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Electric field analysis on the improved skin concentration of benzoate by electroporation

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Abstract

The objective in the present study was to understand the relationship between the increased skin concentration of benzoate as a model drug after topical application of its sodium salt and the electric field intensity produced in the skin barrier, the stratum corneum, by electroporation. A piece of excised abdominal hairless rat skin was set in a Franz type diffusion cell, and 0.5% sodium benzoate and physiological saline were applied to the stratum corneum and dermis sides, respectively. Two needle electrodes made of Ag were connected to an electrical power source, which produced exponentially decaying pulses. The electrodes were placed on the skin surface with a distance of 0.5 cm between both electrodes. After the 4 h passive permeation experiment, an electrical pulse was applied to the rat skin at 300 V every minute for 10 min. The skin was then removed from the diffusion cell, and the amounts of benzoate in different positions of the skin specimen were measured. Field intensity generated in the stratum corneum by electroporation was determined by a finite element method using a computer program. The amounts of benzoate at different sites in the skin were almost proportional to the mean field intensity in the corresponding stratum corneum. These results suggested that the enhancing effect of electroporation can be evaluated by the field intensity more directly than the application voltage.

Keywords: Skin permeation; Skin concentration; Electroporation; Electric field analysis

1. Introduction

Electroporation technology, widely used for introducing DNA and RNA into cells and biological tissues (Zimmermann et al., 1973; Neumann and Rosenheck, 1975), can be used to increase transdermal drug delivery (Prausnitz et al., 1993). Since the work by Prausnitz et al., several researchers have evaluated the effects of electroporation on the skin permeation of drugs (Zhang et al., 1999; Lombry et al., 2000; Sharma et al., 2000). However, little information is available regarding the optimum conditions of electroporation, except that application voltage has been shown to be a major factor determining the en-

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hancing effect (Chen et al., 1999; Vanbever et al., 1999). We also investigated the effects of electroporation on the in vitro skin permeation of a non-ionic model material, mannitol (Mori et al., 1999), and an ionic model compound, sodium benzoate (Yoshida et al., 2000). In these studies, anode and cathode positions were also shown to be important determinants for the effect of electroporation in addition to the application voltage. No differences in the skin permeation, however, were observed between active (Ag/AgCl) and inactive (Pt) electrodes.

In the present study, we examined the effect of the electric field intensity in the skin barrier, the stratum corneum, at electroporation rather than the application voltage, using sodium benzoate as a model penetrant according to the previous study (Yoshida et al., 2000). The electric field was analyzed by a computer simulation using the finite element method, and the amount of benzoate in skin at electroporation after application of its sodium salt was measured by the in vitro skin permeation technique using Franz type diffusion cells. The relationship between the electric field strength and amount of benzoate in the skin at various positions was then evaluated.

2. Materials and methods

2.1. Materials

Sodium benzoate was obtained from Wako Pure Chemicals (Osaka, Japan). Other reagents were of analytical grade and were used without further purification. Needle type Ag electrodes (1.0 mm in diameter and 3 cm in length) were prepared from silver rods (Murata Yohaku, Tokyo, Japan) in our laboratory. The tip of the needle electrode was bent to prevent damage of the skin barrier.

2.2. Skin permeation experiment

Male hairless rats (WBN/ILA Ht), 200–230 g, were supplied either by Life Science Research Center, Josai University (Sakado, Saitama, Japan), or Ishikawa Experimental Laboratory (Fukaya, Saitama, Japan). The rats were anesthetized by intraperitoneal injection of the sodium pentobarbital (50 mg/kg), and the abdominal skin was excised. These animal experiments were performed in accordance with the guidelines of the Life Science Research Center, Josai University. After trimming, the fresh excised full-thickness skin was mounted in a vertical Franz type diffusion cell with an effective diffusion area of 7.07 cm^2 and a water jacket connected to a water bath maintained at 37°C (Yukawa et al., 1989). Sodium benzoate solution (4.0 ml) at a concentration of 0.5% was applied on the skin surface, with only physiological saline ($\approx$ 26 ml) on the dermal side. The receiver side was stirred at 1200 rpm with a star head magnetic bar and a magnetic stirrer (Multistirrer, Scinics, Tokyo, Japan).

Two needle electrodes were set on the skin surface. The distance between the two needle electrodes was kept at 0.5 cm. The diffusion cell, the position and size of the electrodes and sampling position of the skin are schematically shown in Fig. 1. These electrodes were connected to a Gene Pulser® (Bio-Rad, Hercules, CA), commonly used for electroporating bacterial and other cell membranes. The power source delivers exponentially decaying pulses. The capacitance of the electroporation apparatus was set at 1 μF. A consecutive run was carried out to measure passive diffusion and the effect of electroporation using the same skin specimen (Masada et al., 1989). Thus, the electrical pulse was applied 300 V every minute

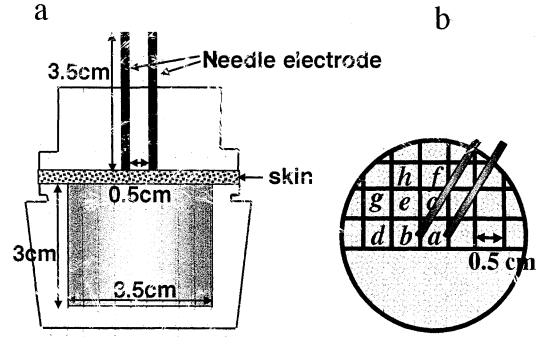

Fig. 1. Schematic illustration of the experimental setting of diffusion cell, electrodes and excised hairless rat skin (a) and each sampling position on the excised skin (b).

for 10 min after a 4 h passive permeation experiment, and the skin was removed from the cell 20 min after electroporation.

2.3. Assay

The drug concentration in each full-thickness skin piece was assayed by HPLC. Each sample was mixed with the same volume of acetonitrile containing 10 μg/ml *p*-ethyl benzoic acid as an internal standard. After centrifugation, the mixed solution was injected into an HPLC apparatus composed of a pump (LC-10AS, Shimadzu, Kyoto, Japan), UV detector (SPD-l0A, Shimadzu), integrator (C-R5A, Shimadzu), system controller (SCL-10A, Shimadzu), auto injector (SIL-10AXL, Shimadzu) and a reverse phase column (Hibar Lichro CART, 4 mm × 250 mm, Kanto Kagaku, Tokyo, Japan). Flow rate was 1.0 ml/min, the mobile phase was acetonitrile: 0.05 M phosphate buffer (1:1) and UV wavelength for detection was 230 nm.

2.4. Two-dimensional electric field analysis

Since the stratum corneum can be treated as dielectrics, the vector of electric field intensity, $\vec{E}$, in the skin barrier can be expressed as follows:

$$\vec{E} = \frac{1}{\varepsilon}\vec{D}, \quad (1)$$

where ε is the dielectric constant and $\vec{D}$ is the vector of electric flux density. In this study, ε was assumed to be constant independent of $\vec{E}$. Using application voltage (300 V) and relative dielectric constants (ratio of ε against ε_0, where ε_0 is the dielectric constant for vacuum air) of the Ag electrodes and hairless rat skin (12.3 and 243), electric field intensity generated on the stratum corneum was determined by a computer simulation using a finite element method (simulation software; PHOTOVOLTST, PHOTON Co., Ltd, Nara, Japan and FEMAP Version 7.0, NST Co., Ltd, Tokyo, Japan) on a personal computer. The mean field intensity in each skin site was calculated using the simulation value.

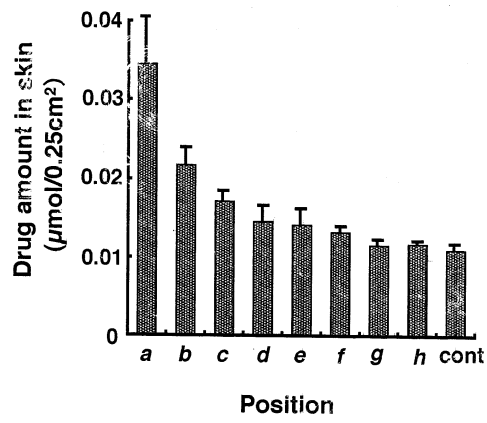

Fig. 2. Benzoate amount in different skin sites at electroporation. Each column represents the mean ± S.E. of 3–5 experiments.

3. Results

Fig. 2 shows the amounts of benzoate at various positions in hairless rat skin. The amounts of benzoate at skin sites *a*, *b* and *c* were 3-, 2- and 1.5-fold higher, respectively, than those without electroporation (control values). The amount of drug in skin decreased with increasing distance from position *a* (Fig. 1), and those at *g* and *h* were not significantly different from those without electroporation (control).

Fig. 3(a) shows the analytical results of field intensity on the stratum corneum, where points *a*–*h* correspond to the skin positions shown in Fig. 1. Values in each square are the mean field intensity for each position. The intensity was highest around the electrodes and decreased with distance from the electrodes, and these were marked differences in the field intensity among the skin sites. Fig. 3(b) summarizes the mean field intensity at each position.

Fig. 4 shows the relationship between the amount of benzoate (Fig. 2) and field intensity (Fig. 3) at each position. Good linearity was observed. The ordinate intercept corresponds to the amount of benzoate in skin due to intrinsic passive diffusion of the drug without electroporation. On the other hand, the effect of electroporation on the amount of drug in skin was proportional to the field intensity. The marked

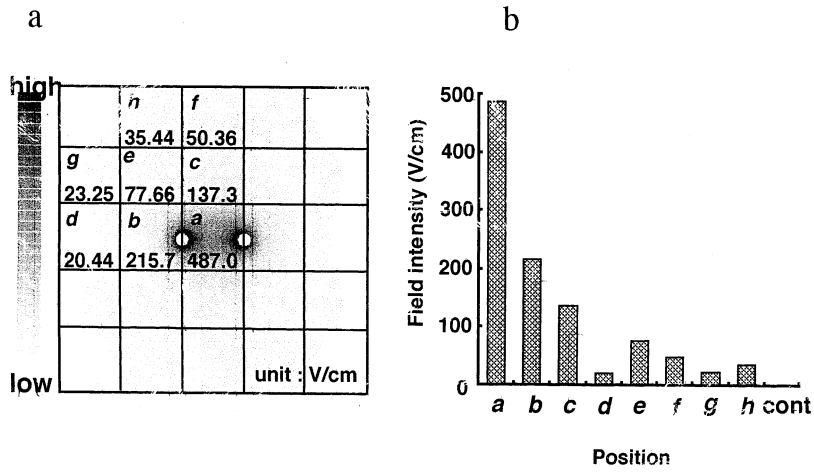

Fig. 3. Analytical profile of electric field intensity generated by electroporation in different skin sites (a) and at each position *a*–*h* (b).

differences in distribution of the electric field (Fig. 3) were probably responsible for the large differences in the amount of the drug in skin among the skin sites (Fig. 2).

4. Discussion

Many previous studies have indicated that the effect of electroporation on the skin delivery of drugs is markedly influenced by the application voltage (Chen et al., 1999; Vanbever et al., 1999). We also investigated the effects of application voltage on the skin permeation of benzoate (Yoshida et al., 2000). Although the application voltage is an important determinant, electric field generated in the skin barrier, the stratum corneum, must be a more direct determinant than the voltage. We thus evaluated the electric field distribution in the stratum corneum by computer simulation. The results obtained showed that the drug distribution from the donor chamber to the skin was due to intrinsic passive diffusion and electrically assisted transport through the skin. In addition, the latter was proportional to the electric field intensity rather than application voltage.

The electric field intensity must be influenced by application voltage and shape and position of electrodes. Even under the same application voltage, the electric field was altered by the position of the electrodes. Shape and position of electrodes were important determinants of the effect of electroporation.

Two methods are mainly used to evaluate the in vitro skin permeation of drugs: one involves periodical measurement of the drug concentration in the receiver chamber of the diffusion cells, and the other involves determination of the amount of drug in skin. Drug distribution (or partition) from the vehicle to skin and drug diffusivity in the skin barrier are two primary determinants of the skin permeation rate, whereas only the partition is a parameter of the skin amount. If electroporation treatment affects only the drug diffusivity in skin

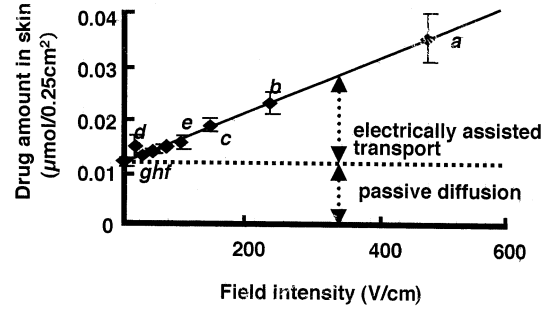

Fig. 4. Effects of field intensity on the benzoate amount in skin at electroporation. Each point represents the mean ± S.E. of 3–5 experiments.

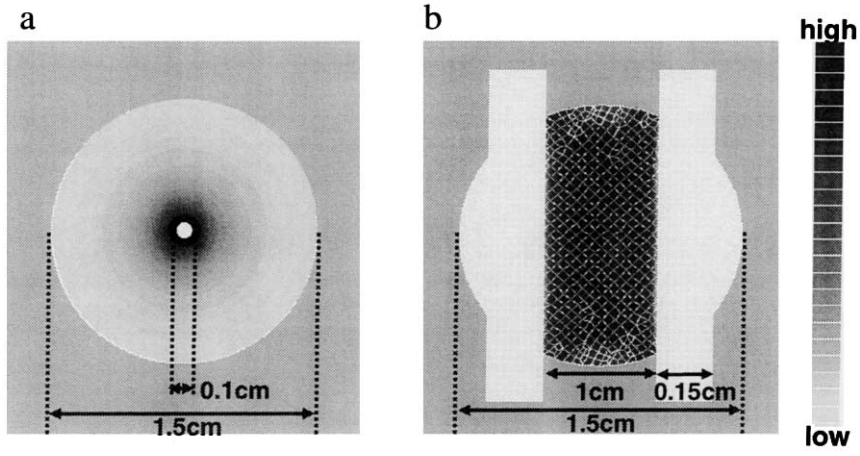

Fig. 5. Analytical profile of electric field intensity generated by electroporation with ring–needle electrode (a) and parallel plate–plate electrode (b) systems.

barrier, no increase in the drug amount in the stratum corneum would be obtained. Since the electroporation treatment used in the present study resulted in a marked increase in the amount of benzoate in the whole skin, electroporation is useful to increase the drug partition from the formulation to the skin barrier. On the other hand, the effect of electroporation on the drug diffusivity was not evaluated in the present study. This technique, however, was also shown to be useful to increase the drug diffusivity in skin by our previous study (Mori et al., 1999).

The needle–needle electrode system used in the present study generated large differences in the electric field among different skin sites. Although a high electric field is preferable to increase the skin permeation, it may cause skin damage. It may be possible to alter the distribution of the electric field to obtain more effective drug permeation without severe skin damage. We then simulated the electric field intensity in the stratum corneum for the needle–ring electrode system (Yoshida et al., 2000) and parallel plate–plate electrode system. Fig. 5(a) and (b) show the results obtained by the computer simulation. The needle–ring electrode showed a lower distribution in the field intensity than the needle–needle electrode, as shown in Fig. 5(a). The parallel electrode system gave an almost even distribution of electric field throughout the stratum corneum between the plate electrodes (Fig. 5(b)). The meander type electrode (Gunter et al., 1995; Zhang et al., 1999) may yield the same results as the parallel plate–plate electrode. At present, the parallel electrode system appears to be the best for electroporation.

In conclusion, skin permeation at electroporation can be explained by two mechanisms; intrinsic passive diffusion and electrically assisted transport. The latter was shown to be proportional to the electric field intensity. Thus, the electric field must be taken into account in addition to the application voltage to optimize the effects of electroporation on the transdermal delivery of drugs.

Acknowledgements

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