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Iontophoretic permeation of sodium cromoglycate through synthetic membrane and excised hairless mouse skin

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Abstract—The iontophoretic transport properties of sodium cromoglycate were characterized using a synthetic membrane and excised hairless mouse skin. The permeation rate of sodium cromoglycate through the synthetic membrane was found to be linearly dependent on the density of electrical current applied. Passive diffusion through the excised hairless mouse skin was not demonstrated for sodium cromoglycate; however, under iontophoresis, an appreciable permeation was exhibited by the drug through the animal skin, which was also found to be a function of the electrical current density.

Sodium cromoglycate is used primarily in the prophylactic treatment of bronchial asthma (Altounyan 1967). Due to its highly polar properties, sodium cromoglycate has poor bioavailability when administered orally or by inhalation. Topical application of sodium cromoglycate was reported to be effective in the treatment of atopic eczema in children (Haider 1977). However, the highly polar nature of the drug makes it difficult for it to be absorbed through skin. Lipophilic prodrugs of cromoglycate have been developed to facilitate the topical absorption of such a polar drug (Bodor et al 1980).

Iontophoresis is a unique transdermal process by which ionic molecules may penetrate through skin. The iontophoretic technique involves transport of selected ions by passing a direct electrical current between a drug solution and the patient's skin using a selected electrode polarity (Banga & Chien 1988). Sodium cromoglycate is an ionic compound which has an aqueous solubility of 100 mg mL^{-1} at 20°C. Taking advantage of the highly polar structure of sodium cromoglycate, iontophoresis may be used to enhance the bioavailability of the drug through transdermal application. The main objective of the present study was to characterize the iontophoretic transport of sodium cromoglycate using a synthetic microporous membrane, and to investigate the effect of iontophoresis on the permeation of such a drug through the excised skin of hairless mice.

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Materials and methods

Materials. Sodium cromoglycate, USP was purchased from Sigma Chemical Company, MO, USA. Sodium chloride and potassium chloride, both reagent grade, were obtained from Merck & Co., NJ, USA. Microporous polyolefin membranes (MSX #640, void volume 60–70%) having pores (maximum effective pore diam. of 0·15–0·2 μm) filled with a hydrophilic urethane polymer and a mean thickness of 25·3 μm were provided by the Health Care Specialties Division/3M Center. MI, USA. The platinum wires (99·9% purity, 0·25 mm in diam.) and silver wires (99·9% purity, 0·5 mm in diam.) were obtained from Aldrich Chemical Co. Inc., WI, USA. Hairless female mice, homozygous, 56–63 days old, and weight 22 g were purchased from Sasco Inc., NE, USA.

Synthetic membrane permeation studies. A side-by-side glass diffusion cell (Crown Glass Co. Inc., NJ, USA) equipped with a plastic membrane holder, specially designed for iontophoresis, was used in all permeating studies. A constant stirring rate of 600 rev min^{-1} in both half-cells was provided using a revolving Teflon-coated magnet and an electrical drive unit. The orifice opening between two diffusion cells was 1·7 cm in diameter, which provides a membrane surface area of 2·27 cm^2, exposed to the solution in both donor and receptor cells. Each side of the diffusion cell contained 9·0 mL of solution. Oval-shaped platinum loops, formed using 10 mm × 5 mm (i.d.) platinum wires, were used as electrodes. A constant current was applied through the two electrodes from a constant current source (Keithley 224 programmable current source, Keithley Instrument, Cleveland, OH, USA). The cathode was placed in the donor cell which contained the sodium cromoglycate solution and the anode was inserted in the receptor cell which held the sodium chloride solution. The concentration of sodium chloride in both cells was 0·02 M. The synthetic membrane was soaked in deionized water at room temperature (21°C) overnight to allow complete hydration of the membrane before use. A 2·0 mL sample solution was taken from the receptor cell at 10 min intervals for 1 h. An equal volume of fresh receptor solution was replaced after

each sample withdrawal. The absorbance of the solution was measured at 329 nm using a UV spectrophotometer (Perkin-Elmer Lambda 3B). The concentration of sodium cromoglycate in the sample was determined by means of a calibration curve. All experiments were done in triplicate. For the passive diffusion studies, no electrode was placed in the diffusion cells and no electric current was applied.

Hairless mouse skin permeation studies. The skin from the abdominal surface of hairless mice, killed by CO_2 inhalation, was used in this study. The skin was removed from the animal by gently pulling the skin away from the carcass while cutting the loose connecting tissue with a scalpel. Following excision, any remaining fat and viscera on the dermal side of the skin was removed. Visual examination of the removed skin was done to assure that there was no disruption of the skin barrier. The isolated skin was placed in a glass scintillation vial and stored at −70°C until used. A silver/silver chloride electrode was used in this experiment to prevent any subsequent pH change of the solution which might affect the electrical properties of the animal skin. In preparing the silver/silver chloride electrodes, two silver coils were first soaked in a 1·0 M HCl solution for 10 min and then rinsed with deionized water. The cleaned silver coils were subsequently immersed in a 1·0 M KCl solution and connected to a constant current source with the current intensity set at 1·0 mA. The silver coils connected to the anode were then coated with a layer of silver chloride. The coating time was set for 12 h for each electrode. During the iontophoresis experiment the silver/silver chloride electrode was connected to the cathode and placed in the donor cell containing the drug solution facing the epidermal side of the skin. An uncoated silver coil was connected to the anode of the current source and placed in the receptor cell containing the sodium chloride (0·02 M) and the dermal side of the skin. A 1·0 mL sample was withdrawn from the receptor cell at hourly intervals for five hours. For the passive diffusion study, no electrode was placed in the cells and no electrical current was applied.

The concentration of sodium cromoglycate in the sample was determined by a reverse phase HPLC method. The instrument consisted of a single piston pump (Model LC-6A, Shimadzu), a 20 μL loop injector (Model RH-7215, Shimadzu), a variable UV detector (Model SPD-6A, Shimadzu) and an electronic integrator (Model CR601, Shimadzu). A Bakerbond C_{18} column (4·6 × 250 mm, Standard, J. T. Baker) and a mobile phase, composed of 75% (v/v) methanol and 25% 0·1 M acetate buffer at pH 4·8 were used for the HPLC determination of sodium cromoglycate in the sample solution. The flow rate was set at 1·0 mL min^{-1} and the wavelength of the detector was set at 329 nm. The relative retention time for sodium cromoglycate was 6·1 min. A standard curve in the range of 5–1000 μg mL^{-1} was constructed each day for the assay. The concentration of the drug in the sample was determined by comparing the peak area of the drug with the peak area of the external standard from the standard curve.

Results and discussion

Fig. 1 shows the linear regression plot of the passive and iontophoretic permeation (at 0·44 mA cm^{-2} electrical current density) of sodium cromoglycate (1 mg mL^{-1}) through an MSX #640 membrane. In spite of a very brief lag time associated with the passive permeation, both permeation processes reached the steady state within a relatively short time interval. The slopes of the two plots (Fig. 1) represent the passive and iontophoretic permeation rate of the drug through the membrane. Table 1 shows the effect of electrical current density on the iontophoretic permeation rate of sodium cromoglycate through the MSX membrane. An enhancing factor, which was calculated as the ratio between the iontophoretic and the passive permeation rate, is also shown in Table 1 for each electrical current density. The transmembrane permeation rate was shown to increase linearly ($r = 0.983$) with a slope of 0·496 μg cm^{-2} min^{-1} per mA cm^{-2} as the current density increases. The magnitude of permeation enhancement achieved by iontophoresis appears to be relatively

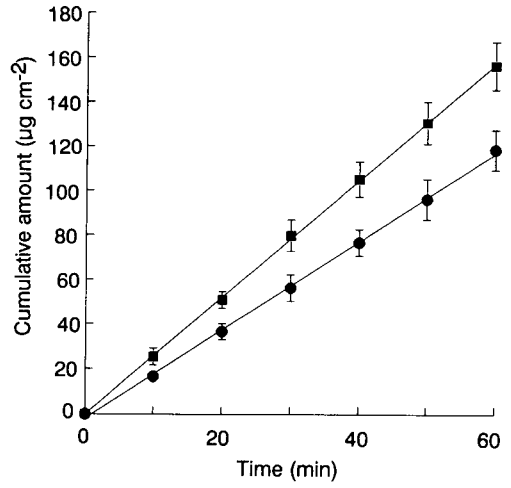

FIG 1. The passive and iontophoretic permeation of sodium cromoglycate through an MSX synthetic membrane. ●, Passive permeation; ■, iontophoretic permeation.

Table 1. The effect of electrical current density on the iontophoretic permeation of sodium cromoglycate (1·0 mg mL^{-1}) through the MSX synthetic membrane.

Current density (mA cm^{-2})	Permeation rate* (μg cm^{-2} min^{-1})	Enhancing factor
0·00	2·01 (0·02)	—
0·11	2·15 (0·22)	1·07
0·22	2·22 (0·17)	1·11
0·44	2·63 (0·17)	1·31
0·88	2·99 (0·13)	1·49

* Mean and standard deviation for three experiments.

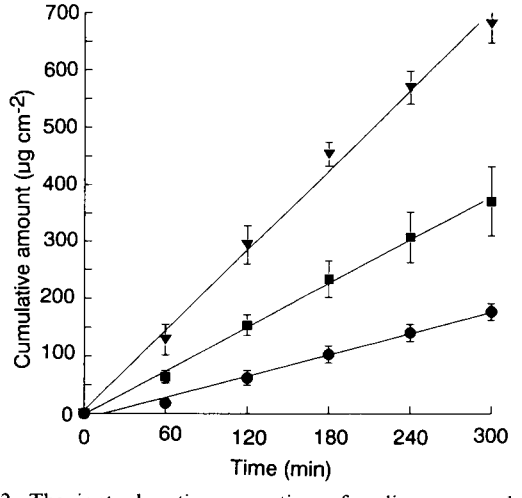

FIG 2. The iontophoretic permeation of sodium cromoglycate through hairless mouse skin. Electrical current density, 0·11 (●), 0·22 (■) and 0·44 (▼) mA cm^{-2}.

Table 2. The effect of electrical current on the iontophoretic permeation of sodium cromoglycate (1·0 mg mL^{-1}) through hairless mouse skin.

Current density (mA cm^{-2})	Permeation rate*
0·11	0·66 (0·03)
0·22	1·28 (0·25)
0·44	2·32 (0·16)

* Mean and standard deviation for three experiments.

low in the present case. This is probably due to the relatively high hydrophilic nature of the membrane used in this study which was shown to result in a relatively high passive transmembrane diffusion of the polar drug molecule (Table 1).

The concentration of sodium cromoglycate in the receptor cell during a 5 h passive diffusion experiment using hairless mouse skin was found to be undetectable using the HPLC method. This result indicates that the passive diffusion of sodium cromoglycate through a lipophilic biological membrane is limited due to its ionic nature. Fig. 2 depicts the iontophoretic permeation profiles of sodium cromoglycate through the mouse skin. It is apparent that the iontophoretic permeation of sodium cromoglycate through the mouse skin exhibited the same linear time-dependent profile as shown for the synthetic membrane permeation (Fig. 1). Table 2 shows the effect of electrical current density on the iontophoretic permeation rate of sodium cromoglycate through the mouse skin. At an electrical current density of 0·11 mA cm^{-2} a drug permeation rate of 0·66 μg cm^{-2} min^{-1} was obtained. The iontophoretic peremeation rate was shown to increase linearly as a function of the electrical current density ($r = 0{\cdot}999$) with a slope of 4·99 μg cm^{-2} min^{-1} per mA cm^{-2}. In comparison with the synthetic membrane permeation data (Table 1), it is evident that iontophoresis produced a more significant permeation enhancement of sodium cromoglycate through hairless mouse skin. Although the hairless mouse skin used in this study was not freshly prepared, and freezing and thawing may have had some effect on the permeability of the animal skin, skin with the same treatment was used in both passive and iontophoretic permeation studies; since the passive permeation data show that the hairless mouse skin was impermeable to sodium cromoglycate it can be concluded that the significant drug permeation enhancement by iontophoresis is attributable to the electrical current effect rather than to skin damage from freezing and thawing.

The results of this study show that iontophoresis facilitates the permeation of cromoglycate through an animal skin. This suggests that the application of this technique may improve the bioavailability of sodium cromoglycate in the prophylactic therapy of bronchial asthma or allergic skin disorders using sodium cromoglycate.

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