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Percutaneous absorption and metabolism of 2-butoxyethanol in human volunteers: A microdialysis study

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Abstract

We determined percutaneous absorption kinetics of 2-butoxyethanol (BE) in volunteers using microdialysis.

Four male volunteers were dermally exposed twice to 90% and 50% aqueous solutions (v/v) of BE for 4.5 h. 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 was reached approximately at 2 h of exposure for both BE concentrations. The maximum dermal flux of 50% BE was higher than that of 90% BE (2.8 ± 0.4, 1.9 ± 0.6 mg cm^{-2} h^{-1}, respectively). The more diluted BE solution showed shorter lag time: 25 min versus 39 min. The amount of BAA was determined in the pooled dialysate samples collected at 4 and 4.5 h. The dermal metabolism seems to be low, the BAA amount ranged from 0.03% to 1.9% of the BE in the same dialysate.

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

Keywords: Percutaneous absorption; Dermal metabolism; Microdialysis; Biological monitoring; 2-Butoxyethanol; Butoxyacetic acid

1. 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

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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 (Johanson and Fernström, 1988; Lockley et al., 2004, 2005) and recently in human volunteers (Jakasa et al., 2004; Kezic et al., 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.

2. Materials and methods

2.1. Chemicals, subjects and experimental design

Four male Caucasian volunteers aged 27–37 years and with no history of dermatological diseases, participated in the study. They were in good health, had no visible skin alterations 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 experiments were performed twice. The period between two dermal experimental exposures was at least 2 weeks. After giving written informed consent 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 h. 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 (i.d.): 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 cm × 0.64 cm) exposure chambers (one for each BE dilution) 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 (i.d.: 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 h. For assessment of dermal metabolism, free butoxyacetic acid (BAA), the main metabolite of BE, was determined in the last two collected dialysates (at 4 and 4.5 h), after exposure to 90% and 50% BE.

In parallel in a separate exposure chamber placed on the left forearm of volunteers in a distance of 5 cm from the upper limb and 3 cm (50% BE) and 6 cm (90% BE) from BE chambers we investigated the percutaneous absorption of pyrene dissolved in ethanol (c = 4 mg/ml) using aqueous ethanol (90/10, v/v) as receptor fluid. To exclude that BAA collected in dialysate samples was systemically metabolised and transported from the liver into microdialysis capillaries we determined BAA also in dialysates from pyrene exposure samples. The experimental design is presented in Supplementary online material (Fig. S1). Urine samples were collected at 4.5 h immediately after end of exposure.

2.2. Analysis of BE and BAA

For the determination of BE in receptor fluid samples 200 μl of receptor fluid was mixed with 800 μl 0.9% NaCl solution, 0.5 g solid NaCl and 100 μl internal standard solution of 2-(2-chloroethoxy)ethanol (c = 2 g/l). The mixture was shaken and added on ISOLUTE HM-N® (Separtis, Grenzach-Wyhlen, Germany) sorbent, packed in glass columns, equipped with polytetrafluoroethylen-frits followed by an extraction with 6 ml of a mixture of dichloromethane and acetone. Gas chromatographic (GC) separation was carried out on an INNOwax® capillary column (Agilent Technologies, Palo Alto, CA) (length 30 m, inner diameter 0.25 mm, film 0.25 μm). The GC analysis was based on a specific time–temperature program and flame ionisation detection (FID). Calibration was carried out with aqueous standard solutions in the range from 0.5 to 200 mg/l with the same sample preparation. The limit of detection (LD) was 0.2 mg/l.

For the analysis of free BAA in urine and dialysate a procedure described in detail by Kezic et al. (2004) was used. The method is based on derivatisation of BAA with pentafluorobenzylbromide and subsequent gas chromatographic–electron capture detection (GC–ECD). For the analysis, 100 μl of urine or 300 μl of dialysate was taken. The LD (three times S.D. of the blank) of the method for the determination of BAA in urine was 0.5 mg/l and the relative coefficient of variation (rCV) was 12%. The LD of the method for the determination of BAA in dialysate was 0.05 mg/l and the rCV was 14%.

2.3. 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 urinary excretion of BAA measured after dermal and a reference