

Design and Feasibility Assessment of Topically Applied Drug Formulations for Electroporation

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Few studies have been reported on the design of topical formulations consisting of electrodes and active drugs for electroporation as a means to increase skin permeability of the drugs, although many studies were reported for the effect of this physical means using aqueous drug solutions. We, therefore, designed a prototypic reservoir and matrix topical formulations that are suitable for electroporation in the present study. Plate-plate Ag electrodes and sodium diclofenac were used as model electrodes and the drug, respectively. The *in vitro* skin permeations of the drug obtained from the reservoir and matrix formulations were slightly higher than that from an aqueous suspension. This may be due to slightly higher electric field in the skin barrier for the presently designed formulations than that for the aqueous suspension. The present feasibility test suggests that these reservoirs and matrix formulations are useful prototypic topical formulations for electroporation application to improve the drug permeability through skin.

Key words electroporation; skin permeation; penetration enhancement; matrix formulation; reservoir formulation

Electroporation has been broadly utilized to introduce genes to several biological cells and bacteria.^{1,2)} The principle of electroporation is to make tiny pores in the biological membranes for the gene introduction by applying a high voltage for a very short period.³⁾ Since Prausnitz *et al.* applied this technique to increase skin permeation of drugs,⁴⁾ the enhancing effect on the skin permeation of several drugs and penetration-enhancing mechanism of electroporation have been broadly investigated.^{5–11)} We previously reported on the mechanism, suitable conditions and simultaneous treatment with iontophoresis of electroporation using mannitol and sodium benzoate as model drugs.^{12,13)} These studies on the electroporation, however, were carried out mostly in simple *in vitro* or *in vivo* experiments using aqueous solutions, not in practical *in vivo* ones using topical formulations.

The skin permeation rate of drugs is generally lower from actual formulations than that from a simple aqueous solution. One possible reason for decreasing skin permeation with the actual formulations is that an additional diffusional barrier may be prepared to decrease thermodynamic activity of the drugs in the formulations. Especially in electroporation, a suitable electric field may not be produced in the skin barrier, stratum corneum after application of the formulations. Therefore, electrodes should be in contact with the skin surface especially for the practical formulations to expect a high enhancing effect on the skin permeation of drugs by electroporation. Since small pores, produced by application of a high voltage, were found to disappear in a short period,⁴⁾ drugs or formulations should be applied immediately after electroporation treatment. These points were considered in the present formulation design for electroporation. Prototypic reservoir and matrix formulations containing sodium diclofenac, as a model drug, for electroporation were designed and evaluated for their drug permeation abilities across the skin membrane. Sodium diclofenac was selected, since it shows a potent anti-inflammatory effect. Furthermore, only a few topical formulations of the drug have been marketed due to its low skin permeability. Agar was selected for preparing

the gel matrix, since it is a nonionic hydrophilic polymer showing a low toxicity and a low interaction with drugs and pharmaceutical additives in the formulations. In addition, a low concentration of agar is enough to make adequately hard gel.

Experimental

Materials Sodium diclofenac was obtained from Sigma Chemical Co., Ltd. (St. Louis, MO, U.S.A.). The JP grade of agar was used for preparing the matrix gel. Silver sheet (40 μm in thickness) as an electrode material was obtained from Murata Yohaku (Tokyo, Japan).

Preparation of Reservoir- and Matrix-Typed Formulations Reservoir- and matrix-typed drug formulations were prepared as follows. Before preparing the reservoir formulation, two silver sheets of 40 μm in thickness, 3.0 mm in width and 50.0 mm in length, as plate electrodes, pasted on electrically insulate double-faced adhesive tape of 0.5 mm in thickness (Nichiban, Tokyo) were again applied on a sheet of porous membrane (Dunapore SVLP, Millipore, Bedford, MA, U.S.A.) with the peripheral adhesive layer had a 6 mm distance between both plate electrodes to make an electrode membrane. The donor cell, as a drug reservoir mold, was fixed to the electrode membrane at the center. Figure 1 illustrates the prepared reservoir formulation (side view and bottom view). Table 1 shows the composition of the reservoir. Two percents of sodium diclofenac solution (suspended solution) (pH 8.0) was used in the reservoir.

For the matrix vehicle, agar was dissolved at a concentration of 1.5% in boiled water, and sodium diclofenac was added to the agar solution at a final concentration of 2% and thoroughly mixed. The resulting drug-suspended agar solution (0.4 g) was filled in a formed cup with a peripheral adhesive layer and cooled to room temperature to make the agar matrix gel. Two silver sheets (the same shape and size used for the reservoir formulations) were applied on the agar matrix as plate electrodes. Electrically insulated double-faced adhesive tape was used to adhere the electrodes to the agar gel and formed cup rims. Figure 2 illustrates the prepared gel matrix formulation. Table 1 also shows the prescription for gel matrix.

Skin Preparation and Permeation Experiments Male hairless rats (WBN/ILA-Ht) weighing between 220 and 240 g were purchased from

Table 1. Composition of Diclofenac Solution, Reservoir and Matrix

Composition (%)	Solution or reservoir	Matrix
Sodium diclofenac	2.0	2.0
Agar	—	1.5
Distilled water	98.0	96.5

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Ishikawa Experimental Animals Laboratories (Fukaya, Saitama, Japan). The abdominal skin was excised from the anesthetized rats by *i.p.* injection of 25% urethane. The excised skin was set on the Frantz type diffusion cell (effective diffusional area, 3.14 cm²).¹⁴ The dermis side was filled with about 17 ml of physiological saline, and 2.0% sodium diclofenac (1.0 ml) (Table 1), reservoir formulation (reservoir volume; 1.0 ml) (Fig. 1) or matrix formulation (matrix amount; 0.4 g) (Fig. 2) was applied to the stratum corneum side. Agar gel matrix could completely cover the effective diffusional surface of skin even when 0.4 g matrix was used, whereas more volume of aqueous solution (1.0 ml) was better to cover the whole skin surface. This is probably due to high surface tension of distilled water. In the application experiment of the aqueous solution, both cathode and anode electrodes (the same shape and size as shown above) were set on the skin surface with a 6 mm distance between both plate electrodes. The upper surface of the electrode sheets to the drug solution (not to the skin surface) was insulated. In the application experiment of the reservoir or matrix formulation, on the other hand, one side of the electrodes was also insulated as shown in Figs. 1 and 2. Common application condition for electroporation was used in the present study^{8,9}; 200 V was applied for 50 ms every hour from 0 to 6 h (total 7 times) using a rectangle-pulsatiled generator (T-820, BTX, San Diego, CA, U.S.A.). The output wave was confirmed to be 200 V by monitoring using a pulse monitor (Enhance 4000, BTX). At predetermined times, 0.2 ml of receiver solution was sampled for analysis of skin permeation of sodium diclofenac and the same volume of fresh saline was added to maintain the receiver volume.

Assay of Diclofenac Diclofenac was determined by HPLC (LC-10Avp, Shimadzu, Kyoto, Japan). ODS column (TSKgel ODS-80TS, 4.6 mm in diameter and 150 mm in length, Tosoh, Tokyo) was used. The mobile phase was methanol: 0.1% phosphoric acid (75:25), the flow rate was 1.0 ml/min, the column temperature was 40 °C and detection was carried out at UV 286 nm.

Results and Discussion

Evaluation of Penetration Enhancing Effect of Electroporation The effect of electroporation on the skin permeation of diclofenac from its simple aqueous solution (suspended solution) was evaluated as a control (without the reservoir or matrix dosage forms). Figure 3 shows the cumulative amount and flux of diclofenac permeated through hairless rat skin with (○) and without electroporation (●). The cumulative amount and flux correspond to that of sodium diclofenac. As shown in the figure, the cumulative amount of the drug permeated through skin over 7 h was $321 \pm 82 \mu\text{g}/\text{cm}^2$ from the aqueous solution with the electroporation, which was about 8-fold higher than that without electroporation ($39 \pm 6 \mu\text{g}/\text{cm}^2$). The flux showed a monotonic increase with time in both cases from the aqueous solution with and without electroporation. Fluxes during the last sampling period (6–7 h) were 94 ± 16 and $11 \pm 1 \mu\text{g}/\text{cm}^2/\text{h}$ with and without electroporation, respectively. In all cases, electroporation showed a marked penetration-enhancing effect when using the simple aqueous solution in the drug donor and plate-plate electrodes adhered on the skin surface.

Preparation of Prototype Formulation for Electroporation At designing the actual formulations for electroporation, the following points were noticed: (i) Electrodes were set directly on the skin surface to effectively apply the voltage on the skin barrier, stratum corneum,¹⁵ and (ii) relatively simple formulations were designed to apply on the skin surface without any drug leakage from the formulations during the shelf life. Figures 1 and 2 show the reservoir and matrix formulation prepared in the present study. When a patch type reservoir is used instead of a donor mold (Fig. 1), a more polished reservoir formulation will be made. Agar gel was used for a matrix formulation, since (i) it shows a low toxicity and a low drug interaction and (ii) the release property of

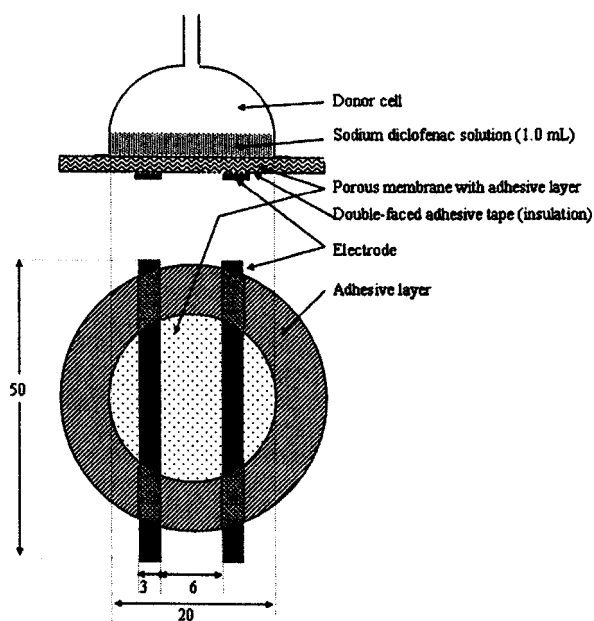


Fig. 1. Schematic Representation of Electrode Porous Membrane and Donor Cells (Reservoir Device) for Electroporation

Each number showing size is in mm.

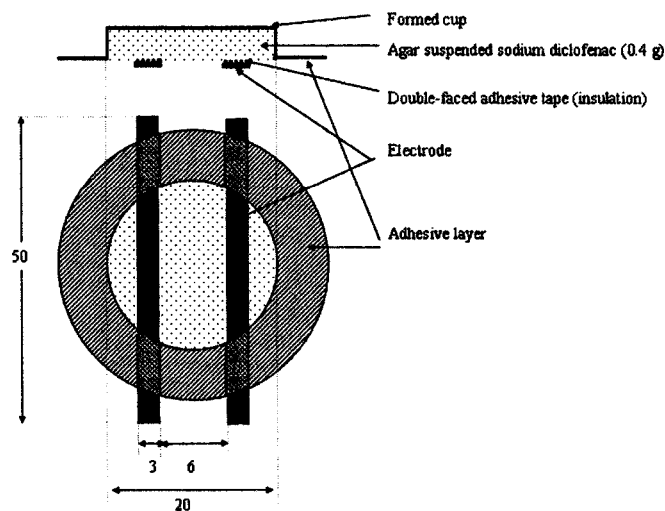


Fig. 2. Schematic Representation of Transdermal Patch with Agar Gel (Matrix Device) for Electroporation

Each number showing size is in mm.

diclofenac was found to be superior to other hydrophilic polymers such as hydroxypropyl cellulose, poly(vinyl alcohol) and carboxyvinyl polymers, from preliminary experiments.

Evaluation of Reservoir- and Matrix-Typed Formulations for Electroporation *In vitro* skin permeation of diclofenac was evaluated using the reservoir- and matrix-typed formulations. Figure 3 also shows the cumulative amount and flux of diclofenac permeated through the skin. The cumulative amount of the drug permeated through skin over 7 h was 504 ± 43 and $648 \pm 104 \mu\text{g}/\text{cm}^2$ for the reservoir (Δ) and matrix formulation (\square), respectively. The corresponding flux during the final sampling period (6–7 h) was 160 ± 11 and $152 \pm 19 \mu\text{g}/\text{cm}^2/\text{h}$, respectively. Interestingly, these values for the reservoir and matrix formulations were slightly higher

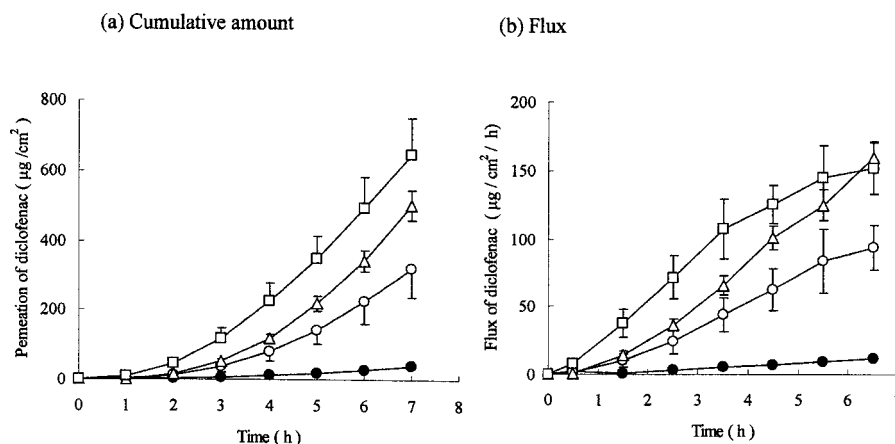


Fig. 3. Effect of Electroporation on the Permeation of Diclofenac from Its Simple Aqueous Solution and Reservoir and Matrix Formulations through the Hairless Rat Skin

●, simple aqueous solution without electroporation; ○, simple aqueous solution with electroporation; △, reservoir formulation with electroporation; □, matrix formulation with electroporation. Each data point represents the mean \pm S.E. of at least three measurements.

than those for the simple aqueous solution with electroporation ($321 \pm 82 \mu\text{g}/\text{cm}^2$ and $94 \pm 16 \mu\text{g}/\text{cm}^2/\text{h}$) as shown above, in spite of no significance. No marked morphological change in the reservoir and matrix formulation when checked after electroporation application. In addition, no clear skin damage was observed for the electroporation.

In general, complex practical formulations show a lower skin permeation than aqueous solutions. The present results shown in Fig. 3, however, revealed an opposite data. This is probably due to the slightly higher electric field in the skin barrier, stratum corneum, for the presently designed formulations than that for the aqueous solution. In the present formulations, the formulation side surface of the electrodes was electrically insulated. The electric current, therefore, was passed through the electrodes themselves that had a thickness of $40 \mu\text{m}$ and probably the hydrophilic region of the skin barrier at electroporation. Therefore, the effective electric field was mainly produced in the stratum corneum at electroporation. On the other hand, the diclofenac solution is easy to penetrate into the gap between the plate electrode and skin surface in the application experiments of the aqueous solution. Thus, a significant current was passed through the applied solution in addition to the stratum corneum to produce a lower electric field in the stratum corneum. Other application conditions of electroporation (*i.e.*, a higher voltage and/or a longer pulse period) could show a similar difference of the penetration-enhancing effects between the aqueous solution and reservoir- or matrix formulation. We previously reported that the electric field is a very important parameter for the penetration-enhancement of the skin permeation of drugs by electroporation.¹² We will discuss this in detail and quantitatively in a separate paper.

In conclusion, the great effect of electroporation was found on the skin permeation of diclofenac from an aqueous

solution as well as the reservoir- and matrix-typed dosage forms in the *in vitro* permeation study. The present studies also suggested that the present prototypic reservoir and matrix formulations are potent and useful topical formulations for electroporation application. Further trials for the formulation design and compact voltage generator will enable the clinical use of electroporation in the near future.

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