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COMMUNICATIONS

Transdermal delivery of insulin by ultrasonic vibration

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Abstract—Ultrasonic vibration has been used to deliver insulin through the skin of hairless mice fasted overnight and partially immersed in an aqueous solution of insulin (20 units mL^{-1}). The skin surface was exposed to ultrasonic vibration in two ultrasonic energy ranges (3000–5000 Pa and 5000–8000 Pa) at 48 kHz for 5 min. Blood glucose concentration was measured before and after exposure to insulin and ultrasonic vibration. In the group subjected to the lower energy vibrations, blood glucose fell rapidly to reach $34 \pm 11{\cdot}9\%$ of control values in 120 min, while when the animals were exposed to higher energy vibrations, the fall in blood glucose was $22{\cdot}4 \pm 3{\cdot}9\%$ of control values at 120 min. The values remained low for the length of the experiment (240 min). Those exposed to insulin alone or ultrasonic vibration alone revealed no significant change in blood glucose concentration. It is postulated that ultrasonic vibration may alter skin permeability resulting in the absorption of insulin. That the blood glucose decrease was greater at the higher of the two energy ranges, suggests this factor could control insulin delivery.

Because the skin serves as a barrier, preventing the percutaneous absorption of most drugs, attempts to alter the stratum corneum by "chemical" and "electrical" (Meyer et al 1988) means have been made. The use of azone (Stoughton & McClure 1983) and iontophoresis (Kari 1985) have produced good results. Siddiqui et al (1987) demonstrated a fall in blood glucose by iontophoresis of insulin in diabetic hairless rats. However, drug permeability was influenced by the pH thus limiting the use of drugs as unstable as insulin. We now report an alternative method for delivering insulin to the body by the use of ultrasonic vibration to the skin surface of hairless mice.

Materials and methods

Hairless mice (8–12 weeks, 25–35 g), obtained from a local supplier, were kept in separate plastic cages to avoid skin damage.

Four experiments were carried out to study the effects of insulin and/or ultrasonic vibration. The mice after being fasted overnight were restrained in a tight cage immediately before each experiment, care being taken to avoid abrasion. In group A ($n = 5$), the mice were immersed hip deep but with the tail clear in a beaker containing 100 mL of an aqueous solution of neutral insulin (20 units mL^{-1}, Insulin Novo Actrapid MC). The beaker was then exposed for 5 min to ultrasonic vibration in an ultrasonic generator water tank $14 \times 25 \times 10$ cm (Cole Parmer Instrument Co. Chicago, Illinois) remodelled to regulate power output. Two energy ranges (3000 5000 Pa and 5000–8000 Pa) were used at a frequency of 48 kHz. The energy of the vibration reaching the skin was measured by a hydrophone (8103 Hydrophone, 2635 Bruel & Kjaer) fixed beside the animal and was reported as pascals. The animal was then removed from the solution. Control experiments were as follows; group B: immersion in insulin alone for 5 min, group C: immersion in saline for 5 min, group D: immersion in saline with ultrasonic vibration at two energy ranges for 5 min, all at an ambient temperature of 37°C. After each test, the skin was rinsed and dried. A 0·05 mL sample of blood was obtained from the tip of the tail. A quick-dry bond (Toa Gouseikagaku Co.) was used to prevent further bleeding after taking the samples. This procedure was repeated for each sample. Samples were taken before and then 5, 15 and 30 min after the tests and then continued every 30 min for the following 3·5 h. Glucose concentration was measured with Ames Dextrostix and an Ames Dextrometer (Ames Co, Elkhart IN). The animals were not anaesthetized.

Various amounts of insulin were also subcutaneously injected into animal groups ($n = 5$) to compare blood glucose and to estimate the uptake of insulin in the main experiment. The measurements were made as described above.

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Results

Fig. 1 shows blood glucose concentrations over 240 min in groups A and B. A rapid fall was observed after exposure to insulin with ultrasonic vibration (group A). Exposure to the lower energy vibrations (3000–5000 Pa) produced a minimum in blood sugar at 120 min of $34{\cdot}1 \pm 11{\cdot}9\%$ of the initial concentration (mean ± s.d.). The value rose to $53{\cdot}5 \pm 9{\cdot}3\%$ at 240 min, one animal dying of hypoglycaemia at 180 min. Exposure to the higher energy vibrations produced a minimum at 120 min of $22{\cdot}4 \pm 3{\cdot}9\%$ of the control value. The glucose concentration rose to $37{\cdot}3 \pm 3{\cdot}2\%$ at 240 min, with two mice dying of hypoglycaemia at 150 min and one at 180 min. Student's *t*-test revealed statistically significant differences ($P < 0{\cdot}05$) between each interval and control groups B (insulin alone) or C or D (insulin-free experiments with or without vibrations), after 5 min. There were no changes ($P > 0{\cdot}05$) in blood glucose concentration compared with initial level in all the control groups (Table 1). There was no evidence of significant cutaneous damage. Fig. 2 shows the decrease of blood glucose in mice subcutaneously injected with insulin. The fall in blood glucose and duration of hypoglycaemia correlated with the dose of insulin.

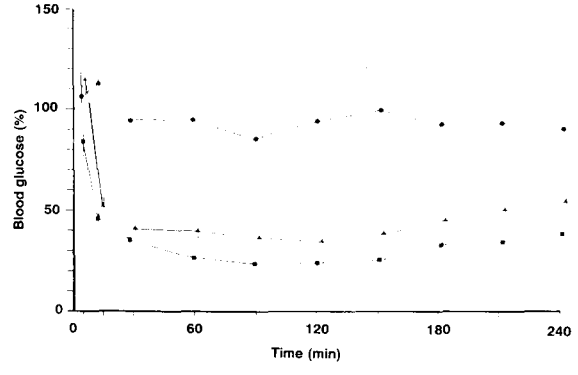

FIG. 1. Blood glucose concentration of hairless mice exposed to insulin alone or insulin plus ultrasonic vibration for 5 min. ●——●, insulin (20 units ml^{-1}) alone; ▲——▲ with 3000–5000 Pa; ■——■ with 5000–8000 Pa.

Table 1. Percentage of initial blood glucose concentration of control groups. (Mean ± s.d.)

Time (min)	Saline	Saline + ultrasound (3000–5000 Pa)	Saline + ultrasound (5000–8000 Pa)
0	100	100	100
5	116·2 ± 8·4	113·4 ± 4·3	121·8 ± 6·4
15	111·8 ± 10·0	104·9 ± 5·3	121·3 ± 2·6
30	106·3 ± 7·7	105·1 ± 4·0	91·3 ± 4·2
60	101·3 ± 4·9	101·4 ± 2·0	102·5 ± 6·6
90	96·0 ± 8·2	113·1 ± 3·4	98·1 ± 4·9
120	92·3 ± 9·5	104·4 ± 5·8	92·1 ± 4·7
150	94·5 ± 8·0	97·5 ± 5·1	94·8 ± 4·1
180	91·5 ± 7·9	92·1 ± 3·6	92·5 ± 5·6
210	91·8 ± 4·8	100·4 ± 5·6	93·4 ± 2·1
240	89·8 ± 4·1	103·1 ± 6·1	103·5 ± 6·8

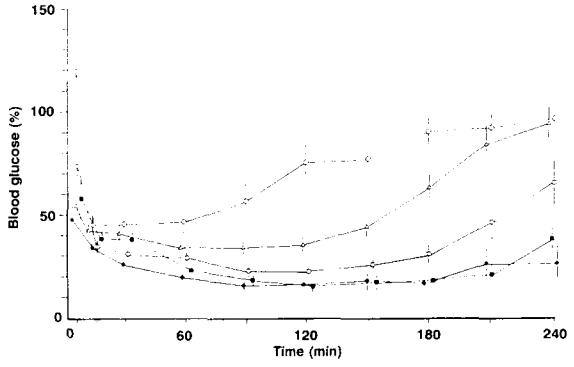

FIG. 2. Blood glucose concentration of hairless mice subcutaneously injected with insulin. O——O 1 unit kg^{-1}; △——△ 2 units kg^{-1}; □——□ 4 units kg^{-1}; ■——■ 8 units kg^{-1}; ▲——▲ 12 units kg^{-1}.

Discussion

Reports on phonophoresis relate to local effects on the skin. However, whether ultrasonic vibration enhances drug absorption remains controversial (Skauen & Zentner 1984; McElnay et al 1985). A low uptake of drugs, different methods, and ultrasonic energies have led to conflicting results (Kost et al 1986; Benson et al 1989; Brucks et al 1989). Furthermore, the output of the generator and the extent of exposure do not always correlate because of the variable efficiency of ultrasonic transmission. In the present study, the size of the water tank made it possible to measure the ultrasonic power near the surface of the skin by a hydrophone, and also helped to keep the availability of insulin at a constant level. Although it is usual for therapeutic exposures of ultrasound frequency to be about 1 MHz, a relatively lower frequency range was used in the experiments to maintain accurate ultrasound energy measurements by the hydrophone (maximum limit 150 kHz).

The results showed a marked decrease in blood glucose in the mice exposed to insulin and ultrasonic vibration, compared with mice in the control experiments. The evidence suggests that ultrasonic vibration had an effect on the skin that resulted in transdermal absorption of insulin.

As shown in Fig. 2, blood glucose remained low for 3 to 4 h after a 5 min exposure to vibration and insulin. Similar results were obtained after subcutaneous injection of insulin, suggesting a slow microcirculation wash-out at the dermo-epidermal junction. This phenomenon has also been reported with iontophoresis (Siddiqui et al 1987). The total amount of insulin absorbed can be estimated by comparing the data obtained from subcutaneous injection (Fig. 2). The exposure to vibration and insulin resulted in a decrease in blood glucose equal to that produced by a subcutaneous injection of 2–4 units kg^{-1} insulin. Furthermore, the more energy applied, the lower the blood glucose thus suggesting that this could be related to the extent of insulin penetration. While, theoretically, changes in energy applied could alter the drug permeability rate, insulin availability, duration of exposure to the energy, total surface area of exposure and skin condition could be factors in the transdermal administration of drugs.

The mechanism and the route of drug delivery into the body through the skin is unclear. The transfollicular "shunt" pathway and "transepidermal" route have been considered by Siddiqui et al (1987). It can be postulated that the energy of ultrasonic vibration enhanced permeability via these routes. Furthermore, the microscopic bubbles produced at the surface of the skin by ultrasonic vibration might generate a rapid liquid current when they implode, thereby increasing the availability of insulin for absorption.

This study demonstrates the effects of ultrasonic vibration on the skin resulting in the absorption of insulin and suggests that changes in ultrasonic energy may be used as a means of regulating the permeability of drugs, through the skin, with the potential of being a useful drug delivery system.

References

Benson, H., McElnay, J., Harland, R. (1989) Use of ultrasound to enhance percutaneous absorption of benzydamine. Phys. Ther. 69: 113–118

Brucks, R., Nanavaty, M., Jung, D., Siegel, F. (1989) The effects of ultrasound on the in vitro penetration of ibuprofen through human epidermis. Pharm. Res. 6: 697–701

Kari, B. (1985) Control of blood glucose levels in alloxan-diabetic rabbits by iontophoresis of insulin. Diabetes 35: 217–221

Kost, J., Levy, D., Langer, R. (1986) Ultrasound effect in transdermal drug delivery. Proceed Intern Symp. Control Rel. Bioact. Mater. 13: 177–178

McElnay, J. C., Matthews, M. P., Harland, R., McCafferty, D. F. (1985) The effect of ultrasound on percutaneous absorption of lidocaine. Br. J. Clin. Pharmacol. 20: 421–424

Meyer, B. R., Kreis, W., Eschbach, J., O' Mara, U., Rosen, S., Sibalis, D. (1988) Successful transdermal administration of therapeutic doses of a polypeptide to human volunteers. Clin. Pharmacol. Ther. 44: 607–612

Siddiqui, O., Sun, Y., Liu, J., Chien, Y. (1987) Facilitated transdermal transport of insulin. J. Pharm. Sci. 76: 341–345

Skauen, D. M., Zentner, G. M. (1984) Phonophoresis. Int. J. Pharm. 20: 235–245

Stoughton, R. B., McClure, W. D. (1983) Azone: a new non-toxic enhancer of cutaneous penetration. Drug Dev. Ind. Pharm. 9: 725–744