Dermal absorption of chemicals through normal and compromised skin
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Percutaneous absorption and metabolism of 2-butoxyethanol in human volunteers: a microdialysis study
G. Korinth, I. Jakasa, T. Wellner, S. Kezic, J. Kruse, K.H. Schaller
(submitted to Arch Toxicol)
Abstract

Human in vivo studies on percutaneous absorption are very scarce although they are needed for the human risk assessment and evaluation of in vitro studies and mathematical predictive models.

In the present study we determined percutaneous absorption kinetics of 2-butoxyethanol (BE) in volunteers using microdialysis technique. This study was performed within a collaborative EU project (EDETOX) and had as the main objective generation of in vivo data for in vitro - in vivo comparison, and assessment of the feasibility of different in vivo methods for determination of percutaneous absorption.

Four male volunteers were dermally exposed on the left forearm to 90% and 50% aqueous solution (v/v) of BE for 4.5 hours. To determine percutaneous absorption kinetics the concentration of BE was measured in the dialysate samples collected at 30 min-intervals throughout exposure. The systemic absorption which is needed to determine recovery of the BE in the dialysate, was estimated from the concentration of the main metabolite of BE, free butoxyacetic acid (BAA) in urine.

A pseudo steady state percutaneous absorption of BE was reached approximately at 2 hours of exposure for both applied concentrations. The maximum dermal flux of 50% BE was higher than that of 90% BE (2.8 ± 1.0 and 1.9 ± 1.7 mg cm$^{-2}$ h$^{-1}$), respectively, and the respective apparent permeability coefficient amounted to 6.1 x 10$^{-3}$ and 2.5 x 10$^{-3}$ mg cm$^{-2}$ h$^{-1}$. The more diluted solution showed the shorter lag time; 25 vs 39 minutes. The average absorption rate into the skin amounted to 3.6 ± 1.3 and 2.3 ± 2.3 mg cm$^{-2}$ h$^{-1}$. In one person we determined the amount of BAA in two dialysates collected at 4 and 4.5 hours in the exposure to 50 % BE and 90 % BE. The dermal metabolism seemed to be low, the amount of BAA was approximately 1% of the amount of BE in the same dialysate.

The presented study demonstrates applicability of microdialysis technique for assessment of percutaneous absorption kinetics and dermal metabolism without interference from the systemic compartment.
Introduction

For assessment of percutaneous absorption of chemicals mostly in vitro experiments using diffusion cells are performed, and in vivo human data are scarce. The lack of in vivo data makes it difficult to judge the validity of the use of in vitro data for the human risk assessment. In vivo studies are considered to be the gold standard for the evaluation of in vitro systems and predictive mathematical models (Howes et al. 1996), however, their wider use is limited due to ethical and practical considerations.

Although microdialysis is not frequently used in percutaneous absorption studies, it has been proposed as a useful method for the determination of in vivo percutaneous absorption of exogenous chemicals (Anderson et al. 1998, Surber et al. 1999). The microdialysis technique can be used in both in vivo and in vitro experiments (Anderson et al. 1991, Boutsiouki et al. 2001, Wellner and Korinth 2004, Klede et al. 2005).

The percutaneous absorption of the glycol ether 2-butoxyethanol (BE) has been studied in vitro (Wilkinson and Williams 2002, Korinth et al. 2005, Wilkinson et al. 2006), in vivo in experimental animals (Lockley et al. 2004, Lockley et al. 2005) and recently in human volunteers (Jakasa et al. 2004, Kezic 2004). The percutaneous absorption of BE was extensively studied within the EDETOX project (Williams 2004) as a model compound for inter-laboratory and in vivo-in vitro comparison.

The aim of the present study was to determine the percutaneous absorption kinetics of BE in volunteers using the microdialysis technique. Additionally, in a limited number of samples dermal metabolism of BE was investigated.

Material and Methods

Chemicals, subjects and experimental design
Four male Caucasian volunteers aged 27-37 and with no history of dermatological disease, participated in the study. They were in good health, had no visible skin damage and used no medication.

Percutaneous absorption of 90% and 50% aqueous solutions (v/v) of BE (≥ 99.8% purity, Fluka® Buchs, Switzerland) was assessed. The period between two dermal experiments exposures was at least two weeks. The volunteers were exposed on the left forearm to both, 90% and 50% BE in different exposure chambers (exposed skin
area of each chamber: 0.64 cm$^2$) for 4.5 hours. Single plasmapheresis hollow fibres (Plasmaflo OP–05(L) separator, Asahi medical, Tokyo, Japan) were used as capillaries for microdialysis. Two capillaries (material: polycarbonate; inner diameter (ID): 340 μm; wall thickness: 50 μm; pore size: 0.3 μm; cut-off: 3000 kDa) per exposure chamber were intradermally inserted without anaesthesia in parallel by linear technique at a length of ~ 2.8 cm in the skin and a distance of approximately 2.2 mm between both capillaries. Two rectangular (1 x 0.64 cm) exposure chambers (one for each concentration) of stainless steel were centred above microdialysis capillaries at a distance of about 3 cm and glued onto the skin surface using UHU®-hart (UHU, Bühl/Baden, Germany). The capillaries were connected by Tygon tubing (ID: 0.381 mm) (Cole-Parmer, Strongsville, OH) to the pulsation-free syringe pump PHD 2000 (Harvard apparatus, Holliston, MA) and perfused with saline as receptor fluid at a flow rate of 8 μl/min. The applied volume of BE solution in the exposure chambers was enough to ensure an infinite dose (200 μl/cm$^2$) and was covered with a foil to prevent evaporation. Receptor fluid samples were collected at 0.5 h intervals up to 4 hours. For assessment of dermal metabolism, free butoxyacetic acid (BAA) was determined in the last two collected dialysates of one subject (at 4 and 4.5 h), after exposure to 90 % BE and 50 % BE. Urine samples were collected at 4.5 h immediately after end of exposure.

**Analysis of BE and BAA**

BE was analysed in dialysates by gas-chromatography using flame ionisation detection. The concentration of BAA in dialysates and in urine samples was analysed by gas-chromatography followed using electron capture detection as described in detail by Kezic et al. (2004).

**Calculation of percutaneous absorption**

To find out the proportion of BE that was recovered in the dialysates relative to the amount absorbed systemically, we measured the concentration of BAA in urine as a biological indicator of exposure. The systemic absorption was estimated using the results from another EDETOX study on percutaneous absorption of BE in volunteers (Kezic et al. 2004) that was based on biological monitoring (BM) method. In that study, the systemic absorption was assessed from the urine excretion of BAA measured after dermal and a reference inhalative exposure of a known dose. The average concentration of BAA in urine sample collected shortly after cessation of 4-h dermal exposure was 121.7 mmol/mol creatinine, and the average systemic uptake was 568 mg.
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The total amount of systemically absorbed BE in the microdialysis experiment was derived from the equation:

$$BE_m^{(systemic~~absorption)} = \frac{BAA_m^{(mmol/mol~~creatinine)}}{BAA_{bm}^{(mmol/mol~~creatinine)}} \times BE_{bm}^{(mg)}$$

where $BAA_m$ is the concentrations of BAA measured in our microdialysis study. $BAA_{bm}$ is the concentration of BAA obtained in the BM study, $BE_{bm}$ is dermal systemic absorption in the BM study. Recovery of BE in the dialysate was calculated for each exposure experiment from the ratio between the total amount of BE measured in the dialysates, and the estimated systemic absorption. Dermal fluxes were calculated for each exposure from the absorbed amount of BE in the dialysate adjusted for recovery divided by sampling period (30 min) and skin area (0.64 cm$^2$) as follows:

$$Dermal~~Flux~~BE \left( mg \cdot cm^{-2} \cdot h^{-1} \right) = \frac{BE_{dialysate}^{(mg)}}{0.5 \left( h \right) \times 0.64 \left( cm^2 \right) \times REC \left( \% \right)} \times 100 \left( \% \right)$$

The maximum fluxes and lag times were determined from the slope of the cumulative amount of BE that was systemically absorbed vs. time. The average absorption rate into the skin (mg cm$^{-2}$ h$^{-1}$) during the exposure period was calculated by dividing the amount of systemically absorbed BE (mg) by the exposed skin area (0.64 cm$^2$) and exposure duration (4 h).

From the results of both replicated dermal exposures, we calculated the intra- and inter-individual variability.

Results

Figure 1 shows the amount of BE in the dialysates collected over 30 min during exposure period, averaged for all volunteers. Dermal fluxes of BE reached plateau between 1.5 and 2 hours after the start of exposure indicating attainment of pseudo steady state absorption (Fig 2), although one volunteer showed in all four experiments a declining absorption rate after a maximum at 3 h exposure.
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The average concentration of the free BAA excreted in the period 0 – 4.5 h after the start of exposure was 3.5 ± 2.0 mmol/mol creatinine. From the concentration of BAA in urine, the systemically absorbed amount of BE was estimated and shown in Table 1. Recovery of the absorbed amount in the dialysate was low and amounted less than 1% of the amount systemically absorbed. The percutaneous absorption parameters i.e. maximum flux, average absorption rate into the skin, permeability coefficient, and lag times are shown in Table 1. In general, 50 % BE showed higher and faster absorption. The lag time was shorter for 50 % BE (25.4 min) than that for 90 % BE (38.9 min). The respective intra-individual and inter-individual variation of the maximum dermal flux of BE amounted to 37 and 49 % for 50 % BE. For 90 % BE, the variation was considerable and amounted to 97 % and 91 %, respectively. This high variation after exposure to 90 % BE was primarily caused by one subject who showed significantly higher absorption.

The ratio of the amounts of BAA and BE in two microdialysis samples was 0.011. When corrected for the different molecular weight of BAA and BE dermal metabolism amounted to approximately 1.0%.

![Fig 1: The amount of 2-butoxyethanol (BE) in the dialysates during 4 hours exposure to 90% and 50% aqueous solution BE (mean ± SD)](image)
**Fig 2:** Dermal flux (mean ± SD) and cumulative absorption of 90% and 50% aqueous solution 2-butoxyethanol (BE)

**Table 1.** The amount of 2-butoxyethanol (BE) recovered in the dialysate, estimated systemic absorption, and the percutaneous absorption parameters for 90% and 50% BE (means ± SD)

<table>
<thead>
<tr>
<th></th>
<th>90% BE (µg)</th>
<th>50% BE (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cumulative amount of BE recovered in dialysate during 4 h</td>
<td>14.5 ± 11.7</td>
<td>30.2 ± 24.6</td>
</tr>
<tr>
<td>Systemic absorption (mg)</td>
<td>6.8 ± 6.6</td>
<td>10.1 ± 3.9</td>
</tr>
<tr>
<td>BAA in urine (mmol/mol creatinine)</td>
<td>3.5 ± 2.0</td>
<td></td>
</tr>
<tr>
<td>Average absorption rate (mg cm⁻² h⁻¹)</td>
<td>2.4 ± 2.3</td>
<td>3.5 ± 1.3</td>
</tr>
<tr>
<td>Maximum dermal flux (mg cm⁻² h⁻¹)</td>
<td>1.9 ± 1.7</td>
<td>2.8 ± 1.0</td>
</tr>
<tr>
<td>Permeability coefficient (cm h⁻¹)</td>
<td>2.5 ± 2.3 x 10⁻³</td>
<td>6.1 ± 2.2 x 10⁻³</td>
</tr>
<tr>
<td>Lag time (min)</td>
<td>38.9 ± 16.1</td>
<td>25.4 ± 10.6</td>
</tr>
</tbody>
</table>
Discussion

Application of the microdialysis technique has not yet attained wide acceptance in the research of percutaneous absorption, and only a few studies investigating occupationally relevant substances by this technique are available (Anderson et al. 1991, Boutsiouki et al. 2001, Klede et al. 2005). An important advantage of the microdialysis technique is that it measures the percutaneously penetrated chemical before entering systemic circulation (Schnetz and Fartasch 2001).

In the present study we have determined percutaneous absorption kinetics of 50% and 90% aqueous BE in volunteers. In addition, we were able, although in a very limited number of dialysates, to measure dermal metabolism.

Penetration of BE through the skin showed to be fast; lag times for 50% and 90% BE were 25 and 39 mins, respectively. Since lag time and diffusion coefficient are interrelated \( t_{\text{lag}} = \frac{L^2}{6D} \) where \( L \) is thickness of the stratum corneum, \( D \) is diffusion coefficient, this implies faster diffusion of 50% BE through the skin than that of 90% BE. Also average absorption rate into the skin and maximum flux were higher for more diluted BE. Average absorption rates amounted to 3.5 and 2.4 mg cm\(^{-2}\) h\(^{-1}\) for 50% and 90% BE, respectively, and the respective maximum fluxes 2.8 and 1.9 mg cm\(^{-2}\) h\(^{-1}\). The apparent permeability coefficients of 50% and 90% BE amounted to 6.1 and 2.5 cm h\(^{-1}\), respectively. The average absorption rates of 50% and 90% BE, in the volunteer study of Jakasa et al. (Jakasa et al. 2004) amounted to 1.3 mg cm\(^{-2}\) h\(^{-1}\) and 0.9 mg cm\(^{-2}\) h\(^{-1}\). In that study also absorption of neat BE was determined, showing five times lower absorption rate than 50% BE. In another volunteer study, an average absorption rate of 3.5 mg cm\(^{-2}\) h\(^{-1}\) for 50% was reported (Kezic et al. 2004), which is very close to the value we obtained in the present study. The maximum flux of 50% BE in vitro in human skin was determined by eight laboratories participating in the EDETOX project, reporting the average value of 1.5 mg cm\(^{-2}\) h\(^{-1}\) (EDETOX, 2004). Also in vitro a marked influence of water on the percutaneous absorption of BE was found. In an in vitro study with human skin, Wilkinson and Williams (2002) reported considerably higher apparent permeability coefficients with aqueous solutions than with neat BE. This effect was assigned to a better partitioning of BE in hydrated stratum corneum. Our results show, that higher content of water increases diffusion of BE which might be caused by the altered structure of lipid bilayers of the stratum corneum. The enhancing effect of water has significant implications. BE is a solvent used on large scale in water based paints, cleaning agents or cutting oils and comes on the market normally as a water formulation. This stresses once again, the
necessity of measurement of percutaneous absorption of a chemical as applied in a formulation rather than that of a neat compound.

It is well known that skin has the capacity for local metabolism of chemicals. For instance, for benzyl alcohol dermal metabolism can reach, even in excised human skin, about 50% of the percutaneously absorbed amount (Bronaugh et al. 1999). Glycol ethers are oxidised by cytosolic alcohol dehydrogenase and aldehyde dehydrogenase (Lockley et al. 2005). Lockley et al. (2004) were not able to detect metabolism of BE in vitro or in vivo in rat skin. The authors attributed this to the rapid penetration of BE through the skin (Lockley et al. 2004), which would reduce the contact of metabolising enzymes of the skin with BE. Our results, although determined only in four experiments, showed that the extent of metabolism in the skin of BE was rather low in relation to the amount which penetrated through the skin. We found that the ratio of BAA and BE in the dialysate was approximately 0.01. Assuming the same recovery of BE and BAA across microdialysis capillaries, BE was dermally metabolised at a percentage of about 1%. Considering the findings of Lockley et al. (2004, 2005) the dermal metabolism of BE seems to be negligible.

One of the main problems of the microdialysis technique is the calibration i.e., the estimation of the penetrated amount recovered in the dialysate. We estimated recovery of BE in the microdialysis capillaries using biological monitoring. From the concentration of BAA measured in the urine sample at the end of exposure we estimated the amount of BE systemically absorbed. For that purpose we used the results of a research group that also participated in the EDETOX project and investigated extensively the excretion kinetics of BAA after dermal and reference inhalative exposure (Jakasa et al. 2004, Kezic et al. 2004). The results of the present study demonstrate that only a small part (less than 1%) of the amount that penetrated was recovered in microdialysis capillaries. In an in vitro microdialysis study with the 50% aqueous BE solution a recovery of approximately 40% (Maas et al. 2004) was reported. However, as pointed out by several authors, (Groth 1996, Stenken 1999), results from in vitro methods are often not reliable indicators for in vivo recovery.

The intraindividual variation in the average absorption rate into the skin of 37% and 49% for 50% BE and 91% BE, respectively and the respective interindividual variations of 97% and 91% was relatively high compared with studies based on biological monitoring. In the study by Jakasa et al. (2004), the coefficient of variation for the intra-individual and inter-individual variation was 20% and 34% when total BAA was used as a biological indicator. The higher variation in the present
microdialysis study was primarily caused by the high values in one person for exposure to 90 % BE. Often, relative large variability is found in percutaneous penetration studies by microdialysis (about 50-100%). Factors which are mostly argued to be responsible for this are insertion depth of microdialysis capillaries, the variations in the blood flow, and flow rate of the perfusate. It has been shown that insertion of the microdialysis capillaries is associated with a temporary increase in cutaneous blood flow, which subsides during exposure (Kreilgaard 2002). In one volunteer, we found that the maximum penetration was achieved fast, however, after 3 hours the penetration rate declined. A possible reason for this might be higher cutaneous flow in the beginning of exposure due to insertion of the microdialysis capillaries, but also to different percutaneous penetration behaviour compared to other volunteers since the kinetics in both duplicate experiments were similar.

The presented study shows some important advantages of the microdialysis technique. The skin penetration kinetics can be studied as a function of time enabling determination of maximum dermal fluxes and in the case of steady state the permeability coefficient. Methods which determine total systemic absorption, yield only average absorption rates into the skin. In addition, by using microdialysis, as shown in our study, the lag time and diffusion coefficient can be easily determined. Besides relevant information on the percutaneous absorption kinetics, the derived parameters enable direct comparison with in vitro experiments.

However, as already discussed, the question of recovery remains important issue. In this study, we determined the recovery using biological monitoring. We collected only one urine sample, directly after end of exposure. It would have been much better if we had measured the cumulative amount excreted; however, this was not possible in this study for practical reasons. However, the obtained percutaneous absorption parameters are consistent with those from volunteer studies (Jakasa et al. 2004, Kezic et al. 2004) and in vitro studies (EDETOX, 2004), so we may conclude that microdialysis is a reliable and useful technique for investigation of percutaneous absorption and dermal metabolism. Microdialysis is particularly suitable when comparative research is needed e.g. for studying influence of vehicle, exposure conditions, damage of the skin etc. In that case the main drawback of the technique – determination of recovery becomes less relevant.
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Acknowledgement
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The experiments comply with the current laws of the countries in which the experiments were performed.

References