*International Journal of Pharmaceutics*, 96 (1993) 249–252



IJP 03237

# Phonophoresis of azidothymidine (AZT)

C. Pelucio-Lopes [a], L. Machet [a], L. Vaillant [a], F. Patat [b], M. Lethiecq [b], Y. Furet [c], L. Pourcelot [b] and G. Lorette [a]

[a] *Department of Dermatology, CHU Trousseau, F37044 Tours cedex (France),* [b] *Department of Biophysics, CHU Bretonneau, F37032 Tours cedex (France) and* [c] *Department of Pharmacology, CHU Bretonneau, Medical University, F37000, Tours cedex (France)*

(Received 16 December 1992)
(Modified version received 5 March 1993)
(Accepted 11 March 1993)

*Key words*: Azidothymidine; Zidovudine; Phonophoresis; Ultrasound; Percutaneous absorption

## Summary

Phonophoresis of zidovudine (AZT, azidothymidine), was studied in vitro, across excised human skin and hairless mouse skin. Sonication was carried out with a continuous mode, at an intensity of 1.5 W/cm$^2$ and a frequency of 1.1 MHz for 20 min. During experiments the temperature in the donor compartment of Franz cells was maintained at 28°C by a cooling coil. The mean flux ($J$) across mouse skin was $4.8 \times 10^{-3} \pm 1.0$ $\mu$mol cm$^{-2}$ h$^{-1}$ for the control cell group and $4.4 \times 10^{-3} + 1.2$ $\mu$mol cm$^{-2}$ h$^{-1}$ for the sonicated cell group. Under the same conditions, percutaneous absorption through human skin was lower, and the mean flux ($J$) was $4.1 \times 10^{-4} \pm 1.5$ $\mu$mol cm$^{-2}$ h$^{-1}$ and $4.4 \times 10^{-4} \pm 1.8$ $\mu$mol cm$^{-2}$ h$^{-1}$ for the control and sonicated groups, respectively. No significant difference was observed on comparison of the mean flux of the sonicated and the control (non-sonicated) groups in mouse and human skin, probably because the thermal effects of ultrasound in the skin were suppressed.

Zidovudine (AZT) is a strong inhibitor of the reverse transcriptase enzyme isolated from the HIV virus and the therapeutic potency on AIDS or AIDS-related complex has been recognized clinically. This virustatic drug, which is administered orally or parenterally, has a short half-life of 1 h. The average bioavailability is about 65%; this is assumed to be result of first-pass metabolism. Since the therapeutic index is low, with a narrow range of plasma concentration of 0.4–4.0 $\mu$mol l$^{-1}$ (Blum et al., 1988), side-effects occur frequently (Richman et al., 1987). Therefore, percutaneous administration of AZT is desirable to obtain zero-order kinetics and could be useful for patients with significant side-effects.

The aim of the present study was to quantify the ultrasound (US) effects on the percutaneous absorption rate of AZT in vitro and to determine the possibility of developing a transdermal system.

Zidovudine (3′-azido-3′-deoxythymidine, AZT, Retrovir®), a hydrophilic molecule with a molecular mass of 267.24 Da, was a gift from The Wellcome Foundation Ltd (Beckenham, U.K.).

The diffusion experiments were performed with specially modified Franz cells which allow

*Correspondence to:* L. Machet, Dermatology Department, CHU Trousseau, 37044 Tours cedex, France.

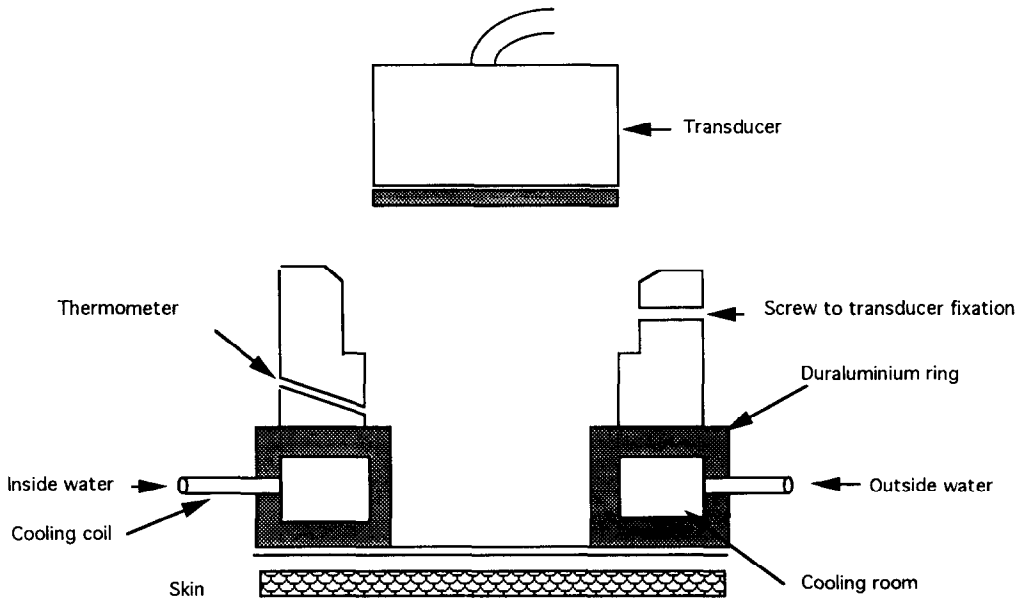


Fig. 1. Donor compartment.

the introduction of an ultrasound probe into the donor compartment (Fig. 1).

The total membrane area available for diffusion was 2.17 $cm^2$. The temperature during sonication was recorded in the donor solution continuously, using a fine rigid wire thermocouple probe and a digital thermometer.

Ultrasound (US) was generated by a 15 mm diameter ceramic transducer (lead titanate zirconate ceramic, P-189 Quartz and Silice Co.), with an output of 90% and area of 1.77 $cm^2$. Ultrasound was applied for 20 min, continuous mode, at an intensity 1.5 $W/cm^2$ and a frequency of 1.136 MHz (Hewlett Packard 3314-A Generator). The cooling coil was constructed with an duraluminium ring around the donor compartment and was filled with water (5°C). There were cylindrical plastic tubes for entry and exit of water to each donor compartment (Fig. 1).

Hairless female mice, aged 6–7 weeks, were killed by cervical dislocation and the skin from the back was removed and trimmed of the fat and other extracutaneous tissues. Human skin was obtained after plastic surgery on the abdominal area (women with a mean age of 30 years) and was stored at −20°C for 2 months. In all cases, the excised skin was initially hydrated in normal saline for 10 h before being used for experiments.

Water at a constant temperature (33°C) was pumped through the receiving compartment in order to warm the receptor solution. The donor compartment was filled with a 4 ml aqueous solution of 12.5 mg/ml of AZT and the receiving compartment was filled with 15 ml of normal saline (0.9%). The contents of the receptors were degassed before experiments for 10 min and were mixed at a controlled speed (300 rpm).

Each diffusion experiment was carried out over 24 h and 500 $\mu$l samples were taken at regular intervals from the sampling port in the receiving compartment. The replacement dilution effects were corrected for in the assay calculations.

Diffusion rates or flux ($J$) were determined from the slope of diffusion curves and expressed as the amount of drug passing across 1 $cm^2$ of skin surface as a function of time ($\mu$mol $cm^{-2}$ $h^{-1}$). The permeability coefficient ($K_p$) was determined from the equation $K_p = J/C_0$ where $C_0$ denotes the initial concentration of drug in the donor compartment. The diffusion rate of AZT,

TABLE 1

*In vitro percutaneous absorption of AZT through hairless mice and human skin*

| Skin | Mean flux ($J$) [a] ($\mu$mol cm$^{-2}$ h$^{-1}$) | | Permability coefficient $K_p$($\times 10^{-3}$) (cm h$^{-1}$) | |
|---|---|---|---|---|
| | Sonicated | Control | Sonicated | Control |
| Hairless mice | $4.4 \pm 1.2 \times 10^{-3}$ | $4.8 \pm 1.0 \times 10^{-3}$ | $0.09 \pm 0.02$ | $0.10 \pm 0.02$ |
| Human | $4.4 \pm 1.8 \times 10^{-4}$ | $4.1 \pm 1.5 \times 10^{-4}$ | $0.01 \pm 0.004$ | $0.009 \pm 0.003$ |

[a] Mean $\pm$ SE (Student's $t$-test).

was determined by radioimmunoassay (ZDV-Trac™, Burroughs Wellcome Co).

Histological examination and electron microscopy of the skin were carried out to reveal any permanent biological lesion after sonication.

In this study, we demonstrated that, when the thermal effects of ultrasound were suppressed, there was no increase in percutaneous absorption of AZT. In the statistical analysis for comparison of the mean flux ($J$) of sonicated and control groups, no enhancement of percutaneous absorption of AZT by phonophoresis was demonstrated in vitro in human skin or hairless skin. Permeability coefficient ($K_p$) and mean flux ($J$), are expressed in Table 1.

Seki et al. (1990) and Wearley and Chien (1990), using a different diffusion cell, demonstrated an increase in percutaneous absorption of AZT with chemical and physical enhancers (Table 2). They hypothesized the possibility of transdermal administration of AZT.

Based on the pharmacokinetic studies of AZT (Klecker et al., 1987), with our mean flux, there was no possibility of obtaining sufficient plasma concentrations in order to develop a transdermal system.

Brucks et al. (1989) adapted a cooling coil in the donor compartment of diffusion cells and they demonstrated that the use of US could increase in vitro penetration of ibuprofen through human skin. In the sonicated group, there was an enhancement of the initial flux of ibuprofen. However, there was no modification in the steady state flux. A significant increase in temperature of 11°C was observed during sonication (mean 42°C). With our cooling coil, the mean temperature was 28°C during sonication (Fig. 2). With simple thermal simulation of electrical resistance,

TABLE 2

*Effects of chemical and physical enhancers in the diffusion rate of AZT through rat and humanskin*

| Enhancers | [AZT] (mg/ml) | Diffusion rate ($\mu$mol cm$^{-2}$ h$^{-1}$) | |
|---|---|---|---|
| | | Human skin | Rat skin |
| Wearley and Chien | | (microtomed skin) | (hairless rat) |
| Water | 25 | $1.9 \times 10^{-2}$ | $1.8 \times 10^{-2} \pm 4.13$ |
| Iontophoresis | 25 | | $5.5 \times 10^{-2} \pm 4.13$ |
| $C_{10}$MSO | 25 | | 4.64 |
| Seki et al. | | (whole skin) | (Wistar rat) |
| Water | 5 | $1.3 \times 10^{-3}$ | $1.5 \times 10^{-3}$ |
| 10% oleic acid (OA) | 5 | $9.2 \times 10^{-3}$ | $4.5 \times 10^{-2}$ |
| Mixed-solvent system [a] | 5 | | $0.63 \times 10^{-2}$ |
| Mixed solvent system [a] | 30 | 0.41 | 4.85 |
| Phonophoresis (this study) | | (whole skin) | (hairless mice) |
| Water | 12.5 | $4.4 \times 10^{-4} \pm 1.8$ | $4.4 \times 10^{-3} \pm 1.2$ |

[a] 10% *N*-methyl-2-pyrrolidone (MP), 10% oleic acid (OA), 5% S-318 (medium chain monoglyceride), 20% propylene glycol (PG).

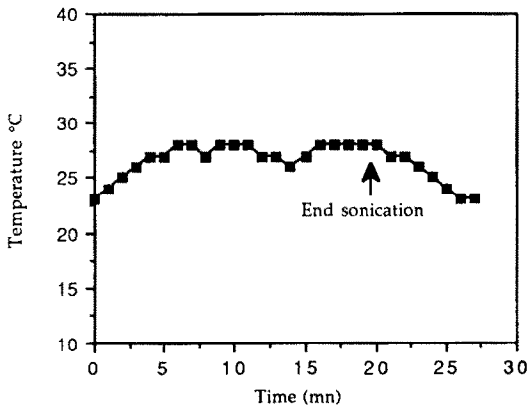

Fig. 2. Temperature control with cooling unit.

we demonstrated that the digoxin flux could be increased in comparable amounts to those obtained by sonication at 3.0 W/cm$^2$ (Pinton et al., 1991).

Clinical studies have demonstrated that US parameters with high intensity could result in burning of the skin (Pinton et al., 1991). However, histological examinations have rarely been performed. In our study, there were no macroscopic or histologic changes in the sample skin at 1.5 and 3.0 MHz, probably because we controlled the temperature thus avoiding permanent alterations or burning.

Enhanced drug penetration is thought to result from thermal, mechanical and chemical alterations in the skin by US (Tyle and Agrawala, 1989). The mechanisms by which the increase in percutaneous diffusion of drugs and the thermal effects of US in the superficial and deep tissues occur are not well known. Knutson et al. (1985) demonstrated that thermal variation in the stratum corneum increased the fluidity of lipids and the diffusion of drugs.

In our study, when the temperature was controlled at the surface of the skin, there was no enhancement in diffusion rates. These results suggest that the thermal effects of US in the stratum corneum are more responsible for the increase in percutaneous absorption than other phenomena induced by ultrasound.

## Acknowledgement

C.P-L. is supported by a grant from C.N.P.q. (Brazil).

## References

Blum, M.R, Liao, S.H.T., Good, S.S. and Miranda, P., Pharmacokinetics and bioavailability of zidovudine in humans. *Am. J. Med.*, 85 (Suppl.) 2A (1988) 189–194.

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

Klecker, R.W., Collins, J.M., Yarchoan R., Thomas, R., Jenkins, J.F., Broder, S. and Myers, C.E, Plasma and cerebrospinal fluid pharmacokinetics of 3′-azido-3′-deoxythymidine: A novel pyrimidine analog with potencial application for the treatment of patients with AIDS and related diseases. *Clin. Pharmacol. Ther.*, 41 (1987) 407–412

Knutson, K., Potts, R.O., Guzek, D.B, Golden, G.M., McKie, J.E., Lambert, W.J. and Higuchi, W.I., Macro and molecular physical-chemical considerations in understanding drug transport in the stratum corneum. *J. Controlled Release*, 2 (1985) 67–87.

Pinton, J., Vaillant, L., Machet, L., Patat, F., Lethiecq, M., Pourcelot, L., Guilloteau, D. and Lorette, G., Does phonophoresis enhance percutaneous absorption? *J. Invest. Dermatol.*, 96 (1991) 650.

Richman, D.D., Fischl, M.A., Grieco, M.H., Gottlieb, M.S., Volberding, P.A., Laskin, O.L., Leedom, J.M., Groopman, J.E, Mildvan, D., Hirsch, M.S., Jackson, G.G., Durack, D.T., Nusinoff-Lehrman, S. and The AZT Collaborative Working Group, The toxicity of azidothymidine (AZT) in the treatment of patients with AIDS and AIDS-related complex. *N. Engl. J. Med.*, 4 (1987) 192–197.

Seki, T., Toeda, C., Kawaguchi, T.,Kazuhiko, J., Sugibayashi, K. and Morimoto, Y., Enhanced transdermal delivery of Zidovudine in rats and human skin. *Chem. Pharm. Bull.*, 11 (1990) 3086–3089.

Tyle, P. and Agrawala, P., Drug delivery by phonophoresis. *Pharm. Res.*, 6 (1989) 355–361.

Wearley L. and Chien, Y.W., Enhancement of the in vitro skin permeability of azydothymidine (AZT) via iontophoresis and chemical enhancer. *Pharm. Res.*, 7 (1990) 34–40.