Iontophoresis-Enhanced Absorptive Flux of Polar Molecules Across Intestinal Tissue *In Vitro*

Maurice Leonard,[1] Elizabeth Creed,[1] David Brayden,[1] and Alan W. Baird[2,3]

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INTRODUCTION

Iontophoretic delivery of drug molecules (1,2) has been successfully demonstrated across various epithelia such as cornea (3) and stratum corneum (4). Until now, the intestinal epithelium has not been investigated as a site of modulation of drug absorption by iontophoresis. Epithelial cells lining the intestinal tract govern the rate of absorption of soluble drugs across the gastrointestinal tract. Systemic availability of hydrophilic and/or macromolecular drugs, particularly peptides, administered perorally is often too low to have any therapeutic effect (5). Drugs are transported across the intestinal epithelium by both passive and active mechanisms. The active transport methods involve carrier-mediated mechanisms or transcytosis whereas passive diffusion may occur via transcellular or paracellular routes (6). We used permeability co-efficient measurements to determine the influence of imposed electrical gradients on absorption of mannitol and the peptide drug, TRH.

MATERIALS AND METHODS

^{14}C-Mannitol (51.5 mCi/mmol) and ^{3}H-TRH (74 Ci/mmol) were purchased from NEN Life Science Products Ltd (Boston, USA). Forskolin and D-glucose were from Sigma (St. Louis, USA). All other chemicals were from Riedel de Haen (Seelze, Germany).

Male Wistar rats (250–300g) were bred and housed in a purpose-built facility following international guidelines for animal care. Animals were sacrificed and sections of isolated colonic mucosae were stripped of underlying smooth muscle layers and mounted between two identical halves of a Sweetana-Grass diffusion chamber (Precision Instruments, Tahoe City, USA) with an exposed window area of 0.65 cm^2 (7) and bathed (10 ml on either side) with physiological solution (113 mM NaCl, 4.7 mM KCl, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4.7H_2O$, 12.1 mM glucose, 25 mM $NaHCO_3$, 1.9 mM $CaCl_2.2H_2O$) and maintained at 37°C. Tissue was continuously gassed with 95% O_2-5% CO_2 to maintain the pH at 7.4, oxygenate tissue and provide a homogenous circulation of the bathing fluids by the gas-lift system.

Tissues were studied under open circuit conditions during basal, test and recovery periods, each of 20 minutes duration. During the test period the tissue was clamped by application of selected levels of charge transfer. Charge (Q) being transferred across the epithelium was calculated for each experiment, its value in equivalents is calculated by Q/F where Q = charge in coulombs [Q = current (amperes) * time (sec)] and F = Faraday constant (96,500). Ag/AgCl electrodes were used to pass and monitor current using a standard voltage clamp apparatus (World Precision Instruments) and the paracellular flux marker ^{14}C-mannitol (m.w. = 181) was used to indicate any absorptive flux enhancement. At time zero ^{14}C-mannitol (3.0 μCi) was added to the apical side of the diffusion chamber. Donor solution samples (100 μl) and recipient solution samples (1000 μl) were taken at 20 minute intervals which were replaced by 37°C physiological solution. ^{14}C-mannitol in the samples was determined using a liquid scintillation counting. The permeability co-efficient (Papp) was measured for mannitol for each sampling period using the following equation: Papp = (dc/dt)/(A.Ci) where dc/dt = transport rate, mol/sec. A = surface area of membrane, cm^2. Ci = initial concentration in donor chamber, mol (8). A simple summary of experimental design is given in Fig. 1.

Measurements of transepithelial electrical resistance (TEER) and short circuit current (SCC) were made using the Ag/AgCl voltage and current electrodes connected via bridges containing 3 M KCl agar gel. The voltage was clamped intermittently at 1 mV and the corresponding deflection in short circuit current used to calculate TEER by applying the Ohmic relationship.

Tissue capacity to respond to the application of the secretagogue (forskolin; 3 and 10 μM), was used to confirm tissue viability at the end of each experiment. Forskolin causes an inward rise in SCC due to net chloride secretion.

RESULTS

After an equilibration period of 20 minutes the permeability coefficient of mannitol absorption across sheets of rat colon *in vitro* was stable over time. For example, the Papp values determined, under open-circuit conditions (i.e. in the absence of applied current) over two consecutive 40 minute intervals were $7.1 \pm 1.3 \times 10^{-6}$ cm.s^{-1} and $6.3 \pm 0.7 \times 10^{-6}$ cm.s^{-1}; n = 5. Since similar values were obtained when mannitol flux was measured in the opposite (basolateral to apical) direction, these data support the hypothesis that mannitol movement across the tissue is due to passive transport. Electrophysiological parameters were also stable in these control experiments. Transepithelial potential difference was 0.35 ± 0.04 mV at the beginning and 0.33 ± 0.07 mV at the end of these experiments. Similarly, TEER values 56 ± 7 ohm.cm^2 (and) 55 ± 7 ohm.cm^2 at the beginning and end of the measurement periods was reproducibly stable; n = 5 throughout.

[1] Elan Pharmaceutical Technologies, Trinity College, Dublin 2, Ireland.
[2] Department of Pharmacology, University College Dublin, Belfield, Dublin 4, Ireland.
[3] To whom correspondence should be addressed. (e-mail: abaird@macollamh.ucd.ie)

ABBREVIATIONS: F, Faraday Constant; Papp, permeability coefficient; PD, potential difference; PKa, dissociation constant; Q, charge; SCC, short circuit current; TEER, transepithelial electrical resistance; TRH, thyrotrophin releasing hormone; Ω, ohms.

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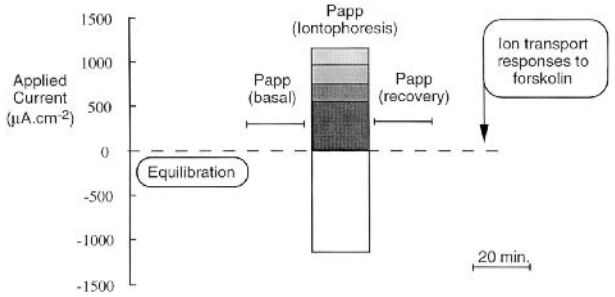

Fig. 1. Schematic illustration of the experimental design employed in these studies. Following an equilibration period, Papp was determined over three consecutive intervals (Basal, Iontophoresis and Recovery). Iontophoresis was performed by applying a range of currents (indicated by shaded bars during the second measurement period), each for 20 minutes. Positive SCC values indicate apical-side negative with respect to the basolateral compartment; the negative value represents 'reverse polarity. The amount of charge transfer was calculated by integration of current and time. At the conclusion of each experiment, tissues were voltage clamped to zero in order to measure SCC responses to the directly acting secretagogue forskolin.

In order to determine the effect of applied current upon mannitol flux, the influence of a range of currents upon Papp was measured (Table I). Mannitol flux was enhanced in response to current and, during a recovery period, the mannitol flux values reverted to levels which were similar to those of control tissues which had not been exposed to exogenous current. Potential difference and TEER values were stable at the beginning and end of each experiment (Table II).

The influence of reversing the polarity of the applied current on the absorptive flux of mannitol across rat colon was also investigated. Papp in the period immediately before application of reverse polarity (lumen cathode) current was $8.5 \pm 0.3 \times 10^{-6}$ cm.s^{-1} compared with $6.8 \pm 0.9 \times 10^{-6}$ cm.s^{-1} during exposure to 22.0 μEq.cm^{-2} of charge (Table I). Similarly, the reverse polarity conditions were without effect upon transepithelial resistance or forskolin-induced SCC responses.

We next applied the model to examine absorptive fluxes of thyrotropin releasing hormone (TRH) across rat colonic tissue. Papp for TRH absorption was $4.4 \pm 0.9 \times 10^{-6}$ cm.s^{-1} which was significantly higher during application of charge (22.0 μEq.cm^{-2}) over the sampling period ($8.6 \pm 0.6 \times 10^{-6}$ cm.s^{-1}). At a pH of 7.4 TRH (pKa = 6.2) is primarily uncharged but is slightly protonated. Therefore, the observed enhanced flux can be attributed to electro-repulsive and electroosmotic effects of the applied current. Following iontophoretic transfer of TRH, ion transport responses to forskolin were not statistically different from corresponding controls.

Table I. Permeability Coefficients for Mannitol and TRH During Passage of Charge Across Rat Colon *In Vitro*

Charge (μ-Eq. cm^{-2})	Mannitol Papp (cm.s^{-1}) × 10^6	TRH Papp (cm.s^{-1}) × 10^6
0	8.5 ± 1.4 (5)	4.4 ± 1.0 (6)
10.5	8.8 ± 1.2 (4)	
14.3	10.0 ± 1.2 (4)	
18.2	12.6 ± 1.8 (4)	
22.0	13.0 ± 0.9[a] (4)	8.6 ± 0.7[a] (6)
−22.0[b]	6.8 ± 0.9 (4)	

[a] = $p < 0.05$ when compared with values obtained from open circuit experiments by Mann-Whitney test.
[b] Influence of reversed polarity (lumen cathode). Numbers of tissues used in each group is given in parenthesis.

Table II. Transepithelial Electrical Resistance Values (A) and Potential Difference Values (B) Were Not Significantly Altered Even During Passage of the Highest Iontophoretic Current Used in These Studies

A. TEER (Ω.cm^2)

	Open circuit (n = 11)	Iontophoresis 22.0 μEq. cm^{-2} (n = 10)
Pre-iontophoresis	63.9 ± 5.9	64.0 ± 7.5
During iontophoresis	67.2 ± 7.8	50.2 ± 6.2
Post-iontophoresis	72.7 ± 9.6	67.5 ± 9.9

B. Potential difference (mV).

	Open circuit (n = 11)	Iontophoresis 22.0 μEq. cm^{-2} (n = 10)
Pre-iontophoresis	1.8 ± 1.1	1.6 ± 0.5
Post-iontophoresis	2.9 ± 1.3	1.6 ± 0.6

At the end of all experiments, tissues were voltage-clamped to zero potential difference by the application of SCC which was continuously monitored. Ion transport responses to the secretagogue forskolin (3 μM and 10 μM) were 34.5 ± 8.3 μA.cm^{-2} and 90.3 ± 32.3 μA.cm^{-2} respectively (n = 11) which were not significantly different when compared with values obtained in tissues which had been exposed to the highest current (22 μEq.cm^{-2}) which were 34.6 ± 20.3 μA.cm^{-2} and 64.9 ± 26.5 μA.cm^{-2} respectively (n = 10).

DISCUSSION

Iontophoresis enhances the delivery of solutes by three mechanisms: electrorepulsion, electroosmosis and electrically induced permeability changes. The establishment of an electrical potential gradient across a tissue can be expected to attract cations from the cathode and anions from the anode as a result of electrostatic repulsion (9). However, a neutral polar compound such as mannitol does not undergo flux enhancement by electrorepulsion but rather as a direct influence of the electroosmotic effect on the convective solvent flow that occurs during iontophoresis. This is reflected in the findings for mannitol. There is a significant enhancement in absorptive flux seen in the anode-to-cathode direction whereas there was no significant influence in flux when polarity was reversed. This electroosmotic effect has been seen for mannitol in other experimental iontophoretic studies (10,11) and is explained by the fact that current is preferentially carried by cations. Under physiological conditions, the intestinal lumen is electrically negative with respect to blood, thus the counterions (i.e. cations) are preferentially attracted into the barrier. In moving under the influence of the applied electric field, the ions collide with the surrounding solvent molecules and transfer a fraction of their momentum. A greater momentum is transferred to the solvent by the cationic mobile species than by the counter anions. As such, there is a net convective flow of volume across the membrane during iontophoresis in the anode-to-cathode direction and consequently an enhanced transport of dissolved polar uncharged

solutes in the same direction but not in the cathode-to-anode direction (12,13). The application of current had no degradative effect on mannitol which was analysed by Nuclear Magnetic Resonance (NMR) spectroscopy (results not shown).

The establishment of a charge-response relationship for electroosmotic flow in the anode to cathode direction (Table I) has illustrated a direct linear dependency between convective transport and current density. It was found that this observed effect could be used to enhance the delivery of a pharmacologically active peptide such as TRH across the colonic epithelial barrier.

Membrane alterations may be induced by the passage of current and this is indicated by the significant change in resistance observed at the time of application of current. Ions move along the pathways of lowest electrical resistance. If pathways are current limited then an increase in transepithelial voltage and/or higher current density might reasonably be expected to increase the amount of material flowing via less resistive pathways. Resistance is often used as a reflection of tight-junction permeability since the tight junction is considered to be rate limiting to paracellular solute movement (14). The slight reduction in TEER during the application of current accompanied by a significant rise in permeability of TRH suggests that the iontophoretic pathway may include the paracellular route. This is supported by a return to normal (basal) resistance values during the recovery period (Table II). However, measuring resistance values as an index of paracellular permeability is an over simplification and there is not always a direct correlation between electrical resistance and permeability to marker molecules (15).

Regarding viability of the tissues, no specific evaluation of tissue damage was performed. However, electrogenic ion transport responses to forskolin, even after application of electrophoretic current, were of a magnitude which was not different from responses obtained in control preparations and are typical of the maximal ion transporting capacity of voltage-clamped rat colon *in vitro*.

These preliminary experiments using intestinal tissue *in vitro* are encouraging in that mannitol and TRH absorption were effectively doubled using this process. As such, our experiments provide proof of the principle that electrophoretic manipulations can modify intestinal absorption of drugs that have otherwise low bioavailability after oral administration. Translation of the idea into a functional apparatus of practical value for drug delivery remains a challenge for future investigations.

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