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An Improved Model for Studies on Transdermal Drug Absorption In-vivo in Rats

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Abstract—In rats, transdermal drug absorption can be studied under physiological conditions by cannulating the peripheral skin vein, draining the area of the skin which is used for drug application, and collecting the blood. This method leads to decreased blood volume, which causes a reduction in skin blood flow and limits the maximal duration of the experiment. We improved the model by replacing the collected blood with blood from donor animals, so enabling the measurement of transdermal absorption over a period of 5 h under near constant conditions of blood pressure, haematocrit and skin blood flow. The model was applied to the transdermal absorption of [^{3}H]prazosin and [^{3}H]scopolamine and their permeability coefficients, fluxes and lag-times were determined. The model is suitable for measurements of transdermal drug absorption under in-vivo conditions, both for comparison of absorption profiles of different drugs and of the same drug in different formulations.

The mechanism by which molecules penetrate the dermal barrier, their transdermal absorption kinetics and their influence on the physicochemical properties of the permeant have attracted considerable interest in the last decade (Guy & Hadgraft 1988; Siddiqui 1989). A particular goal has been to examine approaches by which drug absorption can be effectively and reversibly enhanced in a controlled manner (Walters 1989). Therefore, it is necessary to obtain absorption data of drugs through intact, viable skin. Whether absorption data should be evaluated in animal or human skin or from in-vitro or in-vivo models is discussed by Zesch (1982). According to Zesch (1982), data from animals are not transferable to man, but are useful for investigating the optimal vehicle to use and for the study of absorption enhancers. Thus animal models using hairless species are not necessarily preferable to those using fully haired species. Wester & Maibach (1983) pointed out that skin is a dynamic, living biomembrane with unique properties, imitated best by in-vivo models.

In anaesthetized rats transdermal drug absorption can be studied by cannulating the peripheral skin vein draining the area of skin which is used for drug application and collecting the blood (Dehn et al 1988). This method allows direct measurement of drug absorption in-vivo, which is unaffected by distribution and elimination. A disadvantage is the limited duration of the experiment, caused by the decreasing blood volume resulting in reduction in skin blood flow, and leading to death of the animal within about 180 min. This time is often too short to reach the steady-state of the often slow transdermal absorption processes (Lippold 1984). We modified the model by replacing the blood lost by transfusion of donor blood. Thus, we obtained an experimental model which was stable for at least 300 min, with near constant conditions of blood pressure, haematocrit and skin blood flow. Further, we applied the model to the absorption of the antihypertensive drug, prazosin, in two different vehicles and to the absorption of scopolamine, a drug which has been available in a commercial transdermal therapeutic system since 1981.

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Materials and Methods

Experimental procedure

Male Sprague-Dawley rats, 300 g, were anaesthetized with 1·5 g kg^{-1} urethane intraperitoneally and kept at a body temperature of 37°C by a heating pad (temperature controller with rectal probe CMA/150, Carnegie Medicin, Sweden). The trachea was intubated. The carotid artery and the jugular vein were cannulated for blood pressure measurements (Statham Spectramed, Düsseldorf, Germany) and transfusion of heparinized whole blood (5000 int. units/15 mL) obtained from donor animals, respectively. A branch of the dorsal iliolumbal vein was carefully dissected free (stereomicroscope DAC, 160×, Zeiss, Germany) and a PE 10 catheter (i.d. 0·28 mm, o.d 0·61 mm) was introduced. After heparinization (3500 int. units/300 g, i.p.), this catheter was used for the collection of blood in 10 min fractions throughout the experiment (fraction collector RediFrac, Pharmacia LKB, Freiburg, Germany). The collected blood was replaced by infused heparinized donor blood with an infusion pump (Injectomat cp-IS 50, Fresenius, Bad Homburg, Germany), starting with the fourth fraction. The blood flow and the corresponding infusion rate was 378 ± 85 μL/10 min (mean ± s.d., n = 18). The haematocrit was analysed by centrifugation in haematocrit capillaries. The radioactively labelled drug was applied after the second fraction as a solution of 200 μL in a closed teflon chamber (area of 1·13 cm^2) fixed on the shaved skin (electrical hair clipper Favorita II, Aesculap, Tuttlingen, Germany) with rapid glue (Hypo glue 150, Marston, Zülpich, Germany). The amount of drug absorbed was determined by liquid scintillation counting. At the end of each experiment the drained area of the skin was made visible by administration of a methylene blue solution in the dorsal iliolumbal vein and a central blood sample was taken by heart puncture. Prazosin was applied as an aqueous

solution (2·4 mmol L^{-1} prazosin, about 32 μCi/200 μL) and in ethanol:DMSO 1:1 (12 mmol L^{-1} prazosin, about 20 μCi/200 μL). Scopolamine was applied as an aqueous solution (25 mmol L^{-1} scopolamine, about 25 μCi/200 μL).

Data were analysed by linear regression of the time courses of the mean cumulative amounts of drug in the collected plasma, using the Statistical Consultants Inc. computer package PCNONLIN. The linear regression model was of the form:

$$y = mx + b \quad (1)$$

where x is the time, y is the cumulative amount of drug in the collected blood samples, and m is the slope of the regression line, representing the flux or

$$y = m(x + a) \quad (2)$$

where a is the lag-time until absorption reaches steady-state. The permeability coefficient was calculated as the ratio between the flux and the product of area and concentration (integrated form of Fick's first law of diffusion) (Scheuplein 1967; Flynn & Stewart 1988). The data are expressed with their 95% confidence limits.

Drugs

The drugs used were: urethane (25% in 0·9% NaCl solution, Riedel-de-Haen, Seelze, Germany), prazosin HCl (Pfizer, Karlsruhe, Germany), (−)-scopolamine methyl chloride (Sigma Chemie GmbH, Deisenhofen, Germany), heparin-Na 5000 int. units mL^{-1} (Liquemin 25000, Hofmann-La Roche, Grenzach-Whylen, Germany). The labelled drugs were obtained from Amersham-Buchler (Braunschweig, Germany): (furanyl-5)-[^{3}H]prazosin (25 Ci $mmol^{-1}$), (*N*-methyl)-[^{3}H]scopolamine methyl chloride (83 Ci $mmol^{-1}$), radiochemical purity was 96·2 and 98·1%, respectively, according to HPLC analysis. All other chemicals were of analytical grade (Merck, Darmstadt, Germany).

Results

The model for measuring transdermal absorbtion as described by Dehn et al (1988) allowed experiments with a duration of 120–170 min and showed great interindividual variance (Fig. 1). After that time the skin blood flow decreased rapidly until cardiovascular heart failure occurred. The blood pressure, represented as mean arterial pressure (MAP) from the carotid artery, decreased continuously during the experiment (Fig. 2). The number of surviving animals also decreased with time. The model, when modified by blood replacement, revealed a skin blood flow with an interindividual variance comparable to that obtained without blood infusion, which remained at an acceptable level of skin blood flow for 300 min (Fig. 3). The blood flow did not decrease substantially below 200 μL per fraction. The MAP in the modified model decreased in the first 30 min in the same manner as in the Dehn-model, but following blood

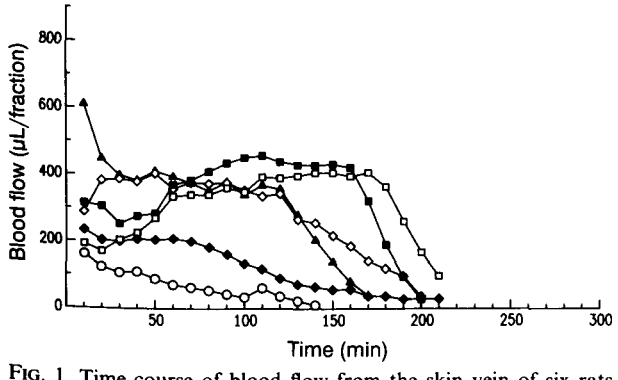

FIG. 1. Time course of blood flow from the skin vein of six rats without blood transfusion.

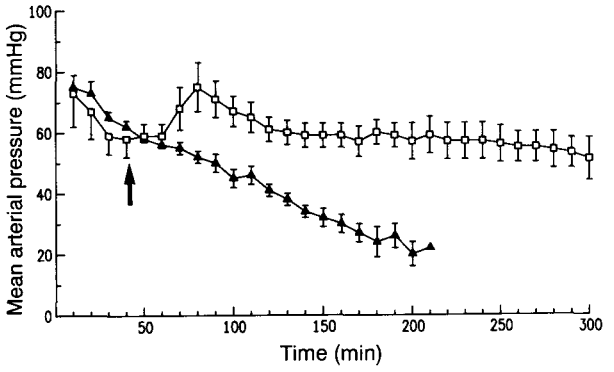

FIG. 2. Time course of the mean arterial pressure (MAP). Data are expressed as mean ± s.d. ▲ MAP without blood transfusion (n = 2–6), □ MAP with blood transfusion (n = 6). Arrow indicates start of blood transfusion.

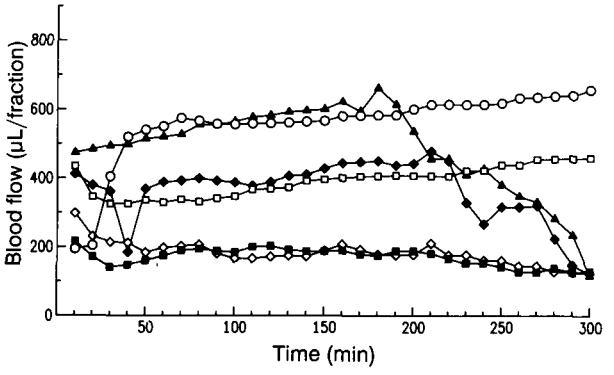

FIG. 3. Time course of blood flow from the skin vein of six rats with blood transfusion. Start of blood infusion followed after 40 min.

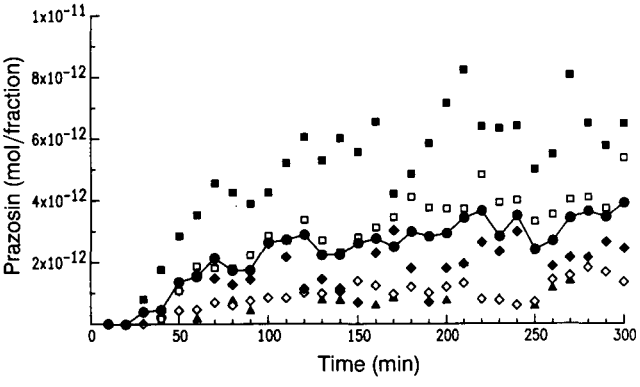

FIG. 4. Time course of the amount of prazosin absorbed in the blood from an aqueous solution. Application followed after 20 min. ●——● Represents the mean values of 5 experiments. The other symbols represent the individual values.

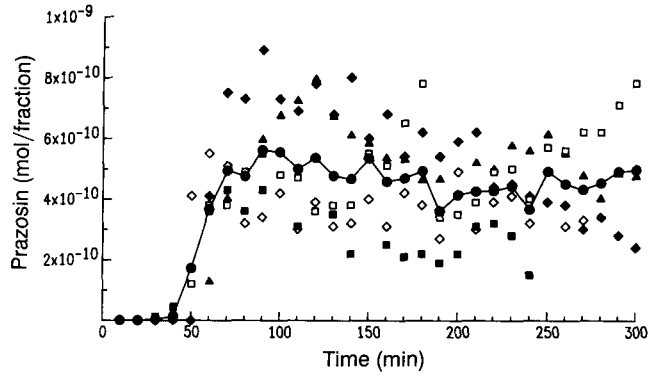

FIG. 5. Time course of the amount of prazosin absorbed in the blood from an ethanol/DMSO solution. Application followed after 20 min. ●——● Represents the mean values of 5 experiments. The other symbols represent the individual values.

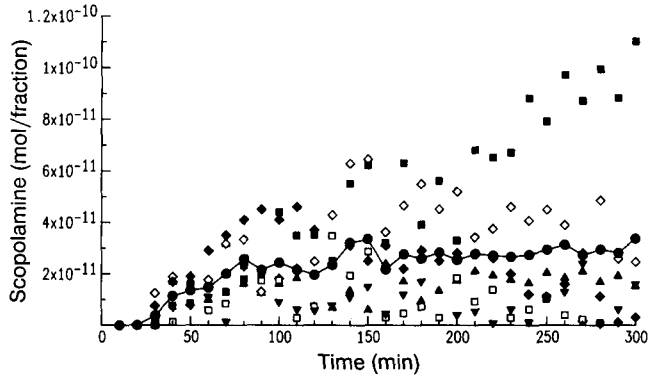

FIG. 6. Time course of the amount of scopolamine absorbed in the blood from an aqueous solution. Application followed after 20 min. ●——● Represents the mean values of 6 experiments. The other symbols represent the individual values.

replacement, the MAP increased within 40 min to the starting level of 75 mmHg. Afterwards, the MAP decreased slightly to about 60 mmHg until the end of the experiments (Fig. 2). The haematocrit obtained from the skin vein was observed at intervals of 40 min and started at 52% decreasing to 40% at the end of the experiment. The haematocrit of the donor blood was 48%.

In the skin permeability experiments using the modified model, the absorption of aqueous [^{3}H]prazosin, applied after collection of the second fraction, increased up to the 10th fraction, where a very low flux of about 3 pmol per fraction was reached (Fig. 4). The experiments with [^{3}H]prazosin in a solution of ethanol: DMSO (1 : 1) showed a very fast absorption with a flux of about 450 pmol per fraction from the 6th fraction onwards (Fig. 5). Scopolamine was absorbed quickly from the aqueous solution, reaching a flux of about 28 pmol per fraction in the 8th fraction (Fig. 6). The values of flux, lag-time and permeability coefficient, calculated by linear regression analysis, are summarized in Table 1.

Blood samples taken at the end of the experiments did not contain labelled drug. This suggests that the total amount of transdermally absorbed drug had been collected with the blood from the peripheral skin vein and that no distribution into the central blood system occurred. The administration of methylene blue solution into the skin vein after each experiment was used to confirm the position of the chamber in the area drained by the iliolumbal vein.

Discussion

The model described by Dehn et al (1988) allows the measurement of transdermal absorption through viable skin under physiological conditions. Therefore, one of the most important parameters reflecting the quality of the model is the blood supply of the skin, evaluated as blood flow in the iliolumbal skin vein. Both in the original and in the modified model, interindividual variance in the blood flow was observed. This variability in cutaneous blood flow was previously reported by Pershing et al (1989), who also observed pronounced day-to-day variation in the same animal. However, the difference between the model of Dehn et al (1988) and the modified model, is that skin perfusion can be obtained in the modified model for 300 min instead of 120–180 min. Further, the constant flow implies good sink conditions of skin for the permeating drug and reduces the risk of artefacts from transdermal absorption measurement, because it is assumed that drug absorption through skin is independent of blood flow (Scheuplein & Bronaugh 1983).

The MAP also reflects the condition of the animal during experimentation. Urethane anaesthesia decreases MAP. In the model of Dehn et al (1988) continuous blood loss results in a continued decrease in MAP and ultimately death by peripheral shock. If the MAP drops below 40 mmHg, the peripheral blood flow decreases in order to compensate for this severe hypotension. (The critical value of the MAP is derived from the combined view of Figs 1, 2.) This change in peripheral blood flow can be avoided by replacement of the lost blood.

The blood collected from the skin catheter and used for the analysis of the amount of drug absorbed plays the important role of acceptor medium. Therefore it is desirable that it should be unchanged during the experiment. In these experiments whole heparinized blood of the same species was used for the substitution, and haematocrit was measured as

Table 1. Absorption of [^{3}H]prazosin and [^{3}H]scopolamine.

	Drug		
Vehicle of application	Prazosin ethanol/DMSO	Prazosin water	Scopolamine water
Flux (nmol cm^{-2} h^{-1})	2·664–2·808	0·0181–0·0191	0·165–0·171
Permeability coefficient (cm h^{-1} 10^{-3})	0·225–0·233	0·0075–0·0079	0·0066–0·0068
Lag-time (min)	17–25	49–57	36–42
C_s (mol)	0·012	0·0024	0·025

Data are expressed as 95% confidence limits.

an indicator that the cellular components of blood were within the normal range.

When the modified model was applied to the absorption of [^{3}H]prazosin, aqueous prazosin was poorly absorbed, as expected, because of its poor water solubility resulting in a low application concentration, and of its low lipophilicity preventing favourable partition into the stratum corneum. However, fluxes of 3 pmol/10 min can still be measured with this method. In a solution of ethanol/DMSO, however, prazosin appeared in blood very rapidly, resembling infusion kinetics. A nearly 30-fold enhanced permeability coefficient in comparison with application in water was observed. The reason for that enhancement is the ability of DMSO, especially in combination with ethanol, to disrupt the skin's barrier function as reported by Barry (1987) and Sugibayashi et al (1988).

For the absorption of scopolamine, steady-state was reached faster in our experiments than in man (Muir & Metcalf 1983) where a patch with scopolamine base was used and steady-state was reached in the 8 h after application, but the extent of absorption was comparable (about 200 pmol in the 5th hour vs about 170 pmol in our experiments). The good absorption of *N*-methyl scopolamine can be explained by the formation of an ion pair by the quaternary ammonium salt with free fatty acids from the skin allowing penetration through the lipid bilayers of the stratum corneum, as suggested by Neubert & Amlacher (1991) for compounds like neostigmine and pyridostigmine.

We only detected prazosin and scopolamine in the blood collected from the skin vein and not in the central blood, thus ensuring that the results were unaffected by distribution and elimination in the central circulation. Further, the administration of a methylene blue solution at the end of each experiment into the skin vein showed good conformity with the application area.

In conclusion, the model of Dehn et al (1988) has been improved by the described modification because it enables us to measure transdermal absorption profiles of different drugs or of the same drug in different formulations under physiological conditions over a 5-h time period.

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