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RESEARCH ARTICLE

Effect of ionization and vehicle on skin absorption and penetration of azelaic acid

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Abstract

Objective: The aim of this study is to investigate the effect of ionization and vehicle of topical formulations on skin absorption and penetration of azelaic acid (AZA).

Materials and methods: *In vitro* transport of AZA was determined for two topical formulations containing AZA with pH values of 3.9 and 4.9, respectively. FINACEA® (15% AZA gel), a US Food and Drug Administration approved drug for treatment of acne and rosacea, was also used for comparison. Release profile and flux of AZA were determined in an *in vitro* hairless mouse skin model using Franz Diffusion Cell.

Results: The data have shown that a higher concentration of AZA is retained in the epidermis/dermis layer and the whole skin for the formulation with pH = 4.9 as compared to that with pH = 3.9 at an active loading level of 2.82 mg/cm^2. In addition, the flux of ionized species of AZA in the pH 4.9 formulation (128.4 ± 35.9 μg/cm^2/h) is approximately five-fold greater than that in the pH 3.9 formulation (27.7 ± 4.0 μg/cm^2/h). The results suggest that the ionized AZA penetrates through the skin and accounts for majority of the total flux.

Discussion and conclusion: This study has demonstrated that the penetration and absorption of AZA show a strong pH- and vehicle-dependency. Solubilization is the rate-limiting step in percutaneous absorption of AZA.

Keywords: Azelaic acid, ionization, pH dependent, percutaneous absorption, vehicle effect

Introduction

Azelaic acid (1,7-heptanedicarboxylic acid, AZA) is a saturated, straight-chained C_9-dicarboxylic acid. It is the active pharmaceutical ingredient (API) in a number of prescription drugs for treatment of rosacea and acne[1], for example, FINACEA® contains 15% of AZA. Chemically, it is a diprotic acid with pK_a values of 4.53 and 5.33[2]. AZA has a limited solubility in water (about 0.24 g per 100 g of water at 25°C). At this concentration, it is therapeutically ineffective. As a result, dosage forms and matrix design of AZA could have a significant effect on its skin absorption and penetration, and consequently its therapeutic efficacy[3]. Prescription drugs containing AZA are generally in form of a gel or cream in which AZA is present as a suspended solid.

Theoretically, for a given molecule, degree of its topical absorption would be likely dominated by diffusion and permeation of the non-ionized species of that molecule, which is less polar than the corresponding ionized species. However, it has been reported that maximum flux through skin tissues could occur at a pH level where ionization is high[4]. Aqueous solubility of an ionized species is generally higher than its non-ionized counterpart. The higher solubility could in turn compensate for lower permeability of the ionized species. Furthermore, ionized species could permeate into the stratum corneum (SC) and their permeability could be modeled using equations proposed by Potts and Guy[5]. Effect of ionization on permeation is molecule-dependent. For topical delivery, effect of pH on percutaneous transport has been reported and contributions of ionization, solubility, lipophilicity, and pH are interrelated[6]. For example, Merino et al. reported a study on

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the effect of pH on the passive permeation of 5-fluorouracil (5-FU) through porcine ear skin[7]. Passive flux of 5-FU is increased by 1.6-fold when pH is increased from 5.0 to 7.4. In a study on skin permeation of ibuprofen, at a higher pH, solubility of ibuprofen increases due to higher degree of ionization, but its permeability decreases[8]. In another study using lignocaine, a basic molecule, Valenta et al. reported a good correlation between the pH value and permeability[9]. Based on these reported studies, it seems that permeability of an ionizable molecule is dependent on factors such as ionization, solubilization and intrinsic permeability of ionized species and the parent molecule. For an acidic compound, such as azelaic acid, if the pH partition hypothesis is the dominant mechanism, the non-ionized fraction of AZA can be increased by lowering pH, leading to an increase in the amount of AZA permeated both in terms of skin retention and flux. On the other hand, at higher pH, more AZA molecules will be in ionized form, which will have higher solubility than parent AZA. If solubilization is the dominant mechanism, the amount of AZA permeated should increase as pH value increases.

The vehicle of a topical formulation plays an important role in its therapeutic efficiency. It is generally believed that an API in the solubilized form is more bioavailable compared to that in the suspended form. The solubilized form generally shows a higher degree of absorption and penetration than the suspended solid form of the same species (ionized or non-ionized) due to enhanced solubilization. It has been reported that in a nasal product (Aerodiol) containing 17-β-estradiol, solubilization of 17-β-estradiol through forming a complex with dimethyl-β-cyclodextrin yields significantly greater nasal bioavailability in rabbits (94.6%) than the non-solubilized suspension formulation (25.2%)[10]. In another study, a formulation containing solubilized benzoyl peroxide (BPO) offers a potential to facilitate follicular penetration of BPO, thereby enhancing therapeutic efficacy. In a clinical study, the formulation containing solubilized BPO has been shown to demonstrate superior efficacy over prescription and over-the-counter medications containing BPO solids[11].

In the present study, formulations containing AZA at two pH values (3.9 and 4.9) were evaluated using hairless mouse skin model to investigate the effect of ionization on topical absorption and penetration of AZA. The objective is to understand mechanism of skin penetration and retention of AZA, the role of ionization and solubilization, and contribution of moieties in AZA (alkyl chain and carboxylic group) to its intrinsic permeability.

Materials and method

Materials

Azelaic acid was purchased from ALFA AESAR (Ward Hill, MA, USA). 1,2-Hexanediol was from Sabina Corporation (Piscataway, NJ, USA). Klucel@ MF was obtained from Hercules, Inc. (Wilmington, DE, USA). All other reagents are of high-performance liquid chromatography (HPLC) grade.

Formulations

Formulation F1 was prepared as follows: niacinamide was dissolved in a solution of 1,2-hexanediol in water. Azelaic acid was dispersed in the above solution with a stirrer and the mixture was heated to 45°C. The pH was adjusted to 4.9 ± 0.1 with triethanolamine. Stirring at 45°C was continued until azelaic acid was dissolved. The solution was allowed to cool to room temperature. Klucel® MF (0.75%) was added to the cooled solution while stirring until the solution was gelled. In this formulation, AZA was completely solubilized.

Formulation F2 was prepared as follows: niacinamide was dissolved in a solution of 1,2-hexanediol in water. Azelaic acid was milled to about 5 μm size and dispersed in the above solution. Klucel® MF (0.75%) was added to the mixture while stirring until gelled. The pH of the formulation was about 3.9 ± 0.1. Compositions of the formulations are listed in Table 1. 1,2-hexanediol in the formulations was used to enhance solubilization of azelaic acid in aqueous solution and serve as a moisturizing agent. Niacinamide was present in the formulations to provide anti-inflammation benefits.

Each gram of FINACEA® Gel contains 0.15 g azelaic acid (15% w/w) as the active ingredient in an aqueous gel base containing benzoic acid (as a preservative), disodium-EDTA, lecithin, medium-chain triglycerides, polyacrylic acid, polysorbate 80, propylene glycol, purified water and sodium hydroxide to adjust pH (pH value is 4.9). AZA exists in a suspended form in FINACEA.

Animals

Male hairless mice (30–40 days old) were purchased from Radiation Medicine Institute for Laboratory Animal Research, Chinese Academy of Medical Sciences, Tianjin, China. The abdominal skin was surgically removed from the animal and subcutaneous fat was carefully cleaned. The skin samples were stored at –20°C and used promptly.

In vitro percutaneous absorption and penetration studies

The skin samples were mounted to vertical Franz Cell (diffusion area: 1.77 cm^2) with the SC side facing the

Table 1. Formulations containing azelaic acid.

	Ingredient (g/100 g)				
Formulation	Azelaic acid	Klucel® MF	Niacinamide	1,2-Hexanediol	Final pH
F1	10.0	0.75	4.0	25.0	4.9
F2	10.0	0.75	4.0	25.0	3.9

donor chamber. The receptor chamber (16 ml) was filled with normal saline (0.9% NaCl), which was continuously stirred with a magnetic stirrer setting of 500 rpm. The skin samples were equilibrated with normal saline for 1 h at 37 ± 0.1°C. Infinite doses (50 mg of the formulations F1 and F2, 33.3 mg of FINACEA®, which correspond to 5 mg AZA) were applied to the skin surface. Each experiment was run in six replicates. At time point 8, 12, 16, 20 and 24 h, the skin surface was wiped with cotton ball soaked with phosphate buffered saline (pH 8.0) to recover the formulation left on the skin surface. The tape-stripping method was used to remove the SC layer. The first strip was combined with the soaked cotton ball. The combination was digested with 1.0 M NaOH, and then neutralized to pH 5.0 using glacial acetic acid, filtered, and ready for HPLC analysis. Repeated tape-stripping was continued until SC layer was disappeared (average 10 strips)[12]. All the strips were collected, combined, and digested in 10 mL 1.0 M NaOH. The mixture was then neutralized to pH 5.0 using glacial acetic acid, filtered, and ready for analysis. AZA retained in the epidermis/dermis layer was collected by methanol extraction method. After tape-stripping removal of SC layer, the skin samples were minced, vertexed with 1 ml methanol and centrifuged, and the supernatant was removed. The extraction step was repeated three times. The supernatants were combined, filtered and ready for analysis.

HPLC analysis

HPLC analysis was carried out with an Agilent 1100 HPLC system. A 250 mm × 4.6 mm stainless steel C_{18} column (5 µm, Thermo Fisher Scientific, Waltham, MA, USA) was used. The mobile phase was a mixture of ammonium acetate/acetic acid buffered solution (pH = 5.0, 10 mM) and methanol at 60:40, v/v[13]. The flow rate was 1 ml/min. The ultraviolet detector was set at a wavelength of 210 nm and the temperature of the column was maintained at 30°C.

Statistical analyses and data presentation

The flux of AZA through the skin into the receptor fluid was determined from slopes of plots of concentration in the receptor phase vs. time and expressed as µg / cm^2 / hour. The lag time, t_L, was determined from the x-intercept of the slope at the steady state. Statistical analyses were performed using Excel software. Student's t-test was performed to calculate the statistical significance. Values are given as means ± SD.

Enhancement ratio (ER) was calculated by using the following Eq. (1)[14]:

$$ER = \frac{Flux\ for\ formulation(solubilized\ AZA)}{Flux\ for\ formulation(suspended\ AZA)} \quad (1)$$

The relative contribution of ionized and non-ionized species to total observed flux (F_{total}) was calculated using Eq. (2)[15]:

$$F_{total} = \alpha F_i + (1-\alpha) F_u \quad (2)$$

where F_i is the flux of the ionized fraction of AZA, F_u is the flux of the non-ionized fraction, and α is the degree of ionization.

Results

In the present study, percutaneous absorption and penetration of AZA in topical formulations were investigated in an *in vitro* skin model of hairless mouse. Hairless mouse skin tends to be thinner and has fewer layers in the SC than human skin. Therefore, the hairless mouse skin is more permeable than human skin[16]. However, the mouse hairless skin is still widely used as an *in vitro* model for studying percutaneous absorption.

Effect of ionization

The effect of ionization on the topical absorption and penetration of AZA through hairless mouse skin was studied at two different pH values, 3.9 and 4.9. At pH value of 3.9 (formulation F2), AZA was in the form of a solid and was not solubilized due to low water solubility of parent AZA. On the other hand, at pH value of 4.9 (formulation F1), AZA was completely solubilized. AZA is a dicarboxylic acid with pK_a of 4.5 and 5.3 and a log *P* value of 1.48. Generally speaking, at a pH value greater than 7.5, an acid molecule is predominately negatively-charged[17]. As shown in Table 2, Figure 1, and Figure 2, non-ionized fraction of AZA gradually decreases with an increase in pH, from 0.820 at pH 3.9 to 0.314 at pH 4.9. As a result, there is a higher concentration of ionized species of AZA in F1 than in F2. The total flux of F1 (pH 4.9) is similar to that of F2 (pH 3.9), while the ionic flux of F1 (128.4 ± 35.9 µg/cm^2/h) is almost five times greater than that of F2 (27.7 ± 4.0 µg/cm^2/h). The ionic flux increases as the pH values because the solubility of azelaic acid is greater at higher pH values. For F1, Fi (flux of the ionized fraction of AZA) and Fu (flux of the non-ionized fraction of AZA) were calculated to 128.4 and 58.8 µg/cm^2/h, respectively. Thus, the Fi value was more than twice

Table 2. Effect of pH on the fraction non-ionized and flux of AZA penetrating through hairless mouse skin. mean ± SD, $n = 6$.

			Flux (µg/cm^2/h)		
Formulation	pH	Fraction non-ionized[a] (α)	F_{total}	F_u	F_i
F1	4.9	0.314	187.2 ± 52.8	58.8 ± 16.6	128.4 ± 35.9
F2	3.9	0.820	154.1 ± 22.2	126.4 ± 18.2	27.7 ± 4.0

[a]Fraction non-ionized = 1/[1 + antilog (pH – pK_a)], calculated by considering its pK_a value of 4.5.
Where F_{total} is the total flux, F_i is the flux of the ionized fraction of AZA, F_u is the flux of the non-ionized fraction, and αis the degree of ionization.

Table 3. The penetration data of AZA delivered from gels through hairless mouse skin, mean ± SD, n = 6.

Formulation	Dose	Total accumulation in 24h (μg)	Lag time (h)	Flux (μg/cm²/h)	ER
F1	5 mg	3448.9 ± 187.2	0.51 ± 0.10	187.2 ± 52.8	1.21
F2	5 mg	2916.6 ± 294.7	0.71 ± 0.22	154.1 ± 22.2	–

ER, enhancement ratio.

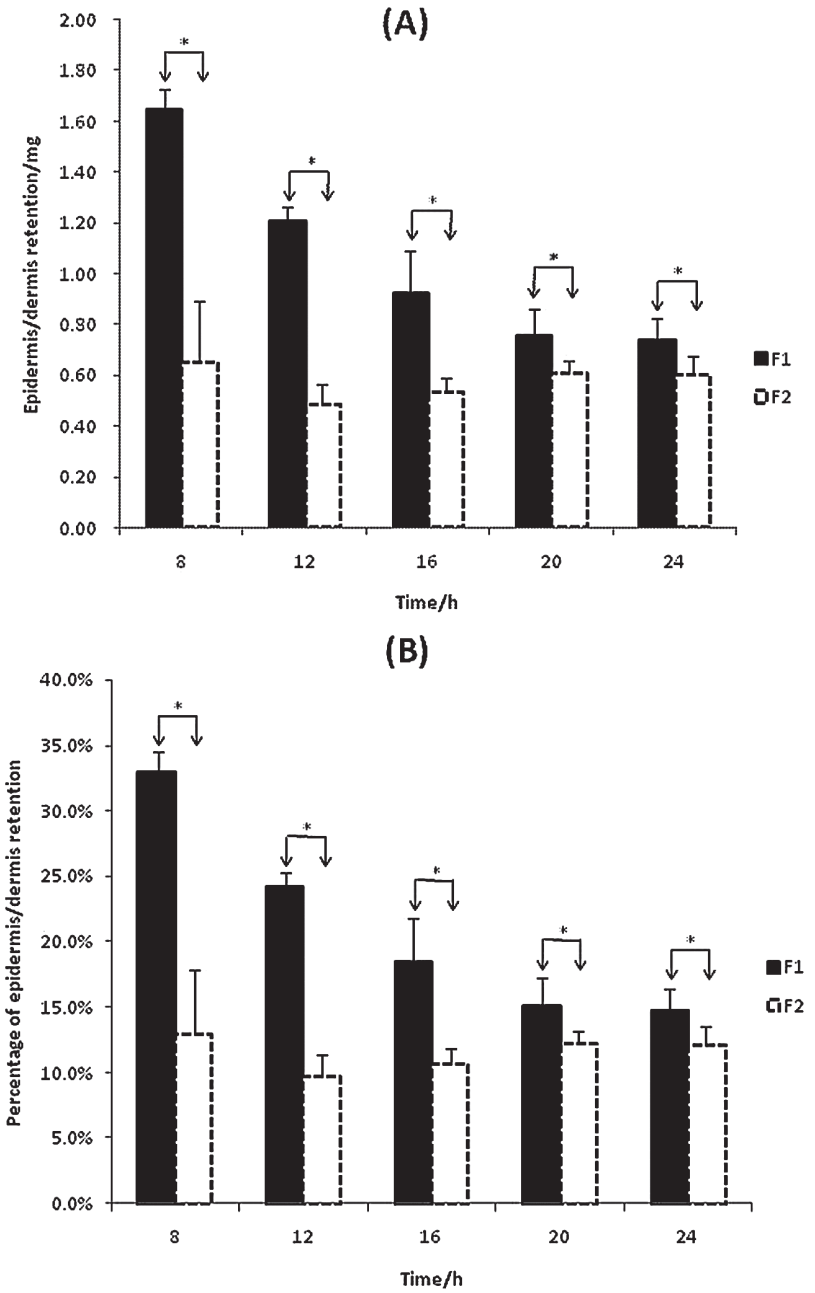

Figure 1. (A) Epidermal retention of AZA: formulation F1 vs. F2, mean ± SD, $n = 6$. (B) Percent of the applied dosage (at 5 mg dose): formulation F1 vs. F2, mean ± SD, n = 6. *$P < 0.05$ and **$P < 0.01$.

that of Fu. In the case of F2, Fi and Fu were 27.7 and 128.4 μg/cm²/h, respectively. The contribution of Fi was one-fifth of Fu.

The flux value, lag time and total amounts accumulated over a period of 24 h for F1 and F2 are shown in Table 3. The value of ER is 1.2, indicating that AZA in F1 penetrates faster than in F2. Furthermore, in addition to slower lag time, higher amount of AZA was accumulated and observed in the receptor medium over a period of 24 h for F1 (3448.9 ± 187.2 μg, 0.51 ± 0.10 h) than in F2 (2916.6 ± 294.7 μg, 0.71 ± 0.22 h).

Effect of vehicle

As shown in Figure 3, the percentage of epidermal retention of AZA for F1 is about 35% at 8 h, which is significantly higher than that for FINACEA® (8%). It is

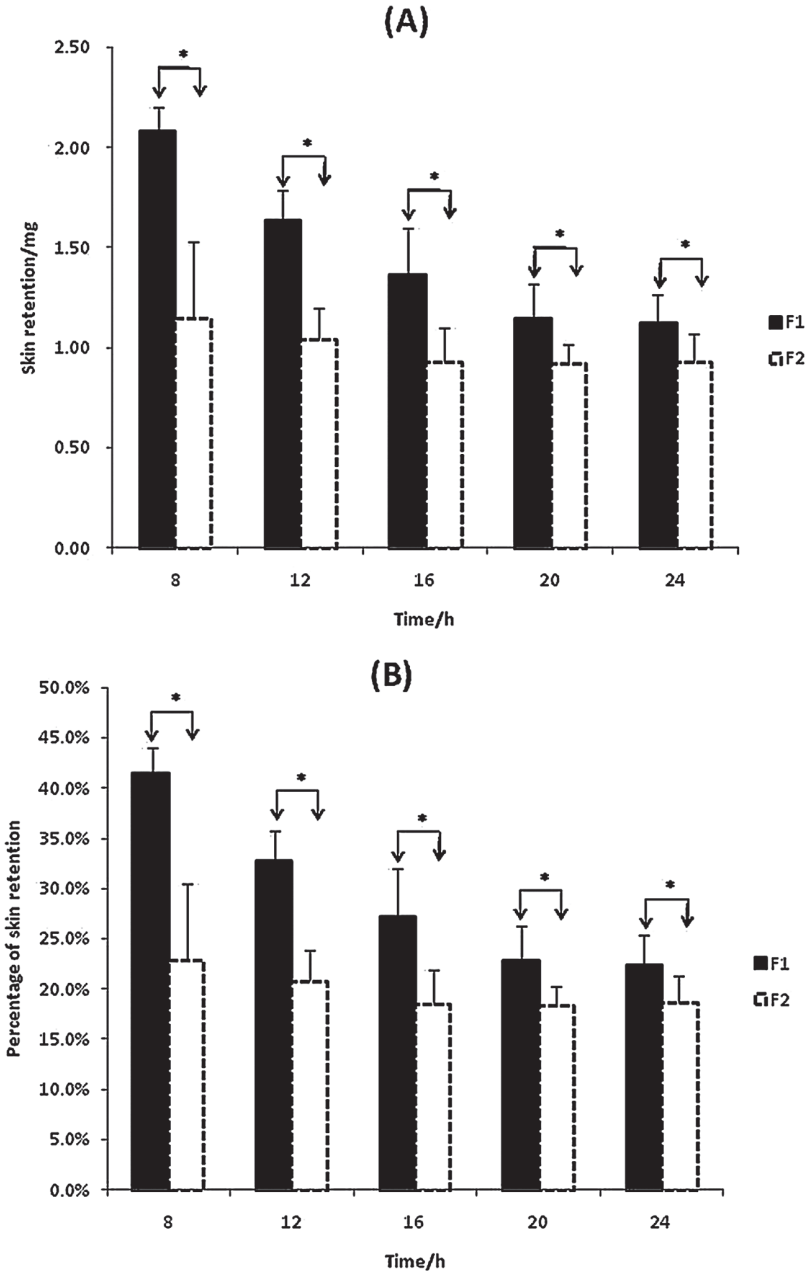

Figure 2. (A) Total skin retention of AZA: formulation F1 vs. F2, mean ± SD, $n = 6$. (B) Percent of the total skin retention (at 5 mg dose): formulation F1 vs. F2, mean ± SD, n = 6. *$P < 0.05$ and **$P < 0.01$.

interesting to note that although the retention of AZA for F1 decreases at the 24-h mark, there is still about 15% of the applied dosage retained in epidermis/dermis layer. That is approximately two times more than that for FINACEA®.

Figure 4 shows the total skin retention (sum of the SC and epidermis/dermis layer) for F1 and FINACEA® at various time points. The data show a similar trend as in the case of epidermal retention discussed above. Skin retention for F1 (from 2.0 mg at 8 h to 1.1 mg at 24 h) is consistently greater than that for FINACEA® (steady at about 1.0 mg over a period of 24 h).

The flux value, lag time and total amounts accumulated over a period of 24 h for F1 and FINACEA® are shown in Table 4. The flux value for F1 (187.2 ± 52.8 μg/cm²/h) is increased by 2.33-fold (ER value) when compared to that for FINACEA® (80.5 ± 14.7 μg/cm²/h). The lag time for F1 (0.51 ± 0.10 h) decreases by more than 10 times when compared to FINACEA® (5.77 ± 2.23 h), which reveals that AZA in F1 is rapidly accumulated in the skin tissue.

Discussion

Effect of ionization

Treatment of dermatological disorders relies on the ability of active agents to effectively penetrate the SC from applied formulations and reach the dermatologically

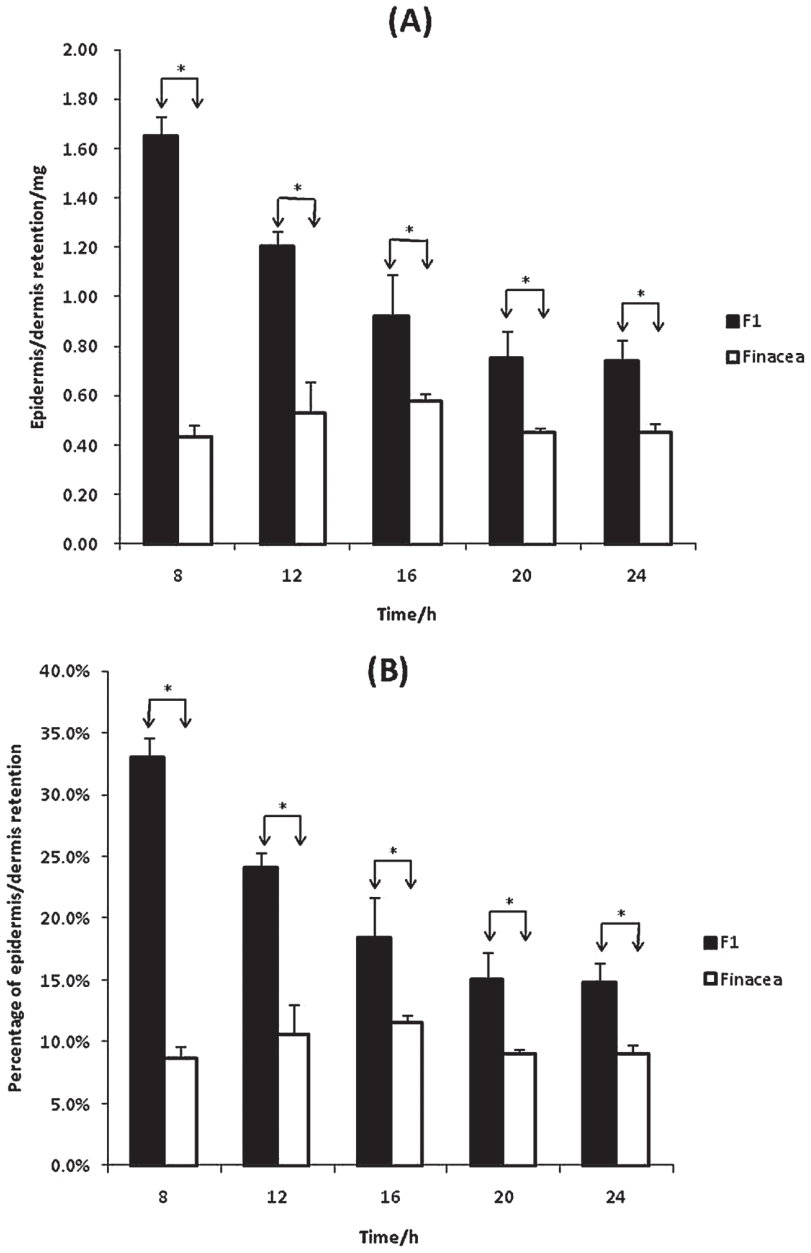

Figure 3. (A) Epidermal retention of AZA: formulation F1 vs. FINACEA®, mean ± SD, $n = 6$. (B) Percent of the total skin retention (at 5 mg dose): formulation F1 vs. F2, mean ± SD, n = 6. *$P < 0.05$ and **$P < 0.01$.

viable skin layers such as epidermis and dermis[18]. Thus, the skin retention of AZA plays an important role in its therapeutic efficacy. This study has shown that the absorption of AZA is strongly dependent on pH value and its ionized fraction in the topical formulations.

SC layer is the barrier to skin absorption and penetration and is hydrophobic in nature. Generally speaking, the ionized species of a molecule, being more polar, tends to have lower skin permeability than its non-ionized counterpart. There are two potential dominant mechanisms in skin absorption and penetration of AZA: solubilization-dominant and intrinsic permeability-dominant. For an AZA formulation, if difference in intrinsic permeability of AZA species, ionized or non-ionized, is dominant, one would expect that a decrease in epidermal and skin penetration and retention with an increase in pH from 3.9 to 4.9 (i.e. an increase in non-ionized fraction). However, if solubilization is dominant, it is expected that an increase in epidermal, skin penetration, and retention accompany an increase in pH from 3.9 to 4.9 (i.e. AZA is more soluble at higher pH). Surprisingly, it was discovered in the present study that AZA has higher skin retention at pH 4.9 as compared to pH 3.9, even though ionized species of AZA is predominant at pH 4.9. The results clearly suggest that the greater solubility of AZA at higher pH levels is more than enough to compensate for the lower permeability of the ionized AZA species, thereby confirming the significant role played by the ionized species in the skin penetration and retention of AZA.

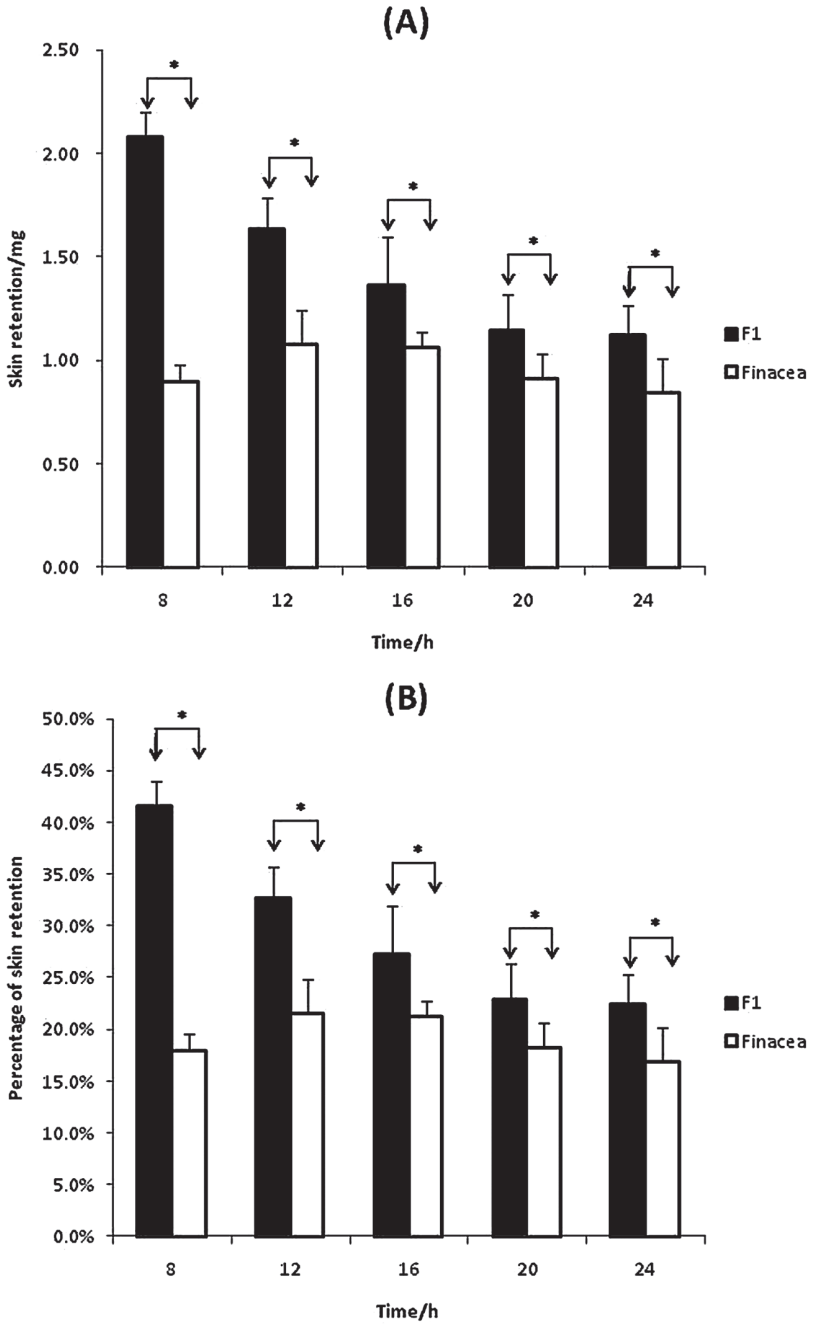

Figure 4. (A) Total skin retention of AZA: formulation F1 vs. FINACEA®, mean ± SD, n = 6. (B) Percent of the total skin retention (at 5 mg dose): formulation F1 vs. FINACEA®, mean ± SD, n = 6. *$P < 0.05$ and **$P < 0.01$.

Table 4. The penetration data of AZA delivered from gels through hairless mouse skin, mean ± SD, $n = 6$.

Formulation	Dose	Total accumulation in 24 h (μg)	Lag time (h)	Flux (μg/cm²/h)	ER
F1	5 mg	3448.9 ± 187.2	0.51 ± 0.10	187.2 ± 52.8	2.33
FINACEA®	5 mg	1726.6 ± 57.2	5.77 ± 2.23	80.5 ± 14.7	–

ER, enhancement ratio.

For an ionogenic compound, both ionized and non-ionized species are present in a formulation at a given pH. The total penetration flux is sum of the contribution from both species[19]. For a topical formulation, it is difficult to predict the effect of ionization on skin absorption and penetration. The general belief is that ionized species are considered to have lower permeability in spite of their higher solubility. A large number of published reports suggest that contribution of ion species to the total flux is generally very small[20–25]. However, a few reports do suggest that penetration of the ionized species of a few weak acids and bases contributes considerably to the total flux[26,27]. For example, the permeation of lidocaine (LC) from buffer solutions follows the

pH partition hypothesis. When the non-ionized fraction of LC increases by adjusting the pH, the amount of LC permeated into the skin also increases[28]. The penetration of ionized species of chlorpheniramine is very limited and it was found that the ratio of non-ionized vs. ionized chlorpheniramine that penetrated through human skin was about 200[21]. The non-ionized vs. ionized ratio of Indomethacin penetrated through various tissues was 100 for shed snake skin[22] and hairless rat skin[23], and 10,000 for human skin[20]. The ratio of the permeability coefficients (non-ionized vs. ionized) of oxycodone through mouse, rat and rabbit was 4:20[27]. In a study by Sudhir, it was reported that the ionized species of benztropine yielded a high partition coefficient, which was reflected by its relatively high skin permeability[26].

In the present study, there is a much higher concentration of ionized species of AZA in F1 (the ratio of non-ionzed fraction is 0.314) than in F2 (the ratio of non-ionzed species is 0.820). Surprisingly, not only is the total flux of F1 is larger than that of F2, there is also a much higher skin and epidermis/dermis retention rate in F1. In addition, Fi (flux of the ionized fraction of AZA) for F1 is calculated to be 128.4 μg/cm^2/h in comparison with 27.7 μg/cm^2/h for F2, confirming relatively high skin permeability and retention for ionized species of AZA. The Fu (flux of the non-ionized fraction of AZA) value of F1 (58.8 μg/cm^2/h), however, is only about a half of that for F2 (126.4 μg/cm^2/h). From these results, it is clear that the ionized species of AZA penetrate through the skin and predominantly contribute to the total flux.

Although it is difficult to predict effect of pH on skin penetration and retention of a weak acid or base, ionization of a weak acid tends to result in lower skin permeability. Furthermore, molecules of similar structural class/feature generally show similar trend. Skin permeability might be predicted based on structural similarity. For example, Watkinson et al. reported that at a higher pH, solubility of ibuprofen increased due to higher degree of ionization, while its permeability decreased[8]. Both ibuprofen (a mono-carboxylic acid) and AZA (a dicarboxylic acid) are weak organic acids. By examining chemical structures and the results from Watkinson et al., it is reasonable to predict that ionization of AZA at higher pH would result in even greater drop in its skin permeability when compared to ibuprofen.

Ibuprofen has one carboxylic group and a relatively long and hydrophobic tail containing a benzene ring. The benzene ring, being quite hydrophobic, exhibits good permeability into skin layer. Thus, once ionized, the ibuprofen molecule resembles a surfactant molecule with a polar head and long hydrophobic tail. It is well known that a surfactant molecule with such a structural feature generally penetrates well into skin layers. A good example is sodium dodecyl sulfate. Sulfonic acid is a strong acid and its sulfate is more polar than carboxylate. Due to the hydrophobic nature of the SC layer, with everything else being equal, it is expected that molecule with the less polar carboxylate head group would penetrate more effectively than the more polar sulfate head group.

On the other hand, AZA molecule has two ionizable carboxylic groups at its terminal ends. Whether AZA is mono- or di-ionized, it has two polar groups at both ends. It is proposed that the non-polar moiety of a chain molecule would intercalate into SC layer, leading to penetration into skin layers. For an ionized AZA molecule, the non-polar moiety is the middle 7-carbon chain, bookended by two polar carboxylic groups. Polar ends generally do not intercalate well. These ionized species of AZA resemble less to a surfactant molecule than ionized ibuprofen. In addition, the 7-carbon chain is too short to form a hydrophobic loop structure. A molecule with terminal polar groups generally does not have higher permeability. By examining chemical structures of ibuprofen and AZA, it is expected that once ionized, AZA molecule would be more polar than ibuprofen. Given the published data for ibuprofen, it is quite reasonable to assume that ionization of AZA would lead to more diminished skin permeability when compared to ibuprofen. The findings in the present study are exact opposite to the predication, which is unexpected.

One explanation to the observed results is that when ionized (mono- and/or di-), polar groups are at both ends of AZA molecule. Even though the 7-carbon chain could not form a hydrophobic loop structure, with the 7-carbon hydrophobic alkyl chain in the middle, the ionized species of AZA resembles, to a certain degree, a bi-layer structure. The bi-layer of ionized species could still be able to intercalate into SC lipid structure. Therefore, ionization of AZA at higher pH makes AZA much more soluble and is considerably more dominant relative to the increase in its polarity. In other words, the potential retardation effect caused by ionization at higher pH is insignificant when compared to the enhancement effect resulted from the increase in solubility.

The data from current study also provides a better understanding of the intrinsic permeability of AZA and relative contribution of each part of an AZA molecule. When the pH increases from 3.9 to 4.9, the ionized fraction of AZA increases from 0.180 to 0.686, which is about 3.8-fold increase. If the carboxylic groups and 7-carbon alkyl chain contribute equally to permeability of AZA, the increase in Fi (flux of ionized species) should mirror the increase in the fraction of ionized species. The data show a 4.6-fold increase in Fi. A smaller increase (3.8-fold) in fraction of ionized species has resulted in a greater increase in Fi (4.6-fold). Thus, it is reasonable to assume that in terms of intrinsic permeability of AZA, the 7-carbon alkyl chain makes a greater contribution to permeability of AZA than the carboxylic groups.

A study by Hadgraft and Valenta suggests that a significant mechanism of skin permeation of the ionized species may be through a lipophilic pathway, possibly as a result of ion pairing[4]. However, the permeation of ionized

species of AZA is not likely to occur through a lipophilic pathway because it seems that no suitable counter ions exist in the lipid layer of skin tissues. Thus, the increase in flux of AZA at pH 4.9 may have been contributed by transport of the ionized species mainly through the intercellular pathway. As a result, the greater flux of AZA through the hairless mouse skin occurs at a pH where level of ionization is higher.

It is highly unlikely that AZA solids could penetrate into skin layers directly. Molecular AZA species, either ionized or non-ionized, is responsible for skin absorption and penetration. If difference in intrinsic permeability between ionized and non-ionized species is more significant than difference in their solubility, intrinsic permeability determines skin absorption and penetration. In such a case, one would expect higher flux and skin retention for F2. It is known that the solubility of the ionized AZA is significantly higher than the non-ionized. If greater solubility of ionized AZA species is more than enough to compensate for its lower permeability, higher flux and skin intention would be observed for formulations with higher concentration of ionized species. Clearly, the results support the second assumption. It can be concluded that the transport of AZA through the skin is associated with pH-dependent partitioning and solubility.

Effect of vehicle

The AZA molecule is in the solubilized form in formulation F1. By contrast, AZA in FINACEA® is in the form of suspended solids. The results from this experiment clearly suggest that dissolution of the suspended AZA in the formulation is the rate-limiting step for the formulations containing suspended active ingredient. Dissolution requires either modification of the solid phase to reduce lattice energy (lower melting point) or modification of the formulation vehicle to break up hydrogen bonding between water molecules[29]. Solubilized AZA is in molecular form, which could diffuse across SC barrier. Thus, when a formulation with the suspended AZA is applied to skin tissue, dissolution from solids becomes a rate-limiting step. Due to its lower solubility in aqueous environment (about 0.24 gram per 100 gram of water at 25°C), the dissolution step could be a very slow process. AZA in solubilized form (as in formulation F1) reaches a high percentage (greater than 30%) of penetration in epidermis/dermis layer when compared to F2 and FINACEA® (at about 10%) at 8 h. Availability of AZA in epidermis/dermis layer (as measured by retention in the layer) increases significantly when the active agent (AZA) is in a solubilized form. This increased availability may possibly result in higher therapeutic efficacy.

Conclusions

The present study demonstrates that *in vitro* percutaneous absorption and penetration of AZA are strongly dependent on ionization and vehicle of the formulations. The study provides a clear mechanistic understanding of the importance of ionization and solubilization on skin penetration and retention. It also delineates contribution of each moiety of AZA molecule on its intrinsic permeability, which sheds light on the relationship between molecular structure and skin permeability. The ionized species play dominant role in its permeation through hairless mouse skin. AZA molecule with two terminal ionized/polar groups actually show increased skin permeability at higher pH (i.e., higher degree of ionization), suggesting important role played by the 7-carbon alkyl chain. It can be concluded that (a) solubilization is the rate-limiting step in skin absorption and permeation of AZA, and (b) the alkyl chain in AZA plays more significant role than carboxylic groups in determining intrinsic permeability of AZA.

Formulation of AZA containing higher level of solubilized AZA offers greater degree of skin penetration and retention when compared to the suspended form. A practical significance of this finding is to allow researchers to develop a clear gel dosage form of AZA where all ingredients are solubilized. A clear gel dosage form is generally considered cosmetically pleasing and user friendly, which in turn might lead to better patient compliance. The study lays ground work for developing superior AZA formulations which could lead to improved therapeutic efficiency and patient compliance.

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Declaration of interest

The authors report no declarations of interest.

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