Transfer of Diclofenac Sodium across Excised Guinea Pig Skin on High-Frequency Pulse Iontophoresis. II. Factors Affecting Steady-State Transport Rate

Tamotsu KOIZUMI,* Masawo KAKEMI, Kazunori KATAYAMA, Hirohiko INADA, Kazuyoshi SUDEJI and Masao KAWASAKI

Faculty of Pharmaceutical Sciences, Toyama Medical and Pharmaceutical University, 2630 Sugitani, Toyama 930-01, Japan. *Received August 4, 1989*

Some of the factors affecting the steady-state transport rate of diclofenac sodium across excised guinea-pig skin during high-frequency pulse iontophoresis were examined. The same mathematical expression was employed for enhancement ratio as a function of applied voltage as the one derived for direct-current iontophoresis. The effective voltage drop across the skin was only 1.1% of the applied voltage. The steady-state flux value increased with increase of the donor concentration.

Keywords iontophoresis; pulse iontophoresis; diclofenac sodium; skin; applied voltage; donor concentration; guinea pig; steady-state flux

Establishment of iontophoresis as means for systemic drug delivery still requires extensive research to elucidate the mechanisms more thoroughly and to ensure that the skin is not burned or irreversibly altered in any way.[1)]

The equivalent circuit model proposed in the preceding paper[2)] facilitates mathematical expression of the voltage-drop time course across the skin or the donor solution. The aim of the present study was to identify some of the factors that affect the transport of diclofenac sodium (DF) through excised guinea-pig skin during high-frequency pulse iontophoresis.

Experimental

Materials DF was used as received from the manufacturer. Solutions were prepared with deionized water. To simplify the system, other electrolytes were not used in the donor solution. To aid preservation of the skin function, MEM culture fluid (pH 7.4, Nissui Seiyaku Co., Tokyo) was used as the acceptor solution.

Membrane All diffusion studies involved abdominal skin, freshly excised from 7- to 8-week-old, Hartley male guinea pigs (Shizuoka Jikkendobutsu Co., Hamamatsu) after removal of hair using electric hair clippers and an electric shaver.

Power Source The iontophoretic device (Advance, Tokyo) was conditioned to provide a pulse of 3 to 10 V at 40 kHz with 30% duty (7.5 μs) followed by a 70% depolarizing period (17.5 μs).

Diffusion Cell A schematic diagram of the diffusion cell utilized in these studies was shown in the previous report. Excised guinea pig skin was sandwiched between donor and acceptor chambers of horizontal diffusion cells, with the stratum corneum facing the donor compartment. The total area available for transport was 3.14 cm^2. The surface area of the platinum electrode was 0.5 cm^2. The donor and acceptor electrodes were positioned 7 and 17 mm from the skin surface, respectively. The cell halves were held together with a pinch clamp, and the entire assembly was maintained at 37 °C by immersion in a thermostatically controlled water bath. For the purpose of mixing, the acceptor solution was recirculated at 3 ml/min. At appropriate intervals, samples (1 ml each) were removed from the acceptor compartment and the concentration of DF was determined by high performance liquid chromatography (HPLC). The acceptor compartment was supplemented with MEM culture fluid immediately after sampling.

Assay Assay of DF was carried out by HPLC on a Shimadzu LC-6A apparatus equipped with a 4.0 mm × 15 cm stainless steel column packed with Merck Lichrosorb RP-18 (5 μm) and an absorbance detector set at 280 nm. The mobile phase (MeOH : acetonitrile : acetate buffer (0.02 M, pH 7.0) = 25 : 20 : 55) was pumped at 0.9 ml/min.

Calculations Least-squares parameter estimations were carried out using the MINSQ program (MicroMath Science Software, Salt Lake City) on an IBM PC computer.

Results and Discussion

Effect of Applied Voltage Cumulative amounts of DF appearing in the acceptor chamber were plotted as a function of time as shown in Fig. 1. Steady-state transport rates were calculated from the slope of the linear portions of these plots. As shown in Table I, a linear relationship was observed between steady-state transport rate and applied voltage. An exceptionally high flux value (not shown), obtained at 5.0 mg/ml donor concentration with an applied voltage of 12 V, probably implies skin damage.

In the study of direct-current iontophoresis, the enhancement ratio, *i.e.*, the ratio of steady-state flux with applied voltage $J(V)$ divided by steady-state passive flux $J(0)$ is expressed by Eq. 1.[3)]

$$J(V)/J(0) = KV/(1-\exp(-KV)), \quad K = zF/RT \tag{1}$$

where V is the voltage drop across the skin, z the charge on the drug ion, F the Faraday constant, R the gas constant and T the absolute temperature.

The data in Table I were obtained during pulse ionto-

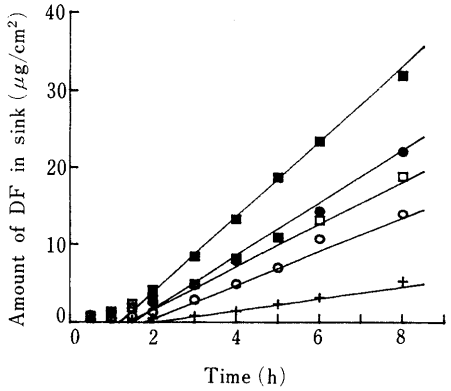

Fig. 1. Time Courses of Cumulative Amount of DF Appearing in the Acceptor Chamber

Donor concentration: 10.0 mg/ml. Applied voltage: +, 0; ○, 3; □, 5; ●, 7; ■, 10 V.

TABLE I. Steady-State Transport Rate of DF

Applied voltage	Steady-state flux (μg/h/cm^2) Donor concentration (mg/ml) 5	10
0	0.907 ± 0.219	0.927 ± 0.160
3	2.27 ± 0.26	3.16 ± 0.67
5	3.46 ± 0.53	5.39 ± 0.53
7	2.73 ± 0.21	6.37 ± 2.89
10	4.73 ± 0.29	9.00 ± 2.00

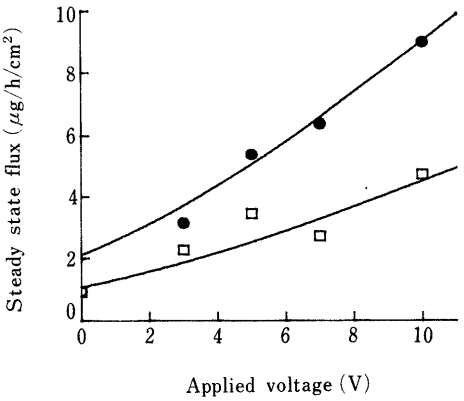

Fig. 2. Steady-State Flux as a Function of Applied Voltage
Donor concentration: □, 5.0; ●, 10.0 mg/ml.

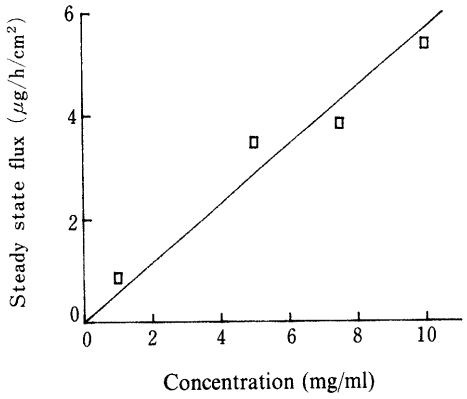

Fig. 3. Steady-State Flux as a Function of Donor Concentration (applied voltage: 5 V).

phoresis and, therefore, the voltage drop across the skin was not constant but undulatory, as shown in the previous report. For 30% of the period, the applied potential was charged on the skin and for the rest of the period, the skin was discharged. Nevertheless, least-squares adaptation of Eq. 1 to the data of Table I was attempted, using applied voltages for V. Calculated $J(V)$'s are shown in Fig. 2 as solid curves.

The K value obtained by least-squares adaptation was $0.42153\,V^{-1}$. Since the K value of Eq. 1 is $37.44\,V^{-1}$ at 37 °C, the effective voltage drop across the skin was only 1.1% of the applied voltage. In the previous report, it was estimated that the electrical resistance of the donor and acceptor solutions relative to the skin resistance was roughly unity. Consequently, the voltage drop across the skin was about 50% of the applied voltage, which was charged for 30% of the period. Thus, about 15% of the applied voltage is charged on the skin. The more than ten-fold discrepancy remains to be elucidated.

Effect of Donor Concentration With an applied voltage of 5 V, steady-state transport rates were plotted against donor concentration as shown in Fig. 3. The steady-state flux value increased with the increase of donor concentration.

Bellantone *et al.*[4] observed, in a study on direct-current iontophoresis, that increasing the concentration of sodium benzoate in the donor chamber produced a slight, apparently linear increase in benzoate flux and that the effect was relatively small over the concentration range examined: a 20-fold increase in benzoate content produced but a 1.7-fold increase in flux. Their explanation was that the fractional contribution of benzoate ion transport to the total current was increased with greater concentrations of sodium benzoate. Although the experimental conditions of the present study, *i.e.*, high-frequency pulse iontophoresis and constant applied voltage, were different from those of Bellantone *et al.*, *i.e.*, direct-current iontophoresis and constant current, the same explanation could apply.

References

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