Available online at www.sciencedirect.com
SCIENCE DIRECT
Environmental Toxicology and Pharmacology 21 (2006) 199–203
ENVIRONMENTAL TOXICOLOGY AND PHARMACOLOGY ETAP
www.elsevier.com/locate/etap

In vitro studies—how good are they at replacing in vivo studies for measurement of skin absorption?

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Available online 29 August 2005

Abstract

Measures of percutaneous penetration are required for risk assessment of exposure of man to chemicals. In vitro approaches and QSAR predictions can be used and reduce the use of in vivo animal experiments. The OECD Guidelines on in vitro dermal absorption studies were recently accepted but progress was hampered by a lack of direct in vitro/in vivo comparisons in humans or in rodents. Either flow through diffusion or static cell systems with full thickness, dermatomed skin or membranes can be used. In a study of the robustness of in vitro techniques, inter-skin variability was greater than inter-laboratory or between cell variability. Recent studies with a number of chemicals have shown a reasonably good prediction but the difference between in vitro and in vivo results was greater for lipophilic molecules as lipophilic molecules which were retained in the stratum corneum. The experimental flux obtained in vitro using conditions that reflect the potential occupational exposure may be the most appropriate figure for risk assessment purposes. A database of in vitro and in vivo dermal penetration has been established.

Dermal absorption data using infinite doses has been combined in a number of databases used for predictive QSAR modelling approaches to dermal absorption. However, absorption values derived from QSAR may over estimate the actual absorption from a finite dose.

Keywords: In vitro; In vivo; Absorption; Skin

1. Introduction

Data on percutaneous absorption of chemicals is required for risk assessment for exposure to man. Chemicals that come in contact with the skin have the potential for absorption locally or to the systemic circulation. The skin acts a barrier to absorption and it comprises an outer non-viable layer (the stratum corneum 10–20 μm thick, the viable epidermis (approximately 100 μm thick) and the inner layer, the dermis which is perfused by blood in the capillary circulation. The mechanism by which chemicals cross this barrier is by diffusion and the rate of passage is described by Ficks Law which states that the rate of passage is proportional to the concentration gradient.

The most useful data on dermal absorption is obtained from studies in humans in vivo, such as volunteer studies and occupational monitoring. Much of toxicology has been derived from rodent models and regulatory bodies in the USA recommend the use of in vivo rodents for dermal absorption studies (Zendzian, 2000). However, it is well documented that absorption through rodent skin is greater than through human skin due to the potential for compounds to obtain rapid passage down the hair follicle and the relative thickness of the stratum corneum. Although it is generally accepted that human skin is the most appropriate in vitro model, pig skin has also been promoted on the basis of its structural equivalence to human skin.

Europe has recommended in vitro approaches to dermal absorption studies (Guidelines and Guidance Documents, EEC, 2002, SCCNFP, 2003, ECB) in line with the three Rs approach to reduction in the use of animals. An area of particular importance now involves the Registration, Evaluation and Authorisation of Chemicals Regulations (REACH) that require toxicity data to be generated for 30,000 chemicals. It is not appropriate or feasible to do extensive in vivo studies in rodents and this goes against the ethics of the EU. There is a need to use in vitro approaches and/or structure activity relationships (QSAR) and modelling to obtain appropriate information.

Dermal absorption is an area in which in vitro approaches have a significant role to play as skin is a relatively easily accessible tissue. However, it has taken 10 years of discussion to agree and accept the OECD Guidelines on in vitro dermal absorption studies. (OECD, 2004). Progress has mainly been hampered by a lack of direct in vitro/in vivo comparisons with the same dose application in humans or in rodents to support the acceptance of the in vitro approach for risk assessment.

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doi:10.1016/j.etap.2005.07.009

In Europe, without the acceptance of dermal absorption data generated by in vitro approaches, risk assessment has been based on route-to-route extrapolation or default assumptions that in the worst-case scenario have been 100% dermal absorption even for chemicals which do not penetrate the skin well. Reproducible and reliable absorption data is required to predict accurately the risk of exposure to chemicals in a number of areas, such as the workplace, agrochemicals, household products and cosmetics.

In this review, I have concentrated on examples of recent studies and general comments about in vitro systems rather than a comprehensive review of all literature.

2. Experimental designs

The recent European Project, Evaluation and Predictions of Dermal Absorption of Toxic Chemicals (EDETOX) (Williams, 2004a,b) and European Chemical Industry Council (CEFIC) report (Jones, 2004), have addressed areas concerning the design of in vitro experiments. Also, a number of in vivo/in vitro comparative studies were carried out during the EDETOX project. Data are presented as percent dose applied measured in the different compartments (unabsorbed, membrane and absorbed) and/or as flux. Permeability coefficient (K_p) values are only presented when an infinite dose was applied (i.e. the maximum absorption rate was achieved).

Either flow through diffusion systems or static cell systems can be used. The flow through system has the advantage that it more closely mimics the in vivo situation as receptor fluid (generally tissue culture medium) flows below the skin which can remain viable for up to 48 h so that local metabolism can be studied. However, this system has the disadvantage of dilution of the absorbed material limiting sensitivity. With the static system, it is necessary to ensure sink conditions in which the concentration in the receptor fluid is not sufficient to inhibit absorption. However, in the robustness study recently published by van der Sandt et al. (2004) in which comparison of absorption measurements between several research laboratories for three marker chemicals were conducted, the design of the diffusion cell (static or flow through) did not appear to be a significant source of variation. Inter-individual differences in permeability of human skin used in the in vitro studies were critical variables (Van der Sandt et al., 2004; Wilkinson et al., 2005). However, a multi-centre study in which skin was replaced by an artificial membrane reported a four-fold variation in average flux values from 16 different laboratories, when saturated aqueous methyl paraben was used as a model penetrant (Chilcott et al., 2005). It seems likely that the differences in temperature of the different parts of the diffusion cell contributed to variation. Variability up to eight-fold for testosterone penetration has been shown for human skin due to inter-individual differences in the characteristic if the skins although the factors that contribute to this have not been defined. The source of skin from humans, e.g. breast skin or abdominal skin, fresh or cadaver, may also influence the result (Lee et al., 2001).

Absorption studies in vitro have used full thickness skin, cut to approximately 300 μm with a dermatome or epidermal membranes. Absorption through full thickness skin in vitro may potentially differ from that in vivo due to a lack of microcirculation within the upper dermis. The dermis can, therefore, act as a reservoir reducing absorption to the receptor fluid. Studies recently reported within EDETOX (Wilkinson et al., 2005) showed that particularly for lipophilic molecules, use of full thickness skin resulted in lower absorption to the receptor fluid than the split thickness skin and that the total distribution of absorbed material indicated a reservoir in the skin.

3. Aspects of comparisons between in vitro and in vivo studies

3.1. Predictive studies

For acceptance of in vitro dermal absorption data, it is important to consider directly comparable in vivo and in vitro studies. It was felt at the Organisation for Economic Co-operation and Development (OECD) meeting in 2000 that there were insufficient directly comparable studies either in the rodent or in man for acceptance of in vitro approaches. Recently, a number of studies have been conducted that have shown that the in vitro approach can predict absorption in vivo. These studies include those by our group in the rodent using fluazifop butyl (Clark et al., 1993; Ramsey et al., 1994), testosterone (Lee et al., 2001), triclosan (Moss et al., 2000), phenoxyethanol (Roper et al., 1997, 1998), butoxyethanol and ethoxyethanol (Lockley et al., 2003, 2004). Recent studies with glycol ethers indicate that a reasonably good prediction of in vivo penetration of chemicals in man can be obtained with human skin in vitro and supports the acceptance of in vitro data for risk assessment. The in vivo percutaneous absorption rate was calculated from blood concentrations after dosing using linear-system dynamics and point area de-convolution methods (Jakasa et al., 2004). It was important to determine the levels and fate of chemicals remaining in the skin and a full mass balance in the in vitro studies (Wilkinson and Williams, 2002). When butoxyethanol was applied to full thickness skin from young rat, the flux to receptor fluid (1.8 $\mu g/cm^2/h$) was slightly higher than in the in vivo flux (1.3 $\mu g/cm^2/h$). The influence of dilution with water on the flux of butoxyethanol through skin in vivo was well predicted by the in vitro approach with rat skin and human skin.

The percutaneous absorption of caffeine using excised human skin was compared with that in human volunteers (Meuling et al., 2002). Caffeine was applied to split thickness human abdomen skin in static diffusion cells (with saline as receptor fluid) or to the volar forearm in water vehicle at either 10 or 100 $\mu g/cm^2$, the exposure time was 4 h in each case, after which the test compounds was removed by washing. Monitoring of blood, urine and faeces or receptor fluid continued for 72 h after dosing. Amounts of caffeine measured in receptor fluid correlated well with the amount excreted in urine and faeces in vivo, especially, at the lower dose. In both models, relative absorption was markedly reduced with the higher dose level and the species difference in vitro at this dose was much less marked than in vivo. A marked skin reservoir was measured in rat skin, especially in vivo, but this was not detected with human skin. Direct absorption of benzopyrene, pyrene and butoxyethanol through rat skin in vitro