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Notes

Influence of temperature on the percutaneous absorption for lipophilic and hydrophilic drugs across the rat skin pretreated with oleic acid

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Abstract

The combined effect of pretreatment with oleic acid and temperature on the rat skin permeation of lipophilic and hydrophilic drugs was examined. Prednisolone was used as a lipophilic penetrant which would penetrate through the lipid route of the skin. On the other hand, glucose was used as a hydrophilic penetrant which would permeate through the polar pathway. The steady-state permeability coefficients (P) of these penetrants were determined employing two-chamber diffusion cells in which excised rat abdominal skin was mounted. On skin pretreated with 3% oleic acid in 30% ethanol for 2 h, the P values of both penetrants abruptly increased with increase in temperature. On the other hand, these penetrants did not permeate through skin pretreated with 30% ethanol alone, even though a high temperature was applied.

Keywords: Temperature effect; Percutaneous absorption; Oleic acid; Arrhenius plot; Enhancement action; Skin permeation

Many studies concerning penetration enhancers have been performed in order to reduce the barrier function of the skin and to increase the percutaneous absorption of drugs. Oleic acid has widely been examined as a potential skin penetration enhancer for many kinds of drugs (Niazy, 1991; Ruland and Kreuter, 1992; Koyama et al., 1994; Ruland et al., 1994a,b). A number of studies have demonstrated that the lipid-associated transition temperature in the stratum corneum was lowered by treatment with oleic acid (Francoeur et al., 1990; Ongpipattanakul et al., 1991). Furthermore, oleic acid induced morphological changes in skin structure (Green et al., 1988).

In previous work (Ohara et al., 1994, 1995), we demonstrated the combined effect of pretreatment of skin with *d*-limonene and temperature. The rat skin permeation of drugs such as ketoprofen, prednisolone, glucose and isoniazid was markedly enhanced with increase in temperature when the skin was pretreated with *d*-limonene. Subsequently, synergy between the pretreatment with *d*-limonene and applied heat was clearly observed in the case of lipophilic drugs. The

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purpose of this study was to clarify the combined effect of pretreatment with oleic acid and temperature on the skin permeation of lipophilic and hydrophilic penetrants.

Oleic acid was of extra pure reagent grade and was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Prednisolone was obtained from Wako Pure Chemical Industries Ltd (Osaka, Japan). D-Glucose (anhydrite) was purchased from Daiichi Pure Chemicals Co., Ltd (Tokyo, Japan). Other chemicals used were of reagent grade.

Skin permeation: Full-thickness abdominal skin was excised from male Wistar rats weighing 180–200 g, the hair of which had been previously removed with an electric animal clipper. The excised skin was used as a permeation membrane for the in vitro study. Two-chamber diffusion cells (available diffusion area, 0.785 cm^2; volume of each half-cell, 3.0 ml) with a water jacket were employed. The donor cell was filled with a pretreatment solution composed of 30% ethanol-phosphate buffer solution (pH 7.0) containing 0 or 3% oleic acid and the receiver cell was filled with phosphate buffer solution (pH 7.0). Both cells were stirred by a magnetic stirrer for 2 h. After pretreatment, the solutions of donor and receiver cells were removed and washed several times with phosphate buffer solution. The penetrants suspended in a 30% ethanol-phosphate buffer solution were then applied to the donor cell. The receiver cell was again filled with phosphate buffer solution. The penetrants in the media were kept in a suspended condition throughout the experiment. Pretreatment of skin and in vitro permeation were performed at 20–40° C. At appropriate intervals, aliquots (20 μl) were withdrawn from the receiver cell. Leakage of endogenous glucose in the skin into the receiver solution was determined in the same way as described above except for using 30% ethanol-phosphate buffer solution as the donor phase instead of the glucose suspended solution.

Determination of prednisolone: The sample solution (20 μl) in the skin permeation study was thoroughly mixed with methanol (200 μl) containing 1 μg/ml methyl *p*-hydroxybenzoate as an internal standard. These mixtures were filtered using a disposable filter unit (Ekikuro-Disk 3CR, Gelman Science Japan, Ltd, Tokyo, Japan), and the concentration of prednisolone in the filtrate was determined using an HPLC apparatus (Model LC-3A, Shimadzu Corp., Kyoto, Japan). Ultraviolet detection (Model SPD-6A, Shimadzu Corp., Kyoto, Japan) at 254 nm was employed; the column (4.6 mm × 150 mm) was packed with A-302 S-5 120A ODS (YMC Co., Ltd, Tokyo, Japan); elution was performed at room temperature with a mobile phase composed of water and methanol (42:58, v/v); the flow rate was 1 ml/min.

Determination of glucose: The concentration of 20 μl aliquots was determined with a Glucose B-test Wako® (Wako Pure Chemical Industries, Ltd, Osaka, Japan) spectrophotometrically at 505 nm using a U-best 30 spectrophotometer (Japan Spectroscopic Co., Ltd, Tokyo, Japan).

Solubility determination: Prednisolone or glucose suspension (including excess amounts of each drug in the 30% ethanol-phosphate buffer solution) was placed in a water bath (20–40° C) for 24 h with stirring using a magnetic stirrer. The sample was then placed in the solution and the upper layer was filtered through a 0.45 μm membrane filter (Gelman Science Japan Ltd, Tokyo, Japan). The concentration of prednisolone was determined by HPLC as described above. The concentration of glucose in the suspension was determined with the Glucose B-test Wako® using a spectrophotometer.

In general, the routes of skin penetration have been classified into two pathways, i.e., polar and non-polar pathways, in the intercellular domain (Barry, 1991; Koyama et al., 1994). In this study, prednisolone and glucose were chosen as a suitable model penetrant which will permeate through the non-polar and polar pathway in the skin, respectively. The skin was pretreated with 3% oleic acid in a 30% ethanol-phosphate buffer solution for 2 h at 20–40° C. Fig. 1 shows the cumulative amount of penetrants as a function of time. Leakage of endogenous glucose in the skin into the receiver solution was negligible even though the skin was pretreated with 3% oleic acid at 40° C. The permeation of both penetrants was markedly enhanced with increasing temperature. On the other hand, these penetrants did not

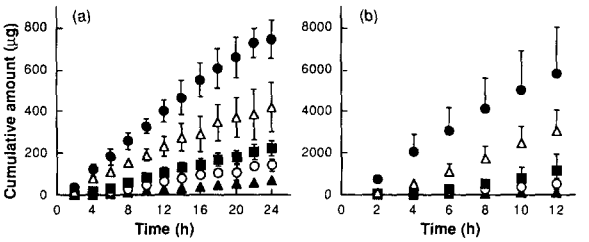

Fig. 1. Combined effect of pretreatment with oleic acid and temperature on the skin permeation of prednisolone (a) and glucose (b). Each point is the mean ± S.D. of three determinations. (●) 40° C, (△) 35° C, (■) 30° C, (○) 25° C (▲) 20° C.

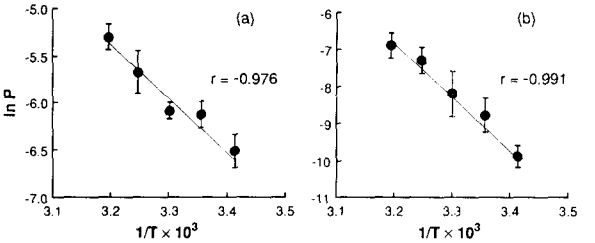

Fig. 3. Arrhenius plot of the steady-state permeability coefficients (P) of prednisolone (a) and glucose (b). Each point is the mean ± S.D. of three determinations.

permeate through the skin which was pretreated with 30% ethanol alone. These findings suggest there exists a combined effect of pretreatment with oleic acid and applied heat on the skin permeation of both lipophilic and hydrophilic drugs. In order to normalize the solubility change of the penetrants in the donor solution by the increase of temperature, the steady-state permeability coefficient (P) was estimated from the solubility and the slope shown in Fig. 1 (Cooper and Berner, 1985). In both penetrants, the P values markedly increased as a function of temperature (Fig. 2), suggesting that the combination of the pretreatment with oleic acid and temperature could directly affect the dense barrier structure of the skin. Fig. 3 shows Arrhenius plots for each penetrant. Good linearity was observed and the activation energy of permeation was estimated to be 43.7 kJ/mol for prednisolone and 114.4 kJ/mol for glucose.

In our previous studies (Ohara et al., 1994, 1995), the Arrhenius plots of lipophilic penetrants exhibited a convex curvature after pretreatment of the skin with *d*-limonene, although the plots obtained with hydrophilic penetrants showed good linearity. Therefore, the barrier function of the non-polar pathway in the skin with *d*-limonene pretreatment might be reduced continuously as a function of temperature, while such a specific phenomenon was not observed with the hydrophilic penetrants. Although oleic acid markedly enhanced the skin permeation of lipophilic and hydrophilic penetrants, its combined effect with applied heat was not synergistic, differing from the result observed with *d*-limonene.

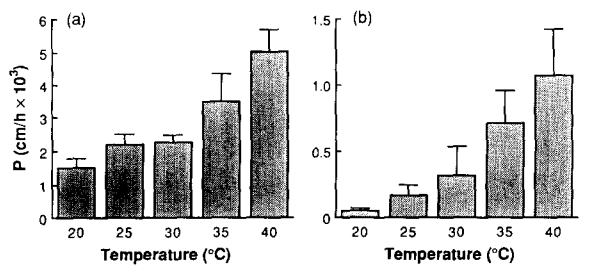

Fig. 2. Steady-state permeability coefficients (P) of prednisolone (a) and glucose (b). Each point is the mean ± S.D. of three determinations.

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