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Transdermal controlled delivery of verapamil: characterization of in vitro skin permeation

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Summary

The objective of this investigation was to determine the feasibility of transdermal delivery of verapamil by studying its in vitro skin permeation characteristics. Skin permeation experiments were carried out using excised skin from hairless mice mounted in an in vitro permeation system. The effects of age, sex and site of skin excised, as well as the influence of pH and of drug concentration in the donor solution on the permeation rate of verapamil were studied. Successive stripping of the stratum corneum was observed to increase the permeation rate until the stratum corneum was completely removed, suggesting that stratum corneum plays a rate-limiting role in the skin permeation of verapamil. The physico-chemical properties of verapamil, viz., diffusivity, partition coefficient and solubility, in stratum corneum and viable skin were determined based on the bilayer skin model.

Introduction

Verapamil is a novel antiarrhythmic and antianginal agent which can also be used in the treatment of hypertension (Singh et al., 1978; McAllister et al., 1983). It is presently available as verapamil hydrochloride in tablet form for oral ingestion and in a solution formulation for i.v. injection. However, the systemic bioavailability of verapamil following oral administration is low due to substantial hepatic first-pass metabolism (Singh et al., 1978), thus necessitating a much higher oral dose and frequent administration.

The above shortcomings of oral verapamil therapy may be overcome by administering it through the transdermal route. Transdermal drug delivery systems have the advantages of bypassing hepatic first-pass metabolism and providing controlled delivery of the drug for an extended period (Chien, 1982, 1983). Development of a transdermal delivery system for verapamil would facilitate the treatment of hypertension by increasing bioavailability and allow once-a-day or less frequent dosing (Shah et al., 1986a,b).

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In the development of a transdermal drug delivery system, it is desirable to evaluate the skin permeation characteristics of the drug in vitro before conducting in vivo studies in human volunteers. A number of factors can affect the transdermal permeation of drugs, including pH of the solution, drug concentration and partition coefficient, source of skin samples, etc. (Chien, 1982). Hairless mouse skin has frequently been used as a model for human skin for in vitro percutaneous absorption studies (Bronaugh et al., 1982). Drug permeation through hairless mouse skin may be influenced by several factors including sex, age and site of skin excised (Behl et al., 1984a).

The objective of this investigation was to determine the feasibility of transdermal delivery of verapamil by studying its in vitro permeation characteristics across hairless mouse skin.

Materials and Methods

Materials

PEG 400, citric acid, phosphoric acid, boric acid, sodium hydroxide, acetonitrile and sulfuric acid were purchased from Fisher Scientific. Silicone fluid (Medical Fluid 360) was supplied by Dow Corning, U.S.A. Verapamil was used as the free base (Knoll Pharmaceuticals, NJ).

Permeation membrane

Full-thickness skin sections, freshly excised from hairless mouse HRS/J strain (Jackson Laboratories, ME), were used in most of the experiments. For some experiments, the skin surface while still on the animal carcass was stripped repeatedly of its stratum corneum using adhesive tape (SCOTCH tape). In all cases, except where specifically noted, abdominal skin from female hairless mice, 6–8 weeks old, was used.

Diffusion cells

A skin permeation system (Chien and Valia, 1984), whose hydrodynamic characteristics were previously calibrated (Tojo et al., 1985a), was used in this investigation. The temperature in both donor and receptor half-cells was maintained at 37°C.

Skin permeation procedure

Hairless mice were killed by cervical dislocation just before the experiment. Square sections (1.5 cm^2) of skin were surgically removed from the animal, and adhering fat and other visceral debris were carefully cleaned. Each skin specimen was then mounted between the half-cells of the skin permeation system.

The donor fluid consisted of a saturated solution of verapamil in either aqueous buffer solutions (citrate-phosphate or citrate-phosphate-borate) at various pH values or silicone fluid. Excess verapamil was added to the donor compartment to ensure constant thermodynamic activity of drug throughout the experiment. In order to study the effect of drug concentration on the permeation rate, appropriately diluted solutions of verapamil in citrate-phosphate buffer (pH 6.15) were used. The receptor fluid was a 40% aqueous solution of PEG 400. At predetermined time intervals, 50 μl of the receptor solution was sampled, diluted with 1 ml of HPLC grade water and assayed for verapamil concentration by HPLC. All experiments were carried out in triplicate. The permeation rate (flux) was calculated as the slope of the plot of the cumulative amount permeated as a function of time.

Solubility determination

The solubility of verapamil in the various vehicles was determined by equilibrating an excess amount of drug with the solvent in a shaking water bath (Fisher Model 129) at 37°C. In the case of aqueous buffer solutions, care was taken to adjust the pH to the desired value. At the end of 24 h, 1-ml aliquots were withdrawn, diluted with the appropriate solvent, and analyzed by HPLC. In the case of silicone fluid, verapamil was extracted from the 1-ml aliquots into 10 ml of methanol using a wrist action shaker (Burrell Model 75) for 12 h. The methanol extracts were then appropriately diluted and analyzed by HPLC.

Analytical method

The samples were analyzed for their verapamil content using a modification of the HPLC procedure developed by Harapat and Kates (1980). A Hewlett-Packard Model 1084B HPLC was used

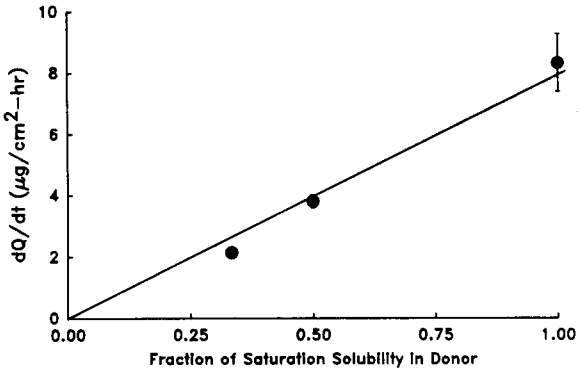

Fig. 1. Effect of drug concentration in the donor solution (pH 6.15, C_s 5.2 mg/ml) on the skin permeation rate of verapamil ($n = 3$; error bars represent SD).

for the analysis. A Waters μBondapak C_{18} column (15 cm × 3.9 mm i.d.) was used with a mobile phase consisting of water (adjusted to pH 2.4 with sulfuric acid) and acetonitrile, in the proportion 35:65. The flow rate was 2 ml/min and the eluent was monitored for verapamil at 232 nm.

Results and Discussion

Effect of drug concentration in the donor solution

Fig. 1 shows a plot of permeation rate vs verapamil concentration in the donor phase. Since the cumulative amount of verapamil permeated was less than 1% of the initial concentration in the donor phase during these experiments, constant donor concentrations can be assumed. It can be seen that the permeation rate is approximately proportional to verapamil concentration in the donor solution.

In case of passive diffusion, the steady-state flux through unit area of a membrane is given by Fick's Law,

$$J = P \cdot (C_d - C_r) \qquad (1)$$

where J is the flux per unit area, P represents the permeability coefficient and C_d and C_r are the concentrations in the donor and receptor solutions respectively. In the case where sink conditions are maintained on the receptor side, $(C_d - C_r)$ is replaced by C_d, the concentration of drug in the donor solution.

$$J = P \cdot C_d \qquad (2)$$

The permeability coefficient, P, is constant for a given drug under the same experimental conditions. Therefore, there should be linear relationship between the flux and donor concentration. As shown in Fig. 1, skin permeation of verapamil obeys Fick's Law and undergoes concentration-dependent passive diffusion through hairless mouse skin.

Effect of skin stripping

Within the last 10–15 years it has become clear that it is the stratum corneum which is the principal barrier to the movement of molecules across the skin. The effect of serially stripping the skin of its stratum corneum with cellophane tape on the permeation of verapamil through abdominal skin of female hairless mice was investigated. The donor phase used in these experiments was a saturated solution of verapamil in citrate-phosphate buffer, pH 6.15. The solubility of verapamil in this buffer solution was 5.2 mg/ml. Both the rate of skin permeation and the lag time were markedly influenced by the number of strippings. Fig. 2 depicts a plot of the enhancement factor, E, as a function of the number of strippings, n.

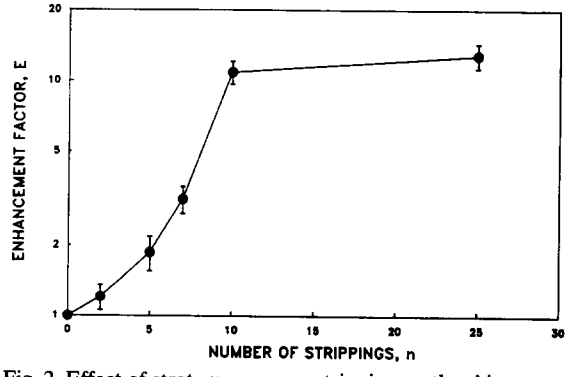

Fig. 2. Effect of stratum corneum stripping on the skin permeation rate of verapamil ($n = 3$; error bars represent SD).

The enhancement factor, E, is defined by the following relationship:

$$E = \frac{(\text{skin permeation rate})_{n=i}}{(\text{skin permeation rate})_{n=0}} \quad (3)$$

where n is the number of strippings and $i = 2,5,7,\ldots$ Fig. 2 shows that the enhancement factor increased with the number of strippings and then reached plateau at about 10 strippings. Further removal of skin layers by stripping did not increase the permeation rate any more, indicating that for verapamil the stratum corneum is a significant barrier to permeation.

Effect of biological factors

A number of factors including age, site of skin excised (anatomical location) and gender have been reported to influence skin permeation through both human and animal skin (Behl et al., 1984a). In order to make valid comparisons of the permeation data, it was necessary to study the effect of these factors on the in vitro permeation of verapamil through hairless mouse skin. The results from these experiments would then be used for carefully controlling the experimental conditions during the in vitro evaluation of the verapamil transdermal delivery system(s).

Fig. 3 shows the effect of age of hairless mouse on the in vitro skin permeation rate of verapamil through abdominal skin sections obtained from female mice. The permeation rate appears to be

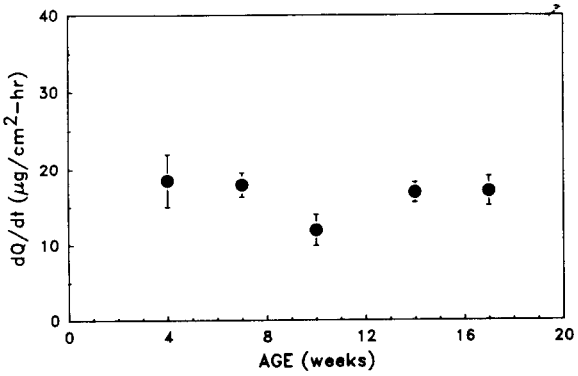

Fig. 3. Effect of age of hairless mouse on the skin permeation rate of verapamil ($n = 3$; error bars represent SD).

TABLE 1

Effect of site of hairless mouse skin on the in vitro skin permeation rate of verapamil ($n = 3$; mean ± SD)

Site	Skin thickness (mm)	Permeation rate ($\mu g/cm^2$ per h)
Abdominal	0.217 ± 0.06	11.97 ± 2.06
Lateral	0.283 ± 0.03	9.39 ± 2.43
Dorsal	0.330 ± 0.04	8.40 ± 0.59

constant over a range of 4–17 weeks. Qualitatively, these results are similar to those obtained with *n*-alkanols (Behl et al., 1984b), hydrocortisone (Behl et al., 1984a) and phenol (Behl et al., 1983). However, in the latter studies it was found that the permeation rate was constant beyond an age of 7 weeks, while in this study it was observed to remain constant beyond 4 weeks of age. The permeation rate is a function of the histological cycle of mouse skin and the difference could be due to differences in the histological cycles as different strains of mice were used for the experiments (SKH for Behl et al., vs HRS/J for our studies).

Table 1 gives the permeation rates of verapamil through full-thickness skin sections obtained from the abdominal, lateral and dorsal sites of 6–8 weeks old female hairless mice. The observed permeation rates appeared to decrease going from the abdominal to the dorsal site. Considering the variability normally encountered with in vitro skin experiments, this difference may not be significant. Table 1 also lists the thicknesses of these skin sections and there was an appreciable difference in thickness associated with the anatomical site. This agrees with the data of Behl et al. (1984b) who reported that the dorsal skin was consistently thicker than abdominal skin over a wide range of age of hairless mice. Fig. 4 illustrate the permeation rate of verapamil through skin sections obtained from female and male mice respectively, as a function of pH of the donor solution. The profile is very similar in both cases, indicating that in the case of verapamil, the sex of hairless mouse does not affect the permeation rate.

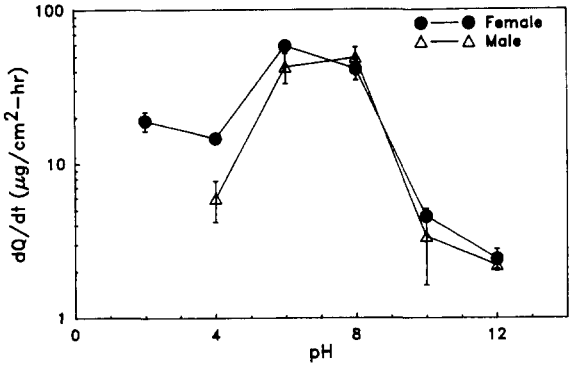

Fig. 4. Effect of sex of hairless mouse and pH of donor solution on the skin permeation rate of verapamil ($n = 3$; error bars represent SD).

Influence of pH on permeation kinetics

Verapamil is a weak base with a pK_a of about 8.7 (Hasegawa et al., 1984). Consequently, as the pH of the solution decreases from pH 12 to 2, the total solubility (ionized + nonionized forms) should increase. However, the amount of nonionized drug in solution (the intrinsic solubility) should be constant. Fig. 5 shows the effect of pH of buffer solution on the solubility of verapamil in citrate-phosphate-buffer solutions adjusted to different pH values. The effect of pH of the donor solution on the permeation rate of verapamil is shown in Fig. 4. Maximum permeation rates were attained at pH 6–7. The permeability coefficient

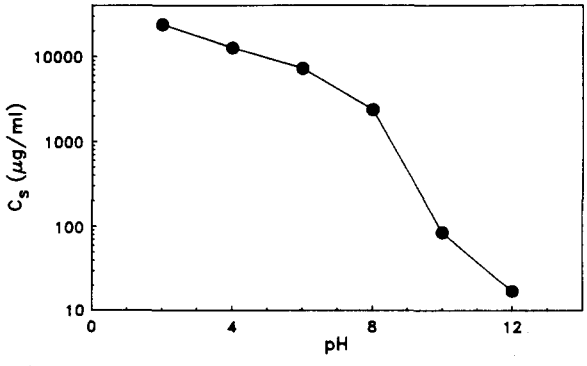

Fig. 5. Effect of pH of buffer solution on the aqueous solubility of verapamil.

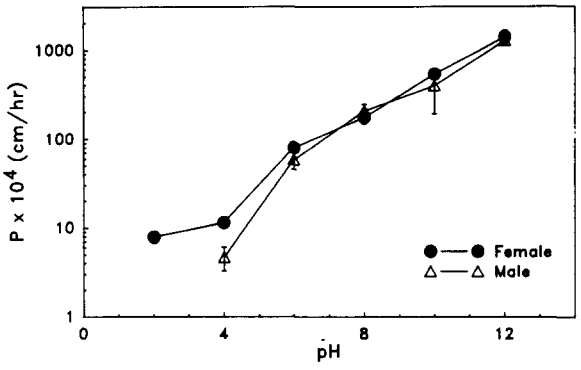

Fig. 6. Effect of pH of donor solution on the permeability coefficient of verapamil ($n = 3$; error bars represent SD).

of verapamil can be calculated through rearrangement of Eqn 2:

$$P = \frac{J}{C_d} \tag{4}$$

Fig. 6 demonstrates the effect of pH on the permeability coefficient of verapamil through skin sections obtained from female and male hairless mice, respectively. There was a log-linear increase in the permeability coefficient with increase in pH. At low pH values, a significant proportion of the total amount of verapamil in solution is the ionized form. As the pH is increased up to and above the pK_a, the proportion of the ionized form decreases. The results show that the nonionized form has a much higher permeability coefficient as compared to the ionized form. The method of Swarbrick et al. (1984) was used to calculate the specific permeabilities of the individual (ionized and nonionzed) species. The specific permeabilities were determined to be 5.3 and 8.0×10^{-3} cm/s for the nonionized and ionized species, respectively. The ratio of the permeabilities is 656. This is in agreement with the pH-partition hypothesis, according to which the permeability of the nonionized form should be higher as it is more lipophilic and hence more soluble in the skin. These results are similar to those for the effect of pH (5, 6 and 7) on the skin permeation of verapamil HCl through an artificial membrane reported by Santi et al. (1991). They found that the flux was highest at pH 6,

whereas the greatest permeability coefficient was attained at pH 7 (the highest pH studied).

Silicone fluid as vehicle

Silicone fluids are chemically inert oils which have a low surface tension and no physiologic activity. As discussed in the previous section, it is the nonionized form, i.e., verapamil free base, which has the highest permeability in hairless mouse skin. The solubility of the nonionized molecule in aqueous buffer solution is very low, viz., only about 17 μg/ml. The solubility of verapamil base in Medical Fluid 360 (20 cs viscosity) was found to be 2124.6 μg/ml. Therefore, the silicone fluid might be a better vehicle for the percutaneous administration of verapamil. The permeation of verapamil from a saturated solution in silicone fluid was then studied across both intact and stripped skin tissues of hairless mice. The respective permeation profiles are shown in Fig. 7. The permeation rate and lag time data from these experiments are listed in Table 2.

Tojo et al. (1985b) have proposed a bilayer model of the skin in order to gain a better understanding of the mechanism of drug transport through the skin. This model treats the skin as a two-layer membrane consisting of the stratum corneum (the principal barrier to permeation) and the viable or stripped skin which in turn consists of the viable epidermis and dermis. The diffusivity, partition coefficient and solubility of verapamil in each skin layer were calculated based on the bilayer model, from the permeation rates and lag times listed in Table 2 and the solubility of verapamil in the silicone fluid (2124.6 μg/ml). These physico-chemical properties of verapamil in hairless mouse skin are listed in Table 3. The diffusivity of verapamil was found to be about three orders of magnitude higher in the stratum corneum than in the stripped skin. This result supports the conclusion that a major barrier resisting the skin permeation of verapamil resides in the diffusion of drug across the stratum corneum. The partition coefficient of verapamil between the stratum corneum and silicone fluid was about 7-fold greater than that between stripped skin and silicone fluid. This is expected because of the relatively more hydrophilic nature of the stripped skin. Not surprisingly, the solubility of verapamil was much higher in the lipophilic stratum corneum.

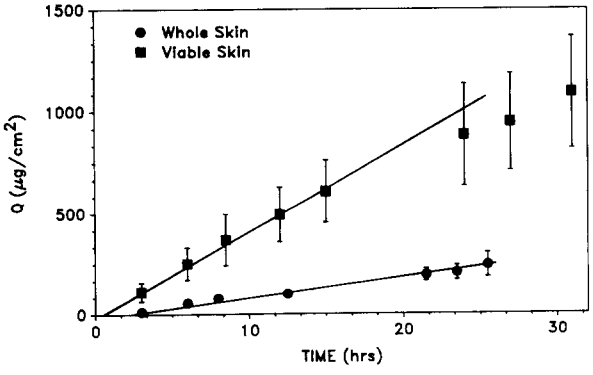

Fig. 7. Permeation profiles of verapamil across whole and stripped skin sections from a saturated solution in silicone fluid ($n = 3$; error bars represent SD).

TABLE 2

Permeation rate and lag time data for verapamil permeation through whole and stripped skin tissues of hairless mouse from a saturated solution in silicone fluid ($n = 3$; mean $\pm$ SD)

Skin tissue	Permeation rate (μg/cm^2 per h)	Lag time (h)
Whole skin	9.96 $\pm$ 2.43	2.04 $\pm$ 0.64
Stripped skin	46.15 $\pm$ 14.79	0.58 $\pm$ 0.22

Conclusions

Verapamil undergoes concentration-dependent, passive diffusion through hairless mouse

TABLE 3

Physico-chemical properties of verapamil in hairless mouse skin, using a saturated solution in silicone fluid as the donor

Parameter	Stratum corneum	Stripped skin
Diffusivity, D (cm^2/s)	1.20×10^{-10}	1.09×10^{-7}
Partition coefficient, K	13.79	2.04
Solubility, C_s (mg/ml)	29.30	4.33

skin, with the stratum corneum being the principal barrier to permeation. The nonionized form of verapamil was found to have a higher permeability through hairless mouse skin from aqueous buffer solutions. Verapamil free base (nonionized) has a much higher solubility in silicone fluid and therefore a transdermal delivery system using silicone elastomer will be developed.

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