External Control of Drug Release and Penetration. VI.[1] Enhancing Effect of Ultrasound on the Transdermal Absorption of Indomethacin from an Ointment in Rats

Shozo MIYAZAKI,* Hideyuki MIZUOKA, Yumi KOHATA, and Masahiko TAKADA

Faculty of Pharmaceutical Sciences, Higashi-Nippon-Gakuen University, Ishikari-Tohbetsu, Hokkaido 061-02, Japan. *Received February 29, 1992*

The effect of an ultrasound (1 MHz) on transdermal absorption of indomethacin from an ointment was studied in rats. Ultrasound energy was supplied for between 5 and 20 min at a range of intensities (0.25, 0.5, 0.75, and 1 W cm^{-2}), energy levels commonly used for therapeutic purposes. For evaluating skin penetration of indomethacin, the change of plasma concentration was measured. The pronounced effect of ultrasound on the transdermal absorption of indomethacin was observed at all ultrasound energy levels studied. The intensity and the time of application were found to play an important role in the transdermal phonophoretic delivery system of indomethacin; 0.75 W cm^{-2} appeared to be the most effective intensity in improving the transdermal absorption of indomethacin, while the 10 min ultrasound treatment was the most effective. Although the highest penetration was observed at an intensity of 0.75 W cm^{-2}, 0.5 W cm^{-2} was preferred because intensities of less than 0.5 W cm^{-2} of ultrasound for 10 min did not result in any significant skin temperature rise nor did it have any destructive effect on rat skin. Progressively more skin damage was noted as the intensity and the time of application of ultrasound increased. When used at a proper intensity and time of application, ultrasound appears to be a safe technique for enhancing the passage of various drug molecules through human skin.

Keywords transdermal absorption; ultrasound; indomethacin; rat skin (*in vivo*); skin temperature; skin damage; phonophoresis

Ultrasound has long been used for medical diagnosis and therapeutic purposes. In addition to clinical applications, the use of ultrasound in drug delivery systems is rapidly increasing and has been reviewed recently.[2] We have previously shown the feasibility of pulsed drug delivery from implantable devices controlled by external ultrasound.[3]

Recently, much research has focused on the discovery of methods for improving the transdermal absorption of drugs. Many reports have described efforts to change skin permeability using chemical enhancers, because of the low permeability of drugs across the skin. In addition to use of chemical enhancers, it is possible to increase the transdermal absorption by use of physical methods[4] such as iontophoresis, *i.e.*, by electric fields, or by phonophoresis, *i.e.*, ultrasound.

Phonophoresis has long been used for treating localized skin condition, and for delivering drugs to inflamed joints.[5] Recently, phonophoresis has been explored for its utility in delivering drugs systemically. There are several reviews of transdermal drug delivery by phonophoresis.[4,6,7] However, there is limited quantitative information on the effect of ultrasound irradiation on the delivery of a transdermally administered drug. Brucks *et al.*[8] estimated the effect of ultrasound on *in vitro* penetration, but not *in vivo*, and they show that ultrasound can increase the *in vitro* penetration of ibuprofen through human skin. For evaluating ultrasound effects on drug absorption, pharmacodynamic assays have been used in most investigations, for example, the blanching assay,[9] skin prick test,[10] and vasoconstrictor assay.[11] Few studies, however, have assessed the *in vivo* effect of ultrasound on the transdermal delivery systems,[12] and no pharmacokinetic parameters are available quantifying the efficacy of ultrasound.

In the previous paper,[13] it was demonstrated that therapeutic ultrasound could enhance the transdermal absorption of indomethacin from an ointment. The purpose of the present paper was to study the effect of operation parameters, such as irradiation intensity and duration of application, on the ultrasound-facilitated transdermal delivery of the model drug from an ointment. The area under the plasma drug concentration–time curve (AUC) was used to assess the transdermal absorption of the drug. In addition, the skin damage evoked by ultrasound irradiation was microscopically investigated, considering safety to the skin.

Experimental

Materials Indomethacin was purchased from Sigma Chemical Co. (St. Louis, MO). Gel ointment used was the commercially available Inteban ointment from Sumitomo Pharmaceutical Co. (Osaka), which contains 1% (w/w) indomethacin. Other reagents used were of analytical grade.

***In Vivo* Transdermal Absorption Experiments** Male Wistar rats weighing 250—350 g were used. The day before the experiment the hair of the abdominal parts was carefully removed with an electric clipper and a razor without breaking the skin. On each study day the rats were anesthetized by intraperitoneal injection of sodium pentobarbital (40 mg/kg), and the indomethacin ointment (1 g) was applied to a 3 cm diameter circular site on the abdominal skin of the rats. The area around the application site was covered with Saran Wrap film (Asahi-Dow, Tokyo), followed by 6 g of ultrasonic gel (Echo Jelly, Aloka Co., Tokyo). Ultrasonication was produced by a commercially available 1 MHz ultrasound system (Model AU-1, Asahi Denshi Kogyo Co., Osaka) approved for human use. The treated area was irradiated for 10 min at therapeutic intensities (0.25, 0.5, 0.75, and 1 W cm^{-2} being employed as continuous irradiation). Control animals were treated by the procedure described above, except that the ultrasonic probe was not applied. Blood samples (0.7 ml) were taken by cardiac puncture at hourly intervals after drug administration. The assay of indomethacin was performed using high-performance liquid chromatography as described previously.[14]

Measurement of Skin Temperature Surface skin temperature was recorded on a thermister (D613, Takara Kogyo Co., Tokyo) after ultrasound application by placing a temperature coupler (Takara SZL-64) on the surface of the treated rat skin. The temperature of the hypodermis was also measured by means of a small incision in the rat abdominal skin and insertion of the temperature coupler into the hypodermis.

Histological Examination of the Skin Tissue The effect of ultrasound on the skin tissue was checked by microscopic observation. The tissue samples excised after ultrasound treatment were fixed in formalin by the standard procedure,[15] stained with hematoxylin–eosin, and observed with a microscope (model PM-10ADS, Olympus, Tokyo). Untreated skin served as a control.

Data Analysis The AUC up to 4 h post-administration was calculated by moment analysis.[16] The Student's t-test was utilized to estimate the significant differences between each ultrasound treatment group and the control group.

Results

Effect of Ultrasound Intensity on the Transdermal Absorption For studying the effect of ultrasound intensity on the drug absorption, energy was supplied for 10 min at a range of intensities (0.25, 0.5, 0.75, and 1 W cm^{-2}), energy levels commonly used for therapeutic purposes.

Figure 1 shows the mean plasma level profile of indomethacin obtained and Table I summarizes the *AUC* value up to 4 h post-administration. It was evident that there was a distinct difference in plasma concentration response with and without ultrasound treatment. The pronounced effect of ultrasound on transdermal absorption was observed for all four intensities studied, but the effect was not proportional to the intensity; the 0.75 W cm^{-2} appeared to be the most effective intensity in improving transdermal absorption of indomethacin. As shown in Table I, the mean *AUC* value (33.22 $\mu g \cdot h \cdot ml^{-1}$) after irradiation at this intensity was 3.4 times greater than the control value (9.70 $\mu g \cdot h \cdot ml^{-1}$).

Effect of the Time of Ultrasound Application on the Transdermal Absorption The effect of the duration of treatment on the transdermal absorption of indomethacin was also studied. In this study, ultrasound was applied continuously at fixed intensities (0.5 and 1 W cm^{-2}), while the duration of ultrasound application was varied: 5, 10, and 20 min.

Figures 2 and 3 show the mean plasma level profile of indomethacin obtained at 0.5 and 1 W cm^{-2}, respectively. Tables II and III summarize the effect of the duration of phonophoresis treatment on the *AUC* values. The time of ultrasound application was also found to play an important role in the transdermal phonophoretic delivery of indomethacin. The magnitude of enhancement in the transdermal absorption of indomethacin following phonophoresis treatment was not proportional to the time of application. *AUC* values increased up to 10 min and then declined as exposure time lengthened (20 min) at intensities of either 0.5 (Table II) or 1 W cm^{-2} (Table III).

Effect of Ultrasound on the Skin Temperature Ultrasound may have harmful effects on the skin if it is used

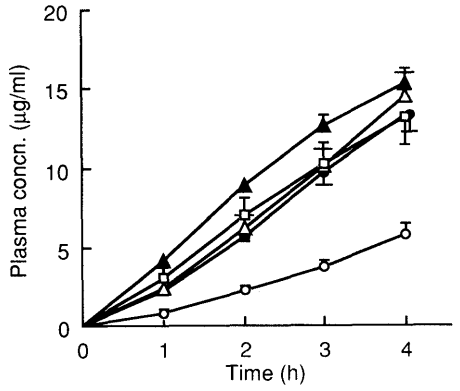

Fig. 1. Effect of Ultrasound Intensities on the Plasma Levels of Indomethacin from an Ointment in Rats

Each rat was irradiated with 1 MHz ultrasound for 10 min at the intensity levels of 0 (○), 0.25 (●), 0.5 (△), 0.75 (▲), and 1 (□) W cm^{-2}. Each value is the mean ± S.E. of 4—9 experiments.

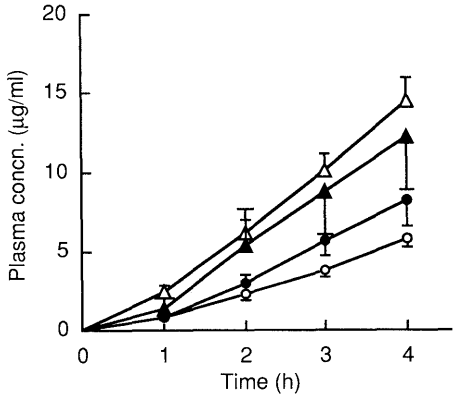

Fig. 2. Effect of the Time of Application of Ultrasound on the Plasma Levels of Indomethacin from an Ointment in Rats

Each rat was irradiated with 1 MHz ultrasound at the intensity level of 0.5 W cm^{-2} for 0 (○), 5 (●), 10 (△), and 20 (▲) min. Each value is the mean ± S.E. of 3—9 experiments.

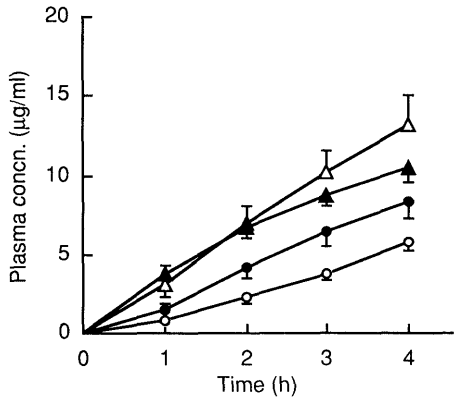

Fig. 3. Effect of the Time of Application of Ultrasound on the Plasma Levels of Indomethacin from an Ointment in Rats

Each rat was irradiated with 1 MHz ultrasound at the intensity level of 1 W cm^{-2} for 0 (○), 5 (●), 10 (△), and 20 (▲) min. Each value is the mean ± S.E. of 3—9 experiments.

TABLE I. *AUC* Values Following Transdermal Absorption of Indomethacin in the Absence and Presence of Ultrasound Treatment with Each Intensity

Intensity (W cm^{-2})	n	*AUC*[a] ($\mu g \cdot h \cdot ml^{-1}$)	Enhancement factors[b]
0	9	9.70 ± 1.07	1.0
0.25	5	24.20 ± 1.58[c,d]	2.5
0.5	6	25.85 ± 2.77[c,e]	2.7
0.75	6	33.22 ± 1.49[c]	3.4
1	4	26.94 ± 3.62[c]	2.8

a) Each value is the mean ± S.E. *b*) Enhancement factor relative to *AUC* from the control. *c*) Significantly different from the control ($p<0.001$). Significantly different from the value for 0.75 W cm^{-2}, *d*) $p<0.01$; *e*) $p<0.05$.

TABLE II. *AUC* Values Following Transdermal Absorption of Indomethacin in the Absence and Presence of Ultrasound Treatment with Each Time of Application at 0.5 W cm^{-2}

Time of application (min)	n	*AUC*[a] ($\mu g \cdot h \cdot ml^{-1}$)	Enhancement factors[b]
0	9	9.70 ± 1.07	1.0
5	4	13.46 ± 2.23[e]	1.4
10	6	25.85 ± 2.77[c]	2.7
20	3	21.60 ± 7.20[d]	2.2

a) Each value is the mean ± S.E. *b*) Enhancement factor relative to *AUC* from the control. Significantly different from the control, *c*) $p<0.001$; *d*) $p<0.02$. *e*) Significantly different from the value for 10 min ($p<0.02$).

TABLE III. *AUC* Values Following Transdermal Absorption of Indomethacin in the Absence and Presence of Ultrasound Treatment with Each Time of Application at 1 $W cm^{-2}$

Time of application (min)	n	$AUC^{a)}$ ($\mu g \cdot h \cdot ml^{-1}$)	Enhancement factors[b)]
0	9	9.70±1.07	1.0
5	4	16.16±2.49[d,e)]	1.7
10	4	26.94±3.62[c)]	2.8
20	3	24.52±2.32[c)]	2.5

a) Each value is the mean±S.E. *b*) Enhancement factor relative to *AUC* from the control. Significantly different from the control, *c*) $p<0.001$; *d*) $p<0.02$. *e*) Significantly different from the value for 10 min ($p<0.05$).

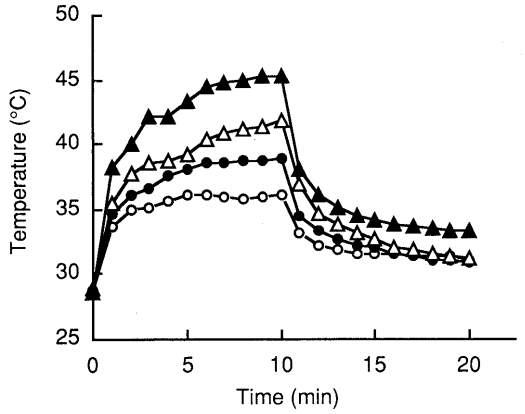

Fig. 4. Effect of Ultrasound on the Surface Skin Temperature of Rats

Each rat was irradiated with 1 MHz ultrasound for 10 min at the intensity levels of 0.25 (○), 0.5 (●), 0.75 (△), and 1 (▲) $W cm^{-2}$. Each value is the mean of 3 experiments.

improperly.[7] Care must be taken to avoid excessive exposure which might sometimes cause skin burning. The temperature of the skin is one indicator of overexposure. Therefore, the effect of ultrasound on the temperature of rat abdominal skin was determined.

Figure 4 shows the change of the skin surface temperature during ultrasound irradiation at different power levels for 10 min. Just after application of the drug ointment and ultrasonic gel to the rat abdominal skin, the surface temperature decreased from the normal temperature, 34.7 °C, to 28.8 °C. Application of ultrasound irradiation increased the temperature of the skin surface proportionally to the ultrasound intensity; the temperature decreased rapidly when the ultrasound irradiation was discontinued. Surface skin temperatures after the ultrasound application at 0.25, 0.5, 0.75, and 1 $W cm^{-2}$ for 10 min were 36.1, 38.9, 41.9, and 45.3 °C, respectively. Higher ultrasound intensity and longer treatment sometimes cause skin burning. Progressively more skin burning was noted in this study as the intensity was increased. The edema of skin ranged from small (0.75 $W cm^{-2}$) to large (1 $W cm^{-2}$), up to 5 mm in diameter. No significant burning was observed in the animals treated with 0.25 and 0.5 $W cm^{-2}$ for 10 min.

Figure 5 shows the change in hypodermic temperature during exposure to ultrasound for 20 min at the different intensities. The changes in hypodermic temperature were similar to those of the skin surface temperature; as the intensity of the ultrasound was increased, greater rises in skin temperature were noted. Hypodermic temperatures

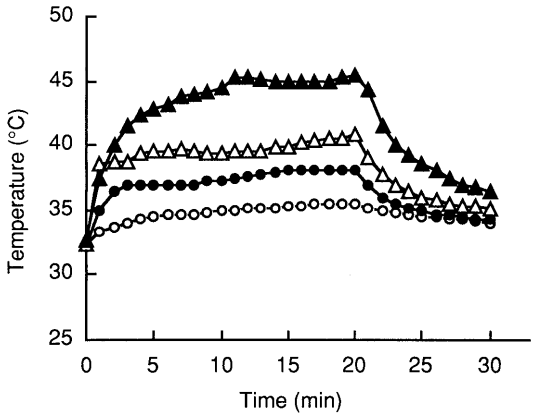

Fig. 5. Effect of Ultrasound on the Hypodermic Temperature of Rats

Each rat was irradiated with 1 MHz ultrasound for 20 min at the intensity levels of 0.25 (○), 0.5 (●), 0.75 (△), and 1 (▲) $W cm^{-2}$. Each value is the mean of 3 experiments.

were 35.4, 38.1, 40.9, and 45.6 °C when ultrasound energy was applied for 20 min to rats at 0.25, 0.5, 0.75, and 1 $W cm^{-2}$, respectively.

Evaluation of Skin Damage In order to examine the effect of ultrasound on the skin, histological comparisons were made between rat skin tissue with and without ultrasound. Figure 6 shows photomicrographs of rat skin at 10 min after application of ultrasound. No change of skin tissue (epidermis, dermis, and hypodermis) while exposed to ultrasound of 0.25 and 0.5 $W cm^{-2}$ (Fig. 6B and 6C, respectively) was observed as compared with the control (Fig. 6A). On the other hand, rat skin treated with 1 $W cm^{-2}$ ultrasound showed atrophy in the epidermis as well as collagen fiber degeneration in the dermis (Fig. 6E). In addition, necrosis was observed in the epidermis and dermis under this condition. The skin damage due to 0.75 $W cm^{-2}$ and 10 min-irradiation was not serious as a whole (Fig. 6D). However, some swelling in the epidermis and interstitum degeneration in the dermis was observed.

Discussion

The purpose of the present study was to examine the effect of therapeutic 1 MHz ultrasound on transdermal drug absorption. In general, the preferred range of intensity used for medical purposes is between 0.001 and 2 $W cm^{-2}$ and the preferred range of exposure is between 5 and 20 min.[8] The present study demonstrated that therapeutic ultrasound could enhance the transdermal absorption of indomethacin to a statistically significant extent from an ointment in rat. The most interesting point is that the effect of ultrasound on the transdermal absorption of indomethacin was not proportional to the intensity and duration of irradiation. The maximum effect was obtained at 0.75 $W cm^{-2}$ and 10 min-irradiation (Fig. 1). This is due to more skin damage at a higher intensity (1 $W cm^{-2}$) which may decrease the diffusivity of the drug through skin tissues. Serious skin damage was found in rats treated with 1 $W cm^{-2}$ for 10 min (Fig. 6E). Progressively more skin damage was noted as the intensity and duration of the treatments increased.[17]

The energy of ultrasound should be high enough to obtain the desired absorption enhancement and should be low enough not to cause any significant elevation in skin temperature and skin damage. The maximum limit of

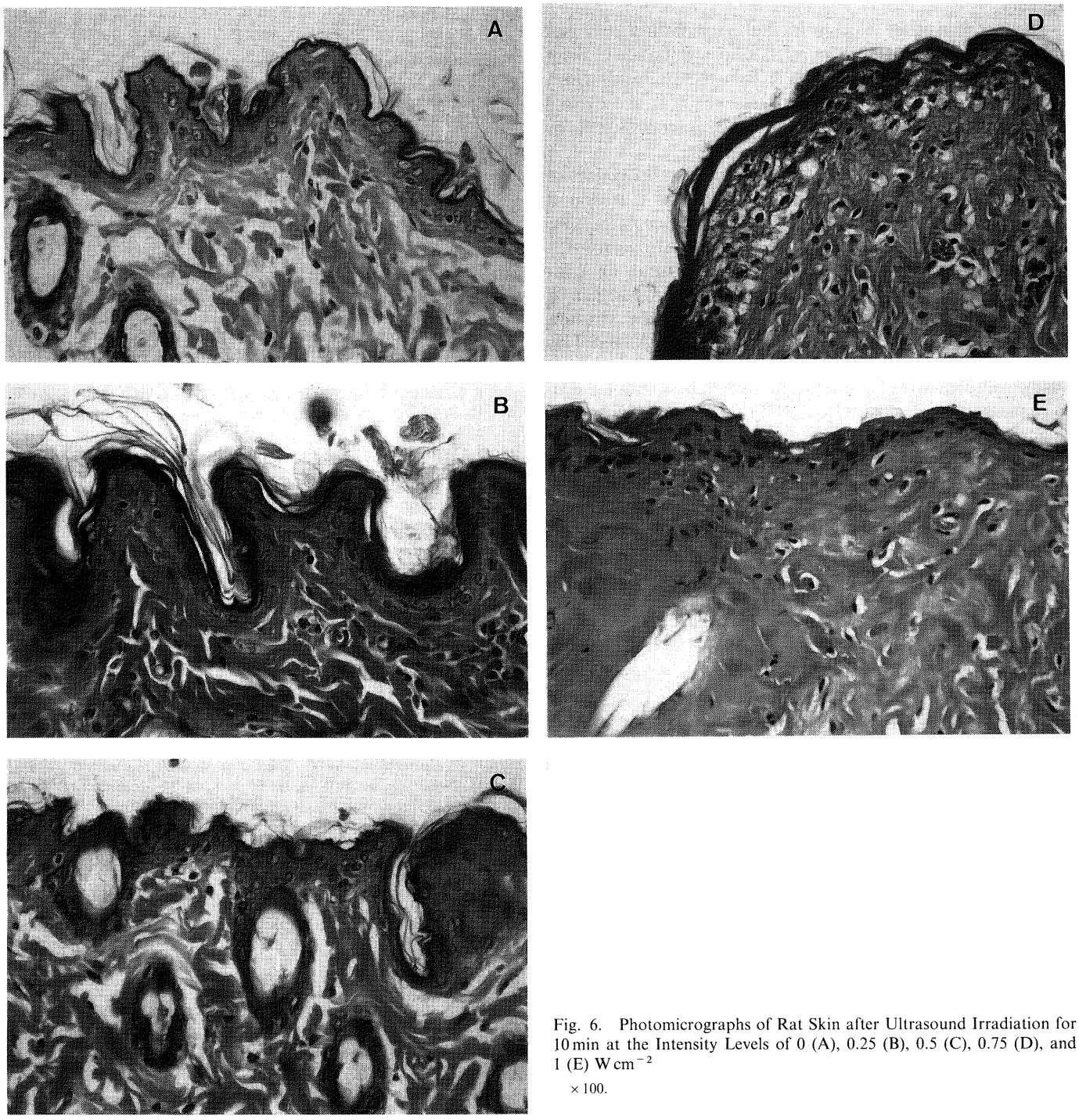

Fig. 6. Photomicrographs of Rat Skin after Ultrasound Irradiation for 10 min at the Intensity Levels of 0 (A), 0.25 (B), 0.5 (C), 0.75 (D), and 1 (E) W cm^{-2}
× 100.

exposure should be determined by measuring the skin temperature. Although the highest penetration was observed at an intensity of 0.75 W cm^{-2}, 0.5 W cm^{-2} was preferred because intensity of less than 0.5 W cm^{-2} of ultrasound for 10 min at a frequency of 1 MHz did not results in a siginificant temperature rise (Figs. 4 and 5) nor did treatment at this intensity have any destructive effect on rat skin (Fig. 6). Minor subcutaneous edema was sometimes observed following exposure to ultrasound to 0.75 W cm^{-2} for 10 min.

When used at a proper intensity and duration of application, ultrasound appears to be a safe technique for enhancing the passage of various drug molecules through human skin. A 1 MHz frequency was used in this study since a variable-frequency generator was not available. Different frequencies may also alter the amount of drug diffused through the skin.[6] Both the intensity and time of application were found to play an important role in the transdermal phonophoretic delivery system of indomethacin. The required length of time and intensity of ultrasound exposure are dependent on a number of factors including frequency, irradiation mode (continous wave or pulsed mode), and the physicochemical properties of both the drug and the formulation. More extensive investigations on various aspects of this system are in progress to obtain optimum parameters for transdermal phonophoretic

delivery.

The mechanism by which ultrasound irradiation increases absorption is not well understood. Enhanced drug penetration by phonophoresis is thought to result from thermal, cavitational, and mechanical effects.[6,7,12,17] These mechanisms may affect the vehicle, diffusion coefficient, or biological membrane itself.

The rate-limiting step in the percutanous absorption of drugs is passage across the stratum corneum. Ultrasound may affect the stratum corneum itself. We speculated that a proposed mechanism for enhanced transdermal absorption by ultrasound vibration is *via* structural or conformational changes of the stratum corneum, which in turn may decrease the barrier function of the stratum corneum and facilitate drug permeation. An ATR-FTIR study[18] has suggested that conformational changes of the lipids and proteins in the stratum corneum of human skin take place upon exposure to 1 MHz ultrasonic energy.

Some localized heat is produced by ultrasonic irradiation. This may also have played a role in the enhanced drug absorption noted in the present study.[19] However, our current experiments show that heat alone has no effect on the transdermal absorption of indomethacin from an ointment.[20] In this experiment, changes in temperature during the heat application were adjusted by heated water to a magnitude similar to those manifested with the ultrasound experiment ($0.5\,W\,cm^{-2}$, 10 min). The increase in skin temperature observed after the ultrasound application is not likely to enhance the transdermal absorption at this ultrasound energy level.

The effect of ultrasound on a biological system may also be associated with cavitation: small gaseous bubble formation.[21] However, cavitation does not take place *in vivo* at therapeutic doses, nor is it produced *in vivo* with the intensities used in this experiment.[6,17]

Current thinking suggests that an induced radiation pressure may be responsible for the successful administration of drugs transdermally by ultrasound.[5] Further studies are needed in order to elucidate the mechanism involved in the enhanced absorption noted with ultrasound.

Acknowledgements This study was partly supported by the Akiyama Foundation. The authors are very grateful to Dr. M. Takeuchi, Sapporo General Pathology Lab. Co., Ltd., for valuable advice in the histopathological study of skin.

References

1) Part V: S. Miyazaki, H. Mizuoka, M. Oda, and M. Takada, *J. Pharm. Pharmacol.*, **43**, 115 (1991).
2) J. Kost and R. Langer, "Pulsed and Self-Regulated Drug Delivery," ed. by J. Kost, CRC Press, Boca Raton, Ann Arbor and Boston, 1990, pp. 3—16.
3) *a*) S. Miyazaki, W.-M. Hou, and M. Takada, *Chem. Pharm. Bull.*, **33**, 428 (1985); *b*) S. Miyazaki, C. Yokouchi, and M. Takada, *J. Pharm. Pharmacol.*, **40**, 716 (1988).
4) D. Rolf, *Pharm. Technol.*, **12**, 130 (1988).
5) D. M. Kauen and G. M. Zentner, *Int. J. Pharmaceut.*, **20**, 235 (1984).
6) P. Tyle and P. Agrawala, *Pharm. Res.*, **6**, 355 (1989).
7) S. Singh and J. Singh, *Drug Design and Delivery*, **5**, 259 (1990).
8) R. Brucks, M. Nanavaty, D. Jyng, and F. Siegel, *Pharm. Res.*, **8**, 697 (1989).
9) J. C. McElnay, T. A. Kennedy, and R. Harland, *Int. J. Pharmaceut.*, **40**, 105 (1987).
10) H. A. E. Benson, J. C. McElnay, and R. Harlan, *Int. J. Pharmaceut.*, **44**, 65 (1988).
11) H. A. E. Benson, J. C. McElnay, R. Harlan, and J. Hadgraft, *Pharm. Res.*, **8**, 204 (1991).
12) D. Levy, J. Kost, Y. Meshulam, and R. Langer, *J. Clin. Invest.*, **83**, 2074 (1989).
13) S. Miyazaki, H. Mizuoka, M. Oda, and M. Takada, *J. Pharm. Pharmacol.*, **43**, 115 (1991).
14) S. Miyazaki, C. Yokouchi, T. Nakamura, N. Hashiguchi, W.-M. Hou, and M. Takada, *Chem. Pharm. Bull.*, **34**, 1801 (1986).
15) S. Miyazaki, T. Nakamura, C. Yokouchi, and M. Takada, *Chem. Pharm. Bull.*, **35**, 1243 (1987).
16) K. Yamaoka, Y. Tanigawa, T. Nakagawa, and T. Uno, *J. Pharmacobio-Dyn.*, **4**, 879 (1981).
17) J. W. Cowden and M. R. Abell, *Exp. Mol. Pathol.*, **2**, 367 (1963).
18) M. Nanavaty, R. Brucks, H. Grimes, and F. P. Siegel, *Proc. Int. Symp. Control. Rel. Bioact. Mater.*, **16**, 310 (1989).
19) H. Sasaki, J. Nakamura, J. Shibasaki, Y. Ishino, K. Miyasato, and T. Ashizawa, *Chem. Pharm. Bull.*, **35**, 4883 (1987).
20) S. Miyazaki, Y. Kohata, and M. Takada, *Yakuzaigaku*, **52**, in press (1992).
21) G. R. Haar and S. Daniels, *Phy. Med. Biol.*, **26**, 1145 (1981).