

Effect of Pressure on *In vitro* Percutaneous Absorption of Caffeine

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The effect of increased pressure, which is a mechanical property of massage, was investigated on the percutaneous absorption of an amphiphilic compound (caffeine) *in vitro* on Franz diffusion cells, using excised human skin. 50 µl of either a 320 µg/ml or a 15 mg/ml acetone solution of caffeine were pipetted onto the surface of each skin sample, which represented caffeine skin deposits of 5 µg/cm² and 240 µg/cm² respectively. During each experiment, a pressure device delivering 0.25 bar over the atmospheric pressure was applied for the first 30 min on half of the cells. At 2, 4, 6, 8, 12 and 24 h the aqueous dermal bathing solution, containing 14 g/l albumin, was removed and chromatographed. With the applied dose of 5 µg/cm² no statistical difference was found between the cumulated absorbed amount under atmospheric pressure and increased pressure. On the other hand, with the applied dose of 240 µg/cm², the permeation of caffeine was 1.8 times higher under increased pressure than the permeation under atmospheric pressure ($p < 0.05$). This enhancing effect of increased pressure was probably connected to either an improved transappendageal route during the percutaneous absorption process or a higher stratum corneum filling-up. **Key words:** Massage; Human skin.

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It is well accepted that the stratum corneum is the major rate-limiting barrier to molecular diffusion through the epidermis (1). Because most drugs do not permeate the skin in amounts sufficient to allow a therapeutic efficacy, chemical and physical approaches have been examined to lower the skin barrier properties and enhance the transepidermal permeation. Although most chemicals are well known for their penetration enhancer properties (2), several physical ways can be used, as an alternative, to increase the percutaneous absorption. Ultrasound or phonophoresis (3), iontophoresis (4), stripping of the stratum corneum (5), occlusion especially for lipophilic compounds (6,7) and long-wave ultraviolet radiation A (UVA) for small polar alcohols (8) have been demonstrated to increase the transepidermal permeation of a chemical.

Among penetration enhancers, massage is of interest since it corresponds to the common practice of favouring skin penetration of a compound. *In vitro* massage involves at least two different mechanisms, skin heating and pressure. Literature relating to pressure as a potential penetration enhancer remains scanty. The purpose of this study was to evaluate the effect of increased pressure on the *in vitro* percutaneous absorption of caffeine, whose transepidermal penetration char-

acteristics are clearly defined (9,10), and on which HPLC assessment is easily performed.

MATERIALS AND METHODS

Chemicals

Caffeine (1,3,7-trimethylxanthine) was obtained from Sigma (St. Louis, MO, USA). Acetone and analytical solvents were purchased from Carlo Erba (Milano, Italy). Human albumin[®] solution (20%) was obtained from the Merieux Institute (Lyon, France).

Skin preparation

Human abdominal skin taken from surgical specimens of women aged 38–54 years were immediately stored in a frozen state at –18°C for periods not exceeding 3 months. Approximately 12 h before use, the skin samples were placed in aluminium foil at 4°C, and the subcutaneous fat was carefully removed prior to the setting down on the diffusion cells.

In vitro percutaneous penetration

The skin samples were mounted on a series of 6 Franz diffusion cells (11), type FDC 200 from Crown glass (Somerville, NJ, USA) at ambient temperature (20–23°C). The cells allowed 3.14 cm² skin to be exposed to the chemical. The dermis was bathed in an aqueous dilution of a 20% human albumin solution, 1:14, which represented an aqueous solution of 14 g albumin/l, which is found in interstitial fluid in humans (12). The receptor solution (volume approximately 9 ml) was stirred by a teflon-coated magnetic bar at 400 rpm and maintained at 37°C in a water jacket. In this study, two different amounts of caffeine were investigated. Fifty µl of either a 320 µg/ml or a 15 mg/ml acetone solution of caffeine were pipetted onto the surface of each skin sample, which represented caffeine skin deposits of 5 µg/cm² and 240 µg/cm². The amounts applied were chosen to generate easily detectable drug concentrations in the receptor solution (7) and were in the same range as previously published (13).

Pressure device

After acetone evaporation, in each experiment, on 3 cells, a pressure device was applied on the outer site of the skin sample for the first 30 min and then removed. The pressure was monitored with a manometer and set at 0.25 bar over the atmospheric pressure. This increased pressure was obtained with the aid of an air pump, so that the skin sample was only slightly deformed (concave aspect) and massage effect on the skin was simulated. The other 3 cells remained under atmospheric pressure.

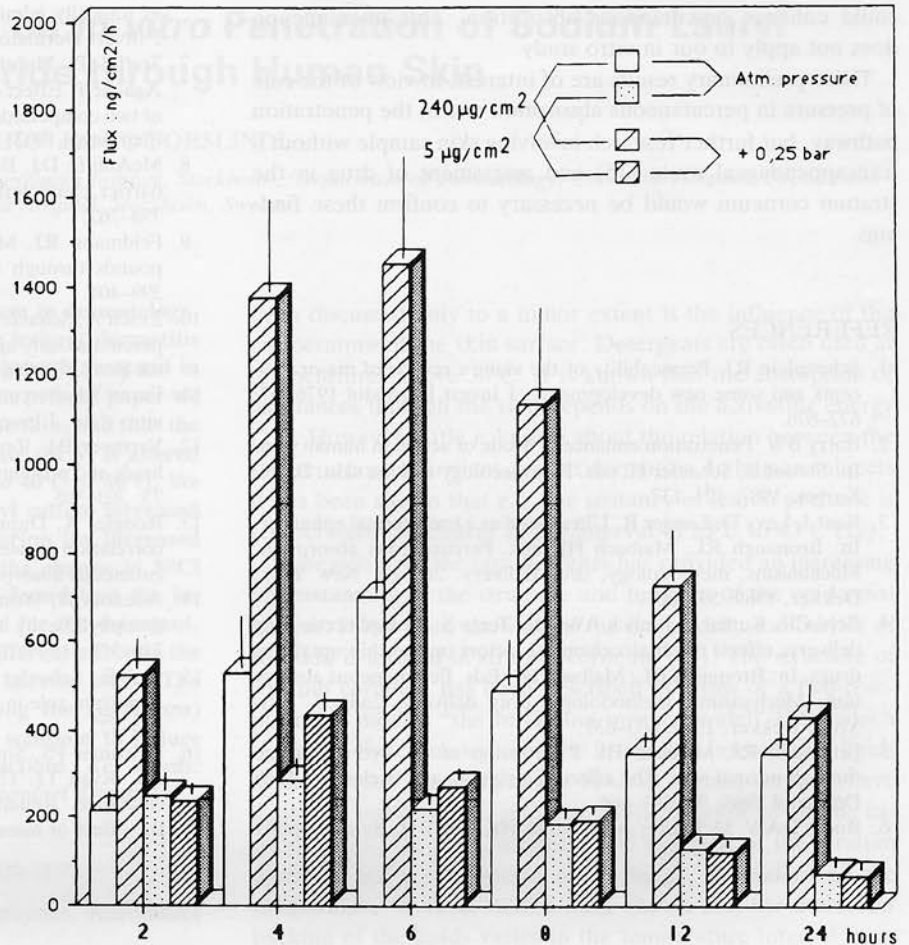
Sample handling and analysis

The receptor solutions were sampled at 2, 4, 6, 8, 12 and 24 h after the deposits and immediately replaced by new ones. The caffeine concentration was assessed as described in our previous paper (7). At the end of each experiment, the last receptor solution was replaced by distilled water and 300 µl of a 0.1% aqueous methylene blue solution were pipetted onto the skin samples. One hour later we considered the absence of coloration in the dermal bathing solution as evidence of the integrity of the skin barrier. Following this, one skin sample had to be discarded.

Statistics

The hourly fluxes (ng/cm²/h) were calculated at each sampling time with and without increased pressure and compared on the basis of the

Fig. 1. Hourly fluxes of caffeine permeation. The results are expressed as a mean \pm SE. Applied dose of $5 \mu\text{g}/\text{cm}^2$, $n=6$; applied dose of $240 \mu\text{g}/\text{cm}^2$, $n=9$ under increased pressure and $n=8$ under atmospheric pressure.



cumulated absorbed amounts in 24 h with the two-tailed Mann-Whitney test.

RESULTS

Data of caffeine permeation are presented in Fig. 1. With the applied dose of $5 \mu\text{g}/\text{cm}^2$, no statistical difference was found between the cumulated absorbed amounts over 24 h. Expressed in ng/cm^2 , they were 1631 ± 146 (SE) and 1788 ± 138 (SE) under atmospheric pressure and increased pressure respectively. However, the caffeine permeation under increased pressure seemed to be greater at 4, 6 and 8 h, although the overall difference was not significant.

On the other hand, with the applied dose of $240 \mu\text{g}/\text{cm}^2$, the cumulated absorbed amounts over 24 h were 1.8 times higher with the increased pressure than under atmospheric pressure ($p < 0.05$); expressed in ng/cm^2 , the results were 17044 ± 3382 (SE) and 9421 ± 2159 (SE) respectively.

DISCUSSION

This study demonstrates a pressure effect on the percutaneous absorption of caffeine when applied in great amounts. Only with caffeine deposits of $240 \mu\text{g}/\text{cm}^2$, did we observe a significant enhancing effect (1.8 times) by increased pressure, compared to atmospheric pressure. This difference between the applied doses was probably caused by the fact that high ap-

plied doses generate high percutaneous absorption levels, which could enhance the effect of pressure. The pressure device was applied only for the first 30 min of the experiment, in agreement with studies by Rougier et al. (13), who demonstrated that 30 min are sufficient to fill up the stratum corneum. In spite of the short application time of the pressure device, caffeine hourly fluxes throughout the experiment remained greater than those obtained without initial supplementary pressure. No steady state flux was achieved; the flux rose to a peak ($1450 \text{ ng}/\text{cm}^2/\text{h}$) and then decreased quickly. Thus, we assumed that pressure induced an increased filling of the stratum corneum, although high skin concentrations do not always generate a high transcutaneous flux (14). Since the behaviour of a molecule (e.g. solubility) depends on pressure, the solubility of caffeine in stratum corneum might be increased under pressure. Another explanation could involve an increase in transappendageal penetration. The area of hair follicles and sweat pores represents 0.1% of the skin surface (1, 15), and approximately 65% of the caffeine amount could penetrate the skin through the transappendageal way (15). Under increased pressure, the role of this route could explain, at least in part, the observed increased in caffeine percutaneous absorption. The holes in the skin surface could probably grow bigger, which favoured the transappendageal diffusion. In vivo massage increases blood flow and lymph flow (16), which increase the resorption capacity of the skin and

could enhance percutaneous absorption. This phenomenon does not apply to our in vitro study.

These preliminary results are of interest, in view of the role of pressure in percutaneous absorption and in the penetration pathway, but further research involving skin sample without a transappendageal route (15) and assessment of drug in the stratum corneum would be necessary to confirm these findings.

REFERENCES

1. Scheuplein RJ. Permeability of the skin: a review of major concepts and some new developments. *J Invest Dermatol* 1976; 67: 672-676.
2. Barry BW. Penetration enhancers. Mode of action in human skin. In: Shroot B, Schaefer H, eds. *Pharmacology and the skin*. Basel: Karger, 1987: 121-137.
3. Kost J, Levy D, Langer R. Ultrasound as a transdermal enhancer. In: Bronaugh RL, Maibach HI, eds. *Percutaneous absorption. Mechanisms, methodology, drug delivery*. 2nd ed. New York: Dekker, 1989: 595-601.
4. Behl CR, Kumar S, Malick AW, Del Terzo S. Iontophoretic drug delivery: effects of physicochemical factors on the skin uptake of drugs. In: Bronaugh RL, Maibach HI, Eds. *Percutaneous absorption. Mechanisms, methodology, drug delivery*. 2nd ed. New York: Dekker, 1989: 603-631.
5. Feldmann RJ, Maibach HI. Penetration of ¹⁴C hydrocortisone through normal skin. The effect of stripping and occlusion. *Arch Dermatol* 1965; 91: 661-666.
6. Bucks DAW, McMaster Jr, Maibach HI, Guy RH. Bioavailability

- of topically administered steroids: a "mass balance" technique. *J Invest Dermatol* 1988; 90: 29-33.
7. Treffel P, Muret P, Muret-D'Aniello P, Coumes-Marquet S, Agache P. Effect of occlusion on in vitro percutaneous absorption of two compounds with different physicochemical properties. *Skin Pharmacol* 1992; 5: 108-113.
8. McAuliffe DJ, Blank IH. Effects of UVA (320-400 nm) on the barrier characteristics of the skin. *J Invest Dermatol* 1991; 96: 758-762.
9. Feldmann RJ, Maibach HI. Absorption of some organic compounds through the skin in man. *J Invest Dermatol* 1970; 54: 399-404.
10. Zesch A, Schaefer H, Stuttgen G. The quantitative distribution of percutaneously applied caffeine in the human skin. *Arch Dermatol Res* 1979; 266: 277-283.
11. Franz TJ. Percutaneous absorption. On the relevance of the in vitro data. *J Invest Dermatol* 1975; 64: 190-195.
12. Vermeer BJ, Reman FC, van Gent CM. The determination of lipids and proteins in suction blister fluid. *J Invest Dermatol* 1979; 73: 303-305.
13. Rougier A, Dupuis D, Lotte C, Roguet R, Schaefer H. In vivo correlation between stratum corneum reservoir function and percutaneous absorption. *J Invest Dermatol* 1983; 81: 275-278.
14. Adenola JJ, Wester RC, Maibach HI. Cutaneous metabolism of theophylline by the human skin. *J Invest Dermatol* 1992; 98: 310-314.
15. Illel B, Schaefer H, Wepierre J, Doucet O. Follicles play an important role in percutaneous absorption. *J Pharm Sci* 1991; 80: 424-427.
16. Mortimer PS, Simmonds R, Rezvani M, Robbins M, Hopewell JW, Ryan TJ. The measurement of skin lymph flow by isotope clearance. Reliability, reproducibility, injection dynamics, and the effect of massage. *J Invest Dermatol* 1990; 95: 677-682.

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Abstract. The effect of pressure on the percutaneous absorption of caffeine was studied in vivo. The pressure was applied only for the first 30 min of the experiment. The results showed that the percutaneous absorption of caffeine was significantly increased by the application of pressure. The increase was more pronounced when the pressure was applied for a longer period of time. The results suggest that pressure may be a useful adjuvant in the treatment of skin diseases.

Key words: caffeine, percutaneous absorption, pressure, skin, stratum corneum.

Introduction

The percutaneous absorption of drugs is a complex process involving several factors such as the physicochemical properties of the drug, the characteristics of the skin, and the route of administration. The stratum corneum, the outermost layer of the skin, is the main barrier to the entry of drugs into the body. The permeability of the stratum corneum is determined by its thickness, the degree of hydration, and the presence of skin appendages. The percutaneous absorption of drugs is generally low, and the development of new drugs with improved percutaneous absorption is a major challenge in drug development.

One of the most common methods for improving the percutaneous absorption of drugs is the use of penetration enhancers. These are substances that increase the permeability of the stratum corneum, thereby allowing a greater amount of drug to enter the body. Penetration enhancers can be classified into several groups, including surfactants, solvents, and chemical enhancers. The use of penetration enhancers is often limited by their toxicity and their ability to irritate the skin.

Another method for improving the percutaneous absorption of drugs is the use of physical enhancers. These are physical agents that increase the permeability of the stratum corneum, thereby allowing a greater amount of drug to enter the body. Physical enhancers can be classified into several groups, including heat, ultrasound, and pressure. The use of physical enhancers is often limited by their ability to cause skin damage and their lack of specificity.

Pressure is a physical agent that has been shown to increase the percutaneous absorption of drugs. The mechanism of action of pressure is not fully understood, but it is thought to involve the compression of the stratum corneum, thereby increasing its permeability. The use of pressure as a physical enhancer has been studied in several studies, and the results have been generally positive.

In this study, we have investigated the effect of pressure on the percutaneous absorption of caffeine in vivo. Caffeine is a commonly used drug, and its percutaneous absorption is well characterized. The results of this study suggest that pressure may be a useful adjuvant in the treatment of skin diseases.

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