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Ethanol Absorption across Human Skin Measured by *In vivo* Microdialysis Technique

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Microdialysis, a new bioanalytical sampling technique enables measurement of substances in the extracellular space. This initial study investigates the technique's usefulness in the field of percutaneous absorption of solvents, using ethanol as test substance. Microdialysis probes are equipped at the tip with a semi-permeable polycarbonate membrane which permits passive diffusion of substances. Ethanol does not damage the membrane. In vitro recovery for ethanol is good. Probes were inserted via a guide into the skin of the ventral forearm in 7 volunteers. 99.5% ethanol was applied to the skin in excess in a glass reservoir. The probe was perfused at a flow of 1 μl/min. 50 μl samples were analysed by gas chromatography. Absorption of ethanol was demonstrated in all subjects. Values from the 9 probes inserted ranged from 10 μg/ml to 800 μg/ml. The variation may be explained by inter-test or inter-individual variability in ethanol absorption. Individual metabolic capacity may be of importance. The method opens new possibilities in the investigation of skin barrier function in man. ***Key words: Organic solvents; Skin penetration; Barrier.***

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Microdialysis technique was originally developed in experimental neuropharmacological animal models (1). A probe can be placed in a tissue and perfused with a physiological solution which is then collected for analysis (2). The perfusate is contained inside a semi-permeable polycarbonate membrane located at the tip of the probe. Substances in the extracellular fluid will diffuse into the perfusate while substances included in the perfusate will diffuse into the tissue. The technique offers many advantages over earlier methods for following biochemical events in the extracellular space. This paper, in which we have tested the practicability of microdialysis for the study of percutaneous absorption of organic solvents in man, reports part of a project to apply the technique dermatologically.

The question of systemic toxicity from percutaneous absorption of organic solvents is of concern especially in the workplace (3,4). Previous studies on the subject have most often been performed in experimental animals (5,6) or on in vitro samples of animal or human skin (7). Microdialysis should allow the study of absorption kinetics from application of relatively small amounts of solvents. For practical reasons (acceptable toxicity and a known behavior of the polycarbonate dialysis membrane), this initial study has been performed using the hydrophilic solvent ethanol.

MATERIALS AND METHODS

Equipment

The basic equipment necessary for the performance of microdialysis is a pump, a probe and a collector:

The perfusate (in the present experiment a physiological Ringer solution) is pumped through the system by a CMA/100 Microinjection Pump (Carnegie Medicine) which is capable of perfusion speeds from 1 nl/min to 1 ml/min.

The probe (CMA/10, Carnegie Medicine) has similar dimensions to a standard intravenous cannula (Fig. 1). The length of the probe shaft varies from 14 mm to 100 mm, and the dialysis membrane from 1 mm to 16 mm. In the present study, probes with shaft length 70 mm and membrane length 10 mm were used. The steel shaft of the probe has an outer diameter 0,64 mm. The diameter at the tip of the probe, where the membrane is located, is 0.5 mm. The standard membrane is permeable to molecules up to 20 000 Dalton, although more freely so to substances of lower molecular weight.

The perfusate is carried to the tip of the dialysis tubule by a fine steel cannula inside the probe to reach the space in contact with the dialysis membrane where the diffusion of molecules from the extracellular space to the perfusion fluid takes place. The perfusate flows back between the

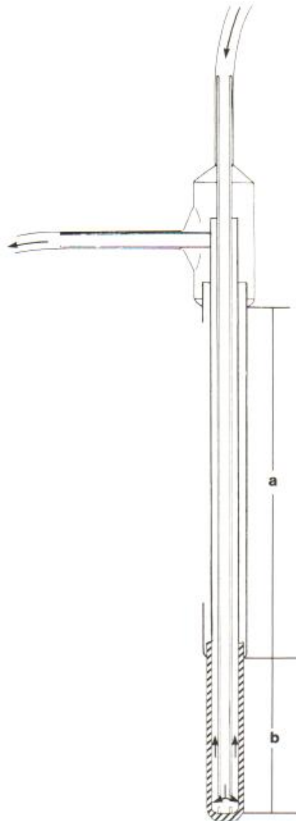

Fig. 1. Diagram showing microdialysis probe and perfusate flow direction. The probe can be made with a variable shaft length (a) from 14 mm to 100 mm. The length of the semipermeable membrane (b) at the tip can also be varied, from 1 mm to 16 mm. Probes with a = 70 mm and b = 10 mm were used in this study. The probe diameter is 0.5 mm at the tip. The shaft diameter is 0.64 mm. The perfusate is carried to the tip of the probe by an inner cannula to reach the space in contact with the dialysis membrane where diffusion takes place. The perfusate flows back between the membrane and the inner cannula to the outlet of the probe.

membrane and the inner cannula to the outlet of the probe.
Teflon tubing carries the perfusate to the CMA/140 Microfraction Collector (Carnegie Medicin) which is controlled from the Microinjection Pump. Aliquote size can be varied as necessary.

Relative and absolute recovery

The concentration of a given substance in the microdialysis perfusate relates to the concentration of this substance in the dialysed tissue or fluid . The relationship between the two concentrations is called the *relative recovery* and is expressed in a percent value (<100%). Slower perfusion speeds allow more time for equilibration across the membrane and will thus give higher concentrations in the perfusate and a higher relative recovery. The term *absolute recovery* refers to the absolute amount of substance that is removed by the perfusate during a defined time period. The easiest way of increasing the absolute recovery is to increase the perfusion flow – the perfusate will however, become more diluted.

Insertion of probes

The skin of the volar aspect of the forearm was anaesthetised with a 3 mm bubble of 5% mepivacaine. A blood donor needle (Terumo apheresis needle 16G x 1,25 inch) was inserted parallel to the surface of the skin and as superficially as possible, especially at the limit of the needle. The probe was then introduced the entire length of the guide, after which the guide was withdrawn. The probe tip thus lay approximately 3 cm from the site of insertion. The probe and tubing were taped in place.

Application of ethanol

A circular glass solvent reservoir with a diameter of 1 cm and a depth of 1 cm was placed over the tip of the probe and held in place with tape. The reservoir was filled with 99.5% ethanol and kept "topped up" throughout the experiment.

Analysis of ethanol

Ethanol in the perfusate was analysed by gas chromatography, using a flame-ionisation detector system. The column used in the original recovery experiments and in the human experiments was a packed Porapac Q, kept at a temperature of 170° C and with a nitrogen flow of 25 ml/min. 5 μl of the perfusate was injected into the column. The lowest detectable level of ethanol was approximately 3 μg/ml. A newer column (Carbowax) has improved sensitivity appreciably, especially at lower levels.

Effects of ethanol on membrane

The effect of ethanol on membrane performance was tested by measuring histamine recovery (8) prior to and after exposure to 1% ethanol in vitro for 40 min. The recovery was unchanged, indicating that the membrane was unaffected by ethanol itself.

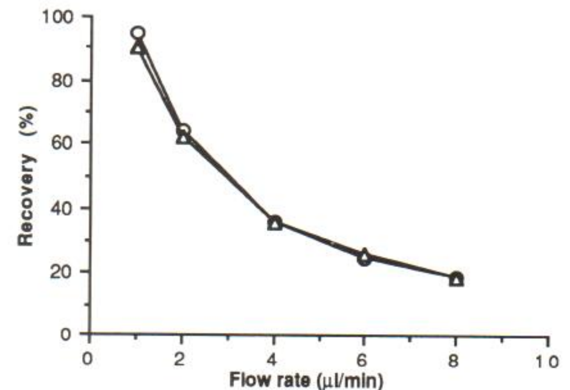

Fig. 2. The in vitro recovery from 0.01% ethanol in water for samples transported at room temperature (○) or on ice (Δ). Analysis of ethanol was performed by gas chromatography using a Carbowax column.

Table I. *Ethanol levels in the dialysate from 9 probes placed in the normal skin of the ventral forearm of 7 individuals (A-G).*

99.5% ethanol was applied in excess in a glass reservoir over the tip of the probe. Samples were collected at a perfusion speed of 1 µl/min for 50 min. Analysis was performed using gas chromatography with a Porapac Q column. (Subjects D, F and G were patients with psoriasis, subject E was a patient with nickel dermatitis. The tested skin sites looked normal to the naked eye).

Subject	Minutes after insertion of probe: 0–50	30–80	50–100
A		330 µg/ml	
B	155 µg/ml		90 µg/ml
A		15 µg/ml	
B		20 µg/ml	
C		30 µg/ml	
D	38 µg/ml		230 µg/ml
E	10 µg/ml		30 µg/ml
F	240 µg/ml		410 µg/ml
G	180 µg/ml		800 µg/ml

Localisation of probes

Ultrasound investigation with a Dermascan A (Sonotron AB) allows the estimation of the thickness of the epidermis plus dermis (9) and the localisation of the probe. The distance to the subcutaneous tissue in the skin close to the tip of the probe was estimated and then the distance to the probe at the tip.

Investigational design

In vitro tests: The recovery characteristics for ethanol were established in in vitro experiments. A 0.01% ethanol in water solution was dialysed. 30 µl samples were collected at flow rates of 1, 2, 4, 6 and 8 µl/min. Four samples were collected at each flow rate. The samples were collected and transported for analysis in capped glass vials, half of them at room temperature, the other half chilled on ice.

In-vivo tests: Volunteers from departmental staff and informed patients were examined. Approval was obtained from the Ethical Committee of the Linköping University Hospital. Nine sterile probes were inserted in 7 individuals. Sterile technique was used throughout the experiment.

After insertion of the probes, 99.5% ethanol was applied to the reservoire, immediately (4 tests) or after 30 min (5 tests). Immediately after application of ethanol 50 µl microdialysis samples were collected at a flow rate of 1 µl/min. In the former group two consecutive samples were obtained. In the latter group one sample was obtained.

Samples were collected and transported at room temperature but were stored chilled prior to analysis.

In two patients ultrasound investigation of skin thickness and probe localisation in the skin were performed.

RESULTS

Fig. 2 shows the in vitro recovery from 0.01% ethanol in water for samples transported at room temperature or on ice, using the new Carbowax column. There was good agreement between the two values. Relative recovery for 0.01% ethanol was more than 90% at 1 µl/min flow (the speed at which the in vivo experiments were performed), decreasing to 20% at 8 µl/min.

Insertion of probes caused relatively little discomfort. Slight echymoses developed in some cases. The total duration of the experiment was at most 2 h, which both patients and volunteers tolerated well.

Table I shows the results from microdialysis samples collected at 1 µl/min for 50 min. Values varied between 10 µg/ml and 800 µg/ml. Where two consecutive samples were obtained the second sample was usually two- or morefold higher (four of five cases).

In the two patients on whom ultrasound investiga-

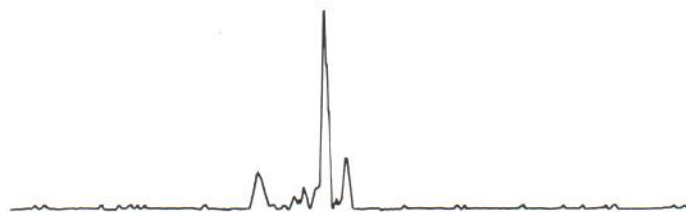

Fig. 3. Dermascan A print-out of probe depth. The initial peak (on left) is the epidermal/air interface, the high peak shows the position of the probe and the small peak on the right the interface between dermis and subcutis.

tion was performed, the probes lay 0.63 and 0.68 mm, respectively beneath the skin surface. Fig. 3 shows the Dermascan A print-out for one of the patients.

DISCUSSION

Human studies involving the subcutaneous use of microdialysis have been reported (10–13) but the present paper is, as far as we know, the first of the technique's use intracutaneously.

The probe membrane gives good recovery for ethanol particularly at lower perfusion speeds. Absorption of ethanol has been demonstrated in all patients in the present study, although a considerable variation in levels has been seen. The variable levels may be explained by inter-test or inter-individual variability in ethanol absorption. Varying metabolic capacity may also play a role. The effect of the depth of the probe also needs to be studied. The present study does not elucidate fully the absorption kinetics of ethanol over human skin. However, since ethanol absorption was higher in the second sample in four of our five cases, the kinetics may be similar to that seen in the guinea pig (6) where n-butanol (another hydrophilic solvent) measured in blood increased in the first hour to reach a plateau in the second hour. Improvement in the sensitivity of our analytical technique by use of the Carbowax column will allow study of shorter sampling intervals and thus give a better picture of absorption kinetics. The absorption characteristics of lipophilic solvents across normal and damaged human skin are also of interest clinically. Collection and estimation of these latter solvents will demand methodological changes, particularly as regards the microdialysis perfusion medium. Ultimately we hope the method will allow assessment of the absorption characteristics of the skin of individual patients and patient categories for a range of substances.

In the animal model used by Boman & Wahlberg (6), it has been shown that mechanical and chemical damage to the epidermis increases the absorption of hydrophilic solvents considerably. We are conducting further investigations to see whether this also occurs in human skin.

We have used the probe in both the present study and in studies where mediators of inflammation have been collected (8), with relatively little discomfort for the patients. Although penetration through the anaesthetized area of skin was painless, some pain occurred as the guide was introduced further parallel to the skin surface. This was more pronounced the more superficial the guide was placed. The same was true for the skin flare (a presumed axon reflex) which was sometimes observed.

In the present experiments we have made two assumptions. Firstly, we have presumed that anaesthetization will not affect results for ethanol absorption, especially as the tip of the probe is around 3 cm from the point of insertion. In situations where anaesthetic may affect results, insertion can be performed without it. The second assumption is that absorption of ethanol will not be affected by the length of the time between probe insertion and measurement of absorption. It is, however, probable that insertion of the probe causes damage to the tissues and that in some situations, a period of tissue "equlibration" after insertion is necessary. The length of this period remains to be determined.

The depth at which the inserted probe lies is of importance for the interpretation of results. The aim has been to insert the probe so that the tip lies as close as possible to the epidermis , but this can be steered only to a certain degree. Ultrasound scanning allows convenient determination of the depth of the probe. Our experience in this and other experiments is that we can in the forearm regularly place probes in the lower dermis (that is at a depth of around 1 mm).

The present study illustrates that microdialysis technique can be used in human skin, with a minimum of discomfort for the patient. Organic solvents are but one group of substances for which transcutaneous absorption kinetics can be studied. We are also using the technique to measure inflammatory mediators (8) in normal skin and contact reactions (14). We feel that microdialysis will greatly increase our ability to measure skin function, not the least barrier function, and that the technique will find many applications in dermatological research, both basic and clinical.

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