Isolated Hemoperfused Porcine Skin as a Valid Model to Assess Percutaneous Absorption

To the Editor:

The passage of topically administered dermatologic drugs across the human skin and consecutive systemic adverse reactions can significantly limit the use of topical drugs and represents one of the main problems in the development of new topical compounds (Zucchi et al., 2001). Also, transdermal absorption is a major pathway for environmental toxins or allergens to reach the systemic circulation, and how drugs such as analgesics may be administered carrier-mediated transport mechanisms and the extent of absorption of drugs and toxins is thus largely determined by their physicochemical properties (Hadgraft and Pugh, 1998). Until now, most studies on skin permeation have been carried out using living laboratory animals (Brown et al., 1999) or humans (Leopold and Maibach, 1999) and, therefore, dermatologic drug development is partly the focus of ethical controversy.

In this study, a model of isolated perfused porcine skin was developed to assess transdermal absorption using porcine forelegs (average organ weight 1514.5 ± 101 g) of eight female pigs. The organs were harvested after desanguination for the collection of autologous blood from commercial abattoir pigs to reduce animal experiments as previously suggested for perfusion studies of the pig ear (VaniRooij et al., 1995). After transfer to the laboratory using cold preservation, the organs were placed in a perfusion system and normothermic pressure controlled perfusion was performed through the brachial artery with a pressure of 100 mmHg and perfusates were periodically collected for analysis. The perfusion system consisted of a blood and a dialysis circuit (Fig LA) The dialyate (composition in mmol per liter: 142.5 Na⁺, 2.9 K⁺, 1.5 Ca²⁺, 0.5 Mg²⁺, 109.9 Cl⁻, 37 HCO₃⁻, 2.5 CH₃COO⁻, 3.55 glucose) reservoir with a capacity of 10 l was permanently enriched

REFERENCES


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*The authors have declared not to have a conflict of interest.

The passage of topically administered dermatologic drugs across the human skin and consecutive systemic adverse reactions can significantly limit the use of topical drugs and represents one of the main problems in the development of new topical compounds (Zucchi et al., 2001). Also, transdermal absorption is a major pathway for environmental toxins or allergens to reach the systemic circulation, and how drugs such as analgesics may be administered carrier-mediated transport mechanisms and the extent of absorption of drugs and toxins is thus largely determined by their physicochemical properties (Hadgraft and Pugh, 1998). Until now, most studies on skin permeation have been carried out using living laboratory animals (Brown et al., 1999) or humans (Leopold and Maibach, 1999) and, therefore, dermatologic drug development is partly the focus of ethical controversy.

In this study, a model of isolated perfused porcine skin was developed to assess transdermal absorption using porcine forelegs (average organ weight 1514.5 ± 101 g) of eight female pigs. The organs were harvested after desanguination for the collection of autologous blood from commercial abattoir pigs to reduce animal experiments as previously suggested for perfusion studies of the pig ear (VaniRooij et al., 1995). After transfer to the laboratory using cold preservation, the organs were placed in a perfusion system and normothermic pressure controlled perfusion was performed through the brachial artery with a pressure of 100 mmHg and perfusates were periodically collected for analysis. The perfusion system consisted of a blood and a dialysis circuit (Fig LA) The dialyate (composition in mmol per liter: 142.5 Na⁺, 2.9 K⁺, 1.5 Ca²⁺, 0.5 Mg²⁺, 109.9 Cl⁻, 37 HCO₃⁻, 2.5 CH₃COO⁻, 3.55 glucose) reservoir with a capacity of 10 l was permanently enriched

Figure 1. Potential cis-acting regulatory regions in phylogenetically conserved regions of the mouse and human plakoglobin genes. This "regulogram" (Jegga et al, in press) shows a comparison of the mouse (top) and human (bottom) plakoglobin gene sequences aligned by BlastZ (Schwartz et al, 2000). Exons are shown as dark boxes. Homologous conserved regions are connected by shaded polygons. Potential cis-elements were identified using MatInspector (Werner, 2000) and the TRANSFAC professional transcription factor binding site database. Cis-elements contained within a 200 bp window of the homologous regions are scored as a hit. Hits per 200 bp window are shown on the Y-axis. Further detailed analyses of implicated cis-elements can be viewed at: http://trafac.chmcc.org/
with 97.5% O\textsubscript{2} and 2.5% CO\textsubscript{2} and adjusted to 38°C by a heat exchanger. The model drug for testing transdermal absorption was fentanyl, which was applied using a transdermal fentanyl system (transdermal therapeutic system, 50 μg per h; Durogesic, Janssen-Cilag, Neuss, Germany). This compound was chosen because of its widespread clinical use (Allan et al., 2001) and the existence of a transdermal application system that ensures a continuous percutaneous administration (Grond et al., 2000). The transdermal therapeutic system patch was placed radially on the foreleg and fentanyl levels were examined using commercial radioimmunoassay and gas chromatography tests (Coat-Account, DPC, Los Angeles, CA).

Besides the assessment of functional parameters for organ viability, oxygen consumption, serum electrolytes (sodium (mmol per liter), potassium (mmol per liter), chloride (mmol per liter), calcium (mmol per liter)), glucose (mg per dl), lactate (mg per dl), pO\textsubscript{2} (mmHg), and pCO\textsubscript{2} (mmHg) were examined.

The perfusion experiments were carried out over a period of 7 h. The sodium and chloride concentrations remained constant during the perfusion period of 420 min ranging from 145.4 ± 2.1 to 150.7 ± 1.9 mmol per liter and 105.3 ± 2.1 to 106.1 ± 2.1 mmol per liter, respectively, whereas there was an increase of potassium and calcium from initially 4.69 ± 0.40 mmol per liter at 60 min to 5.65 ± 0.48 mmol per liter after 420 min and 0.47 ± 0.10 mmol per liter to 0.60 ± 0.11 mmol per liter, respectively. In all experiments, percutaneous fentanyl absorption was detectable with increasing plasma levels from 28.5 ± 16.5 pg at 60 min to 602.3 ± 295.1 pg at 240 min and 1774.3 ± 453.3 pg at 420 min (Fig 1B). The analysis of hemodynamic parameters demonstrated constant perfusion flows ranging from 4.97 ± 0.25 ml per min per 100 mg at 60 min to 4.96 ± 0.25 ml per min per 100 mg at 240 min. Also, the arterial pressure remained constant within limits of 80.6 ± 8.8 mmHg (240 min) to 87.8 ± 9.4 mmHg (420 min). Organ resistance values were found at levels of 17.3 ± 1.8 mmHg × min per 100 g per ml (60 min) to 18.3 ± 2.3 mmHg × min per 100 g per ml (420 min). Whereas the arterial and venous protein parameters remained constant during the perfusions (1.77 ± 0.12 g arterial albumin per dl, 1.77 ± 0.10 g venous albumin per dl at 420 min) metabolic parameters changed; lactate levels increased from 40.1 ± 5.1 mg per dl (arterial) and 47.7 ± 4.1 mg per dl (venous) at 60 min to 85.0 ± 7.2 mg per dl (arterial) and 91.6 ± 7.1 mg per dl (venous) at 420 min. Also glucose levels decreased from 123.3 ± 21.7 mg per dl (arterial) and 115.7 ± 21.6 mg per dl (venous) at 60 min to 62.1 ± 13.7 mg per dl (arterial) and 55.9 ± 11.2 mg per dl (venous) at 420 min. The arteriovenous glucose consumption exhibited a peak at 180 min (Fig 1D), whereas the oxygen consumption resembling the main criteria of organ viability (Adham et al., 1997), decreased slightly from 0.097 ± 0.013 ml per min per 100 g at 60 min to 0.079 ± 0.012 ml per min per 100 g at 420 min (Fig 1C), but stayed within physiologic ranges. Enzyme analysis revealed constant increases for lactic dehydrogenase from 2162 ± 78 U per liter (arterial) and...
2347 ± 289 U per liter (venous) at 60 min to 6171 ± 1871 U per liter (arterial) and 6028 ± 916 U per liter (venous) at 420 min.

The organ-specific data with hemodynamic and hematologic parameters of arterial pressure and flow, organ resistance, hematocrit, albumin, oxygen saturation and consumption, sodium, potassium, lactic dehydrogenase, and glucose consumption illustrate the proximity of this model to established models of isolated perfused organs, such as liver or kidney, which are used for transplantation research (Schon et al., 1993; Zarzuelo et al., 2000). Also, intact skin morphology was revealed (Fig 1E,F) using previously described histology protocols (Fischer et al., 2001; Groneberg et al., 2001).

In summary, the present model of isolated normothermic hemoperfused skin using porcine forelegs allows a continuous assessment of drug or environmental compound absorption within a period of up to 7 h. As shown previously, there is a large variability in the pharmacokinetics of fentanyl absorption (Plezia et al., 1989) despite the rate control membrane that reduces the variation in skin drug transport by 50% (Hwang et al., 1991).

The skin of small laboratory animals possess significant differences in organ function and morphology in comparison with human skin. Therefore, the isolated porcine skin model may be a useful approach to simulate human conditions with an isolated system consisting of the skin and the adjacent musculo-veno-arteral area; however, although porcine skin reacts qualitatively similar to human skin, there may be quantitative differences in the permeability to drugs in the presence of fatty alcohols (Andega et al., 2004). The system seems to be a well-balanced model to investigate percutaneous absorption of drugs as it shares similarities in drug absorption and plasma levels if compared with human conditions of transdermal drug application as shown here for fentanyl (Plezia et al., 2001).

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The ex vivo perfused porcine skin may also be a more suitable model for testing the topical administration of drugs as it is by far more similar to physiologic conditions as compared with in vitro cell culture tests. Future studies on the percutaneous absorption of newly developed compounds in the present model could also include the measurement of transdermal water loss as a marker of permeability barrier function. As the organs and autologous blood can be obtained from commercial abattoirs in high numbers, and the model does not conflict with animal welfare acts, experimental animal numbers can be reduced and thus ethical concerns about dermatologic or cosmetic drug testing may be diminished.

This study was supported by a grant from the German Ministry of Education and Research (BMBF) (0311021). We thank V. Essig and M. Meissler for excellent assistance, and C. Peiser, S. Nagel, and Q.T. Dinh for helpful discussions. We also gratefully acknowledge the organ support by Eberswalder Abattoir Pharmrose Inc., Britz, and technical support by Mediport Biotechnik Inc., Berlin, Germany.

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