Diffusion and Metabolism of Prednisolone Farnesylate in Viable Skin of the Hairless Mouse

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Received May 13, 1993; accepted October 14, 1993

The diffusion and metabolism of prednisolone 21-farnesylate were investigated in viable skin of the hairless mouse *in vitro*. The prodrug ester was extensively metabolized in viable skin, while it was stable in the donor and receptor solutions. The rate of appearance of the prodrug and its metabolite prednisolone was markedly influenced by the direction of the skin placed between the *in vitro* diffusion half-cells. The rate of bioconversion of the prodrug was determined as a function of the distance from the surface of the skin. The prodrug was increasingly metabolized with the distance from the surface of the skin, indicating that the responsible enzymes are enriched in the lower layers of the viable skin. A model with linearly increasing enzyme activity in the viable skin accounts for the *in vitro* profiles of the diffusion/metabolism of the prodrug in the viable skin of hairless mouse.

KEY WORDS: skin metabolism; enzyme distribution; diffusion/metabolism model; percutaneous absorption; prednisolone prodrug; prednisolone farnesylate.

INTRODUCTION

The stratum corneum controls the percutaneous absorption of most drugs that are stable in the skin (1). For drugs that undergo biotransformation, however, skin metabolism may become a rate-limiting step in percutaneous absorption. Recently, enzymatic activity in the skin was exploited for transdermal therapy relying on prodrug bioconversion (2–4). Drustrup *et al.* reported that the skin permeability of morphine can be enhanced by using its ester prodrugs (2). Bonina *et al.* investigated prodrug esters of indomethacin that were efficiently metabolized in human skin (3). Liu *et al.* analyzed the metabolism of β-estradiol in hairless mouse skin *in vitro* assuming a nonhomogeneous distribution of enzyme activity (4). A model assuming that enzymes are present only in the basal cell layer can account for their *in vitro* data (5). Valia *et al.* reported that prodrug esters of estradiol were efficiently metabolized in hairless mouse skin (6). Provitamins for vitamins E and C were metabolized in hairless mouse skin, and vitamin E was further metabolized to its metabolites, while vitamin C was stable in the skin (7). Further, nicotine is slowly metabolized to cotinine in the hairless mouse skin (8). Therefore, the skin may exhibit a first-pass metabolism for drugs applied topically (9). However, the impact of skin metabolism on percutaneous absorption remains unclear.

In this report, we investigate the diffusion and bioconversion of a prodrug of prednisolone in the viable skin of hairless mouse *in vitro*. We also determine the distribution of the rate of bioconversion of the prodrug in the viable skin. Finally, we develop a dynamic mathematical model of prodrug diffusion and metabolism in the skin.

MATERIALS AND METHODS

Materials

Prednisolone (PN) and its prodrug ester, prednisolone farnesylate (PNF), were supplied by Taihou Pharmaceuticals Co., Tokushima, Japan. Figure 1 shows the chemical structures of the compounds. Polyethylene glycol 400, methanol, and water (HPLC grade) were used as obtained. Female hairless mice (Hr-/Kud), 5 to 7 weeks old, were obtained from Kyudo, Tosu, Japan.

Analytical Method

Both PNF and PN were assayed by HPLC (Beckman Pump Module 126 and Detector Module 166). The column was a Shim-pack CLC-ODS-150 mm (Shimadzu). The UV detector was operated at a wavelength of 254 nm. The mobile phase was a mixture of methanol and 20 m*M* KH_2PO_4 buffer solution and the methanol fraction varied from 55 to 100% within 30 min to separate PNF and PN completely.

In Vitro Skin Permeation Cell

A side-by-side *in vitro* skin permeation system was used in this diffusion/metabolism experiment. The effective membrane area and the effective volume are 0.64 cm^2 and 5 mL, respectively (10). The hydrodynamic characteristics in the donor and the receptor compartments were studied from the dissolution experiment of a benzoic acid disk as described previously by the present authors (11). The thickness of the diffusion boundary layer on the surface of the skin was approximately 40 μm, which was found to cause a negligible effect in evaluating the intrinsic rate of permeation (1).

Skin Preparation

In the diffusion/metabolism experiment, a stripped skin sample was used. The hairless mouse was sacrificed just prior to the experiment by cervical dislocation. Immediately following sacrifice, the abdominal skin was stripped 20 times with cellophane tape (Nichiban LP-24) to remove the stratum corneum completely. The thickness of the skin was measured with a micrometer by sandwiching the skin sample between two plastic sheets.

Diffusion/Metabolism Experiment

The stripped skin was mounted between the two half-cells of the skin permeation system. A drug suspension with

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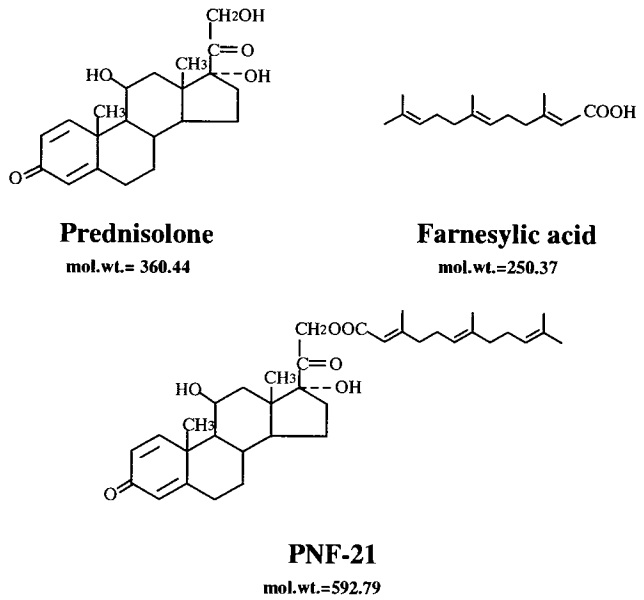

Fig. 1. Chemical structure of prednisolone and its farnesylate ester, PNF ((+)-11β,17α,21-trihydydroxy-1,4-pregnadiene-3,20-dione 21-[(E,E)-3,7,11-trimethyl-2,6,10-dodecatrienoate], prednisolone farnesylate).

a known loading dose in a 40% PEG400 solution was loaded into the donor compartment after a 40% PEG400 solution without drug was charged into the receptor compartment. Polyethylene glycol 400 was added to maintain a sink condition in the receptor solution for PNF and PN and in the donor solution for PN. At each of the predetermined time intervals, a 100 μL sample was withdrawn from both compartments and assayed for the concentration of PNF and PN by HPLC. The same volume of the saturated solution and the fresh solution (without drugs) was added to the donor and the receptor solution, respectively, to maintain a constant volume. To study nonhomogeneous distribution of skin enzymes, the skin sample was mounted both in the forward direction, with the surface layer facing the donor solution, and in the backward direction, with the surface layer facing the receptor solution.

Distribution of Enzyme Activity

The abdominal skin was sliced from the surface of the skin 20 to 21 times with a microtome Cryostat (Milles Sankyo, Tokyo). Three consecutive slices were placed in 5 mL of the saturated 40% PEG400 solution of PNF. After 24 hr of incubation at 37°C, 100 μL of ethanol was added to the sample (300 μL). After filtration, 50 μl of the sample solution was injected into the HPLC to assay PNF and PN concentration. The prodrug and the drug were found to be stable in the elution medium without skin samples.

RESULTS AND DISCUSSION

Diffusion/Metabolism Experiment

Figure 2 shows the time course of the cumulative amount of the prodrug PNF and its metabolite PN following

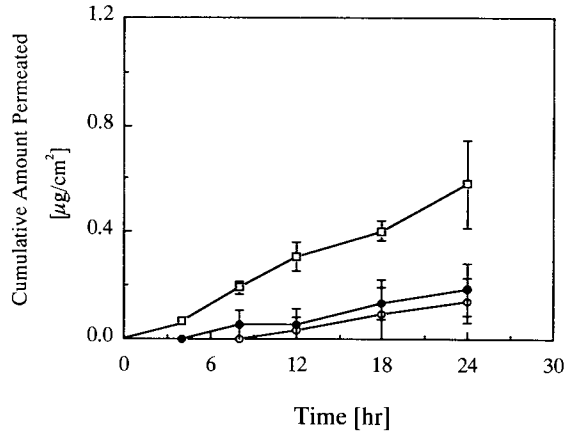

Fig. 2. Time course of the cumulative amount of the prodrug PNF and its metabolite prednisolone (PN) following skin penetration in the forward direction, with the skin surface facing the donor solution. Open circles, PNF in the receptor solution; filled circles, PN in the receptor solution; open squares, PN in the donor solution.

the skin metabolism of PNF when the skin was placed in the forward direction. The metabolite PN appeared not only in the receptor solution but in the donor solution in a greater amount. The approximately linear profiles shown in Fig. 2 indicate that the enzyme activity responsible for the PNF metabolism was constant in the viable skin during the entire period of the *in vitro* experiment (24 hr). However, skin metabolism of PNF to PN was previously found to decrease markedly after 36 hr in the *in vitro* experiment (10). The steady-state rates of permeation of PNF and PN suggest that approximately 80% of the prodrug was metabolized into PN in the viable skin of the hairless mouse.

Figure 3 shows the time course of the cumulative amount of PNF and its metabolite PN when the skin was mounted in the backward direction. The metabolite PN found in the donor solution was twice that in the forward direction. However, little PNF or PN was detected in the receptor solution. These results indicate a nonhomogeneous distribution of the enzymes responsible for skin metabolism

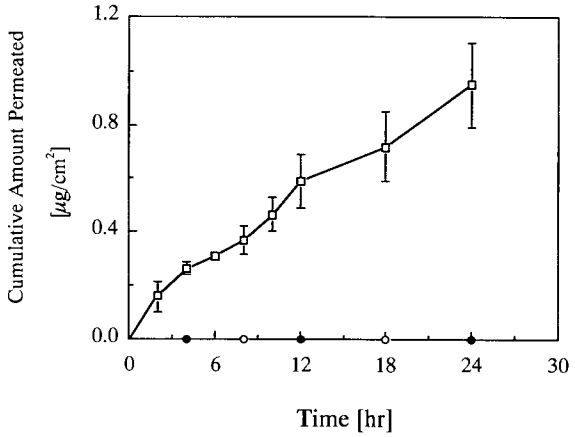

Fig. 3. Time course of the cumulative amount of the prodrug PNF and its metabolite prednisolone (PN) following skin penetration in the backward direction, with the skin surface facing the receptor solution. Symbols are the same as in Fig. 2.

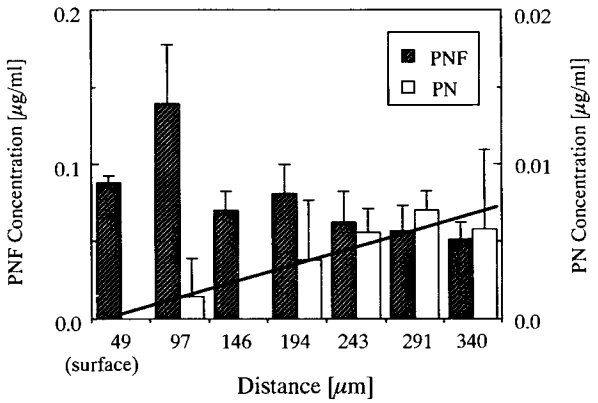

Fig. 4. Concentrations of PNF and PN in the elution medium following 24 hr of incubation with skin layers. Solid line, linearly increasing distribution of enzyme activity [Eq. (1)].

of PNF, with more enzyme in the lower region of the viable skin than in the upper region near the stratum corneum boundary.

Distribution of Enzymes

Figure 4 shows the distribution of both PNF and PN in the viable skin measured by the skin slicing technique following 24 hr of incubation. The concentration of the metabolite PN, not detected near the surface of the skin, gradually increased with increasing distance from the surface of the skin. Correspondingly, the intact prodrug was most concentrated in the upper layers of the skin and decreased gradually in the lower region of the skin, indicating that the enzyme activity is negligible in the upper layers of the skin and gradually increases in the lower layers. This finding supports the observation in the *in vitro* diffusion/metabolism experiment described above (Figs. 2 and 3).

Based on the experimental findings, we assume the first-order rate constant for bioconversion of PNF in the hairless mouse skin as

$$k = 2k_0x \tag{1}$$

for the forward direction of the skin and

$$k = 2k_0(1 - x) \tag{2}$$

for the backward direction of the viable skin, where x is the distance from the surface of the skin and k_0 is the rate constant in the central layer of the viable skin.

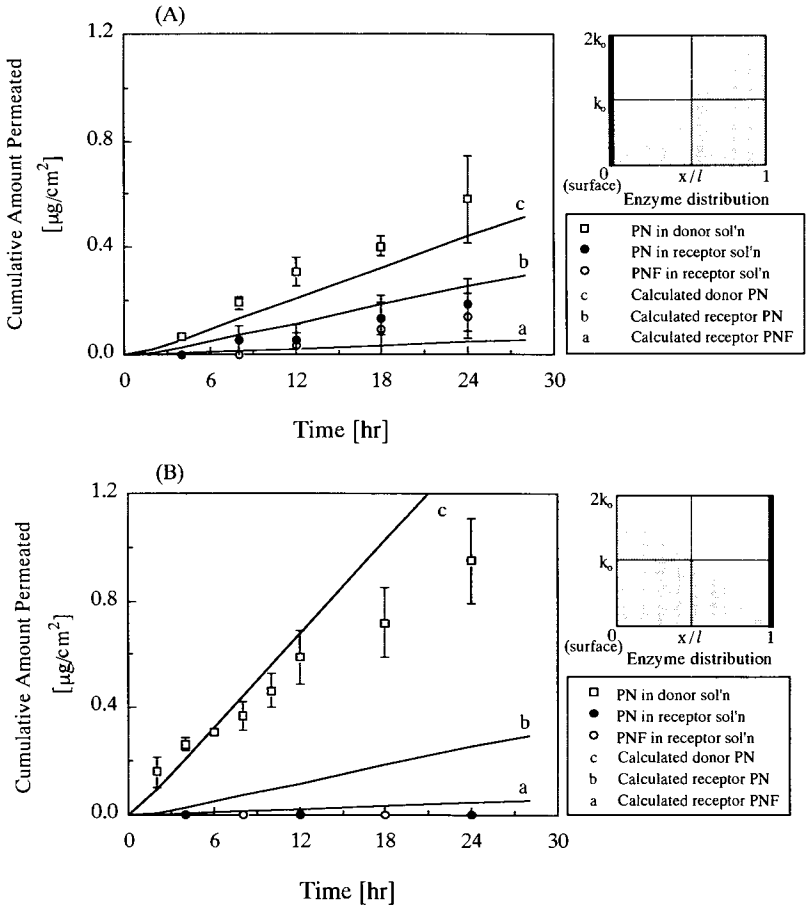

Fig. 5. Comparison of the experimental cumulative amount of PNF and PN following diffusion/metabolism of PNF in viable skin with the calculated profiles based on the linearly increasing model of skin enzymes ($k_0 = 2.4\ \text{hr}^{-1}$). (A) Forward direction; (B) backward direction.

The mass balance equations for the present diffusion/metabolism study are given by

$$\frac{\partial C_1}{\partial t} = D_1 \frac{\partial^2 C_1}{\partial x^2} - kC_1 \quad (3)$$

$$\frac{\partial C_2}{\partial t} = D_2 \frac{\partial^2 C_2}{\partial x^2} + kC_1 \quad (4)$$

where subscripts 1 and 2 refer to the prodrug and the drug, respectively, and D is the diffusion coefficient. The diffusion coefficient of the drug PN was calculated from the time lag (11) in the permeation profile of prednisolone. The same diffusion coefficient was assumed as that for the prodrug PNF because of the similar molecular volume of the steroidal skeleton.

The appropriate initial and boundary conditions are

$$C_1 = C_2 = 0, \quad \text{at} \quad t = 0, \quad 0 \leq x \leq l \quad (5)$$

$$C_1 = KC_S, \quad C_2 = 0, \quad \text{at} \quad t > 0, \quad x = 0 \quad (6)$$

$$C_1 = C_2 = 0, \quad \text{at} \quad t > 0, \quad x = l \quad (7)$$

where l is the thickness of the skin, C_S is the saturated concentration in the donor solution, and K is the skin/donor solution partition coefficient. Since the rate constant k is a function of the distance, we have numerically solved Eqs. (3) and (4) subject to Eqs. (5) to (7). If the enzyme activity is constant throughout the skin layers, Eqs. (3) and (4) can be solved analytically (10). The cumulative amount of PNF and PN either in the donor solution or in the receptor solution can be calculated from Eqs. (8) and (9), respectively:

$$Q_i = \int_0^t D_i \left.\frac{dC_i}{dx}\right|_{x=0} dt \quad (8)$$

$$Q_i = \int_0^t -D_i \left.\frac{dC_i}{dx}\right|_{x=l} dt \quad (9)$$

The method of lines (12) was employed here to solve Eqs. (3) and (4) subject to the initial and boundary conditions. The details of the method of solution have been described elsewhere by the present authors (12). All calculations were carried out on an IBM-compatible personal computer. The Microsoft FORTRAN optimizing compiler (Version 5.0) was used for running the computer program, written in FORTRAN77.

The rate constant k_0 in Eq. (1) was determined from the time profile of the metabolite concentration in the receptor solution with the forward skin direction. The experimental time courses of PNF and PN in the receptor solution or in

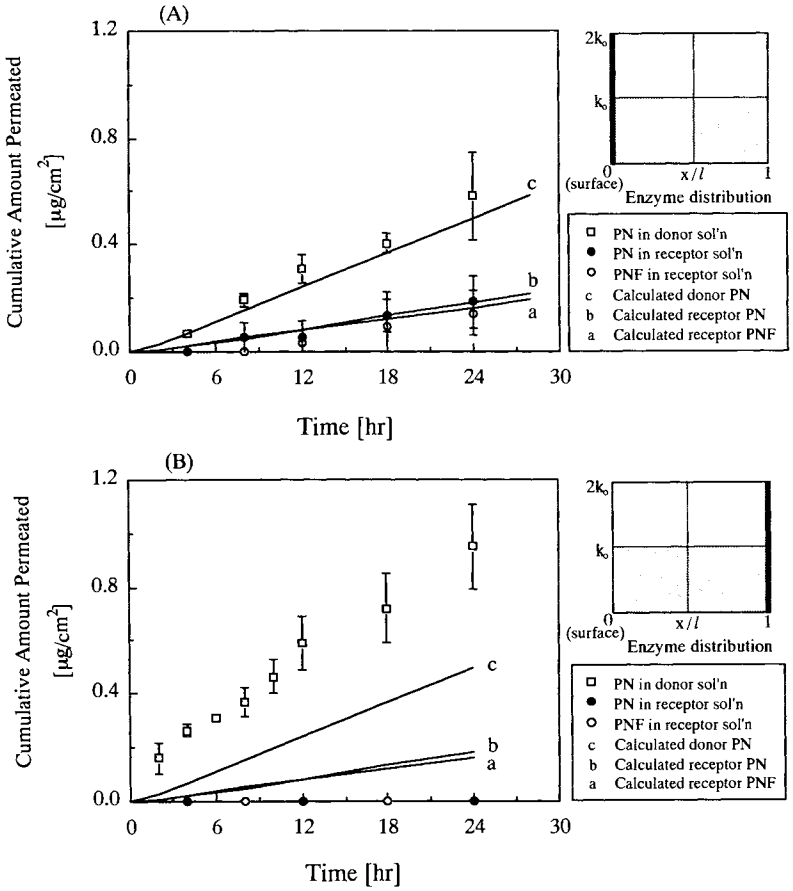

Fig. 6. Comparison of the experimental cumulative amount of PNF and PN following diffusion/metabolism of PNF in viable skin with the calculated profiles based on the homogeneous distribution model of skin enzymes (k = 0.87 hr^{-1}). (A) Forward direction; (B) backward direction.

the donor solution were compared with the calculated values based on the linear distribution model of skin enzymes in Fig. 5, while the analytical solutions based on the homogeneous distribution of enzymes are plotted in Fig. 6. The linear distribution model described reasonably well the experimental data of skin metabolism in the forward direction as well as in the backward direction (Fig. 5). In contrast, the homogeneous distribution model (Fig. 6) accounted for the time profile only in the forward direction; the calculated time courses of the metabolite in the backward direction deviated significantly from the experimental data. These results may be useful in the design of transdermal prodrug development.

ACKNOWLEDGMENTS

We would like to thank Taihou Pharmaceutical Co. and Kuraray Co. for providing the graduate research assistantship for T.H. We are grateful to Mr. Akira Ohtori and Dr. Akihiro Yamashita for their technical assistance.

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