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Combined effect of ultrasound and chemical enhancers on the skin permeation of aminopyrine

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Abstract

The combined effect of 150 kHz ultrasound with 111 mW/cm^2 intensity and chemical enhancers on the skin permeation of aminopyrine (AMP) was investigated using excised hairless rat skin. Monoterpenes (L-menthol, L-calvone and D-limonene), laurocapram (Azone®), glycerol monocaprylate (Sefsol-318®), isopropyl myristate and ethanol were selected as enhancers. Combined application of ultrasound and enhancers increased the skin permeation rate (flux) of AMP compared with ultrasound or enhancers alone. Better effects were obtained by the combination with monoterpenes. The influence of detailed conditions of ultrasound and enhancer applications on the AMP flux was further investigated using L-menthol. The enhancement effect by this combination was increased with an increase in ultrasonic application duration and L-menthol concentration, suggesting that these conditions might be used to achieve the controlled drug delivery. A pretreatment experiment with ultrasound or L-menthol was carried out, and L-menthol content in the skin and the skin permeation of deuterium oxide (D_2O), used as a donor vehicle, were measured to understand the role of ultrasound in the combined effect. Application of ultrasound to the L-menthol-pretreated skin increased the AMP flux, while the effect of L-menthol on ultrasonic-pretreated skin was similar to that of L-menthol alone. The ultrasound increased the L-menthol content in the skin as well as the skin permeation of D_2O from a vehicle with L-menthol. These results suggested that simultaneous application of ultrasound and enhancers is essential to obtain the pronounced effect. Ultrasound application also strongly assisted migration of L-menthol into skin, which increases the enhancing action on the skin permeation for a drug.

Keywords: Skin penetration enhancement; Phonophoresis; Ultrasound; Chemical enhancers; L-Menthol; Combined effect

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1. Introduction

In the development of a transdermal drug delivery system (TDS), the skin penetration enhancement of the drug is a key factor because of the high barrier properties of the stratum corneum, the outermost layer of skin, to drug permeation. Alterations in physicochemical properties of a drug by structural modification were attempted and characteristics of skin barrier by chemical enhancers were extensively studied (Sloan, 1992; Walters and Hadgraft, 1993). Iontophoresis, which delivers ionic species into the body by electric potential, may also offer the possibility of developing an intelligent TDS. This can provide pulsatile drug delivery by turning the potential on and off (Brand and Guy, 1995; Nakakura et al., 1995). On the other hand, phonophoresis, skin penetration enhancement by ultrasound, has been accepted for clinical therapeutics, and is known to practically increase skin permeation of steroids and promote therapeutic efficacy for a variety of muscular and arthritic conditions (Skauen and Zentner, 1984; McElnay et al., 1987). Trials to employ phonophoresis for a systemic drug delivery have also been performed recently (Miyazaki et al., 1992; Mitragotri et al., 1995).

We previously investigated the effect of 150 kHz ultrasound with a relatively low intensity, 111 mW/cm^2, on the skin permeation of nine drugs having different polarities, using excised hairless rat skin (Ueda et al., 1995). When water was used as a vehicle in the donor compartment, the application of ultrasound increased the diffusivity of the drugs in the skin by disorganizing the stratum corneum lipid packing to enhance skin permeation of polar compounds (Ueda et al., 1996). The enhancing effect obtained from the ultrasonic irradiation for 60 min, however, might be insufficient for systemically achieving transdermal drug delivery. The enhancement of skin penetration by ultrasound is influenced by the alteration of the ultrasonic frequency, intensity, duration and wave mode (continuous or pulsed wave) (McElnay et al., 1993). Therapeutic ultrasound, e.g., 0.5–5 MHz frequency and 0–3 W/cm^2 intensity, was generally used in many cases of phonophoresis. The use of high ultrasound intensity for phonophoresis may result in damage to the skin or subcutaneous tissue by cavitational or thermal effect (Suslick, 1988). If a combination of low ultrasound intensity with other promoting techniques such as use of chemical enhancers can raise the transdermal delivery rate of a drug, the benefits of phonophoresis will be even greater than at present.

Objectives in the present study were to examine the combined effect of 150 kHz ultrasound application with an intensity of 111 mW/cm^2 together with chemical enhancers on the skin permeation of aminopyrine (AMP). Terpenes (L-menthol, L-carvone and D-limonene), laurocapram (Azone®), glycerol monocaprylate (Sefsol 318®, S-318), isopropyl myristate (IPM), and ethanol (EtOH) were used as enhancers, and have been well studied in the development of TDS. AMP was used as a model drug, having an intermediate polarity ($\log K_{ow} = 0.38$) among the drugs used in the previous study (Ueda et al., 1995). The combined effect of the ultrasound and enhancers was evaluated based on the in vitro permeation rate (flux) of AMP through excised hairless rat skin. The influence of ultrasonic application duration and enhancer concentration on the skin permeation of the drug was also estimated when used in a combination with L-menthol. L-Menthol content in skin and the skin permeation of deuterium oxide (D_2O) from the donor vehicle were also measured to evaluate the role of ultrasound in the combined system.

2. Materials and methods

2.1. Equipment

The equipment previously described (Ueda et al., 1995) was used for the in vitro phonophoretic experiment. A continuous ultrasound generator (Dai-Ichi High-Frequency, Tokyo, Japan) connected to an ultrasonic transducer with 150 kHz frequency and an effective irradiation area of 3.14 cm^2 was used. The power of the ultrasound from the transducer measured by the radiation force balance method was 111 mW/cm^2 (Rooney, 1973).

2.2. Materials

AMP, L-carvone, D-limonene and EtOH were purchased from Wako Pure Chemical Industries (Osaka, Japan), IPM from Tokyo Kasei Industries (Tokyo), and D_2O from Merck (Darmstadt, Germany). L-Menthol (JP grade) was obtained from Hoei Pharmaceutical (Osaka). Azone was supplied by Nelson-Sumisho (Tokyo), and S-318 by Nikko Chemicals (Tokyo). Other chemicals and solvents were of reagent grade and obtained commercially.

2.3. Animals

Male hairless rats (WBN/ILA-Ht strain) weighing 160–180 g (7–8 weeks old), supplied by Life Science Research Center of Josai University were used in all experiments.

2.4. Combined effect of ultrasound and chemical enhancers

L-Menthol (5%), L-carvone (5%), D-limonene (5%), S-318 (5%), Azone (3%), IPM (10%) and EtOH (40%) were used as chemical enhancers. Each concentration was selected based on the previous studies for skin penetration enhancement (Sato et al., 1988; Okumura et al., 1991; Sugibayashi et al., 1995; Morimoto et al., 1992; Kobayashi et al., 1994). These enhancers were emulsified by 0.1% Tween 20 aqueous solution. Excised abdominal hairless rat skin was mounted on a vertical diffusion cell (donor and receiver volume, 5 and 12.5 ml; effective diffusion area, 4.91 cm^2) with a water jacket connected to a water bath at 32°C. The receiver compartment (dermis side) was filled with distilled water and stirred with a star-head magnetic bar driven by a constant speed motor (MC-301, Scinics, Tokyo) at 1200 rpm. AMP solution at a concentration of 1% containing each enhancer was added to the donor compartment (stratum corneum side), and then the ultrasound was immediately irradiated to the compartment for 60 min. An adequate amount of sample was withdrawn from the receiver compartment at predetermined times to measure the AMP concentration. The same volume of distilled water was added after sampling to keep a constant volume. An increase in temperature of the donor solution during ultrasonic irradiation (3–4°C) (Ueda et al., 1995) was prevented by controlling the temperature of the water bath.

2.5. Effect of ultrasonic application duration and L-menthol concentration on combined application of ultrasound and L-menthol

The hairless rat skin was mounted on the vertical diffusion cell, as described above. To measure the effect of ultrasonic application duration, 1% AMP solution containing 5% L-menthol was added to the donor compartment, followed by an ultrasonic application duration of 10, 30 or 60 min. An ultrasound application duration of 60 min was used when applying 1 or 2.5% L-menthol-loaded 1% AMP solution. Other procedures were as described above.

2.6. Effect of pretreatment with ultrasound or L-menthol on the skin permeation of AMP

Fig. 1 shows time schedules of the pretreatment. For the pretreatment with ultrasound (Fig. 1a), 1% AMP solution without L-menthol was first added to the donor compartment, and the ultrasound was applied to the compartment for 60 min from 4 h after beginning of the experiment. The donor solution was then replaced with 1% AMP solution with 5% L-menthol. For the pretreatment with 5% L-menthol (Fig. 1b), 1% AMP solution containing 5% L-menthol was first applied to the donor compartment, and the ultrasound was irradiated to the compartment for 60

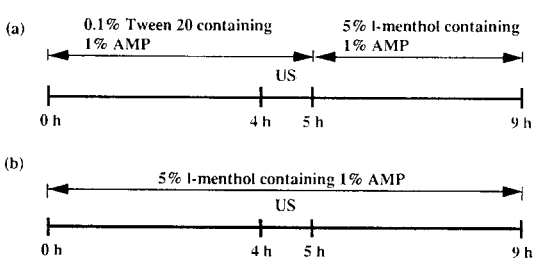

Fig. 1. Time schedules for pretreatment experiment with ultrasound or L-menthol, US, ultrasound.

min from 4 h after the start of the experiment. Then, the donor solution was replaced with a new solution, and the subsequent procedures were as described above.

2.7. Determination of L-menthol content in the skin

The hairless rat skin was mounted on the vertical diffusion cell, as described above, and 1, 2.5 or 5% L-menthol solution and distilled water were added to the donor and receiver compartments, respectively. After 4 h, the donor compartment was irradiated by the ultrasound for 60 min, and the skin was then removed from the cell. After rinsing the skin surface with distilled water three times and blotting off excess water, the effective diffusion area (4.91 cm^2) of the skin was cut off and weighed. The resulting skin tissue was immersed in a test tube containing 0.1% L-carvone as an internal standard in 1,4-dioxane, and sonicated (Branson 5200, Yamato, Tokyo) for 20 min to extract L-menthol (recovery ratio over 99%). After centrifugation, the concentration of L-menthol in the supernatant was determined using a gas chromatograph flame ionization detector (GC-FID). A calibration curve was constructed with 1,4-dioxane containing 0.1% L-carvone, a known amount of L-menthol, and excised hairless rat skin tissue.

2.8. Combined effect of ultrasound and L-menthol on the skin permeation of D_2O

L-Menthol in D_2O at a concentration of 5% and distilled water were added to the donor and receiver compartment, respectively. The ultrasound was then applied to the donor compartment for 60 min. Other procedures were as described above.

2.9. Analysis

Acetonitrile (500 μl), containing 2.5 μg of butyl *p*-benzoate as an internal standard, was mixed with the same volume of sample solution. After centrifugation, the AMP concentration in the supernatant was determined by high performance laser chromatography (HPLC). An HPLC system with a pump (LC-6A, Shimadzu, Kyoto, Japan), a UV spectrophotometric detector (SPD-6A, Shimadzu) and an integrator (C-R 6A, Shimadzu) were used for analyzing AMP. Acetonitrile: 0.1% phosphoric acid (pH 2.0) (45:55) containing 5 mM sodium dodecylsulfate (Tokyo Kasei Industries, Tokyo) was used as a mobile phase at a flow rate of 1.2 ml/min. A 4.6 × 250 mm stainless steel column packed with Nucleosil $5C_{18}$ (Macherey Nagel, Germany) and a wavelength of 254 nm were selected to assay AMP.

The L-menthol concentration in skin was determined with a GC-FID (GC-14A, Shimadzu) (Sugibayashi et al., 1995). Conditions were as follows: column, OV-17 (GL Science, Tokyo); column, injection and detection temperatures, 120, 160 and 160°C, respectively; carrier gas, N_2; and flow rate, 50 ml/min.

D_2O was quantified from the intensity of the OD stretching vibrational band at 2512 cm^{-1} (Hatanaka et al., 1993). The absorbance of the sample in a calcium fluoride cell (0.025 mm thick) was determined with an infrared spectrophotometer (260-30, Hitachi, Tokyo).

3. Results

3.1. Combined effect of ultrasound and chemical enhancers

Table 1 shows AMP fluxes at a pseudo steady-state (6–8 h) after application of each enhancer alone (J_{en}) and combined application of 1 h ultrasound and each concentration of enhancer (J_{com}). A permeation experiment using 0.1% Tween 20 was carried out as a control (without enhancer), since the influence of Tween 20 on the skin permeation of drugs can be ignored, compared with general enhancers (Morimoto et al., 1986). Steady-state flux for Tween 20 without ultrasound application (J_{cont}) was 5.13 ± 0.22 $\mu g/cm^2$ per h, and that with 1 h ultrasound (J_{us}) was 9.00 ± 0.90 $\mu g/cm^2$ per h, suggesting that the ultrasound effect was only 1.8-fold. Enhancing ratio for the combined application, E_{com} (ratio of J_{com} to J_{cont}), was calculated for comparison (Fig. 2, shaded

Table 1
Comparison of AMP flux treated with enhancer alone or with combinations of enhancer and ultrasound

	AMP flux (μg/cm^2 per h)	
	J^a_{cont}	J^b_{us}
0.1% Tween 20	5.13 ± 0.22	9.00 ± 0.90
	J^c_{en}	J^d_{com}
5% L-menthol	33.06 ± 2.27	219.32 ± 11.35
5% L-carvone	22.90 ± 0.97	148.68 ± 11.08
5% D-limonene	55.06 ± 2.10	159.85 ± 9.60
3% Azone	27.56 ± 1.97	69.09 ± 7.66
5% S-318	18.05 ± 1.06	21.79 ± 1.49
10% IPM	7.76 ± 0.58	18.60 ± 1.09
40% EtOH	5.69 ± 0.34	6.12 ± 0.27

[a] AMP flux of control without ultrasound.
[b] AMP flux of control with ultrasound.
[c] AMP flux of enhancer alone.
[d] AMP flux of combined application.
Each datum represents the mean ± S.E. of three experiments.

bar). Combination of ultrasound with S-318, IPM, and EtOH had a low enhancing effect on the AMP permeation (E_{com} value of 1.2–4.2), while combination with terpenes had a pronounced effect (E_{com} value of 29–42). The J_{com}/J_{en} ratio for each enhancer, E_{us}, was also calculated to compare the enhancement by ultrasound alone

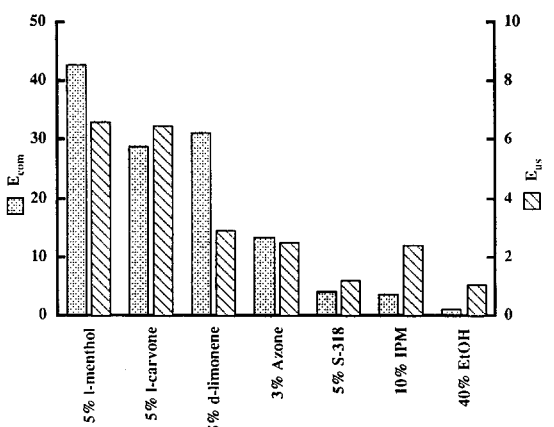

Fig. 2. Enhancement ratio for the combination of ultrasound and chemical enhancers, and ultrasound alone. E_{com}, ratio of J_{com} to J_{cont}; E_{us}, ratio of J_{com} to J_{en} for each enhancer.

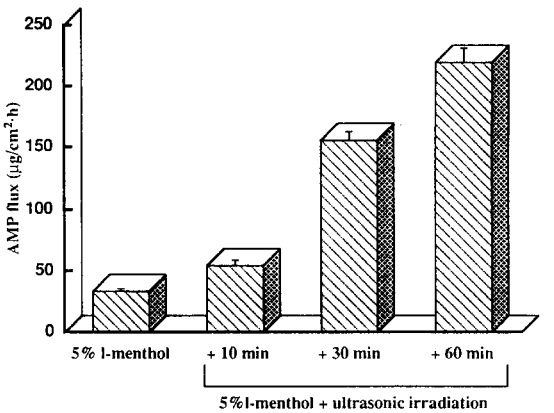

Fig. 3. Effect of ultrasonic application duration on the combination of ultrasound and L-menthol. Each datum represents the mean ± S.E. of three experiments.

with the combination of ultrasound and enhancer (Fig. 2, hatched bar). The E_{us} value was dependent on the enhancer used in this study, and those for L-menthol and L-carvone were higher than the others.

3.2. Effect of ultrasonic application duration and L-menthol concentration

Fig. 3 shows the influence of ultrasonic application duration on the combined effect of ultrasound and 5% L-menthol. AMP flux increased with increases in the application duration from

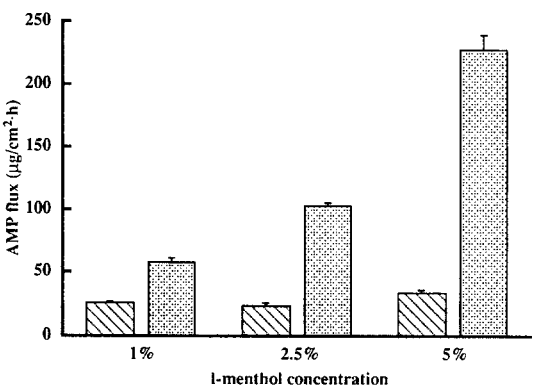

Fig. 4. Effect of L-menthol concentration on the combination of ultrasound and L-menthol. Dotted and hatched bars mean AMP flux with and without ultrasound, respectively. Each datum represents the mean ± S.E. of three experiments.

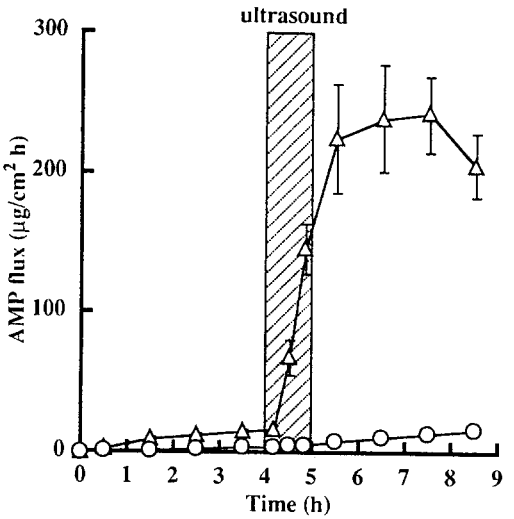

Fig. 5. Time course of AMP flux in the pretreatment experiment with ultrasound or L-menthol. (○), pretreatment with ultrasound; (△), pretreatment with L-menthol. Each data point represents the mean ± S.E. of three experiments.

10, 30 and 60 min. Fig. 4 shows the influence of L-menthol concentration in the donor compartment on the combination effect (ultrasonic duration was fixed at 1 h). AMP flux with the combined application was higher than that with L-menthol alone at each L-menthol concentration (1, 2.5 or 5%). Although the effect of L-menthol alone was comparable in all concentrations, the combined effect was dependent on the concentration of L-menthol.

3.3. Effect of pretreatment with ultrasound or L-menthol

Fig. 5 shows time courses of AMP flux from pretreatment with ultrasound or L-menthol. When L-menthol was used at a concentration of 5% on skin pretreated with ultrasound for 1 h, AMP flux was comparable to that of application with 5% L-menthol alone (○). On the other hand, 1 h application of ultrasound to the skin pretreated with 5% L-menthol induced a marked increase in AMP flux (△). Simultaneous application of ultrasound and L-menthol was necessary to obtain a substantial enhancement of AMP flux.

Table 2
Comparison of L-menthol content in the skin after treatment with L-menthol

	L-Menthol content (mg/g skin)		
	1%[a]	2.5%[a]	5%[a]
Non-US	3.24 ± 0.19	2.66 ± 0.30	4.84 ± 1.33
US	6.10 ± 0.97*	7.51 ± 1.15*	8.15 ± 1.03*

US, ultrasound. Each datum represents the mean ± S.E. of three to six experiments.
[a] Concentration of l-menthol used in treatment.
* $P<0.05$ (compared with non-US).

3.4. L-Menthol content in the skin

Table 2 shows L-menthol content in skin after treatment with either the ultrasound–L-menthol combination or L-menthol alone. This content seemed to depend on the L-menthol content applied in the donor phase, although the content at 2.5% without ultrasound was lower than that at 1%. Additionally, at the same L-menthol concentration in the donor compartment, the skin content was increased 1.7–2.8-fold by ultrasound at all levels of L-menthol concentration. To estimate the relationship between the L-menthol skin con-

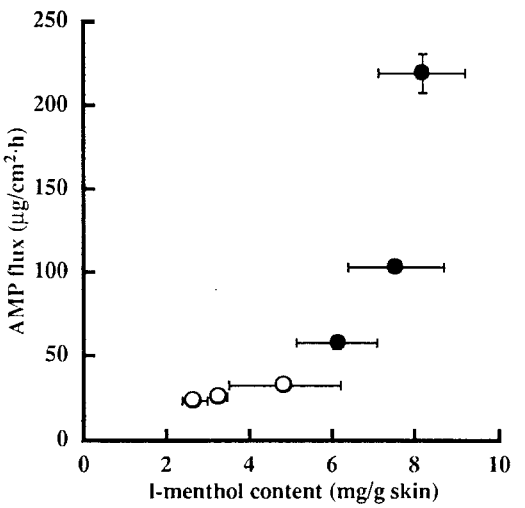

Fig. 6. Relationship between L-menthol content in the skin and AMP flux through the hairless rat skin. (○), without ultrasound; (●), with ultrasound. Each data point represents the mean ± S.E. of three to six experiments.

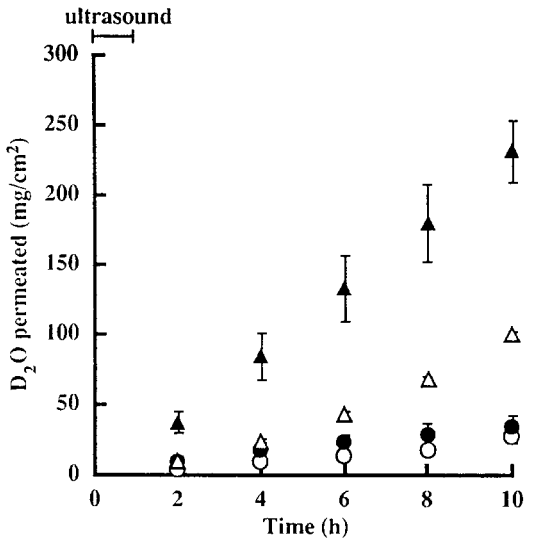

Fig. 7. Combined effect of ultrasound and L-menthol on the skin permeation of D_2O. (○), 0.1% Tween 20 without ultrasound; (●), 0.1% Tween 20 with ultrasound; (△), 5% L-menthol without ultrasound; (▲), 5% L-menthol with ultrasound. Each data point represents the mean ± S.E. of three experiments.

tent and AMP flux, the two are plotted in Fig. 6. It was found that the AMP flux was dependent on the L-menthol content in skin and was greatly increased when the content was more than about 6 mg/g skin.

3.5. Combined effect of ultrasound and L-menthol on the skin permeation of D_2O

Fig. 7 shows the permeation profiles of D_2O used as an aqueous donor solvent. An application of L-menthol alone increased the skin permeation of D_2O compared with that of control (0.1% Tween 20 without ultrasound), whereas an application of ultrasound alone did not. Combined application of the two, in contrast, greatly increased the skin permeation of D_2O.

4. Discussion

Several multi-component enhancement systems — L-menthol and ethanol (Morimoto et al., 1993), monoterpenes and ethanol (Obata et al., 1991), and D-limonene and temperature (Ohara et al., 1994) — were recently studied in search of a higher enhancing effect. We previously studied the effect of 150 kHz ultrasound with a relatively low intensity (111 mW/cm²) on the skin permeation of several low molecular drugs from an aqueous vehicle through hairless rat skin. Maximum skin penetration enhancement by this ultrasound, however, was of the order of 10-fold (Ueda et al., 1995, 1996). In the present study, a combined effect of low intensity ultrasound and chemical enhancers on the skin permeation of AMP was investigated. AMP flux was promoted by the combinations of ultrasound and monoterpenes, whereas the combined effect with S-318, IPM and EtOH was not as pronounced (Fig. 2).

As shown in Figs. 3 and 4, the combined effect with the L-menthol system which has the maximum E_{com} and E_{us} values was influenced by the conditions such as ultrasonic application duration and L-menthol concentration in vehicle, showing that regulation of these factors in the vehicle may aid in controlled drug delivery with a higher enhancement level than that of ultrasound alone.

The effect of pretreatment with ultrasound or L-menthol on the skin permeation of AMP was then compared, and L-menthol content in skin after treatment with the combination or with L-menthol alone was measured to understand the role of ultrasound in the combined system. The results obtained (Fig. 5) indicate that their simultaneous application was required for the pronounced increase in the drug flux. In addition, the promotion of L-menthol migration into skin by ultrasound (Fig. 6 and Table 2) may be related to their combination effect.

Williams and Barry (1991) reported that skin penetration enhancement by monoterpenes occurred through an increase in diffusivity of polar compounds in the stratum corneum. Our previous analysis using a two-layer skin model also indicated that L-menthol increased the migration of aqueous donor solvent into the stratum corneum (Kobayashi et al., 1994). In the present study, ultrasound obviously increased the skin permeation of D_2O from the donor vehicle with L-menthol, although did not have an enhancing effect on D_2O permeation from the donor vehicle with-

out L-menthol (Fig. 7). Therefore, ultrasound multiplies the enhancing action of L-menthol in such a way as to increase the migration of aqueous donor solvent into the stratum corneum.

We showed here that the simultaneous application of ultrasound and chemical enhancers achieved a great increase in skin penetration of AMP, even though the intensity of ultrasound was low. When enhanced drug delivery by ultrasound and chemical enhancers is anticipated, selection of the best combination becomes the key to assuring high ultrasonic enhancement, because the skin permeation rate of a drug is influenced by ultrasonic conditions such as duration. Combinations of ultrasound with L-menthol or L-calvone may be useful for effective skin delivery of a drug. Increase in enhancer content in skin to assist the action of enhancers may be one reason for the effectiveness of ultrasound. The combined effect of ultrasound and enhancers, however, may be largely dependent on the potential of an enhancer molecule and the amount of enhancer distributed into skin which is promoted by ultrasound. These factors may explain the difference in E_{us} values due to the kind of enhancers used. Systematic in vivo studies on the combination of ultrasound and enhancers to seek maximal enhancing effect, and to determine the mechanism, as well as to gain information on the causes of skin irritation, are necessary for optimal application to TDS.

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