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Prediction of the In-vitro Human Skin Permeability of Nicorandil from Animal Data

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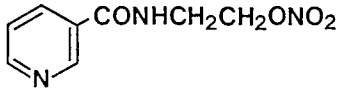
Abstract—A method for estimating the in-vitro permeability of human skin to drugs, based on in-vitro permeation studies using animal skins, has been developed. The skins from hairless rats, guinea-pigs, dogs and pigs were used, with nicorandil and deionized water as model drug and solvent in a drug-donor compartment. Diffusion coefficients through the skin barrier, D, and partition coefficients from the drug-donor compartment to skin, K, of the drug, in each species, were calculated by curve-fitting the in-vitro permeation data to a diffusion equation describing the drug permeation through a homogeneous membrane, using a non-linear least squares method. Each barrier thickness, L, was measured microscopically from microtomed skin sections. A positive relationship was found between the skin permeability, K_p, and K value among the four species, but species differences in the D and L values were small in spite of the K_p values being different among the four species. A positive correlation was also observed between the calculated and experimental K values among the four species, and hence it was suggested that the main factor for the species difference in the skin permeability of nicorandil would be the difference in partitioning of the drug from vehicle to stratum corneum. As a result, it has become feasible to predict and estimate skin permeability of nicorandil in humans by substituting each parameter, extrapolated from the animal skin permeation data and partition experiments, in the diffusion equation.

In-vitro permeation experiments using excised animal skin have been widely used for estimating the skin permeabilities of drugs. These studies have been useful for comparison of skin permeability of a drug from several vehicles (Huang et al 1985; Sheth et al 1986), and for the analysis of the effect of percutaneous absorption enhancers (Mirejovsky & Takruri 1986; Yamada & Uda 1987; Sugibayashi et al 1988). However, differences exist in the skin permeabilities of drugs between animals and man (Bronaugh et al 1982; Barry 1983), and hence skin permeation data from animals are not directly applicable to the estimation of human drug permeation behaviour. To overcome this, various comparative studies with animal and human skin have been done. Bartek et al (1972) concluded that the miniature swine would be a good animal model, and Wester & Maibach (1975, 1976) found in monkeys that the percutaneous absorption of several drugs was similar to that in man. However, most reports go no further than mentioning agreement or difference of the skin permeabilities of selected drugs between animals and man.

We have attempted to predict the in-vitro permeability of human skin to nicorandil (I), a coronary vasodilator (Nakagawa et al 1979), from skin permeation data produced for the drug in animals.



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Materials and Methods

Materials

Nicorandil, N-(2-hydroxyethyl) nicotinamide nitrate, was supplied by Nisshin Flour Milling Co. (Tokyo, Japan). Trypsin (Type II, from porcine pancreas) was purchased from Sigma Chemical Co. (St Louis, MO, USA). All other chemicals and solvents were reagent grade, obtained commercially, and used without further purification.

Excised skin

Abdominal skin of hairless rats (WBN/kob, 150–200 g), guinea-pigs (Hartley, 300–350 g), dogs (beagles and mongrels, 10–15 kg), and back skin of immature pigs (LWD, 2–6 kg, 4–35 days old) were used. The abdominal or back regions were carefully shaved and excised under anaesthetic. The subcutaneous fat was removed and stored at −20°C until used (usually within 1 month). Human chest skin (female, age 27–62) from surgical procedures in Sagamihara National Hospital was also stored at −20°C until used. Each piece of skin excised was of full-thickness (i.e. epidermis with stratum corneum + dermis).

Skin thickness measurement

Stratum corneum thickness was determined microscopically from microtomed sections after they had been stained with haematoxylin-eosin.

In-vitro skin permeation studies

For skin permeation studies, sections of full-thickness were mounted between the two halves of diffusion cells, each half having a volume of 2·0 mL and an effective diffusion area of 0·636 cm^2 (Morimoto et al 1986). The receiving compartment (dermis side of the skin) of each half cell was filled with 2 mL of saline (0·9 % NaCl) and the donor compartment (stratum corneum side of the skin) was filled with 2 mL of aqueous

suspension of the drug. The cells were maintained at 37°C in a water bath. Both compartments were stirred throughout with Teflon stirrers driven by a 150 rev min^{-1} constant speed motor. At appropriate times, 100 μL of sample was withdrawn from the receiver compartment, and 100 μL of internal standard solution (ethyl *p*-hydroxybenzoate, 1 μg mL^{-1} in methanol) was added. After sampling, 100 μL of saline was added to the receiver compartment to keep the volume constant.

Stratum corneum preparation

Stratum corneum sheets were prepared from each animal skin by trypsin treatment (Knutson et al 1985). Full-thickness skin was soaked in 1 (w/v)% trypsin solution (10000 ATEE units mL^{-1} in PBS, at pH 7·4) at 37°C for 8 h. Stratum corneum was separated from the epidermis, and rinsed with deionized water. Human stratum corneum (callus) was obtained from the plantar area by trimming the cornified layer. Stratum corneum samples were dried and stored in a desiccator at room temperature (20°C) until used.

Partition coefficient between stratum corneum and water

Partition coefficients for nicorandil between the stratum corneum in each species and water were determined from the loss in concentration of the solution phase as described by Scheuplein (1965) and Saket et al (1985). One mL of 0·05 (w/v)% nicorandil aqueous solution was added to each dry stratum corneum sample, approximately 10 mg accurately weighed, in a glass stoppered tube. After being equilibrated by vigorous stirring of the mixture at 37°C for 24 h, the sample was centrifuged. The concentration of the drug in the aqueous layer was determined by HPLC. The partition coefficient of nicorandil between stratum corneum and water was calculated as:

$$\text{Partition coefficient} = \frac{C_{in} - C_{eq}}{C_{eq}} \times \frac{1000}{W_{sc}}$$

where C_{in}, C_{eq} and W_{sc} are initial concentration of nicorandil in water (mg mL^{-1}), equilibrium concentration in water (mg mL^{-1}) and weight of stratum corneum (mg), respectively.

HPLC conditions

In all experiments, the concentration of nicorandil was determined by HPLC (LC-6A, Shimadzu, Kyoto, Japan) under the following conditions: column, 4·6 mm × 250 mm stainless column packed with Nucleosil 5C18 (Macherey Nagel, Germany); mobile phase, water:acetonitrile (6:4); detector, UV 254 nm.

Results and Discussion

Nicorandil was used as a model drug because a skin permeation study of it through excised hairless rat skin had already been carried out (Sato et al 1988). To minimize the influence of the solvent (vehicle) and/or drug concentration in the permeation experiments, nicorandil aqueous suspensions prepared in deionized water were used as donor vehicles. Full-thickness skin of adult hairless rats, guinea-pigs and dogs was prepared from the abdominal region because of the ease of shaving and excision, while the back region of immature pigs was used, because the skin permeabilities of drugs in immature or weanling pigs are known to be close to those in man (Galey et al 1976; Hawkins & Reifenrath 1986); also the abdominal, rather than the back, region of pigs is easily damaged during breeding.

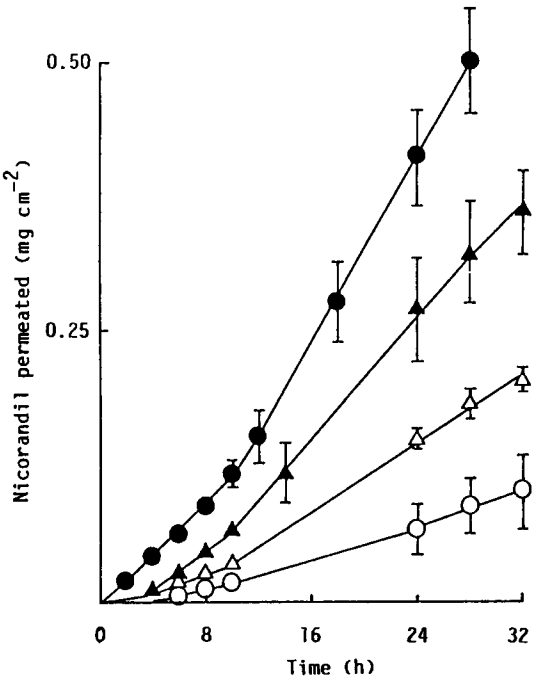

FIG. 1. Permeation of nicorandil across the excised animal skin from aqueous suspension. (●) Hairless rat; (▲) guinea-pig; (○) dog; (△) pig. Each point represents the mean ± s.e. of 4–8 experiments.

Fig. 1 shows the cumulative amount of nicorandil per unit area that crossed the skins to give a ranking: hairless rat > guinea-pig > pig > dog.

The permeability coefficient, K_p, and lag time in each species are listed in Table 1. K_p was calculated by dividing the steady state flux in Fig. 1 by the solubility of nicorandil in water. The intercept indicated the lag time when a steady state plot was extrapolated back to time axis. As shown in Table 1, the barrier function of the hairless rat skin against nicorandil permeation was the lowest of the four species. The deviations in the K_p and lag time in each species were relatively small. This surprising reproducibility might be attributed to the physicochemical properties of nicorandil, since it shows high solubility both in water and organic solvents, such as chloroform, and its molecular weight is small, properties that could favour steady skin permeation.

Table 1. Permeability coefficient, K_p and lag time for skin permeation of nicorandil from aqueous suspension in several species.

Species	n[a]	K_p (cm h^{-1}, × 10^4)	Lag time (h)
Hairless rat	5	7·27 ± 0·77	3·51 ± 0·33
Guinea-pig	5	5·08 ± 1·23	5·27 ± 0·46
Dog	4	1·69 ± 0·30	6·53 ± 0·93
Pig	8	2·85 ± 0·15	5·91 ± 0·48

[a] Number of determinations.
K_p was calculated from individual steady state flux in Fig. 1 and solubility of nicorandil. Lag time was individual data in Fig. 1. Each value represents the mean ± s.e.

For the analysis of drug permeation in this experiment, the skin was assumed to be a homogeneous barrier membrane. The cumulative amount of drug, Q_t, which permeates per unit area through the skin membrane at time t from the donor solution (where the drug concentration, C_v, is constant) is calculated using the following equation:

$$Q_t = \frac{DKC_v}{L}\left(t - \frac{L^2}{6D}\right) - \frac{2KC_vL}{\pi^2}\sum_{n=1}^{\infty}\frac{(-1)^n}{n^2} \cdot \exp\left(\frac{-n^2\pi^2D}{L^2}t\right) \quad (1)$$

where D = diffusion coefficient of the drug in skin barrier, K = partition coefficient of the drug between skin and donor vehicle, L = thickness of skin barrier and C_v = saturated concentration of the drug in the donor vehicle (Crank 1975). To determine each parameter (D, K and L), DKC_v/L and D/L^2 were replaced with P(1) and P(2), respectively, where P(1) and P(2) were calculated by curve-fitting of individual in-vitro permeation data in Fig. 1 to the theoretical equation (eqn 1) using a computerized non-linear least squares method MULTI (Yamaoka et al 1981). The mean and standard errors of each calculated P(1) and P(2) in four species are listed in Table 2. By employing the individual calculated P(1) and P(2) values, D and K in each species could be calculated from the equations:

$$D = P(2) \times L^2 \quad (2)$$

$$K = P(1) \times L/D \times C_v \quad (3)$$

where the solubility of nicorandil in water at 37°C, Cv was measured as 26·7 mg mL^{-1} and each thickness of the stratum corneum, L, was determined microscopically from microtomed sections. Table 3 shows the calculated D and K values (mean and standard error) in four species, together with each L value. The mean calculated K values were widely different among the four species, e.g. the value in hairless rats was about 3 times that in dogs, while the D and L values were similar to each other.

Table 2. P(1) and P(2) values in several species.

Species	P(1) ($\mu g\,cm^{-2}\,h$)[a]	P(2) (h^{-1})[b]
Hairless rat	19·40 ± 2·04	0·05 ± 0·005
Guinea-pig	13·55 ± 3·28	0·03 ± 0·004
Dog	4·52 ± 0·80	0·03 ± 0·005
Pig	7·50 ± 0·34	0·03 ± 0·002

[a] $DKC_v\,L^{-1}$.
[b] $D\,L^{-2}$.
P(1) and P(2) were calculated by curve-fitting of individual data in Fig. 1. Each value represents the mean ± s.e.

Table 3. Diffusion coefficient, D, partition coefficient, K and barrier thickness, L in several species.

Species	D ($cm^2\,h^{-1}$, $\times 10^7$)	K	L (cm, $\times 10^3$)
Hairless rat	1·164 ± 0·108	9·68 ± 0·67	1·54 ± 0·33
Guinea-pig	1·138 ± 0·125	8·61 ± 2·37	1·86 ± 0·12
Dog	1·093 ± 0·195	3·44 ± 0·94	1·99 ± 0·43
Pig	0·905 ± 0·074	5·68 ± 0·51	1·75 ± 0·24

L was determined microscopically and individual D and K were calculated with P(1), P(2) in Table 2 and equations 2, 3. Each value represents the mean ± s.e.

To clarify the relationships between these calculated parameters and the skin permeability, or, more specifically, to analyse the effect of each parameter (D, K or L) on the species difference of the skin permeability of nicorandil, K_p values in four species were plotted against the corresponding D, K or L value (Fig. 2). It is apparent that a positive relationship between the K and K_p values is present among the four species (r = 0·970), and that the D and L values would be similar in animals despite widely differing values of K_p.

Several reports have suggested that skin permeation is related to the partition coefficient of drugs between the stratum corneum and vehicle (Poulsen et al 1968; Ostrenga et al 1971; Okamoto et al 1986). Therefore, partition coefficients of nicorandil between the stratum corneum and vehicle (water) were experimentally measured to clarify this relationship.

Table 4 shows the partition coefficients of nicorandil between the dry stratum corneum, in each species, and water. Deviation of the partition coefficients was relatively large compared with the K_p values in Table 1, especially in dog. One of the reasons for such deviation might be due to the difference in water uptake of the stratum corneum among four species. It is known that the stratum corneum slowly takes up water and ultimately absorbs up to six times its dry weight (Scheuplein 1965). However, the direct measurement of the partition coefficient from vehicle to the stratum corneum is difficult. In the present study, dry stratum corneum samples were used and the influence of extent of water uptake on the species difference in the partition coefficient was not considered.

Fig. 3 shows a relationship between calculated (from Table 3) and experimental (from Table 4) K values. Since a positive correlation was observed between the calculated and experimental K values among the four species (r = 0·945), this strongly suggested that the main factor for a species difference in the permeability of nicorandil across animal skins, is the difference in partitioning of the drug from the vehicle to stratum corneum. Komatsu & Kurihara (1985) have also reported a high degree of correlation between drug permeabilities and partition coefficients for butylparaben in guinea-pigs and man.

Equation 1 indicates that the cumulative amount of permeation of drugs, Q_t, can be calculated from the parameters D, K, L and C_v. To predict drug permeation behaviour in man, these parameters for nicroandil in man (Table 5) were estimated as follows. The calculated K value in humans was estimated by substituting the experimental partition coefficients (in Table 4) into the linear regression in animal data as shown in Fig. 3. The mean D of the four species (in Table 3) was adopted as D value in humans, since diffusion coefficients were not significantly different in the four species. The L value for human skin was determined by measuring stratum corneum thickness in a similar manner as in animals.

By substituting these values, as shown in Table 5, into each parameter in equation 1, the skin permeation profile of nicorandil in humans could be estimated. Fig. 4 shows the predicted skin permeation in man together with the observed permeation data using excised human skin. The observed permeation data correlated well to the predicted values,

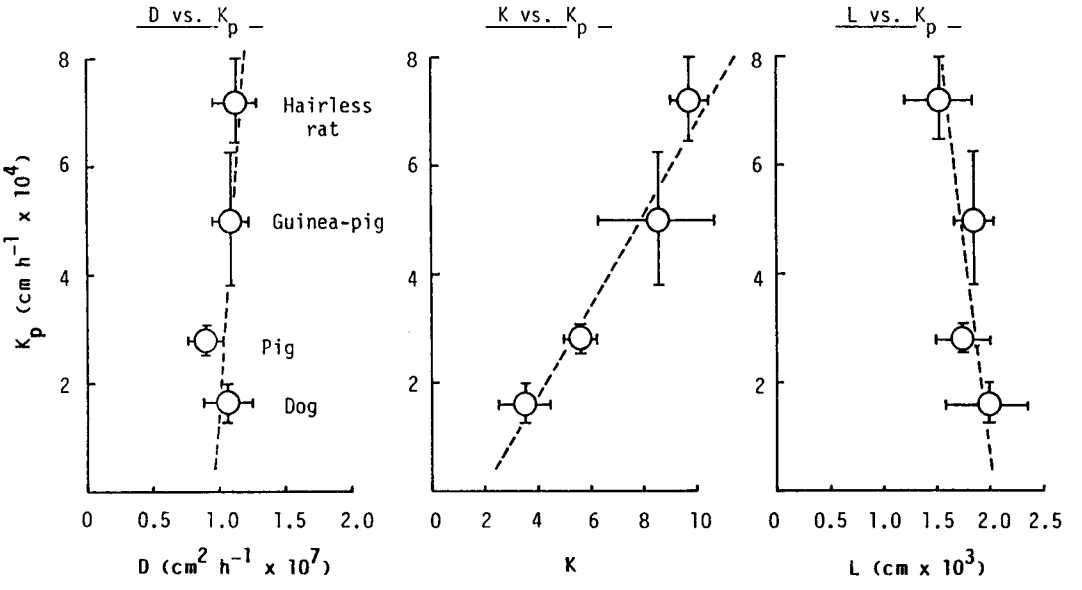

FIG. 2. Effect of diffusion coefficient, D, partition coefficient, K and barrier thickness, L, on the permeability, K_p, of nicorandil. K_p was from Table 1 and D, K and L were from Table 3. Each point represents the mean ± s.e.

Table 4. Partition coefficient of nicorandil between stratum corneum and water in several species.

Species	n[a]	Partition coefficient at 37°C (stratum corneum/water)
Hairless rat	4	10·38 ± 0·94
Guinea-pig	5	8·55 ± 0·99
Dog	3	4·48 ± 1·63
Pig	4	7·90 ± 0·69
Human	5	7·25 ± 1·25

[a] Number of determinations.
Each value represents the mean ± s.e.

Table 5. Estimated diffusion coefficient, D, partition coefficient, K, and barrier thickness, L, in humans.

Parameter	Estimated value
D ($cm^2 h^{-1}$)	$1·075 \times 10^{-7}$ [a]
K	6·15[b]
L (cm)	$1·82 \times 10^{-3}$ [c]

[a] The mean of D values of other species (hairless rat, guinea-pig, dog, pig: in Table 1).
[b] Estimated by substituting the experimental partition coefficient (in Table 4) into the linear regression in animal data (in Fig. 3).
[c] Microscopically determined.

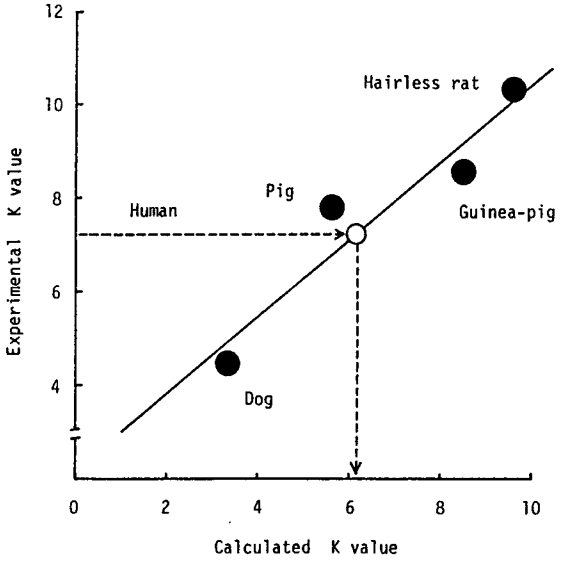

FIG. 3. Relationship between the calculated and experimental partition coefficients of nicorandil. Experimental and calculated K vaues were quated from Tables 4 and 3, respectively.

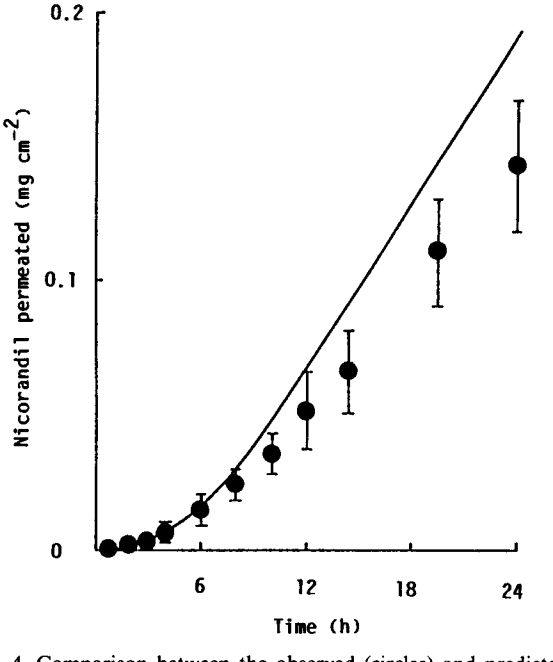

FIG. 4. Comparison between the observed (circles) and predicted (solid line) skin permeations of nicorandil in humans. Each point represents the mean ± s.e. of 3 experiments.

particularly up until 8 h. It seems reasonable, therefore, to estimate the skin permeation profile in man by employing the diffusion equation (eqn 1) and three parameters (D, K and L), obtained by the above method. Although there exists a tendency for the observed and predicted values to deviate with increasing time, the deviation was only about 30% even at 24 h. Such deviation might be attributable to the skin hydration and/or the difference in skin samples in the two experiments, that is, the chest skin in the permeation experiments and the callus from the plantar area in the partition experiments. Since the skin hydration may increase the thickness of the skin barrier, the skin permeation may be overestimated when using the value of thickness measured by a microscopic sectioning technique. As to the latter, Wester & Maibach (1985) have also reported that the partition coefficients of hydrocortisone between stratum corneum powder from the plantar and water were about 1·6 times larger than those between abdominal stratum corneum sheet and water. Hence, it seems necessary to take into account overestimation or underestimation resulting from the differences in application sites between the two experiments.

A long-term object of these skin permeation studies was the development of topical formulations for humans. However, the procurement of excised human skin for the in-vitro permeation experiments is currently difficult. Data about the permeability of nicorandil in human skin would provide valuable information to assist the production of new and more effective topical formulations. The method herein described provides information on drug permeation that can be extrapolated to apply to human skin without using human skin.

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