Penetration and Bioconversion of Drugs in the Skin

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The dynamic phenomena of skin diffusion/bioconversion of a provitamin have been described by assuming a bilayer skin consisting of the stratum corneum as a main diffusion barrier and the viable skin as a major site of bioconversion. The mathematical model adequately describes the time courses of the in vitro appearance profiles of the provitamin and its metabolites, vitamins C and E, in the hairless mouse skin. The present model can be used not only to investigate transdermal delivery of prodrugs but also to elucidate mechanisms of skin detoxification of xenobiotics entering the skin.

Introduction

Biochemical functions of the skin are utilized to develop a prodrug transdermal delivery system\(^{(14)}\). The parent drug is chemically modified to improve partitioning toward the stratum corneum, the outermost layer of the skin and a major barrier to diffusion. After penetrating the stratum corneum, the prodrug is enzymatically bioconverted to its active drug in the viable skin. Biochemical functions of the skin are also related to detoxification of environmental pollutants entering the skin.

Several prodrug approaches have been developed for enhancing the skin permeation of polar drugs using lipophilic derivatives which undergo bioconversion to the parent drug in the skin. Various drugs, including vidaravine\(^{(15)}\), cromolyn\(^{(3)}\), acetylsalicylic acid\(^{(5)}\) and theophylline\(^{(3)}\) have been the subjects of recent investigations.

In spite of the fact that many researchers have published papers on percutaneous absorption during the last two decades\(^{(1,3,4,11)}\), dynamic phenomena of skin diffusion of a drug accompanied by an enzymatic reaction have not yet been elucidated. We still lack much of the information needed to understand the kinetics of skin penetration and bioconversion.

We have recently reported diffusion and bioconversion of a provitamin for vitamins C and E in the hairless mouse skin using an in vitro side-by-side diffusion cell\(^{(13)}\). Vitamin C was found to appear quickly in the receptor solution after bioconversion of the provitamin. Vitamin E, on the other hand, appeared negligibly during the initial 24 hours after the onset of an in vitro experiment and thereafter appeared gradually. These findings implied that vitamin C distributing endogenously might cause bursting release initially, while vitamin E might be further metabolized.

In this communication we analyze the time course of appearance profiles of the provitamin and vitamins C and E in the receptor solution, using a mathematical model which assumes a bilayer skin in which both diffusion and bioconversion take place. The rate constant for the bioconversion of the provitamin is then determined from mathematical simulation.

1. Mathematical Model

Assuming a bilayer skin which consists of the stratum corneum, a main barrier to diffusion, and the viable skin, a metabolic barrier against the entry of xenobiotics (Fig. 1), a mathematical model for diffusion/bioconversion was developed. The reaction scheme of bioconversion of the provitamin, EPC, for vitamins C and E is illustrated in Fig. 2. First-order reactions are assumed for the skin bioconversion of the provitamin and the vitamins. The chemical structure of the provitamin is shown in Fig. 3. The physicochemical properties of the provitamin and the vitamins were given in our previous paper\(^{(13)}\).

The mass balance of the provitamin (EPC) and the vitamins over a differential volume element of the skin yields:

\[
\frac{\partial C_p}{\partial t} = \frac{\partial}{\partial x} \left( D_p \frac{\partial C_p}{\partial x} \right) - k_1 C_p
\]

for the provitamin,
Fig. 1. Schematic diagram of skin penetration and bioconversion of provitamin (EPC) to vitamins C and E. Thicknesses of stratum corneum (h) and whole skin (H) of the hairless mouse are 0.0010 cm and 0.0380 cm respectively.

Fig. 2. Reaction scheme of skin bioconversion of provitamin (EPC), vitamin C and vitamin E.

Fig. 3. Chemical structure of provitamin (EPC).

\[
\frac{dQ}{dt} = -D_i \left( \frac{\partial C_i}{\partial x} \right)_{x=H} \tag{5}
\]

The cumulative amount of each compound appearing in the receptor solution is calculated by

\[
Q_i = \int_0^t \left( \frac{\partial Q}{\partial t} \right) \, dt \tag{6}
\]

Equations (1) to (3) were solved under appropriate initial and boundary conditions as follows. Initial conditions \((t = 0)\):

\[
C_p = \begin{cases} C_{p0} & (x = 0) \\ 0 & (0 < x \leq H) \end{cases} \tag{7}
\]

\[
C_e = \begin{cases} C_{e0} & (0 \leq x \leq H) \end{cases} \tag{8}
\]

\[
C_c = \begin{cases} \frac{C_{c0} P_c}{C_{c0} + P_c} & (0 \leq x \leq h) \\ \frac{C_{c0}}{h} & (h < x \leq H) \end{cases} \tag{9}
\]

Boundary conditions:

\[
x = 0: \quad C_p = C_{p0} = K_p C_d \tag{10}
\]

\[
\frac{dC_e}{dx} = \frac{dC_c}{dx} = 0 \tag{11a}
\]

\[
C_c = C_e = 0 \tag{11b}
\]

\[
x = h: \quad C_{c1} = P_c C_{c2} \tag{12}
\]

\[
D_{c1} \frac{dC_{c1}}{dx} = D_{c2} \frac{dC_{c2}}{dx} \tag{13}
\]

\[
x = H: \quad C_i = 0 \tag{14}
\]

Equation 11a is applied to the system in which the metabolites (vitamins C and E) do not diffuse back into the donor solution. Otherwise, Eq. 11b can be used, since the diffusion coefficient in the stratum corneum is usually so small that the concentrations of the metabolites in the donor solution are usually negligible compared to the solubilities. Therefore, Eq. 11b can be used for the present in vitro experiment. The mathematical model was numerically solved by a method of lines procedure\(^{10}\).

2. Diffusion Coefficient and Partition Coefficient

The diffusion coefficient of various progestins in the skin was almost independent of the lipophilicity or hydrophilicity of the penetrants\(^{12}\). The diffusion coefficient of testosterone derivatives through silicone membranes was found to be independent of the length of the side chain attached to the steroidal skeleton\(^{9}\). This finding indicated that the membrane diffusivity of a drug with two major portions may be influenced by the volume of the larger portion of the penetrant. The diffusion coefficients of various drugs that are stable in the skin were determined by conducting the penetration experiment using both the stripped skin.
and the intact skin on the basis of the time-lag method developed previously. The diffusion coefficients for the drugs (13 compounds including vitamins C and E) were found to be of the same order of magnitude; the average value of the diffusion coefficient for drugs with molecular weights ranging from 170 to 490 was $6.4 \times 10^{-11}$ cm$^2$/s in the stratum corneum and $9.5 \times 10^{-8}$ cm$^2$/s in the viable skin. The diffusion coefficient of the provitamin cannot be determined by a simple time-lag method because of the bioconversion in the membrane. Therefore, we used the average of diffusion coefficients obtained previously for various drugs as that for the present provitamin by assuming that the skin diffusion coefficient of the provitamin is of the same order of magnitude as that of vitamins C and E.

The partition coefficient of the provitamin between the stratum corneum and the viable skin was estimated by interpolation based on the experimental partition coefficients of vitamins C and E together with the octanol/water partition coefficients for these three compounds. The stratum corneum/viable skin partition coefficients for the provitamin and vitamins C and E are 0.51, 0.28 and 70, respectively.

3. Activity of Enzymes

Enzymes (esterases) responsible for bioconversion of estradiol esters were previously found to decrease the activity during the in vitro penetration experiment. The rate constants for bioconversion for the estradiol esters were well described by the following exponential decay law:

$$k = k_0 \exp(-At)$$

where $k_0$ is the intrinsic rate constant and $A$ is the degradation rate constant. In the present study, the rate constant for bioconversion of the provitamin is assumed to follow Eq. (15), because the experimental conditions were the same as those for the estradiol esters with respect to the animal model and the in vitro system employed. The intrinsic rate constant $k_0$ can be determined from the penetration (appearance) profiles during the initial 24 hours, for which the activity of enzymes remains virtually unchanged. Having the intrinsic rate constant, we can evaluate the decay rate constant $A$ from the profile during the late period of the penetration experiment.

4. Initial Tissue Concentration of Vitamin C

Since vitamins C and E are essential in our body, the skin may contain these vitamins endogenously. The initial tissue concentration of vitamin C was estimated by analyzing the penetration profile of both radiolabeled and nonlabeled vitamin C ($^{14}$C-ascorbic acid). The penetration profile of a radio-labeled compound is generally independent of the initial tissue concentration since the vitamin distributing endogenously is nonlabeled, while the penetration profile of a nonlabeled compound may be affected by the tissue concentration. The tissue concentrations of vitamin C in the viable skin and in the stratum corneum were found to be 2.7 $\mu$mol/ml and 1.4 $\mu$mol/ml, respectively. The initial tissue concentration of vitamin E, on the other hand, was found to be negligible. The rate constant of bioconversion of the provitamin $k_1$ was determined by comparing the appearance profiles of the provitamin with those of vitamin C, which was little metabolized in the skin. The rate constant $k_2$ for bioconversion of vitamin E was then evaluated.

5. In Vitro Penetration/Bioconversion Experiment

A full-thickness abdominal skin was freshly excised from a female hairless mouse (5–7 weeks old, HRS/J strain). A square section of the skin specimen (3 cm x 3 cm) was removed surgically and its dermal surface was cleaned carefully. This skin sample was then mounted between the half-cells of the in vitro side-by-side diffusion chamber. The hairless mouse skin was found to contain enzymes (esterases) responsible for bioconversion of prodrug esters which are also found in the human skin. The donor solution was prepared by dissolving 13 mg/ml of the provitamin in distilled water. The receptor solution was 50% glycerin aqueous solution for vitamin C, 5 mM Tween-80 aqueous solution for vitamin E and water for the provitamin, respectively. These solutions were selected to minimize degradation of the vitamins in the elution media. At appropriate time intervals, 30 $\mu$L samples were withdrawn from the receptor solution and assayed by HPLC. The detailed assay procedure was described elsewhere. The temperature in both donor and receptor compartments was maintained at 37°C.

6. Results and Discussion

Figure 4 shows the appearance profiles of the provitamin, vitamin C and vitamin E after skin bioconversion. Negligible amounts of the provitamin and vitamin E appeared in the receptor solution during the initial 24 hours. Thereafter, both compounds appeared gradually. Vitamin C, on the other hand, appeared in the receptor solution immediately after initiation of the penetration experiment. After about three hours, the rate of appearance reached a steady state. The significant difference in the appearance profiles between the provitamin and vitamin C during the initial 24 hours may suggest that the provitamin was almost entirely bioconverted to vitamin C and E in the viable skin, while the difference in the penetration profiles between vitamins C and E may indicate that vitamin E was further metabolized in the skin. From the penetration experiment using a
radiolabeled vitamin E, the time-lag for skin penetration was found to be about 2 hours, which was close to that for radiolabeled vitamin C. Therefore, we conclude that the long time-lag appearing in the profile of vitamin E (Fig. 2) was due to skin metabolism of vitamin E. The gradual increase in the concentration of vitamin E after about 36 hours indicates deactivation of enzymes in the excised hairless mouse skin under in vitro conditions. Prompt appearance of vitamin C after bioconversion of the provitamin is attributable to bursting release of the initial tissue concentration of vitamin C.

Skin diffusion and bioconversion of the provitamin to vitamins C and E were analyzed by the present mathematical model. The experimental profiles of the cumulative amount of the provitamin and the vitamins appearing in the receptor solution are compared with the calculated ones in Fig. 5. The dotted line for vitamin C was obtained by assuming no initial tissue concentration ($C_{0} = 0$) and constant activity of enzymes ($A = 0$). The bold solid lines were calculated assuming the exponential decay law for rate constants (Eq. (15)). The model parameters used in this calculation are given in the legend of Fig. 5. The present mathematical model well described the time courses of the cumulative amount of the provitamin and the vitamins appearing in the receptor solution. The model can be used to gain better understanding of the kinetics of prodrug transdermal delivery as well as the mechanisms of detoxification of xenobiotics entering the skin from the environment. Since human skin may differ from hairless mouse skin with respect to activity and distribution of enzymes, the model parameters obtained from the mouse skin must carefully be extrapolated to the human skin. The present model, however, will be useful for exploring the kinetics of diffusion and bioconversion of drugs in the skin as well as for simulating clinical situations based on the in vitro animal data.

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Nomenclature

- $A$ = decay rate constant of enzyme activity [s$^{-1}$]
- $C$ = concentration [μmol/ml]
- $C_{0}$ = tissue concentration of vitamin C in viable skin [μmol/ml]
- $C_{0}$ = provitamin concentration on skin surface [μmol/ml]
- $D$ = diffusion coefficient of penetrant [cm$^{2}$/s]
- $h$ = thickness of stratum corneum [cm]
- $H$ = thickness of whole skin [cm]
- $k$ = overall rate constant of skin bioconversion [s$^{-1}$]
- $k_{0}$ = intrinsic rate constant of skin bioconversion [s$^{-1}$]
- $k_{1}$ = rate constant for bioconversion of provitamin [s$^{-1}$]
- $k_{2}$ = rate constant for bioconversion of vitamin E [s$^{-1}$]
- $k_{3}$ = rate constant for bioconversion of vitamin C [s$^{-1}$]
- $K_{p}$ = partition coefficient of provitamin between stratum corneum and donor solution
- $P_{c}$ = partition coefficient of vitamin C between stratum corneum and viable skin
- $Q$ = cumulative amount appearing in receptor solution [μmol/cm$^{2}$-s]
- $t$ = time [s]
- $x$ = distance from surface of skin [cm]
\begin{align*}
\text{Subscripts} \\
\text{c} & = \text{vitamin C} \\
\text{d} & = \text{donor solution} \\
\text{e} & = \text{vitamin E} \\
\text{p} & = \text{provitamin} \\
\text{l} & = \text{stratum corneum} \\
\text{2} & = \text{viable skin}
\end{align*}

Literature Cited