Lin et al., 1992, 1993). After absorption in the bone, alendronate has an estimated terminal half-life of 10 years (Gertz et al., 1993).

Alendronate prevents bone loss in women without osteoporosis when taken at an oral dose of 5 mg daily and increases BMD at the hip and spine in women with osteoporosis at an oral dose of either 5 or 10 mg daily (Liberman et al., 1995; Black et al., 1996). Once a week administration of a single dose of alendronate has been developed. It has been studied that one single 70 mg pill has the same efficacy on bone turnover and BMD as that of the conventional daily dose of 10 mg (Schnitzer et al., 2000).

Address for Correspondence: Hyesun Gwak, College of Pharmacy and Division of Life and Pharmaceutical Sciences, Ewha Womans University, 11-1 Daehyun-Dong Seodaemun-Gu, Seoul 120-750, Korea. Tel.: +82-2-3277-4376; Fax: +82-2-3277-2851. E-mail: hsgwak@ewha.ac.kr

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Alendronate is known to exhibit esophageal toxicity due to its acidic nature. There have been reports of severe gastrointestinal (GI) tract adverse events in clinical use including GI bleeding, ulcer (gastric, peptic, duodenal, and esophageal), GI symptoms (vomiting and nausea), abdominal pain, esophageal reflux, gastritis, and esophagitis (Shakweh et al., 2007). To avoid these symptoms, patients were advised to take their medication with at least one glass of water, not to chew or allow the pill to dissolve in the mouth, and to stay in the upright position for 30 min after the intake of the drug without eating or drinking (Lambrinoudaki et al., 2006).

Because the transdermal delivery reduces the GI problem, an alternative route to oral delivery has been recognized" to "it has been recognized as an alternative route to oral delivery (Ansel et al., 1995). In addition, the advantages of the transdermal delivery are avoidance of metabolism by oral administration and constant maintenance of plasma drug concentration.

Amines have been used to enhance permeability of acidic drugs. Aungst et al. (1990) studied the enhancing ability of dodecylamine for skin permeation, and Kadono et al. (1998) and Megwa et al. (2000) reported that amines enhanced percutaneous penetration by ion pair formation with salicylate. Cheong and Choi (2002) conducted a research of enhanced percutaneous absorption of piroxicam via salt formation with ethanolamines. Therefore, it is expected that the addition of amines would increase the solubility or partitioning of alendronate, which is an acidic drug.

In our previous *in vitro* study, 6% caprylic acid in propylene glycol (PG) showed relatively high permeation flux (Choi et al., 2008). Based on the results from the previous studies, the objective of this study was to examine the effects of amines at various concentrations on the permeation of alendronate in PG containing 6% caprylic acid using solution and pressure-sensitive adhesive (PSA) alendronate transdermal delivery system (TDS).

Material and Methods

Animals

Male hairless mice aged 6 weeks and male Sprague-Dawley rats weighing 250 g were obtained from Orient Bio Inc. (Gapyeong, Korea). The animal studies were carried out according to the Principles for Biomedical Research Involving Animals developed by the Council for International Organizations of Medical Sciences.

Materials

Alendronate sodium trihydrate and pamidronate sodium (internal standard, IS) were kindly supplied by Whanin Pharmaceutical Company (Seoul, Korea) and Hallim Pharmaceutical Company (Seoul, Korea), respectively. Ethanol, PG, toluene, calcium chloride, sodium dihydrogen phosphate, sodium hydroxide, hydrochloric acid, potassium phosphate, sodium acetate, acetic acid, sodium citrate, citric acid, monoethanolamine (MEA), diethanolamine (DEA), triethanolamine (TEA), diethylamine (DEYA), and triethylamine (TEYA) (Duksan Pure Chemical Co., Ansan, Korea) were used. Caprylic acid and 9-fluorenyl methylchloroformate (FMOC) were purchased from Sigma Chemical Co. (St. Louis, MO). The DEYA solid-phase extraction (SPE) cartridges were used (Strata, Phenomenex, Torrance, CA). Acetonitrile and methanol used were of high-performance liquid chromatography (HPLC) grade. Acrylic PSA 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) was obtained from National Starch and Chemical Company (Bridgewater, NJ). Other reagents were of analytical grade.

HPLC analysis

Samples were analyzed by HPLC. The HPLC system consisted of a pump (CBM-20A, Shimadzu, Kyoto, Japan), with a detector (RF-10AXL, Shimadzu, Kyoto, Japan) set at 260 and 310 nm for excitation and emission, respectively. An ODS column (Capcell Pack C18, Shiseido, Tokyo, 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 solutions A and B at the ratio of 28:72 (v/v, %) and delivered at a flow rate of 1.5 ml/min; solutions A and B were made 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 held 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.

Preparation of samples

Samples were analyzed using the method by Ptáček et al. (2002). Two hundred microliter of IS (30 μg of pamidronate disodium) was added to 3 ml of urine or permeated solution, and the tube was briefly shaken. One hundred microliter of 0.1 M KH_2PO_4 and the same amount of 0.1 M $CaCl_2$ were added and the sample was made alkaline with 400 μl of 1 M NaOH. The sample was centrifuged for 5 min at 10,000 rmp and the supernatant was discarded. The precipitate was completely dissolved in 0.5 ml of 0.2 M acetic acid and 3 ml of water was added. Consequently, the precipitation with sodium hydroxide was repeated twice. The resulting precipitate was finally dissolved in 1 ml of 0.2 M acetate buffer (pH 4.5) and diluted with 2 ml of water. The sample was then loaded on DEYA SPE cartridge prewashed with water. After washing the cartridge, the drug was eluted with 1.6 ml of 0.2 M sodium citrate and an aliquot of the eluate was taken for the derivatization. The derivatization procedure involved addition of 100 μl of 1 M sodium carbonate buffer (pH 11.9) to 270 μl of the sample and subsequent addition of 100 μl of FMOC solution (1 mg in 4 ml of acetonitrile). After 3 min, 100 μl of 1 M citric acid was added to adjust pH and 20 μl of the sample was injected into the chromatographic system.

Calibration curves were constructed by plotting the peak area ratios of alendronate to IS versus the concentrations of alendronate in solution.

Physicochemical tests

Solubility

An excess amount of alendronate sodium trihydrate was added to various vehicles and shaken at 37°C for more than 48 h. The solutions were then centrifuged at 10,000 rmp for 5 min, and the supernatant was assayed by HPLC after appropriate dilution.

Partition coefficient

Fifty milliliter of PG and 10 ml of toluene was saturated with each other for 10 min, and 10 ml was taken from hydrophilic phase. Fifty milliliter of toluene and 5 ml of PG was saturated with each other for 10 min, and 10 ml was taken from lipophilic phase. The composition of hydrophilic/lipophilic phase was PG containing 6% caprylic acid with or without amines/toluene, respectively. Alendronate solution (500 μg/ml) was prepared with hydrophilic phase saturated with lipophilic phase. Five milliliter of this solution was transferred to 10 ml centrifuge tube containing 5 ml of lipophilic phase saturated with hydrophilic phase. The tube was vortex-mixed for 30 min and centrifuged at 10,000 rmp for 5 min. Then, 3 ml of the solution was withdrawn from both phases. After pretreatment, the drug concentrations in both phases were determined by HPLC.

Preparation of alendronate PSA TDS

Sodium alendronate 78.06 mg (alendronate 60 mg) was dissolved in 1.4 ml of PG containing 6% caprylic acid with/without amines and then mixed with 3.6 g of Duro-Tak 87-2196. Alendronate PSA TDS was prepared by casting the above solution on a polyester release liner coated with silicone (Gelroflex ALU-PET 100 μ-2S DR, 3M, St. Paul, MN) using a casting knife. The area and thickness of the cast solution was 10 cm × 15 cm and 0.45 mm per 5 g solution, respectively. They were set at room temperature for 10 min to evaporate the solvents, and then dried for 20 min in an oven set at 90°C. The dried film was transferred onto a backing film (Scotchpak 1109, 3M, St. Paul, MN).

Procedure for skin permeation from solution formulation and PSA TDS

After sacrificing with ether, the dorsal skin of each hairless mouse was excised. One milliliter of alendronate solution formulation (saturated or 1 mg/ml) or PSA TDS of 2 cm × 2 cm size was applied to the epidermal side of the skin and mounted on a Franz Cell permeation system (Diffusion Cell Drive System, Labfine, Anyang, Korea); the dermal side was in contact with the receptor compartment. The theoretical amount of alendronate loaded to the epidermal side from PSA TDS was 1.785 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 (4, 8, 12, 24, and 36 h), 3 ml of receptor solutions was withdrawn and replaced simultaneously, and the amount of alendronate permeated was determined by HPLC.

Procedure for pharmacokinetic study in rats

Rats were divided into 17 groups, comprising six rats each. Groups 1 to 17 were administered by oral and TDS 1-16; the oral dose was 25 mg/kg of alendronate. For the administration of TDS, the hairs on the left side between back and abdomen were shaved carefully so that the stratum corneum remained intact. The size of TDS applied to the shaved site of rat was 3 cm × 4 cm. At predetermined time interval, urine samples were collected and analyzed by HPLC.

Data Analysis

Permeation study

The steady-state flux (J_s), lag time (T_L), diffusion coefficient (D), skin/vehicle partition coefficient (K), and apparent permeation coefficient (P_{app}) are defined by following equations (Barry, 1983).

$$J_s = \frac{DKC}{h}$$

$$D = \frac{h2}{6T_L}$$

$$P_{app} = \frac{DKC}{C_s h}$$

A: the effective diffusion area
h: the thickness of skin
C: the constant concentration of the donor solution
C_s: the solubility of the donor solution
$(dQ/dt)_{ss}$: the steady-state slope

Pharmacokinetic study

In pharmacokinetic study, the amount remaining to be excreted (ARE) plot was used to estimate the pharmacokinetic parameters from urine data. The value of ARE at each time was obtained by subtracting the cumulative amount excreted up to that time from the total amount excreted.

From a semi-logarithmic plot of $Ae_\infty - Ae(t)$ against time, the elimination rate constant (k) was obtained with a slope of –k. If the plot did not decline as a single exponential process, we had determined k by initial slope (Shargel et al., 2005). The value for ARE at zero time was assumed to be Ae_∞ according to following equation.

$$\ln[Ae_\infty - Ae(t)] = \ln Ae_\infty - kt$$

where Ae_∞ is the amount remaining to be excreted at time 0 and $Ae(t)$ is the amount remaining to be excreted at time t.

Statistical analysis

All the values were expressed as the mean ± SD. The pharmacokinetic parameters of TDS were compared with Kruskal-Wallis test, which was followed by a posterior with an unpaired *t*-test using the Bonferroni correction. A *P*-value of less than 0.05 was considered significant.

Results and Discussion

Solubility of alendronate was determined in PG containing 6% caprylic acid in the presence and absence of amines as shown in Table 1. The highest solubility was achieved with the formulation containing 10% DEA in PG with 6% caprylic acid, which was 1.7 times that without amines. The solubilities increased as concentrations of amines increased.

To evaluate partitioning to lipid layer of skin, partition coefficient between vehicles used in this study and toluene was measured. Even though *n*-octanol is known to have the similar polarity with skin lipid (Aungst et al., 1990), it was not used in this study because it was miscible with PG. In contrast to small difference in solubility of alendronate, dramatic increase in the partition coefficients was observed by the addition of amines. The maximum partition coefficient was 927.04 ± 161.22 in PG containing 6% caprylic acid with 10% TEA, followed by 6% TEYA (805.5 ± 45.3), 3% TEYA (603.3 ± 51.5), 10% TEYA (577.2 ± 43.9), and 6% TEA (500.5 ± 16.6). The addition of amines revealed 3- to 200-fold higher partition coefficient than that without amine (4.3 ± 1.1). The dramatic increase in partition coefficient by the addition of amines indicated that the drug could distribute through lipid layer more easily. Therefore, alendronate TDS containing amines in PG with 6% caprylic acid was expected to enhance permeation through stratum corneum.

Table 1 summarized permeation parameters of alendronate through excised hairless mouse skin using saturated solutions. TEA and TEYA at 3% concentration and DEA and DEYA at 10% concentration from saturated solutions showed relatively high permeation fluxes (J_s), which were 8.20 ± 0.80, 4.72 ± 0.81, 4.82 ± 0.10, and 7.87 ± 0.18 μg/cm²/h, respectively.

Several mechanisms were suggested for the enhancement of drug permeation through skin. One possible mechanism 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. Ion pairs are used to deliver organic ions across hydrophobic membranes more efficiently. It can enhance the skin transport of ionic drugs without modification of the drug structure, change in skin barrier function or specific devices (Hatanaka et al., 2000). 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 are used to increase the solubility of basic drugs and vice versa (Aungst et al., 1990).

The effects of amines on the skin permeation of alendronate varied according to the kinds or amounts of amines used in this study as described in Table 1. The addition of MEAs at various concentrations failed to change diffusion coefficient (*D*) or skin/vehicle partition coefficient (*K*), resultant to almost same values of J_s

Table 1. Permeation parameters of alendronate through excised hairless mouse skin from saturated solution formulation containing 6% caprylic acid in PG in the presence and absence of amines.

Amines	J_s (μg/cm²/h)	T_L (h)	D (cm²/h, x10⁴)	K	Solubility (μg/mL)
None	1.11 ± 0.25	12.0 ± 0.79	0.17 ± 0.012	1.37 ± 0.35	1.67 ± 0.13
3% MEA	1.13 ± 0.41	7.89 ± 0.95	0.26 ± 0.034	1.30 ± 0.32	1.14 ± 0.091
6% MEA	1.30 ± 0.16	9.46 ± 0.67	0.22 ± 0.016	1.10 ± 0.21	1.93 ± 0.25
10% MEA	1.31 ± 0.60	12.5 ± 0.42	0.16 ± 0.0050	1.15 ± 0.51	2.43 ± 0.36
3% DEA	0.31 ± 0.0092	0.10 ± 0.0010	20.42 ± 0.023	0.0070 ± 0.0010	1.24 ± 0.27
6% DEA	0.52 ± 0.71	3.86 ± 5.30	10.34 ± 14.25	0.12 ± 0.17	1.68 ± 0.11
10% DEA	4.82 ± 0.10	2.59 ± 1.39	0.92 ± 0.49	0.76 ± 0.39	2.79 ± 0.12
3% TEA	8.20 ± 0.80	6.15 ± 6.96	0.93 ± 1.05	4.69 ± 5.14	1.74 ± 0.25
6% TEA	0.51 ± 0.098	5.96 ± 0.98	0.35 ± 0.057	0.24 ± 0.0064	1.83 ± 0.079
10% TEA	0.30 ± 0.078	5.27 ± 3.05	0.38 ± 0.23	0.15 ± 0.093	2.14 ± 0.083
3% DEYA	1.24 ± 0.057	7.85 ± 1.83	0.26 ± 0.063	0.96 ± 0.27	1.72 ± 0.021
6% DEYA	2.04 ± 0.66	4.82 ± 2.69	0.50 ± 0.28	1.07 ± 0.86	1.74 ± 0.040
10% DEYA	7.87 ± 0.18	2.99 ± 0.27	0.69 ± 0.061	2.67 ± 0.30	1.75 ± 0.12
3% TEYA	4.72 ± 0.81	10.6 ± 2.44	0.20 ± 0.046	4.08 ± 1.61	2.14 ± 0.0036
6% TEYA	2.16 ± 0.67	9.14 ± 0.72	0.22 ± 0.017	1.52 ± 0.46	2.17 ± 0.041
10% TEYA	0.98 ± 0.16	9.01 ± 0.38	0.23 ± 0.010	0.74 ± 0.14	2.22 ± 0.066

Data were expressed as the mean ± SD (n = 3).
MEA = monoethanolamine, DEA = diethanolamine, TEA = triethanolamine, DEYA = diethylamine, TEYA = triethylamine, J_s = steady state flux, T_L = lag time, D = diffusion coefficient, K = skin/vehicle partition coefficient.

as control (no amines). In the case of DEAs, it was found that as DEA concentrations increased, solubility and *K* of alendronate increased while *D* decreased. The penetration enhancement factor of 4.34 was obtained with the addition of 10% DEA, compared with control. On the contrary, the increase in TEA concentrations lowered permeation fluxes due to the decrease in *K*. Among various amines added to PG containing 6% caprylic acid, 3% TEA achieved the highest flux of 8.20 ± 0.80 μg/cm²/h.

As DEYA concentrations increased, both *D* and *K* increased whereas solubility was not affected, resulting in higher permeation rates with higher concentrations of DEYA. TEYAs showed the opposite tendency to DEYAs, in which higher concentrations revealed lower permeation rates due to the significant decrease in *K*, compared with relatively constant values of *D* and solubility. Most saturated solutions showed shorter lag times than no amine, especially, the addition of DEA shortened lag times, significantly.

Figure 1 shows permeation profiles from saturated solution formulations containing 6% caprylic acid in PG in the presence of DEYA. Saturated DEYA 10% solution initially presented a very high permeation rate followed by a swift decrease until 36 h diffusion. The decrease in permeation rate with time may result from the rapid reduction in driving force owing to initial high permeation rate, for example prompt drop in alendronate concentration in the donor compartment.

Unlike saturated solution formulation, when alendronate was used at a fixed concentration (1 mg/ml) below solubility, most amines failed to enhance permeation fluxes of alendronate. According to the equation derived by Barry (1983), permeation fluxes of alendronate from fixed concentration solutions would not be affected by solubility, if the concentrations are below solubility, but by skin diffusivity or partitioning. Only three amines, MEA, DEYA and TEYA, at 10% concentration showed higher permeation flux than control, which were 0.95 ± 0.15, 1.79 ± 0.28 and 1.72 ± 0.72 μg/cm²/h, respectively.

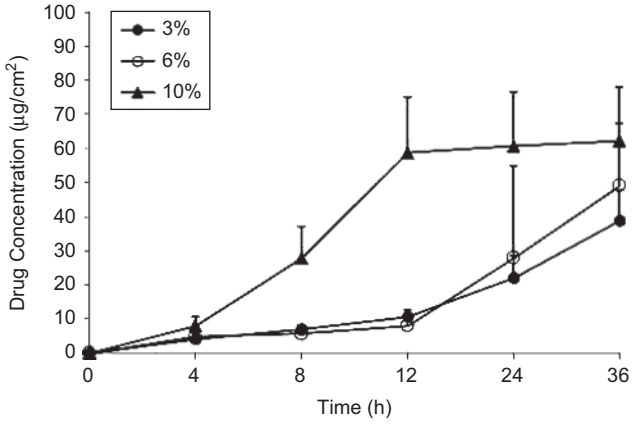

Figure 1. The permeation-time profiles for alendronate from saturated solution formulations containing 6% caprylic acid in propylene glycol in the presence of various concentrations of diethylamine.

Figure 2 depicts the apparent permeability coefficients of alendronate from 1 mg/ml solution formulations containing 6% caprylic acid in PG in the absence and presence of DEA and DEYA at 3, 6, and 10% concentration. DEYA dramatically increased the apparent permeability coefficient of alendronate as amine concentration increased, but DEA decreased it. The lag times of DEA and DEYA at 3, 6, and 10% concentrations were 2.65 ± 0.71, 0.20 ± 0.051, and 0.18 ± 0.31 h, and 3.58 ± 3.22, 7.02 ± 2.95, and 7.66 ± 0.46 h, respectively. Considering that *D* values are inversely correlated with lag times, the increased or decreased permeation fluxes by the change of amine concentrations were attributable to the changes of *K*.

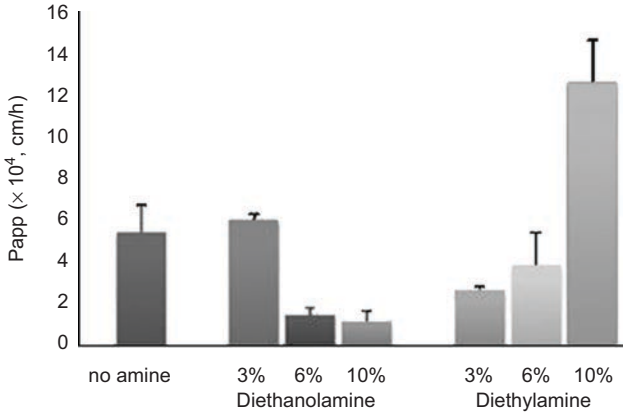

Figure 2. The apparent permeability coefficient of alendronate from 1 mg/ml solution formulation containing 6% caprylic acid in propylene glycol in the absence of amine, presence of 3, 6, and 10% diethanolamine and diethylamine, respectively.

Table 2. Permeation parameters of alendronate pressure-sensitive adhesive transdermal delivery systems through excised hairless mouse skin from TDS containing 6% caprylic acid in PG in the presence and absence of amines.

Amines	J_s (μg/cm²/h)	T_L (h)	P_{app} (× 10⁴, cm/h)
None	0.858 ± 0.165	7.49 ± 4.04	5.15 ± 0.988
3% MEA	0.786 ± 0.344	7.25 ± 3.93	6.89 ± 3.02
6% MEA	0.799 ± 0.256	5.17 ± 4.47	4.14 ± 1.32
10% MEA	1.59 ± 0.758	8.47 ± 1.27	6.56 ± 3.12
3% DEA	0.829 ± 0.165	7.25 ± 1.74	6.67 ± 1.33
6% DEA	0.739 ± 0.179	4.85 ± 4.21	4.39 ± 1.06
10% DEA	1.01 ± 0.540	6.50 ± 1.65	3.60 ± 1.93
3% TEA	1.13 ± 0.0702	11.3 ± 1.28	6.47 ± 0.403
6% TEA	0.776 ± 0.453	4.46 ± 5.40	4.24 ± 2.48
10% TEA	0.970 ± 0.299	8.83 ± 3.48	4.53 ± 1.40
3% DEYA	0.929 ± 0.113	9.65 ± 1.50	5.38 ± 0.653
6% DEYA	0.841 ± 0.274	3.86 ± 1.26	4.84 ± 1.58
10% DEYA	0.465 ± 0.0209	4.97 ± 4.34	3.28 ± 0.147
3% TEYA	1.02 ± 0.297	6.89 ± 2.95	4.77 ± 1.39
6% TEYA	0.846 ± 0.254	3.79 ± 0.906	4.09 ± 1.23
10% TEYA	0.676 ± 0.0251	2.47 ± 2.88	3.04 ± 0.113

Data were expressed as the mean ± SD ($n = 3$).
MEA = monoethanolamine, DEA = diethanolamine,
TEA = triethanolamine, DEYA = diethylamine,
TEYA = triethylamine, J_s = steady state flux, T_L = lag time,
P_{app} = apparent permeability coefficient.

The effects of amines on the percutaneous absorption of alendronate PSA TDS were investigated to identify the optimum permeation enhancers and to compare their effects with the results obtained from solution formulations. Based on the previous study (Choi et al., 2008), Duro-Tak 87-2196 was used as PSA, which is an acrylic adhesive matrix.

The permeation parameters of alendronate such as J_s, T_L, and P_{app} from formulated PSA TDS including 6% caprylic acid in PG with/without amines are listed in Table 2. The highest enhancement factor of 1.85 was attained with the addition of 10% MEA compared with no amine. The order of alendronate permeation rates from PSA TDS was ranked as 10% MEA > 3% TEA > 3% TEYA > 10% DEA > 10% TEA > 3% DEYA > no amine > 6% TEYA > 6% DEYA > 3% DEA > 6% MEA > 3% MEA > 6% TEA > 6% DEA > 10% TEYA > 10% DEYA. Although the order of permeation fluxes from PSA TDS formulations were quite different from solution formulations, TEA and TEYA at 3% concentration showed high permeation fluxes (1.13 ± 0.070 and 1.02 ± 0.29 μg/cm²/h, respectively), which were similar to saturated solution formulations. The addition of 10% MEA in PSA TDS formulations showed the highest permeation flux of 1.59 ± 0.76 μg/cm²/h whereas it did not reveal significant permeation enhancing effects in solution formulations. In addition, the overall permeation rates were lower than those from solution formulations. These differences between solution and PSA TDS formulations were thought to be probably due to the decrease in solubility, diffusivity, or partitioning to skin of alendronate in the PSA layer.

Table 3. Pharmacokinetic parameters of alendronate oral administration and transdermal delivery systems containing 6% caprylic acid in PG in the presence and absence of amines.

	$t_{½}$ (h)	Ae(t) (μg)	Ae_∞ (μg)
Oral alendronate	18.2 ± 6.85	6.50 ± 3.15	7.11 ± 3.26
None	28.3 ± 9.46	4.84 ± 3.33	8.71 ± 5.57
3% MEA	22.9 ± 3.76	4.80 ± 1.29	11.5 ± 2.72
6% MEA	33.3 ± 5.96	3.82 ± 1.06	6.10 ± 2.37
10% MEA	30.8 ± 3.53	12.7 ± 4.70	19.5 ± 6.93
3% DEA	28.0 ± 8.57	3.11 ± 1.40	6.51 ± 2.41
6% DEA	23.8 ± 2.36	3.10 ± 1.73	7.96 ± 5.58
10% DEA	34.3 ± 4.54	11.6 ± 2.08	17.3 ± 7.24
3% TEA	51.5 ± 10.1	7.18 ± 1.62	11.0 ± 2.21
6% TEA	30.2 ± 7.58	3.55 ± 1.83	8.35 ± 5.49
10% TEA	29.9 ± 5.66	5.36 ± 3.00	8.93 ± 5.99
3% DEYA	50.6 ± 10.0	9.45 ± 3.70	14.7 ± 7.38
6% DEYA	34.3 ± 15.6	4.81 ± 2.58	12.0 ± 8.50
10% DEYA	26.9 ± 3.52	3.92 ± 2.02	8.44 ± 4.36
3% TEYA	48.4 ± 21.1	4.63 ± 1.88	6.56 ± 4.81
6% TEYA	24.8 ± 2.94	2.96 ± 2.06	7.27 ± 4.97
10% TEYA	24.2 ± 5.40	3.74 ± 1.60	7.91 ± 3.10

Data were expressed as the mean ± SD (n = 5 or 6).
MEA = monoethanolamine, DEA = diethanolamine, TEA = triethanolamine, DEYA = diethylamine, TEYA = triethylamine, $t_{½}$ = elimination half-life, Ae_∞: amount remaining to be excreted at time 0, Ae(t): amount remaining to be excreted at time t.

Table 3 shows the pharmacokinetic parameters of alendronate after oral and various TDS administrations. ARE plot requires an accurate estimate of Ae_∞, so there has to be complete urine collection for at least four half-lives. In this study, mean half-life was 26 h among TDS containing amines and our last collection time was 156 h. Therefore, it was assumed that the urine was collected until no more alendronate is excreted.

The Ae_∞ and Ae(t) differed among TDS and oral delivery significantly ($P < 0.01$). The highest maximum Ae_∞ was 10% MEA (19.5 ± 6.93 mg h/ml), which was 2.7- and 2.2-fold, compared with oral and no amine administration, respectively (Figure 3), but there were no significant differences. The Ae(t) value from PSA TDS containing 10% MEA was the highest (12.7 ± 4.70 mg h/ml), which was significantly higher than no amine administration ($P < 0.05$).

Although the permeation data obtained from saturated alendronate solutions suggested that the

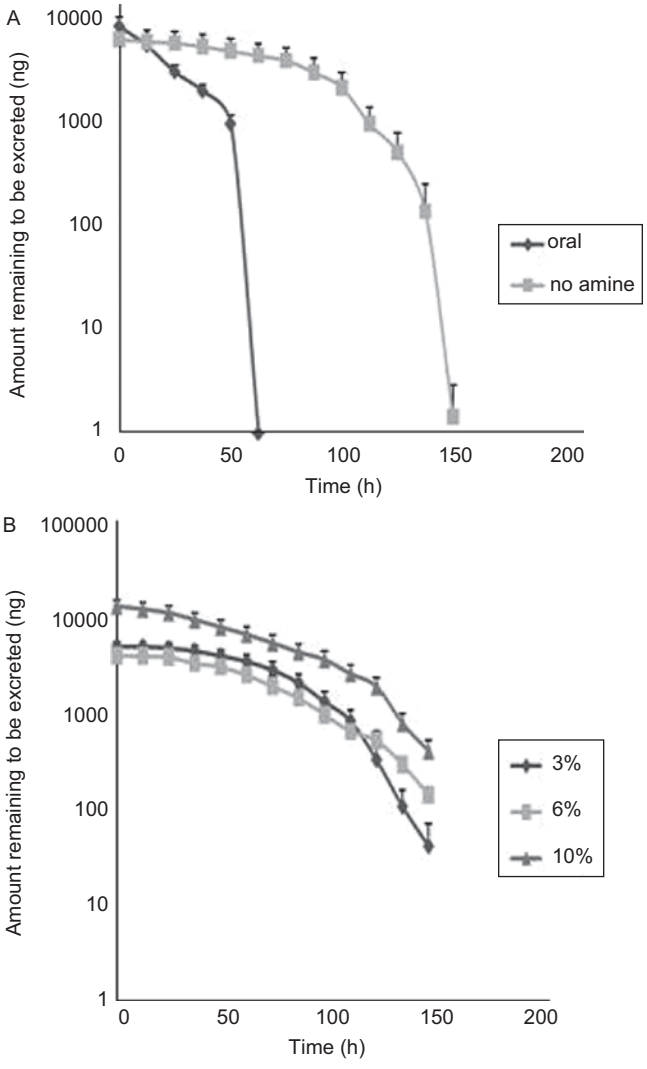

Figure 3. Amount remaining to be excreted (ARE) plot for alendronate from oral administration and pressure sensitive adhesive (PSA) transdermal delivery system (TDS) containing 6% caprylic acid in PG in the absence of amines (A) and PSA TDS containing 6% caprylic acid in PG in the presence of MEA (B).

alendronate TDS in the presence of 10% DEA is a better candidate than that in the presence of MEA, following *in vitro* experiments using PSA TDS showed that 10% MEA is better than 10% DEA in enhancing the permeation of alendronate. Therefore, it seems likely that the data obtained from *in vivo* study are more correlated with *in vitro* results using PSA TDS. MEA at 10% concentration showed the maximum enhancing effect of permeation in both *in vitro* and *in vivo* study of TDS. Therefore, TDS with 10% MEA in PG containing 6% caprylic acid could be a good candidate for the alendronate TDS.

Declaration of interest

The authors declare no conflict of interest.

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