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Influence of artificial sebum on the dermal absorption of chemicals in excised human skin: A proof-of-concept study

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ABSTRACT

In an initial diffusion cell study, the influence of artificial sebum on dermal penetration and intradermal reservoir of ethanol and toluene was investigated in comparison with the effects of a skin cream (o/w- and w/o-emulsion) and untreated (control) skin. Human skin was exposed to neat ethanol and toluene for 4 h, respectively. During the experiments, the penetration of the compounds was assessed in the receptor fluid. The amounts of the test compounds in the skin were determined at the end of exposure. In the control experiments, 42% of the total resorbed ethanol amounts were found in the intradermal reservoir after 4 h, whereas 82% of the toluene amounts were found in the skin compartments. The treatment with artificial sebum showed no significant differences in dermal absorption of both test compounds compared to control skin. In contrast, the treatment with skin cream increased the percutaneous penetration ($p < 0.001$) and the intradermal reservoir of ethanol ~2-fold but not of toluene. In all exposure scenarios, a relevant intradermal reservoir was formed. The results indicate that sebum does not influence the percutaneous penetration and the intradermal reservoir of epidermally applied chemicals, whereas the application of skin creams may increase the dermal penetration of the compounds.

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1. Introduction

The epidermal barrier function is of interest in occupational medicine and hygiene, since in certain scenarios, the skin may be the predominant absorption pathway for several chemicals at workplaces (WHO, 2006). The lipid matrix of stratum corneum represents the main skin barrier against the systemic uptake of chemicals into the body (de Jager et al., 2006). It is reported that the skin appendage route, such as hair follicles (Liu et al., 2011) and glands (Schaefer and Redelmeier, 1996), also contributes to cumulative absorption of chemicals.

Several functions of the sebaceous glands interact with skin protection (Zouboulis et al., 2008). The human skin surface lipid film mainly consists of sebum, a mixture of predominantly neutral lipids. The main function of sebum in fur-bearing animals is to form a hydrophobic barrier against water. However, little is known about the influence of sebum on the skin barrier function against a dermal absorption of chemicals in humans. The sebum amount on the skin surface ranges from 0.2 to 130 μg/cm^2 (Wilhelm et al., 1991); the thickness of the sebum film varies from <0.5 μm in most body areas to >4 μm in the sebum-rich face skin (Sheu et al., 1999). Some components of the human sebum, like fatty acids and cholesterol (Stefaniak and Harvey, 2006), are main constituents of the stratum corneum lipid matrix as well (Jungersted et al., 2008) and are therefore involved in building the skin barrier.

Percutaneous penetration data of chemicals is mostly obtained by diffusion cell studies using human skin from plastic surgeries (van de Sandt et al., 2004; OECD, 2011). However, experimental treatment of skin surface with 70% aqueous isopropyl alcohol demonstrated an initial removal of sebum of >90% in vivo (Rode et al., 2000). Thus, it may be supposed that the integrity of the natural sebum film is affected by pre-surgical disinfection, too.

Therefore, the question arises whether the lack of a topical sebum film can affect the results of dermal penetration experiments based on human skin ex vivo. The aim of this proof-of-concept study was to evaluate the influence of artificial sebum on the percutaneous penetration behaviour of two test compounds with different physicochemical properties, compared to the results for untreated skin. Moreover, the comparison with the effects of a regular skin cream application was included in the study, as it was reported that topical skin cream application can enhance the dermal absorption of the chemical compounds (van der Bijl et al., 2002; Korinth et al., 2003, 2008).

Abbreviations: CAS, chemical abstract service; CV, relative coefficient of variation; GC, gas chromatography; KOH, potassium hydroxide; LogP, decadic logarithm of the octanol/water partition coefficient; NaCl, sodium chloride; o/w, oil in water emulsion; SD, standard deviation; SEM, standard error of the mean; w/o, water in oil emulsion.

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2. Materials and methods

2.1. Test compounds

Ethanol (CAS no. 64-17-5) and toluene (CAS no. 108-88-3) were used as test compounds for percutaneous penetration experiments. Ethanol (purity: absolute GR for analysis) and toluene (purity: SupraSolv® grade) were purchased from Merck (Darmstadt, Germany).

2.2. Preparation of the artificial sebum and ingredients of the skin cream

Artificial sebum was prepared, considering the data on human sebum composition from literature (Stefaniak and Harvey, 2006). Fig. 1 shows the composition of artificial sebum. Squalene (CAS no. 111-02-4), lanolin (CAS no. 8006-54-0), glyceryl tripalmitate (tripalmitin; CAS no. 555-44-2), glyceryl tristearate (tristearin; CAS no. 555-43-1), stearic acid (CAS no. 57-11-4), palmitic acid (CAS no. 57-10-3), cholesteryl oleate (CAS no. 303-43-5) and free cholesterol (CAS no. 57-88-5) were purchased from Sigma-Aldrich (Munich, Germany); oleic acid (CAS no. 112-80-1) was supplied by Fisher Scientific (Schwerte, Germany). The purity of palmitic acid, cholesteryl oleate and free cholesterol was ≥99%; the purity of squalene was ≥98%, of stearic acid ≥98.5% and of tripalmitin ~90%. Tristearin was of technical grade purity (usually ≥90%), lanolin for laboratory research purposes (usually ≥95%) and oleic acid of general purpose grade (usually >95%). Considering the saturation state, equal amounts (v/v) of saturated (stearic acid:palmitic acid, 25:25%) and unsaturated (oleic acid, 50%) free fatty acids were chosen.

The ingredients for artificial sebum were weighted using an analytical precision balance (XA105DU; Mettler Toledo®, Giessen, Germany), mixed and heated in a water bath at 60 °C for ~10 min under continuous stirring at 250 rpm (IKA® RET basis; IKA®-Werke, Staufen, Germany) until the mixture became a fluid consistence. Subsequently, the artificial sebum was cooled down over night to room temperature and stored at 4 °C for 27 days according to the literature (Wertz, 2009).

The skin cream (Basiscreme DAC; NRF 11.104) was obtained from the pharmacy of the University Hospital Erlangen, which prepared it according to the German Drug Codex (DAC, Deutscher Arzneimittel Kodex, Eschborn, Germany). The cream consisted of glycerol monostearate 60 (4%), cetyl alcohol (6%), medium-chain triglycerides (7.5%), petrolatum (25.5%), polyethylene glycol (PEG)-1000–glycerol monostearate (7%), propylene glycol (7%) and purified water (40%).

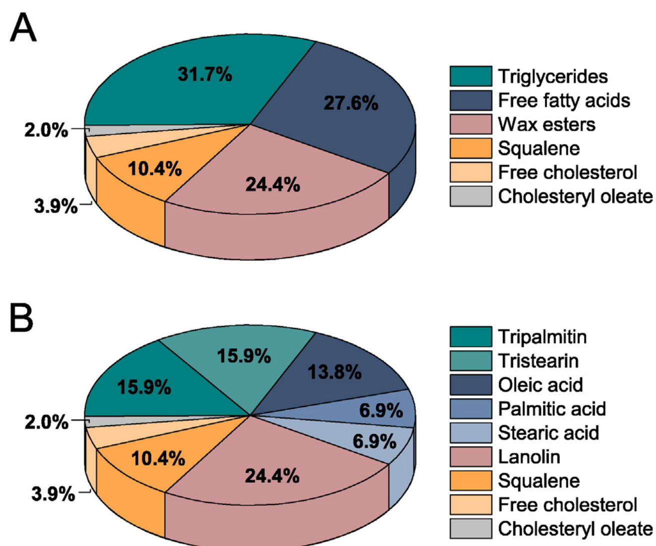

Fig. 1. Comparison of the composition of human (A) and our artificial sebum (B). *The composition of human sebum was obtained from Stefaniak and Harvey (2006). The amounts of the ingredients of both sebum types are expressed as a percentage. To represent the triglyceride fraction of human sebum, equal amounts of tristearin and tripalmitin were chosen. The fraction of free fatty acids is represented by equal aliquots of saturated (stearic:palmitic acids, 25:25%) and unsaturated (oleic acid, 50%) compounds.

2.3. Dermal application of the artificial sebum and the skin cream

Both artificial sebum and the skin cream were applied on the skin surface by gentle wipes with one cotton swab per exposure area. They were coated with artificial sebum by wiping the sebum surface. The skin cream was applied on cotton swabs in a volume of 20 µl (weight (mean ± SD): 19.39 ± 0.63 mg) using a 1 ml syringe (Omnifix® — F 1 ml; B. Braun, Melsungen, Germany).

The amounts of the artificial sebum and the skin cream applied on the skin surface were determined from the weight difference of the cotton swabs (before and after application). The weight difference was determined using an analytical precision balance (XA105DU; Mettler Toledo®, Giessen, Germany).

2.4. Percutaneous penetration experiments

In this study, excised human skin of 4 female donors (age range: 30–59 years, mean age: 41 years) was used, which was anonymously obtained from a local clinic after reduction of abdominoplasty, according to the ethical guidelines of our university. After removing subcutaneous tissue with a scalpel, the skin was wrapped in aluminium foil, put in plastic bags and stored at −20 °C for up to 3 months until the beginning of diffusion cell experiments, as proposed in the study protocol of van de Sandt et al. (2004). Similar skin preparation and storage are proposed by Dennerlein et al. (2013) and OECD (2011). For the experiments, the skin was thawed at room temperature. It was prepared with a scalpel to a thickness of 0.9–1.2 mm (n = 48), using a precision vernier calliper before mounting on diffusion cells. The preparation of skin is in close agreement with recommendations of OECD (2011) for studies using full thickness skin. The physical integrity of the skin membranes was visually assessed. The skin surface temperature was considered.

Percutaneous penetration experiments were carried out using static PermeGear® diffusion cells (vertical system, flat flange joint; 9 mm orifice; exposure area 0.64 cm^2, receptor chamber volume 5 ml) (SES Analysesysteme, Bechenheim, Germany), which are similar to the model described by Franz (1975). A diffusion cell consists of an exposure chamber (upper compartment) and a receptor chamber (lower compartment), where excised skin can be fixed in between. Receptor chambers of diffusion cells were charged with 0.9% aqueous NaCl solution and heated during the experiments by a thermostatic circulating water bath (MV-4; Julabo®, Seelbach, Germany) at 35 °C. Receptor fluid was continuously stirred (500 rpm) with a teflon-coated magnetic bar. Before the treatment with artificial sebum or skin cream, the skin surface temperature was measured using a digital precision thermometer (GMH 1160 with GOF 500 universal probe, type K; Greisinger electronic, Regenstauf, Germany).

To exclude background contamination, blank receptor fluid samples were taken from each diffusion cell before application of the test compounds. Percutaneous penetration of ethanol and toluene was compared between untreated and treated skin (with artificial sebum or skin cream). Here, the 2 skin membranes of the 4 donors (n = 8 per each exposure scenario) were used in parallel. 10 min after the treatment with artificial sebum or skin cream, 160 µl/0.64 cm^2 (= 250 µl/cm^2) of neat ethanol (dose: 126.3 mg) or toluene (dose: 139.2 mg) was applied on treated and control skin surface under occlusion. Receptor fluid samples of 500 µl (~10% of receptor chamber volume) were taken at 0.5, 1, 2, 3 and 4 h of exposure and stored deep frozen until chemical analyses several days later. The sampled volume was immediately replaced with fresh receptor fluid.

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