Difference in the Enhancing Effects of Ultrasound on the Skin Permeation of Polar and Non-polar Drugs

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The effect of ultrasound (150 kHz, 111 mW/cm^2) on the permeability of isosorbide dinitrate (ISDN) and antipyrine (ANP) through excised hairless rat skin was evaluated using an Arrhenius plot. The permeability coefficients of ISDN across skin (at various temperatures) in the presence and absence of ultrasound were virtually isolinear on the Arrhenius plot. It has been suggested that the temporal increase in the ISDN flux, which was observed when ultrasound was applied in our previous study, was only a result of the thermal effect of ultrasound, *i.e.* an increase in the temperature of the donor solution. On the other hand, ultrasound influenced the Arrhenius plot of ANP, suggesting that the enhancement effect for ANP permeation could be not explained only by the thermal effect of ultrasound. In addition, the effective diffusion (D) and partition coefficients (K) of ISDN and ANP were estimated using their skin permeation profiles across the ultrasonic pretreated skin. The coefficients of ISDN with ultrasonic pretreatment were comparable to those without pretreatment. On the other hand, the D value of ANP with ultrasonic pretreatment was increased about 4 times by ultrasonic pretreatment, in spite of an insignificant change in the K value. These results suggest that the ultrasound used in the present study increased the effective diffusivity across the aqueous region in the stratum corneum to enhance the skin permeation of the polar compound, ANP.

Key words   percutaneous absorption; phonophoresis; ultrasound; Arrhenius plot; skin permeation parameter

The application of an external physical force to improve the low skin permeability of a drug is an attractive development for the transdermal drug delivery system (TDDS). Iontophoresis, which uses electrical current as a physical force, has been extensively studied to achieve controlled drug delivery, and its enhancing mechanisms have been described by many researchers.[1—3] Ultrasound is also available for skin penetration enhancement, and this technique is known as phonophoresis or sonophoresis.[4,5] Although an understanding of the ultrasonic effect on the skin permeation of a drug is important to effectively apply the TDDS, the mechanism of phonophoresis has not been identified. Mitragotri *et al.* have described that a disordering of the lipid bilayers in the stratum corneum (s.c.), which resulted from a cavitational effect (among several ultrasonic phenomena), was responsible for the phonophoretic enhancement.[6,7] However, there has not been much information gathered on the exact mechanism by which ultrasound enhances drug permeation through skin.

In our previous study, the effect of ultrasound (150 kHz, 111 mW/cm^2) on the skin permeation of several drugs was measured. The enhancement effect by this ultrasound technique was more pronounced for polar compounds than for non-polar compounds, and they could be distinguished by reversible or irreversible enhancement effects.[8] The result, estimated from an electrochemical technique, suggested that the latter effect was caused by an enlargement of the aqueous region in the s.c.[9] The former might be related to the thermal effect of ultrasound, because the temperature of the donor solution was increased from 32 °C to 36 °C immediately after ultrasound application and returned to 32 °C after the ultrasound was turned off.

In the present study, our objectives were to elucidate the thermal effect of ultrasound and to evaluate why ultrasound enhances skin permeability to polar com-

pounds to a greater extent than it enhances skin permeability to non-polar compounds. The effect of ultrasound on the skin permeation of drugs was evaluated with an Arrhenius plot of the permeability coefficient (P) using a polar drug, antipyrine (ANP) and a non-polar drug, isosorbide dinitrate (ISDN). In addition, the skin permeation parameters of these drugs, such as the effective diffusion (D) and the partition coefficients (K), were estimated in order to obtain mechanistic information about phonophoretic enhancement.

Experimental

Ultrasonic Equipment We used a continuous ultrasound generator (Dai-Ichi High-Frequency Co., Ltd. Tokyo, Japan) connected to an ultrasonic transducer with a frequency of 150 kHz and an effective irradiation area of 3.14 cm^2. The ultrasound from the transducer was measured at 111 mW/cm^2 by the radiation force balance method.[10]

Materials ISDN was supplied by Toko Pharmaceutical Co., Ltd. (Tokyo). ANP and trypsin (Type II, from porcine pancrease) were purchased from Wako Pure Chemical Industries Co., Ltd. (Osaka, Japan) and Sigma Chemical Co., Ltd. (St. Louis, MO, U.S.A.), respectively. Other chemicals and solvents were of reagent grade and were obtained commercially.

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

Skin Permeation Study for the Arrhenius Plot 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 27, 32, 37 and 42 °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. ANP or ISDN solution, at concentrations of 100 or 0.1 mg/ml, respectively, were added to the donor compartment (s.c. side), and the cell was left for 12 h to achieve a pseudo steady state permeation rate (flux) for the skin. The solutions in both compartments were then replaced with fresh solution, and the donor compartment was irradiated with ultrasound for 2 h. A detailed procedure for the irradiation technique was shown in our previous paper.[8] An adequate amount of sample was withdrawn from the receiver compartment at predetermined times and the same volume of distilled water was added after sampling to keep the volume constant. The temperature

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of the donor compartment was monitored using a thermometer and was exactly maintained by controlling the temperature in the water bath. A control experiment was done using the above mentioned procedure, except for ultrasound application.

Estimation of the Skin Permeation Parameters (D) of Each Drug A skin piece was mounted on the vertical diffusion cell, as described above. After both compartments of the cell were filled with distilled water for 12 h to hydrate and equilibrate the skin, ultrasound was applied to the donor compartment for 1 h. The donor solution was then replaced with ANP or ISDN solution at a concentration of 10 or 0.1 mg/ml. An adequate amount of sample was withdrawn from the receiver compartment at predetermined times to assay the drug concentration. The same volume of distilled water was added after sampling to keep the volume constant. The temperature of the whole experimental set was kept at 32 °C.

The effective diffusion (D) and partition coefficients (K) were calculated by fitting the time course of the cumulative amount of drugs that permeated across the skin, Q_t, to Eq. 1, as described by Scheuplein et al.,[11] using a non-linear least squares program, MULTI.[12]

$$Q_t = AKC_vL[D_t/h^2 - 1/6 - 2/\pi^2 \sum_{n=1}^{\infty} (-1)^n/n^2 \exp(-D_n^2\pi^2 t/h^2)] \quad (1)$$

where C_v, A and L are the drug concentration in the donor solution, the effective diffusion area, and the thickness of the skin barrier (15.4 μm),[13] respectively.

Analysis An adequate amount of acetonitrile solution containing 5 μg/ml methyl or butyl p-benzoate as an internal standard for ANP or ISDN, was added to the sample solution. After centrifugation, the drug concentration in the supernatant was determined by 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) was used for analysis. Acetonitrile:0.1% phosphoric acid (pH 2.0) (30:70) was used as a mobile phase for ANP, and acetonitrile:water (50:50) was used as a mobile phase for ISDN. A flow rate of 1.2 ml/min was used for both mobile phases in the HPLC system.

Table 1. Physicochemical Properties of the Drugs Used in This Study

	Isosorbide dinitrate (ISDN)	Antipyrine (ANP)
M.W.[a]	236.1	188.2
log K_{ow}[b]	1.20	−0.69
C_w (mg/ml)[c]	0.972	634

a) Molecular weight. b) Logarithm of the octanol/water partition coefficient at 32 °C. c) Solubility in water at 32 °C.

A 4.6 × 250 mm stainless steel column packed with Nucleosil $5C_{18}$ (Macherey Nagel, Germany) were selected to assay ANP and ISDN at wavelengths of 254 and 220 nm, respectively.

Results and Discussion

The Effect of Ultrasound on the Arrhenius Plot of Drug P Values The physicochemical properties of ANP and ISDN are shown in Table 1. These drugs were used as polar and non-polar compounds in the previous study, respectively.[8]

First, the Arrhenius plot of P values was analyzed to elucidate the thermal effect of ultrasound and to differentiate the enhancing effects of ultrasound on the skin permeation of polar and non-polar drugs. Figures 1a and b show the time courses of ISDN flux through the skin over 27—42 °C with and without the ultrasound application, respectively. The P values were calculated using the mean value of ISDN flux, and were plotted against the reciprocal of the absolute temperature, $1/T$ (Fig. 1c), because almost all of the fluxes were constant over the experimental period. The Arrhenius plot regression line for ISDN (Fig. 1c) in the absence of ultrasound was consistent with the one for ISDN in the presence of ultrasound. The activation energies (E_a) of ISDN skin permeation were calculated from the slope of each regression line, and these were not found to be significantly different (24.07 and 25.70 kcal/mol for the treatment with and without ultrasound, respectively, 1 cal = 4.184 J). This result indicates that the ultrasound used in the present study has no enhancing effect on the skin permeation of ISDN under the constant temperature condition. A temporal increase in the ISDN flux during the ultrasound application, however, was observed in the previous study, and the flux was 11.2 μg/cm²·h, when the donor concentration was 486 μg/ml.[8] In addition, the temperature in the donor solution during ultrasound application was elevated about 4 °C in the previous study. The ISDN flux of 10.35—11.83 μg/cm²·h was then estimated by substituting this temperature to the regression

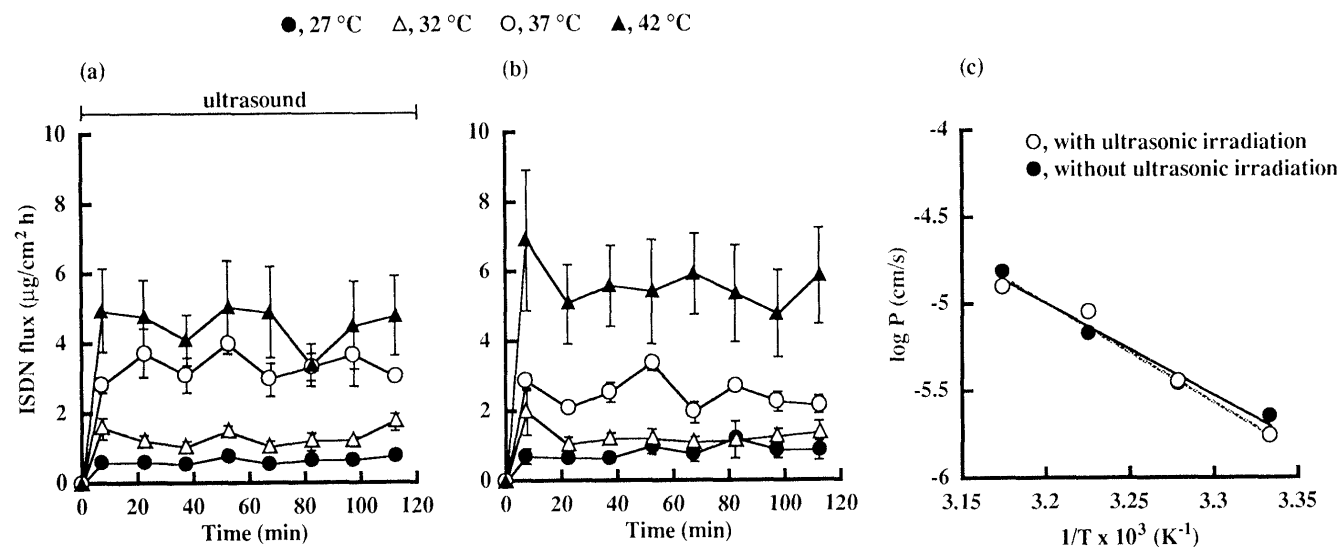●, 27 °C △, 32 °C ○, 37 °C ▲, 42 °C

Fig. 1. Time Courses of ISDN Flux at Each Temperature with (a) and without (b) Ultrasonic Irradiation, and the Arrhenius Plots (c) of the Permeability Coefficients (P) of ISDN

Shaded and solid lines in (c) show the regression lines with and without ultrasonic irradiation. Each data point in (a) and (b) represents the mean ± S.E. of 3—6 experiments.
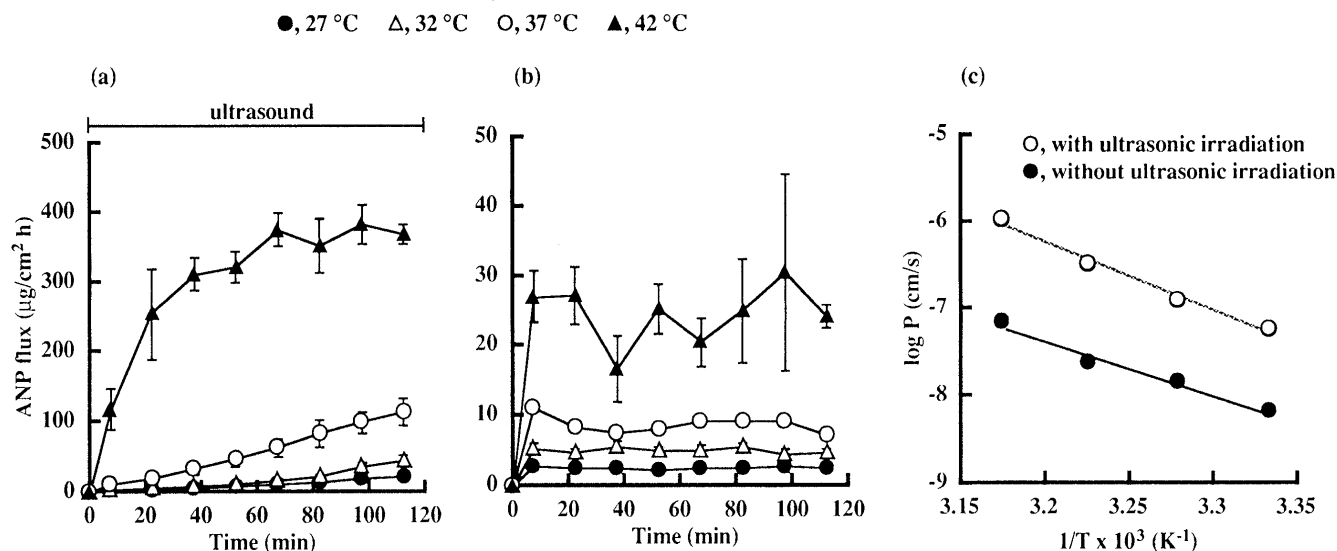
●, 27 °C   △, 32 °C   O, 37 °C   ▲, 42 °C

Fig. 2.   Time Courses of ANP Flux at Each Temperature with (a) and without (b) Ultrasonic Irradiation, and the Arrhenius Plots (c) of the Permeability Coefficients (P) of ANP

Shaded and solid lines in (c) show the regression lines with and without ultrasonic irradiation. The P values of ANP during ultrasonic irradiation were calculated from the flux of 90—120 min. Each data point in (a) and (b) represents the mean $\pm$ S.E. of 3—6 experiments.

Table 2.   Activation Energies of ISDN and ANP for Permeation through Hairless Rat Skin with and without Ultrasound

	Without ultrasound	With ultrasound
ISDN	24.07 ± 10.56	25.70 ± 13.09 (n.s.)
ANP	28.34 ± 12.10	36.18 ± 12.29 (n.s.)

Unit; kcal/mol (1 cal = 4.184 J).   n.s.; not significant compared to the data without ultrasound (p = 0.05).

Table 3.   Permeation Parameters of ISDN and ANP through Hairless Rat Skin with and without Ultrasound

	ISDN		ANP	
	Without ultrasound	With ultrasound	Without ultrasound	With ultrasound
P (cm/s)	3.33×10^{-6}	3.53×10^{-6}	8.76×10^{-8}	3.04×10^{-7}
D (cm^2/s)	1.72×10^{-10}	1.93×10^{-10}	5.93×10^{-11}	20.3×10^{-11}*
K	29.9	28.2	2.27	2.31

* $p < 0.05$ (compared with non-ultrasound).

line without ultrasound. The temporal increase in the ISDN flux previously observed, therefore, was obviously caused only by the thermal effect.

Figures 2a and b show the time course of ANP flux with and without ultrasound. The ANP flux with ultrasound increased with the elapsed time of ultrasonic application, so that the P values were calculated using the ANP flux over 90—120 min and plotted against $1/T$ (Fig. 2c). Their Arrhenius plot for ultrasound treatment was obviously different from that without ultrasound, although the temperature of the donor solution was maintained exactly. This result shows that the non-thermal effects of ultrasound should be taken into consideration for the skin permeation of ANP. In addition, the Arrhenius plots in Figs. 1c and 2c suggest that ultrasound enhances the skin permeability of penetrants across the polar region rather than the non-polar region of the s.c. Mitragotri et al. recently described using a low frequency ultrasound (20 kHz) with which cavitation was the dominant mechanism responsible for phonophoresis.[7] The enhancement effect of 150 kHz ultrasound on the skin permeation of ANP may be related to the cavitational effects of ultrasound.

The Effect of Ultrasound on the Skin Permeation Parameters of Drugs   The skin permeation parameters of ISDN and ANP through ultrasonic pretreated skin were estimated by fitting the permeation amount to Eq. 1. Table 3 shows the obtained permeability (P), diffusion (D), and partition coefficients (K) of ISDN and ANP with and

without ultrasonic pretreatment. No significant changes were found in the P values of ISDN with and without ultrasonic pretreatment. The D and K values with the pretreatment were also comparable to those without the pretreatment. These suggest that the enhancement effect of ultrasound is reversible on the skin permeation of drugs such as ISDN across the non-polar region in the s.c. In the previous study, we observed that ultrasound had only a small effect on the leaching of s.c. lipids, i.e. sterols and ceramides.[9] Therefore, this may be one reason for the temporal permeation enhancement of ISDN only during ultrasonic irradiation by temperature increase.

On the other hand, the D value as well as the P value of ANP was significantly increased by the ultrasonic pretreatment, while no increase was found in the K value (Table 3), suggesting that the enhancement effect of ultrasound may be related to an increase in the diffusivity of drugs across the polar region in the s.c. in addition to the temperature increase. The diffusivity of solutes in an aqueous solution is several orders higher than that in the ordered lipid barrier.[14] Drug diffusivity in the aqueous region in the s.c. is related to the tortuosity of the permeation domain.[15] If the ultrasound enlarges the aqueous region in the s.c. or shortens the diffusion length of the aqueous domain, the effective diffusion coefficient of polar compounds such as ANP, which favors the

aqueous phase in the s.c., is increased. Morphological sites for the aqueous region are still unknown. The aqueous domain in the lipid packing in the s.c., however, may be included in the region. A slight leaching of the s.c. lipids by ultrasound may be why the enlargement of the total aqueous region.

In summary, we showed that the thermal effect of ultrasound was responsible for the reversible enhancement effect on the skin permeation of drugs. In addition, it was suggested that an irreversible enhancement effect of ultrasound was due to an increase in diffusivity across the aqueous region. In addition, differences in the skin penetration-enhancing effects of ultrasound on ISDN and ANP permeation may be related to the enhancing mechanism of phonophoresis. However, a contribution of the mechanical effects of phonophoresis, such as cavitation, on skin permeation has not been identified. An understanding of the mechanical influence of ultrasound on the skin permeation of drugs (in addition to its thermal effect) may enable us to select optimal phonophoretic conditions.

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