Effect of Ultrasound Application on Skin Metabolism of Prednisolone 21-Acetate

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Received December 20, 1997; accepted August 3, 1998

Purpose. The effect of ultrasound on skin penetration and metabolism of prednisolone (PN) and prednisolone 21-acetate (PNA) was investigated in the hairless mouse skin *in vitro*.
Methods. The abdominal skin excised freshly was pretreated under different ultrasound intensities (4.32, 2.88, and 1.50 W/cm^2) for 10, 30, and 60 min. The penetration/metabolism rate of PNA and its metabolite (PN) was then measured using a side-by-side diffusion cell.
Results. The skin penetration of PN was enhanced by the ultrasound pretreatment. This enhancement was attributed to the decrease in the stratum corneum barrier capacity by ultrasound energy. The steady-state appearance rate of PN following the skin bioconversion of PNA decreased appreciably with increasing the product of the duration of pretreatment (D_p, min) and the intensity of ultrasound applied (I_u, W/cm^2). When the product value was less than 40 W/cm^2 · min, the steady-state appearance rate of the PN hardly increased in spite of the penetration enhancement of PNA.
Conclusions. These findings indicated a possible deactivation of the skin enzymes by ultrasound energy.

KEY WORDS: ultrasound pretreatment; skin metabolism; penetration; enzyme deactivation; free radicals.

INTRODUCTION

Ultrasound has been applied to enhance the skin penetration of various bioactive agents (1) including local anesthesia (2,3), antiinflammatory drugs (4–6) and anticancer agents (7). The ultrasound energy may cause the mechanical, thermal and physiological changes in skin tissues; the cavitation induced by ultrasound contributes to the enhancement in the skin penetration of large peptide and protein drugs (8). Ultrasound may also change the structure of stratum corneum (9). Low-frequency ultrasound (20 kHz, 125 mW/cm^2, 100 msec pulses applied every second) which induced little damage to the skin, increased the penetration rate of sucrose as much as 1000-fold higher than that by the higher-frequency ultrasound (1 MHz, 2 W/cm^2, continuous) (10). Coakly *et al.* demonstrated that some enzymes in the solution were deactivated by 20 kHz ultrasound application (11). However, little is known with respect to the effect of ultrasound on the metabolism of drugs in the skin.

In this paper, we have investigated the effect of ultrasound on the skin penetration and metabolism in the hairless mouse skin *in vitro*. Prednisolone (PN) and prednisolone 21-acetate (PNA) are used as the model drug and prodrug, respectively. The skin is pretreated by ultrasound for 10 min. to 60 min. before the penetration/metabolism experiment. We have also studied the effect of free radicals and heating induced by the ultrasound application on the skin metabolism of PNA.

MATERIALS AND METHODS

Materials

Prednisolone (PN) was purchased from Nakarai Tesque, Inc. (Kyoto, Japan). Prednisolone 21-acetate (PNA), HIVISWAKO® 104 and other reagents were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Female hairless mice (Hr$^-$/Kud strain, 8–10 weeks old) were obtained from Kyudo (Tosu, Japan). All animal studies conformed to the "Principles of Laboratory Animal Care" (NIH publication #85-23, revised 1985).

Measurement of Ultrasound Intensity

The ultrasound intensity applied was calorimetrically evaluated by measuring the temperature difference of water before and after the application of ultrasound in the Water Calorimeter (Nakamura Chemical Industries, Tokyo, Japan); under an adiabatic condition, the head of ultrasound probe (Model 6600, Interface Co. Tokyo, Japan) was immersed in a 300 mL distilled water well mixed during the measurement. The temperature of water was measured by the digital thermometer (SK-2000MC, SATO KEIRYOKI MFG CO., LTD, Tokyo).

Ultrasound Pretreatment Experiment

The intact and stripped skin excised freshly from the hairless mouse abdomen was clamped between the two half cells of a modified Franz cell (the effective volume is 11.5 mL and the effective membrane area is 1.77 cm^2) (12). A 0.9 mL hydrogel (1.2% HIVISWAKO® 104, Taihou Pharmaceuticals Co., Tokushima, Japan) without drug was loaded onto the skin, while the receptor solution was a 40% PEG400 solution to keep the sink condition. After setting the ultrasound probe on the hydrogel, the skin was treated by the ultrasound of 1 MHz, 4.3 W/cm^2 and continuous mode for 10 min, 30 min, and 60 min. The distance from the ultrasound probe to the skin surface was controlled to be 5 mm in all experiments. After the hydrogel on the intact and stripped skin was removed completely with cotton buds, the steady-state penetration rate (dQ/dt) and lag-time (td) of PN were measured by using a side-by-side permeation system (the effective volume is 5.0 mL and the effective membrane area is 0.64 cm^2) or the modified-Franz cell with the hydrogel containing 0.87% (w/w) PN as the donor compartment. The stripped skin was used for measuring the penetration/metabolism rate of PNA and its metabolite (PN) in the skin. The concentrations of PNA and PN in the receptor solution were assayed by HPLC (12). The control experiment was also carried out by setting the probe on the skin without ultrasound application.

After the pretreatment of the intact skin with the ultrasound (4.3 W/cm^2) for 30 min in the modified Franz cell, the skin

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0724-8741/98/1100-1680$15.00/0

Table I. The Steady-State Penetration Rate and Lag-Time of Prednisolone Across the Hairless Mouse Skin

	Duration of pretreatment [min]	dQ/dt [μg/cm²/hr]		td [hr]	
		Ultrasound	Control	Ultrasound	Control
Intact	10	15.37 ± 6.21	7.30 ± 1.69	7.90 ± 1.24*	12.76 ± 0.08
Skin	30	47.04 ± 8.38*	10.08 ± 3.59	1.47 ± 0.44***	12.56 ± 0.19
	60	50.43 ± 9.16*	9.95 ± 1.60	0.78 ± 0.75**	12.28 ± 0.07
Stripped	10	36.28 ± 3.71	30.49 ± 9.10	1.18 ± 0.72	1.42 ± 0.56
Skin	30	46.66 ± 18.22	45.52 ± 7.20	0.28 ± 0.19	0.23 ± 0.10
	60	46.91 ± 5.57	48.41 ± 15.13	0.31 ± 0.16	0.30 ± 0.05

Note: Effect of the duration of ultrasound (4.3 W/cm²) pretreatment on percutaneous absorption of PN. Each value is the average ± S.D. Significantly different from control at $p < 0.05$ (*), $p < 0.005$ (**) and $p < 0.001$ (***), which are caluculated using t-test.

surface was observed using CCD camera (CCD-f2 MICRO CCD SCOPE, Shimadzu Co. Ltd., Kyoto, Japan) to assess a possible structural change of the stratum corneum.

Effect of Ultrasound on Skin Enzymes

The effect of free radicals on the skin enzymes was investigated by adding 10 mM vitamin C in the receptor solution as a radical scavenger (13) and then the stripped skin was pretreated with ultrasound (4.3 W/cm² for 30 min). The skin was carefully mounted in the side-by-side permeation system for measuring the penetration/metabolism rate of PNA and its metabolite (PN). The effect of temperature on skin enzymes was also investigated in the modified Franz cell; the stripped skin was directly treated by a disposable thermal patch (Maru-kyu-ban®, Aso Pharmaceuticals Co. Kumamoto, Japan) for 30 min and the temperature of the surface of skin was measured by Adhesive Type Thermocouple K (Model ST-50, RKC, Tokyo, Japan). The skin was then mounted in the side-by-side permeation system for measuring the penetration/metabolism rate. The appearance rate of PNA and its metabolite (PN) was measured in the stripped skin pretreated at three intensity levels: 4.3 W/cm², 2.9 W/cm² and 1.5 W/cm².

RESULTS AND DISCUSSION

Effect of Ultrasound Pretreatment on Skin Penetration

Table I summarizes the steady-state penetration rate (dQ/dt) and the lag-time (td) of PN across the hairless mouse skin pretreated with ultrasound. In the intact skin, dQ/dt increased and td decreased with increasing the duration of ultrasound pretreatment. In the stripped skin, however, dQ/dt increased less significantly. Figure 1 shows the CCD camera image of the surface of skin exposed with the ultrasound (4.3 W/cm²) for 30 min. The skin surface pretreated with the ultrasound (Fig. 1b) has shown several holes which hardly appear in the non-treated (control) skin surface (Fig. 1a). This finding indicates that the stratum corneum barrier capacity to skin penetration was damaged by the present ultrasound energy.

Effect of Ultrasound on Enzyme Activity in Skin

Table II summarizes the effect of ultrasound pretreatment on the steady-state penetration rate of PNA and its metabolite (PN) in the stripped skin, where $(dQ/dt)_p$ and $(dQ/dt)_c$ represent the steady-state penetration rate with ultrasound pretreatment and the control experiment without ultrasound application, respectively. The ratio of $(dQ/dt)_p/(dQ/dt)_c$ for PNA slightly exceeded 1.0. However, the ratio of $(dQ/dt)_p/(dQ/dt)_c$ for the metabolite (PN) decreased appreciably as the duration of ultrasound pretreatment increased. This finding clearly indicates that the enzymes in the skin deactivate after ultrasound application.

Effect of Ultrasound Intensity on Skin Enzyme

Figure 2 shows the ratio of $(dQ/dt)_p/(dQ/dt)_c$ for PNA (a) and its metabolite (b) as a function of the duration of pretreatment of three different ultrasound intensities. The ratio for the metabolite with 1.5 W/cm² for 10 and 30 min. pretreatment

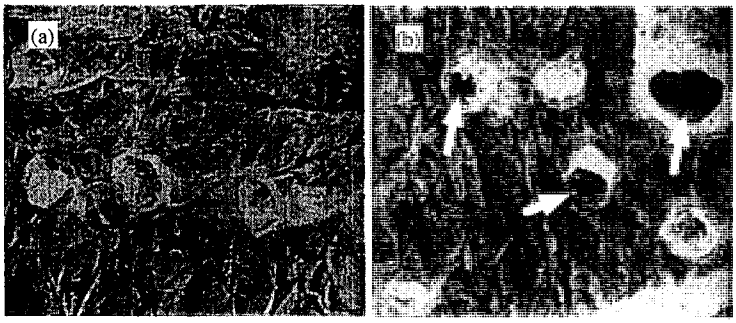

Fig. 1. The surface of hairless mouse skin exposed to ultrasound (4.3 W/cm²) for 30 min. Figure 1(a) shows a non-pretreated skin (control), while Figure 1(b) indicates a pretreated skin. The arrow implies the presence of holes. Magnification ×600.

Table II. The Steady-State Penetration Rate of Prednisolone Acetate (PNA) and Its Metabolite (PN) Across Stripped Skin

		dQ/dt[μg/cm²/hr]		
		10 min	30 min	60 min
Prednisolone Acetate (PNA)	Ultrasound	0.99 ± 0.45	1.50 ± 0.38	0.76 ± 0.03
	Control	0.98 ± 0.12	1.05 ± 0.01	0.69 ± 0.46
	$(dQ/dt)_p/(dQ/dt)_c$	1.01	1.43	1.10
Metabolite (PN)	Ultrasound	0.12 ± 0.01	0.07 ± 0.02*	0.02 ± 0.01*
	Control	0.17 ± 0.02	0.20 ± 0.04	0.12 ± 0.06
	$(dQ/dt)_p/(dQ/dt)_c$	0.65	0.35	0.17

Note: Effect of the duration of ultrasound (4.3 W/cm²) pretreatment on the skin metabolism of PNA. $(dQ/dt)_p$ and $(dQ/dt)_c$ represent the steady-state penetration rate for ultrasound pretreatment and control without pretreatment, respectively. Each value is the average ± S.D. Significantly different from control at $p < 0.05$ (*), which are caluculated using t-test.

was 1.18 ± 0.21 and 1.56 ± 0.18, respectively, and was not significantly different from the control. The ratio of the metabolite at the intensity of 2.9 W/cm² and 4.3 W/cm² clearly decreased with increasing the duration of pretreatment (Fig. 2b), while the ratio of PNA was relatively constant (Fig. 2a), indicating the deactivation of enzymes by ultrasound energy. Figure 3 shows the ratio $(dQ/dt)_p/(dQ/dt)_c$ for the metabolite as a function of the product of the ultrasound intensity (I_u) and the duration of pretreatment (D_p). As can be seen, the ratios were well correlated with the product of I_u and D_p.

Mechanism of Deactivation of Skin Enzymes

Acoustic cavitation due to ultrasound may cause free radicals formation (14) and damage enzymes in the skin. In order to examine the effect of free radicals on skin enzymes, we added vitamin C (ascorbic acid) as a radical scavenger (13) in the receptor solution during ultrasound pretreatment. The ratio $(dQ/dt)_p/(dQ/dt)_c$ for the metabolite increased from 0.35 to 0.68 by adding vitamin C (Table III), suggesting that free radicals appreciably contribute to the enzyme deactivation in this experiment. Acoustic cavitation may also cause temperature increase in the skin (internal heating) and the ultrasound probe itself is heated by sonication (external heating). As a result, the temperature of the skin surface reached about 54°C during the ultrasound pretreatment. By using a thermal patch, Marukyu-ban® (Fig. 4) which simulates the temperature

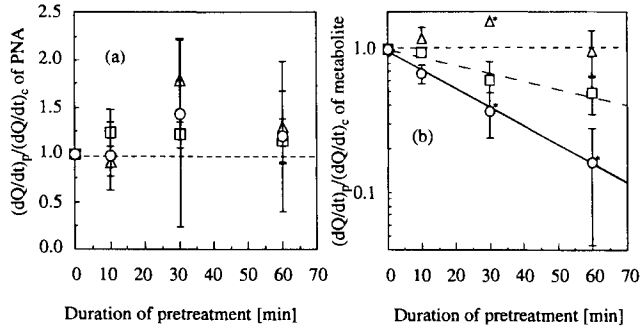

Fig. 2. Ratio of $(dQ/dt)_p/(dQ/dt)_c$ for PNA (a) and its metabolite (b) as a function of the duration of ultrasound application. Ultrasound intensity is 4.3 W/cm² (○), 2.9 W/cm² (□) and 1.5 W/cm² (△). Significantly different from control at $p < 0.05$ (*), which are calculated using t-test.

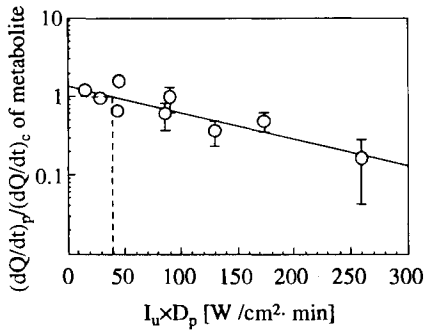

Fig. 3. Ratio of $(dQ/dt)_p/(dQ/dt)_c$ for metabolite (PN) as a function of the product of ultrasound intensity (I_u) and the duration of pretreatment (D_p).

Table III. Effect of Radical Scavenger (10 mM Vitamin C) and Heating (Marukyu-ban®) for 30 min on Steady-State Penetration Rate of Prednisolone 21-Acetate Metabolite (PN)

	dQ/dt [μg/cm²/hr]		
	Ultrasound	Vitamin C	Heating
Pretreatment	0.07 ± 0.02*	0.17 ± 0.04	0.27 ± 0.04
Control	0.20 ± 0.04	0.25 ± 0.05	0.24 ± 0.01
$(dQ/dt)_p/(dQ/dt)_c$	0.35	0.68	1.13

Note: $(dQ/dt)_p$ and $(dQ/dt)_c$ represent the steady-state penetration rate for pretreatment and control, respectively. Each value is the average ± S.D. Significantly different from control at $p < 0.05$ (*), which are calculated using t-test.

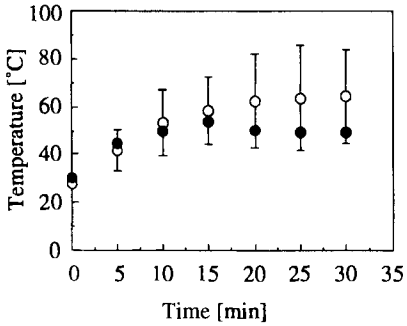

Fig. 4. Temperature profiles of ultrasound probe (○) and Marukyu-ban® (●).

profiles during the ultrasound application, we have found that the elevated skin temperature caused by the present ultrasound energy led to negligible effect on the deactivation in the skin enzymes (Table III).

CONCLUSIONS

The penetration of PN across the intact skin was enhanced by ultrasound pretreatment. However, the penetration rate of PN across the stripped skin was hardly influenced by ultrasound application. These findings may indicate that the ultrasound application damages the stratum corneum barrier capacity, while the effect on the viable skin is negligible with respect to skin permeability. The appearance rate of the PNA metabolite (PN) decreased appreciably with increasing the duration and the intensity of ultrasound pretreatment, indicating that the skin enzymes are deactivated by the ultrasound. Approximately half of the enzyme deactivation was caused by the free radicals generated by ultrasound application and external heating due to ultrasound energy may be negligible to the enzyme denature. The present study also indicates that the deactivation of skin enzymes can be controlled by the duration of pretreatment and the ultrasound intensity.

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