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Percutaneous administration of digoxin across hairless mouse skin and human skin in vitro

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Summary

We investigated in vitro the percutaneous absorption of tritiated digoxin in normal saline using Franz-type diffusion cells. Across mouse skin, the diffusion rate (J) and permeability coefficient (K_p) were respectively equal to 55.4 ± 19 pg/cm^2 per h and $0.14 \pm 0.04 \times 10^{-3}$ cm/h. When PEG 400 and ethanol were added to the receptor compartment, the flux increased to 233.4 ± 109.5 pg/cm^2 per h and K_p rose to $0.60 \pm 0.28 \times 10^{-3}$ cm/h. Under the same conditions, percutaneous absorption across human skin was dramatically lower ($J = 6.0 \pm 1.5$ pg/cm^2 per h, $K_p = 0.016 \pm 0.008 \times 10^{-3}$ cm/h). This is explained by the dermal retention of this lipophilic molecule.

Digoxin administration is a common and effective treatment of congestive heart failure and supra-ventricular arythmias. This drug is available in two forms: oral and intravenous. Since the therapeutic index is low with a narrow range of plasma concentrations (0.5–2 ng/ml), intoxications occur frequently with these forms (Smith, 1973). Despite the long biological half-time (36 h) and low hepatic metabolism, percutaneous administration of digoxin through a controlled-delivery device could be developed in order to decrease the risks of oral administration.

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Based on the values of the permeability coefficient, namely, 1×10^{-3} cm/h across hairless mouse skin and 9×10^{-3} cm/h across human skin, which are comparable to those of scopolamine — that is efficiently delivered by a transdermal system — Cairncross and Ackermann (1985) concluded in a preliminary study that it was possible to develop a transdermal system.

The aim of our investigation was to examine the percutaneous absorption of digoxin in vitro and to determine the possibility of sufficient concentrations being attained in order to develop a transdermal system.

[12α-^{3}H(N)]Digoxin (spec. act. 10 Ci/mmol; purity 99%) was purchased from NEN France. Propylene glycol 400 and dipropylene glycol were

obtained from Merck. Female hairless mice (age: 7–9 weeks) were supplied by Elevages Janvier, Laval, France. Franz-type diffusion cells (diffusion cells were kindly donated by Laboratoires Fournier, Dijon, France), designed with a volume of 15 ml and diffusion area of 2 cm^2, were employed.

The hairless mice were killed by cervical dislocation. The abdominal skin was removed and trimmed of fat. Human skin was obtained after plastic surgery on the abdominal area and was stored at $-20\,^{\circ}C$ for 2 months.

Skin membranes were placed on the receiving chamber and allowed to reach equilibrium overnight. The donor compartment was filled with a normal saline containing digoxin at a concentration of 390 ng/ml or 5 μCi/ml. For assays of diffusion across hairless mouse skin, the receiving chambers were filled with normal saline ($n = 5$), with normal saline plus 20% ethanol ($n = 5$), and normal saline plus 20% ethanol and 20% PEG 400 (n = 10). The latter mixture (60% saline, 20% PEG 400, 20% ethanol) was used for diffusion across human skin (n = 10). 0.5 ml samples of the saline solution in the receiving chamber were taken through the sampling port at regular intervals up to 40 h. Each sample was replaced by an equal volume of free digoxin saline. Samples were placed in 20 ml liquid scintillation vials and 7 ml scintillation cocktail added. All samples were assayed for ^3H label with an Intertechnique® SL 32 liquid scintillation counter.

Diffusion rates or flux (J) were determined from the slope of diffusion curves and expressed as the amount of drug passing across 1 cm^2 of skin surface as a function of time (pg/cm^2 per h). The permeability coefficient (K_p) was calculated from the equation $K_p = J/C_0$ where C_0 denotes the initial concentration of drug in the donor compartment (Dugard, 1986).

Absorption across hairless mouse skin ex vivo: Using a normal saline in the receptor compartment, the diffusion rate was determined to be 55 ± 6 pg/cm^2. On addition of ethanol and PEG 400, the flux increased 3–5-fold (Table 1, Fig. 1). When 5% dipropylene glycol (DPG) was added to the donor side, digoxin absorption decreased dramatically (Fig. 2).

Absorption across human skin ex vivo: The receptor compartment was filled with 20% ethanol, 20% PEG 400 and saline. Under the same conditions, the diffusion rate was 36-fold lower than across hairless mouse skin (Table 1).

We have demonstrated that the permeability coefficient of digoxin, delivered in a saline solution across hairless mouse skin, is comparable that of other drugs which can be administered via the percutaneous route (Cairncross and Ackermann, 1985). However, this coefficient is very low in the case of total human skin.

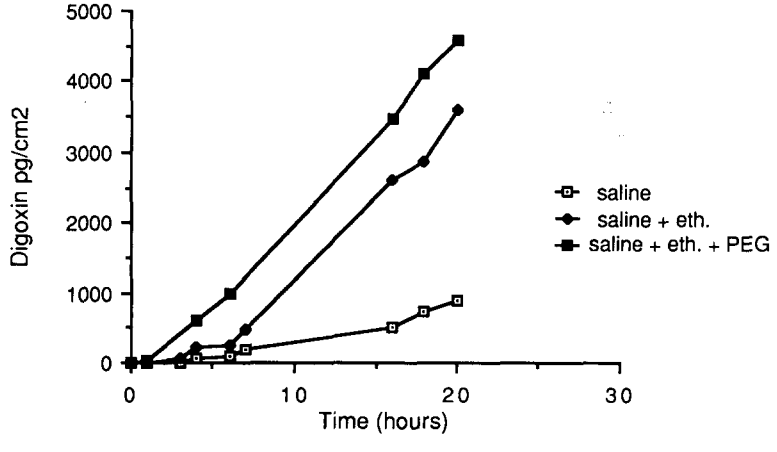

Fig. 1. Diffusion profiles of digoxin across hairless mouse skin according to receptor compartment fluid composition.

TABLE 1

In vitro percutaneous administration of digoxin through hairless mouse and human skin

	Receptor medium	J (pg/cm^2 per h)	K_p ($\times 10^{-3}$) (cm/h)
Hairless mouse skin	saline	55 ± 14.6	0.14 ±0.03
	saline + ethanol	162 ± 65	0.42 ±0.016
	saline + ethanol + PEG	233 ±109	0.60 ±0.28
Human skin	saline + ethanol + PEG	6.19 ± 2.02	0.016 ± 0.005

To increase in vitro percutaneous absorption, addition of lipophilic compounds to the receptor compartment was found to be required in order to diminish dermal retention (Bronaugh and Stewart, 1984). Using both ethanol and PEG 400, the diffusion rate across mouse skin was determined to be 5-fold greater than with normal saline. We also examined the effect of adding the penetration enhancer, dipropylene glycol (5%), to the donor solution, since it is known to be an effective promoter of the percutaneous absorption of estradiol (Hoellgaard and Mollgaard, 1985).

In contrast, the addition of DPG for the case of digoxin decreased the diffusion rate. This could be explained on the basis of the increase in solubility of digoxin, resulting in a lowering of the percutaneous flux (Hoellgaard and Mollgaard, 1985).

Only one study has been published on the percutaneous absorption of digoxin, using a different diffusion cell (Cairncross and Ackermann, 1985). The main difference between the two cell types concerns the sampling procedure. In the latter study, all of the receptor compartment was removed at hourly intervals, while the sample volume in the present investigation was only 500 μl (i.e. 3.33% of the receptor compartment). Using abdominal hairless mouse skin, the K_p value was 4-fold lower in our study, the discrepancy being accounted for by the difference in sampling procedure, since the complete removal of the receptor compartment increases the rate of diffusion of lipophilic compounds. On addition of PEG 400 and ethanol in order to enhance diffusion, K_p fell within the same range of values (Table 2). However, our results in the case of whole human skin differ markedly from those of Cairncross and Ackermann. According to the latter workers, the permeability coefficient was at least 3-fold greater

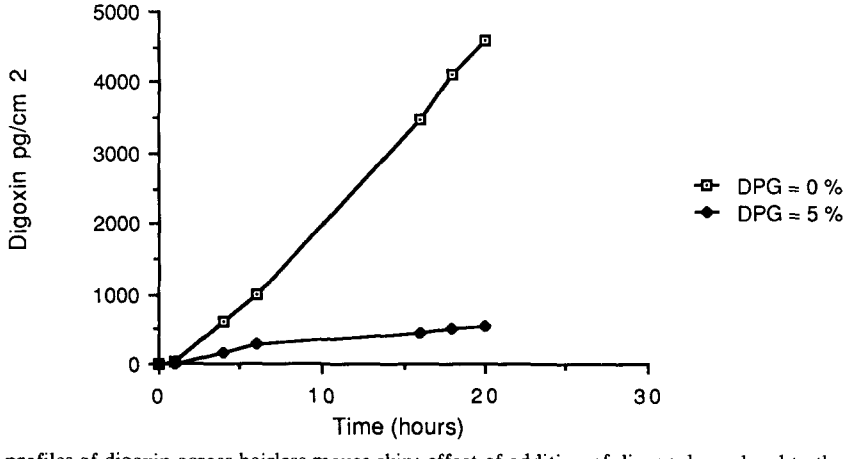

Fig. 2. Diffusion profiles of digoxin across hairless mouse skin: effect of addition of dipropylene glycol to the epidermal side.

TABLE 2

Comparative data on permeability coefficient of digoxin across hairless mouse and human skin [a]

	Medium composition of the two compartments		K_p ($\times 10^{-3}$) (cm/h)	
	Dermal side	Epidermal side	This work	Cairncross and Ackermann
Hairless mouse skin	NaCl [b]	DPG = 0, NaCl	0.244 ± 0.062	1 ± 0.6
	NaCl + EtOH	DPG = 0, NaCl	0.705 ± 0.279	–
	NaCl + EtOH + PEG	DPG = 0, NaCl	1.146 ± 0.364	–
	NaCl + EtOH + PEG	DPG = 5%, NaCl	0.210 ± 0.041	–
Human skin	NaCl + EtOH + PEG	DPG = 0, NaCl	0.032 ± 0.007	–
	NaCl	DPG = 0, NaCl	–	7 ± 4.4

[a] Addition of DPG to the epidermal side decreased K_p.
[b] NaCl in all cases present at 0.9%; EtOH, ethanol.

in whole human skin as compared to hairless mouse skin. In contrast, the value of K_p in our study was nearly 36-fold lower than across hairless mouse skin.

Hairless mouse skin is assumed to be an adequate and very convenient model for percutaneous absorption (Dürrheim et al., 1980), and when differences exist between the two species, human epidermis is less permeable than hairless mouse skin (Wester and Maibach, 1985). Consequently, if whole human skin is being used, the differences should be larger due to dermal retention of digoxin.

Therefore, although our data appear to be more realistic, they lack promise as regards the attainment of therapeutic plasma levels. Indeed, based on a transdermal system of 20 cm^2 area, the rate of absorption through human skin can be calculated (Guy and Hadgraft, 1986), the required value reaching nearly 0.5 $\mu g/cm^2$ per h (Table 3). Our preliminary in vitro data are far from this value.

In order to increase the percutaneous absorption of digoxin, effective penetration enhancers must be sought. DPG was found to be ineffective, and paradoxically, decreased the rate of diffusion. Therefore, future investigations will be directed at other chemical or physical enhancers.

TABLE 3

Calculated zero-order release rate from a digoxin transdermal therapeutic device

Parameter	
K_0	$= (V_d \times K_4 \times C_{ss})/A = 0.54\ \mu g/cm^2$ per h
K_0	= release rate from the transdermal device
K_4	= elimination rate constant = 0.019 h^{-1}
C_{ss}	= steady-state plasma concentration = 1 $\mu g/l$
A	= contact area of the device = 20 cm^2
V_d	= distribution volume = 560 l

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