JPP
Journal of
Pharmacy and Pharmacology

JPP 2010, 62: 721–729

Received October 30, 2009
Accepted February 9, 2010
DOI 10.1211/jpp/62.06.0008
ISSN 0022-3573

Research Paper

Electroosmotic transport of mannitol across human nail during constant current iontophoresis

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Abstract

Objectives This work aimed to elucidate the role of electroosmosis during trans-nail iontophoresis.

Methods Passive and iontophoretic experiments were performed after short hydration (10–15 min) of human nail tips. The electroosmotic fluxes of mannitol were determined during anodal and cathodal iontophoresis and at different pH values. Passive controls were also carried out. Four sets of experiments were performed: (a) three anodal delivery experiments using different nails, at pH 4.0, 5.0 and 7.4, (b) one anodal delivery experiment that kept the same nails across two pH stages, (c) one experiment, comprising an anodal delivery stage (pH 4 and 7.4) followed by a cathodal delivery stage (pH 7.4 and 4), which kept the same nails across the different polarities and pH stages, and (d) a passive experiment keeping the same nails across different pH values (4 and 7.4).

Results The fluxes of mannitol measured were very variable and little difference between passive and electroosmotic transport was observed. Cathodal and anodal fluxes were not always significantly different. Experiments which minimised internail variability suggested that the nails were negatively charged at physiological pH, and that this negative charge was lost at pH 4.

Conclusions These results suggest a modest and highly variable contribution of electroosmosis to the iontophoretic transungual flux.

Keywords electroosmosis; iontophoresis; mannitol; nail; permselectivity

Introduction

The treatment of onychomycosis, which affects 2–13% of the population,[1] is long and complicated due to the adverse effects and drug interactions resulting from systemically administered antifungal drugs.[2] Topically applied therapies are advantageous but have limited efficacy,[3] probably because they fail to deliver sufficient drug to the nail bed where the onychomycosis lies. Different approaches have been considered to enhance drug topical penetration ,[4,5] including iontophoresis, which has received increasing attention lately.[6–10]

Iontophoresis is driven by two main forces, electrorepulsion and electroosmosis. Electrorepulsion results from the interaction of charged molecules with electrodes while electroosmosis is a current solvent flow resulting from the permselective properties of the membrane.[11] Electroosmosis is the only mechanism of transport available for uncharged and zwitterionic molecules. The skin is negatively charged, and therefore permselective to cations at physiological pH. An electroosmotic solvent flow is induced when an electric field is imposed across a permselective membrane; this solvent flow occurs in the same direction as the preferential passage of counterions.[12] Thus, for a negatively charged membrane the electroosmotic flow occurs in the anode-to-cathode direction. The volume flow J_V (volume per unit time per unit area) is predicted to be proportional to the potential gradient established by the electric field, and the molar flux of a solute J_j present at concentration c_j is then $J_j = J_V c_j$.[12]

In the case of transdermal iontophoresis, the electroosmotic contribution to overall transport is much less than the electrorepulsive one, although it is responsible for the transport of uncharged polar molecules and its contribution to the transport of high molecular cationic molecules is not negligible. In the case of transungual iontophoresis the role of the electroosmotic contribution is not completely understood. However, given the physicochemical (molecular weight, ionisation and lipophilicity) properties of antifungal

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drugs it is important to establish whether electroosmosis could contribute to their transport by iontophoresis.

The direction of the electroosmotic flow is directly related to the membrane's charge and does not always occur in the same direction of transport as that of the main charge carriers. For example, the transdermal electroosmotic flow of mannitol remained in the anode-to-cathode direction during experiments in which the lidocaine hydrochloride transport number was 0.1 and the counter ion, chloride, was the main charge carrier.[13] Thus the measurement of electroosmotic flux is crucial to determine the charge of a membrane, and indeed this issue has been investigated in previous work, using different electroosmotic markers and experimental conditions. Murthy *et al*.[9] measured the cathodal and anodal electroosmotic transport of glucose at different pH values across nail tips from human volunteers. The nail tips were pre-hydrated for 6 h before a 0.125 mA constant current was applied for 10 h. These experiments showed the anodal flux to be approximately five to six times the cathodal flux at pH 7 and the cathodal flux to be approximately five to six times the anodal flux at pH 2–3. Both fluxes were equivalent at pH 5, suggesting the isoelectric point of nails to be around 5. These results suggest the nails are negatively charged at physiological pH and have similar properties to the skin, such as being cation permselective and presenting an electroosmotic flux in the anode-to-cathode direction.[14] The iontophoretic transport of griseofulvin (weak acid, pKa ~ 11) also supports this suggestion.[9] For example, the highest fluxes of griseofulvin at pH 3 and 7 were obtained using cathodal and anodal iontophoresis respectively, again indicating that electroosmosis occurs in the cathode-to-anode direction at pH 3 (nail positively charged) and in the anode-to-cathode at pH 7 (nail negatively charged). Griseofulvin fluxes were about a half of those reported for glucose, which could be due to the drug's higher molecular weight, increased lipophilicity or its extensive binding to keratin.

However, this six-fold enhancement of glucose fluxes, caused by the application of 0.125 mA iontophoresis, and the unambiguous distinction between anodal and cathodal fluxes, contradicts other data. Hao *et al*. performed mannitol and urea iontophoresis, with and without chemical enhancers, across cadaver nail plates, which were hydrated for 24 h prior to experiments that were longer.[6–8] A first study found that anodal iontophoresis enhanced mannitol and urea transport compared to passive diffusion, although the level of enhancement was quite moderate (1–1.4-fold at 0.1 mA) and (0.8–2.8-fold at 0.3 mA).[6] Subsequent work by the same group found no difference between the passive and the anodal transport of mannitol, an observation which, according to the authors, was due to the presence of tetraethyl ammonium chloride in the donor solution.[7] In a third paper, the results indicated that anodal and cathodal delivery of mannitol is dominant at pH 7 and pH 3, respectively, and that an inverse relationship exists between mannitol delivery and ionic strength; it was concluded that electroosmosis provides a moderate contribution to the overall transport during transnail iontophoresis.[8] Unfortunately, Hao *et al*.[6–8] reported their results as permeability coefficients, enhancement factors and Peclet numbers, which limited their direct comparison with the fluxes reported by Murthy.[9]

It follows that nail permselectivity has not been demonstrated unambiguously and the aim of the work here reported was to further investigate this phenomenon. In line with Hao *et al*.[6–8] and numerous skin studies[13,14] we have used mannitol, a usual marker for electroosmosis, which is a neutral, polar and exogenous sugar having a molecular weight close to that of glucose. In a key difference to previous work, we have limited prior hydration of the nail to a maximum of 15 min, expecting this short hydration to mimic more closely a practical application of transungual iontophoresis. Secondly, we were interested in the effect of internail variability on electroosmotic fluxes and, ultimately, on the performance of iontophoresis as a nail drug-delivery technique. Indeed, previous work has also differed in the degree of variability observed.[6–10] To this end, the passive, anodal (anode-to-cathode) and cathodal (cathode-to-anode) electroosmotic flux of mannitol were measured at pH 4.0, 5.0 and 7.4. The significance of internail variability was also considered and integrated into the experimental design.

Materials and Methods

Materials

Mannitol, sodium chloride, HEPES free acid, hydrochloric acid and sodium hydroxide were obtained from Sigma Aldrich Co. (Gillingham, UK). ^{14}C-mannitol was obtained from Amersham plc (Little Chalfont, UK). Ultima Gold XR, Hionic Fluor and Solvable were obtained from Perkin Elmer (Waltham, MA, USA). Silver wire, silver chloride powder and platinum wire, used to prepare the electrodes, had a minimum purity of 99.99% and were obtained from Sigma Aldrich Co. (Gillingham, UK). All aqueous solutions were prepared using high-purity deionised water (18.2 MΩ.cm, Barnstead Nanopure Diamond, Dubuque, IA, USA).

Nail tips

Ethical approval was granted by the Bath Local Institutional Review Board and fingernail clippings were obtained from 14 healthy volunteers who gave their written informed consent. The harvested nails were washed with deionised water and kept at room temperature in a desiccator until use. Prior to an experiment, the nail's thickness was measured with a point micrometer (Point Anvil Micrometer, Mitutoyo, Andover, UK) on the portion of the nail that was the closest to the hyponychium. Next, each nail was soaked in deionised water for 10–15 min in order to recover some flexibility, and then placed in 5 mm nail adapters (PermeGear Inc., Bethlehem, PA, USA).

Mannitol iontophoresis

Mannitol experiments were performed in Franz vertical diffusion cells (PermeGear Inc., Bethlehem, PA, USA), presenting a transport area of 0.2 cm^2. To facilitate sampling of the receptor solution, the bottom chamber was used to hold the donor solution while the receptor was placed on the top. The nail adapter was placed so the nail's dorsal layer faced the donor. Experiments were performed with solutions adjusted to an identical pH in both chambers: 4.0, 5.0 or 7.4. To fill the donor chamber (3.6 ml), a 10 mM mannitol solution added to

154 mM NaCl was prepared in HEPES 25 mM. This solution was spiked with 40–50 μl of ^{14}C-mannitol (200 μCi/ml, equivalent to 7.4 Bq/ml) to result in 2.5–3 μCi/cell (around 0.1 Bq/cell). The receptor chamber (700 μl) contained 25 mM of HEPES and 154 mM of NaCl.

Both the receptor and donor chambers of each cell were initially filled with 'cold' (receptor) HEPES buffer at the appropriate pH and, cell by cell, a 0.2 mA current was applied (Kepco APH 1000M, Flushing, NY, USA) until the voltage reached a value close to 30–40 V (~30 min/nail). It was not possible to connect the three cells in series initially as the resulting voltage would exceed the limit of 200 V set on the power supply. This was due to the nails' initial high resistance to the current. Next, the chambers were filled with the appropriate 'hot' donor and 'cold' receptor solutions, and the current (0.2 mA) was started. Homemade Ag/AgCl electrodes were used.[15] Four sets of experiments were performed as described in Figure 1. Set I comprised three independent experiments for study of the effect of pH (4, 5 and 7.4). This experiment suggested the effect of internail variability on mannitol fluxes to exceed that of the other two factors (pH and polarity of delivery) under investigation. Thus, the next sets (II–IV) comprised experiments that modified either pH or polarity sequentially on the same nails. In experiments comprising several pH stages, the donor and receptor solutions were rinsed three times and filled with the new buffer solution before proceeding with the next stage. At the very end of each experiment, the donor and receptor were also rinsed three times with some receptor solution at the appropriate pH. In all cases three replicates were performed.

Passive experiments were performed in the same way except that no current was applied.

Mannitol quantification

The complete receptor solution was sampled at the indicated times (Figure 1) and the chamber refilled with fresh receptor buffer. Each receptor sample was mixed with 15 ml Ultima Gold XR before being assessed for its ^{14}C-mannitol content on a Liquid Scintillation Analyzer Tri-Carb 2800TR equipped with QuantaSmart 4.01 software (Perkin Elmer, Waltham, MA, USA). At the end of each experiment, nails were carefully removed from their adapters, placed in preweighed glass scintillation vials and weighed. Five millilitres of Solvable was added to each nail and the vials were subsequently placed in an oven (Heraeus Function Line, Weiss-Gallenkamp, Loughborough, UK) at 60ºC for nail digestion. The vials were taken out of the oven on complete digestion of the nails (minimum 6 h) and 10 ml Hionic Fluor scintillation cocktail were added once they were at room temperature. The nails' content of mannitol was assessed by liquid scintillation counting (LSC). Appropriate background samples were prepared for each experiment. Three 700 μl samples of buffer solution were mixed with 15 ml of Ultima Gold XR to obtain an average radioactivity background, which was then subtracted from the values for receptor samples. Five millilitres of Solvable combined with 10 ml Hionic Fluor scintillation cocktail was used as background counting for digested nails. Three reference samples were prepared by mixing 100 μl of the spiked donor solution with 15 ml of Ultima Gold XR. The LSC of these references

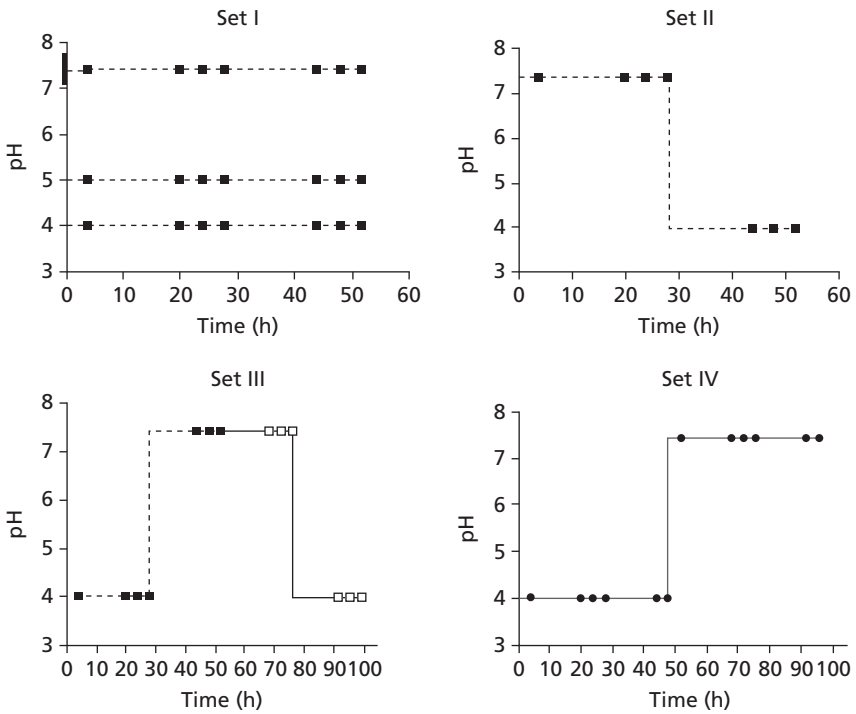

Figure 1 Sequence followed in mannitol experiments. Each line represents one experiment, which used three nails. A dotted line (■) means the mannitol delivery occurred in the anode-to-cathode direction, a black continuous line (□) indicates cathode-to-anode delivery and a light gray continuous line (●) means passive diffusion. Square and circle symbols indicate the sampling times

provided the ratio of hot-to-cold mannitol in the donor solution and allowed estimation of the equivalent cold mannitol present in the samples.

Mannitol binding study

The possible binding of mannitol to the nail material was studied. Three nails (A, B and C) were cut into three pieces (A1, A2, A3; B1, B2, B3; C1, C2, C3). Each piece was weighed and placed for 24 h in a vial containing 2 ml of a 10 mM mannitol solution in a pH 7.4, 25 mM HEPES buffer spiked with ^{14}C-mannitol (200 μCi/ml equivalent to 7.4 mBq/ml). After 24 h, all of the pieces were taken out of the mannitol solution and rinsed twice in 5 ml of a 25 mM HEPES buffer at pH 7.4. Samples A1, B1 and C1 were then mixed with Solvable and placed in the oven for nail digestion and subsequently 10 ml Hionic Fluor were added prior to LSC analysis. The remaining nail samples (A2, A3; B2, B3; C2, C3) were placed in individual vials containing 5 ml of HEPES 25 mM for 1 week. The buffer solution was completely refreshed in the middle of the week. At the end of the first week, the samples A2, B2 and C2 were prepared for digestion and LSC analysis as previously described. Samples A3, B3 and C3 were left in 5 ml of HEPES 25 mM for one more week. The buffer solution was replaced at the end of the first week and in the middle of week two. Finally, samples A3, B3 and C3 were prepared for digestion and LSC analysis at the end of the second week. The rinsing and buffer solutions were also analysed by LSC after addition of 15 ml Ultima Gold.

Data analysis and statistics

All data are presented as mean and standard deviation of at least three replicates. Linear regressions and statistics were performed using GraphPad Prism V5.00 (GraphPad Software Inc., San Diego, CA). When relevant, sets of data were compared by a one-way ANOVA followed by a Tukey's post-test and by a paired *t*-test. Alternatively, non-parametric tests (Kruskall–Wallis followed by Dunn's post-test and Wilcoxon paired *t*-test) were also used due to the small number of replicates. The slopes obtained from the mannitol amounts for each nail were compared at each pH and condition by using the comparison tool in the GraphPad Prism V5.00 software.[16] The level of statistical significance was fixed at $P < 0.05$. All linear regressions were significant ($P < 0.05$) unless otherwise indicated.[16]

Results

Mannitol transport experiments

Set I experiments

Set I comprised three independent experiments which lasted for 52 h (Figure 1). Mannitol was always delivered from the anode. The nails used during these experiments came from female participants (21–44 years old). The average thickness of the nails used at pH 4, 5 and 7.4 was 290 ± 20 μm, 290 ± 80 μm and 220 ± 20 μm, respectively.

Figure 2 shows the cumulative amounts of mannitol delivered to the receptor solution for each nail and pH plotted against time (Figure 2). The total amount of mannitol delivered at each pH stage is shown in Table 1. All the slopes of the linear regressions were significant and were used to estimate an average flux for each nail and pH.

First of all, it was observed that the data were very variable (Figure 2, Table 1). A test for comparison of the slopes[16] indicated that the three slopes (i.e. fluxes) obtained for each pH were significantly different. This variability was particularly manifest for the experiments performed at pH 5 and 7.4. Secondly, the fluxes measured at pH 7.4 (0.43 ± 0.26 nmol/h) were clearly the lowest of the set. An ANOVA indicated that the flux at pH 7.4 was statistically different to that measured at pH 4.0 (5.48 ± 1.46). This trend was unexpected and is in complete contradiction to previous data and to the physicochemical properties of keratin.[4,8,9] Wide internail variability has been described previously,[6–8] although not quantitatively reported. It was then hypothesised that the experiments should be implemented in such a way that internail variability could be separated from the experimental error, thus allowing an improved investigation of the effects of pH and polarity on the delivery.

Set II experiments

All the nails used in this experiment came from the middle finger of female participants (26–44 years old). Their average thickness was 270 ± 10 μm. Mannitol was delivered from the anode at pH 7.4 over 28 h, after which the donor and receptor solutions were replaced by equivalent solutions at pH 4 (Figure 1). The anodal iontophoresis of mannitol at pH 4 was then studied for another 24 h (28–52 h). The cumulative mannitol delivered for each pH stage is shown as a function of time in Figure 3. The fluxes for each nail and pH stage

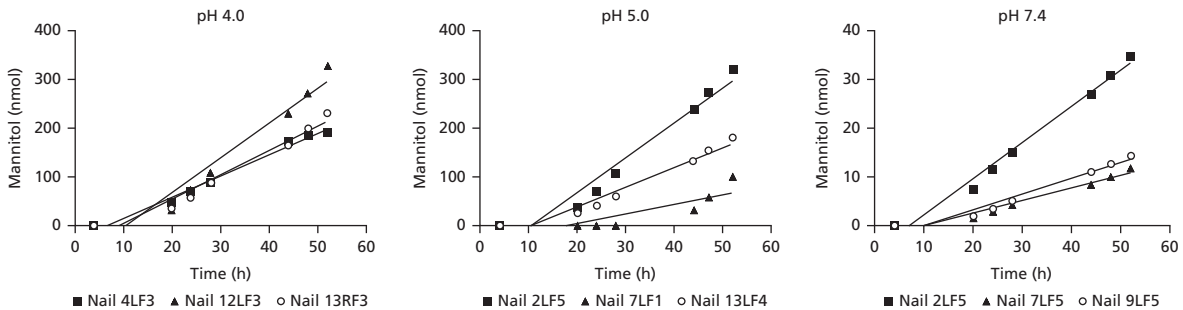

Figure 2 Cumulative amount of mannitol delivered by anodal iontophoresis for each nail and pH during set I experiments. Dotted lines are linear regressions which were all significant. The first digit in the nail code indicates the participant number, L or R indicates left or right hand and F+digit indicates the finger, where F1 is the thumb

Table 1 Total mannitol delivered during the different stages of sets I–IV experiments

	Set I		
	Mannitol delivered (nmol)		
	Anodal pH 4.0	Anodal pH 5.0	Anodal pH 7.4
Nail	192.5 (4LF3) [270]	323.6 (2LF5) [210]	34.4 (2LF5) [220]
Nail	327.8 (12LF3) [290]	101.2 (7LF1) [360]	11.7 (7LF5) [200]
Nail	231.1 (13RF3) [310]	182.4 (13LF4) [290]	14.4 (9RF5) [230]
Mean±SD	250.5±69.7	202.4±112.5	20.2±12.4

	Set II	
	Mannitol delivered (nmol)	
	Anodal pH 7.4	Anodal pH 4.0
2LF3 [270]	44.7	20.7
4RF3 [280]	82.5	19.4
7LF3 [260]	24.0	12.3
Mean±SD	50.4±29.7	17.5±4.5

	Set III			
	Mannitol delivered (nmol)			
	Anodal		Cathodal	
	pH 4	pH 7.4	pH 7.4	pH 4.0
2RF5 [220]	0.96	2.51	2.05	2.86
4LF5 [240]	0.59	0.96	1.31	2.38
13LF5 [240]	0.45	0.89	1.41	2.50
Mean±SD	0.67±0.27	1.45±0.91	1.59±0.40	2.58±0.25

	Set IV – Passive	
	Mannitol delivered (nmol)	
	pH 4.0	pH 7.4
2RF4 [200]	1.92	8.43
4RF4 [240]	0.67	6.45
12RF4 [270]	1.89	8.22
Mean±SD	1.49±0.71	7.70±1.08

Set I comprised three independent experiments each using three different nails. Sets II–IV used three nails each and the pH and polarity were varied sequentially (see Figure 1 for detailed explanation). The nail code provides information about the source for each nail: the first digit in the nail code indicates the participant number, L or R indicates left or right hand and F+digit indicates the finger, where F1 is the thumb. The values in brackets correspond to the thickness of the nail in micrometres.

were obtained from the slope of the respective linear regressions, which were all significant[16] with the exception of nail 2LF3 at pH 4.0. The total amount of mannitol delivered at each pH stage is shown in Table 1.

Again, an important variability was observed at pH 7.4, namely that the three slopes were significantly different; no differences were found between the three slopes corresponding to the pH 4.0 stage. Decreasing the pH from 7.4 to 4.0 clearly reduced mannitol delivery for each nail (Table 1), but no statistically significant differences were found between the mean total mannitol delivery at different pH values (Table 1, Wilcoxon paired test) or between the average mannitol flux at pH 7.4 (1.91 ± 1.04 nmol/h) and at pH 4.0 (0.44 ± 0.13 nmol/h). Nevertheless, the trend observed in Set II was more consistent with a net negative charge for the nail at pH 7.4 and with what is known about keratin (pI ~ 5) and previous data.[4,6–9] Anodal mannitol fluxes slightly decreased (although not to the level of statistical significance) as the bathing solutions become more acidic, indicating the loss of negative charge in the nail. The variability observed at pH 7.4 suggests that the factors determining the magnitude of the convective flow (for example the amount of fixed charge in the membrane and the geometry of the pathway of penetration[12]) may differ considerably among different nails.

Set III experiments

The nails used during this experiment came from the little finger of female participants (21–44 years old) and their average thickness was 230 ± 10 μm. This experiment comprised four stages (Figure 1). Mannitol was delivered from the anode at pH 4.0 during the first stage (0 to 28 h) and at pH 7.4 (28 to 52 h) during the second one. The polarity of mannitol delivery was switched at 52 h and mannitol was subsequently delivered from the cathode at pH 7.4 (52 to 76 h) in a third stage and at pH 4.0

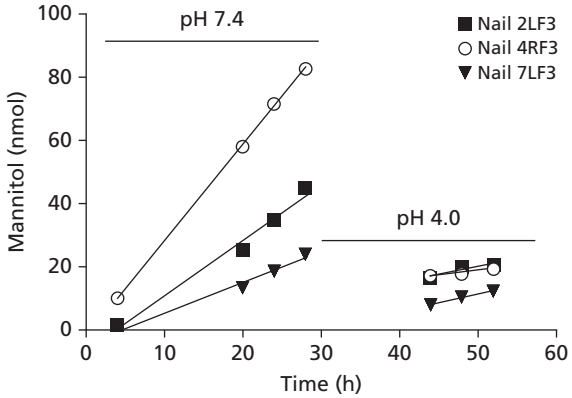

Figure 3 Cumulative mannitol delivered by anodal iontophoresis during the pH 7.4 and pH 4.0 stages of set II experiments. Dotted lines are linear regressions. The linear regression of nail 2LF3 at pH 4.0 was not significant. The first digit in the nail code indicates the participant number, L or R indicates left or right hand and F+digit indicates the finger, where F1 is the thumb

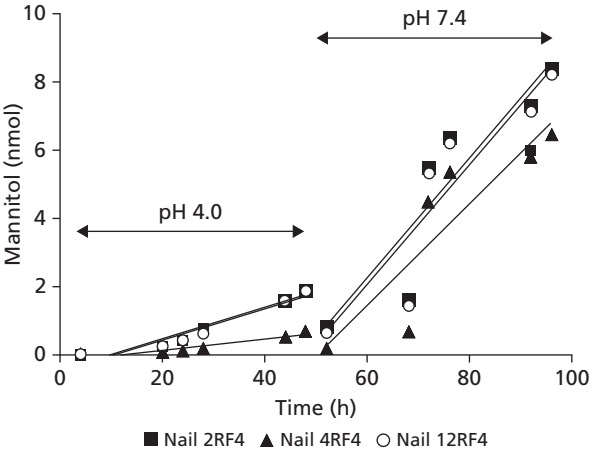

Figure 5 Effect of pH on the passive transport of mannitol across the nail during set IV. Dotted lines are linear regressions which were all significant. The first digit in the nail code indicates the participant number, L or R indicates left or right hand and F+digit indicates the finger, where F1 is the thumb

(76 to 100 h) in a final fourth stage. The amount of mannitol delivered for each pH and polarity is shown as a function of time in Figure 4 and the fluxes for each nail and stage were obtained from the slope of the respective linear regressions. Again the variability was higher for anodal delivery at pH 7.4 but formal comparisons of the slopes were not performed in this case because many slopes were not significant (see Figure 4).[16] The average fluxes show the expected trend: anodal delivery increases from 0.02 ± 0.01 nmol/h at pH 4 to 0.11 ± 0.07 nmol/h at pH 7.4 and cathodal delivery decreased from 0.15 ± 0.02 nmol/h at pH 4 to 0.06 ± 0.01 nmol/h at pH 7.4. However, a non-parametric paired *t*-test (Wilcoxon *t*-test) could only show statistically significant differences for the cathodal fluxes.

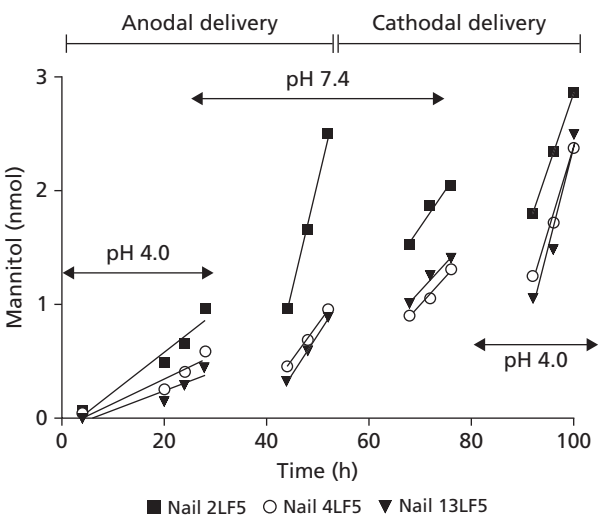

Figure 4 Effect of pH and polarity on the cumulative mannitol delivered across the nail during set III experiments. Dotted lines are linear regressions. The following regressions were not significant: anodal delivery: 13LF5 at pH 4.0 and cathodal delivery: 2RF5 at pH 7.4, 4LF5 at both pHs and 13LF5 at both pHs. The first digit in the nail code indicates the participant number, L or R indicates left or right hand and F+digit indicates the finger, where F1 is the thumb

Passive diffusion experiments

The nails used during this experiment came from the middle finger of the right hand of female donors (22–44 years old). Their average thickness was 240 ± 40 μm. The passive diffusion experiment had a total duration of 96 h and comprised a first stage at pH 4.0 (0 to 48 h) and a second stage at pH 7.4 (48 to 96 h) (Figure 1). The cumulative mannitol delivered during each pH stage as a function of time is shown in Figure 5. The fluxes for each nail and pH were obtained from the slope of the respective linear regressions, which were all significant.[16] In this case the internail variability was greater at pH 4 as the slopes at this pH were significantly different; there were no differences observed among the slopes at pH 7.4. The passive fluxes were significantly higher (paired *t*-test) at pH 7.4 (0.17 ± 0.02 nmol/h) than at pH 4.0 (0.03 ± 0.02). The total amount of mannitol delivered during the passive experiments is reported in Table 1. The cumulative amount of mannitol delivered at pH 7.4 for each nail was higher than at pH 4.0. However, a Wilcoxon paired *t*-test did not reveal any statistical differences between the two means.

Mannitol binding experiments

The amount of mannitol accumulated into the nail just after 24 h of exposure and after 1 or 2 weeks of washing periods was measured. The average nail content in mannitol was 1.77 ± 0.47 nmol/mg, 0.46 ± 0.22 nmol/mg and 0.13 ± 0.04 nmol/mg at 24 h, 1 week and 2 weeks, respectively. The content of mannitol in nails A, B and C decreased to 30, 28 and 18% of the initial value after 1 week and to 6, 5 and 11% after 2 weeks. The amount of mannitol recovered from the second rinses performed at 24 h was much smaller than the amount recovered from the first rinse or from the rinse at the middle of the first week. After 1 week, 0.46 ± 0.22 nmol of

Table 2 Comparison of skin and nail electroosmotic fluxes of mannitol and glucose

		Transungual iontophoresis				Transdermal iontophoresis	
		10 mM mannitol pH 7.4 (this work)		10 mM glucose pH 7.0[9]		1 mM mannitol pH 7.4[17]	
Intensity (mA)		0.2		0.125		0.4	
Current density (mA/cm^2)		1.0		0.5		0.5	
		Fluxes (nmol/h)	J_s (μl/h per mA)	Fluxes (nmol/h)	J_s (μl/h per mA)	Fluxes (nmol/h)	J_s (μl/h per mA)
Anodal	All sets	0.82 ± 0.99	0.41 ± 0.50	3.10 ± 0.32	2.48 ± 0.26	0.65 ± 0.26	1.64 ± 0.66
Cathodal	Set III	0.06 ± 0.01	0.03 ± 0.01	0.28 ± 0.05	0.22 ± 0.04	0.05 ± 0.03	0.12 ± 0.08

Skin and nail electroosmotic fluxes (nmol/h) of mannitol or glucose at pH 4.0, 7.0 and 7.4 are shown together with their corresponding normalised convective solvent flow (J_s = μl/h per mA). Glucose data modified from Murthy *et al.*[9] and transdermal mannitol data modified from Marro *et al.*[17]

mannitol per milligram remained in the nails and 2.55 ± 4.61 nmol was found in the rinsing solutions. By the end of the second week, only 0.13 ± 0.05 nmol of mannitol per milligram remained in the nails and no mannitol was detectable in the rinsing solutions. Overall these results showed that mannitol did not irreversibly bind to nail material.

Discussion

The aim of this work was to investigate the magnitude and the variability of electroosmotic transport during transungual iontophoresis. Thus, it was pertinent to examine all the data gathered in this work and to compare it with the starting hypothesis and previous data. Table 2 compares glucose and mannitol fluxes and the normalised electroosmotic solvent flow measured during transungual iontophoresis in this and in previous work,[9] as well as during transdermal iontophoresis.[17] Overall, the data are consistent with the nail being cation permselective at physiological pH. However, the convective solvent flow deduced from the glucose data, 2.48 and 0.22 μl/h per mA for anodal and cathodal delivery respectively, is higher that measured during transdermal iontophoresis, 1.65 and 0.10 μl/h per mA for anodal and cathodal delivery respectively.[9,17,18] On the other hand, the normalised convective solvent fluxes estimated using mannitol in this work are smaller than those reported for the skin. It is not clear at this point the reason for these differences; both Murthy[9] and ourselves used nail clips from healthy volunteers, but the experiments presented here used a much shorter hydration time before iontophoresis application.

Figure 6 shows the passive and iontophoretic mannitol fluxes measured during the four sets of experiments, according to the pH and polarity of delivery. On the one hand, Murthy's data indicate the following patterns: (a) passive flux smaller than anodal iontophoretic fluxes at pH > 6 and than cathodal iontophoretic fluxes at pH < 5, (b) cathodal flux greater than the anodal at pH 2.0–4.0, (c) anodal flux greater than the cathodal at pH 6.0–7.4 and (d) clear enhancement of glucose transport (approximately five-fold) caused by iontophoresis; overall these data suggest the nail behaves like the skin.[9] On the other hand, Hao *et al* observed a much more moderate enhancement effect of pH and polarity of the delivery on mannitol and urea transport.[6–8]

In this work, the experiments with minimised internail variability (sets II and III) also point to the nails being cation permselective at pH 7.4 and this net negative charge reversing at acidic pH. Electroosmotic flow may therefore occur as result of nail permselectivity, as is the case with the skin.[12,14,17] However, Figure 6 suggests this factor to be overwhelmed by others when a wider population of nails is considered. For example, consideration of set III on its own shows that cathodal delivery is higher than anodal delivery at pH 4.0, suggesting that the nail is anion permselective at this pH, which is probably true. However, anodal delivery at pH 4 for other nails (sets I and II) provides a higher flux than cathodal delivery at pH 4 (set III). Similarly, while the results from set III indicate that anodal iontophoresis at pH 7.4 is superior to

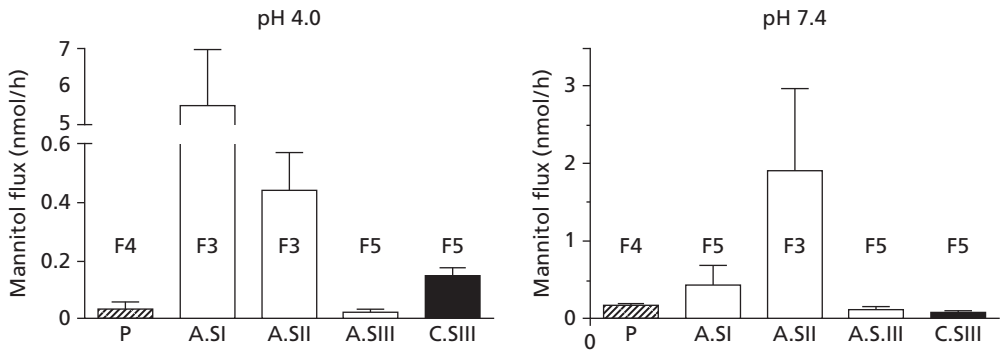

Figure 6 Iontophoretic and passive mannitol fluxes at pH 4.0 and 7.4. Left panel, pH 4.0; right panel, ph 7.4. Hatched, empty and filled bars correspond to passive (P), anodal (A.SI, A.SII and A.SIII) and cathodal (C.SIII) delivery experiments. F+digit indicates the finger from where the nail tips were sourced, F3, F4 and F5 being middle, ring and little finger, respectively

cathodal transport for this group of nails, overall these fluxes are relatively low compared to passive conditions (set IV).

Thus, while nail tips are possibly negatively charged at physiological pH, there is a huge internail variability in the magnitude of the electroosmotic fluxes caused by iontophoresis. The reasons for this variability are not yet understood. Previous studies did not report any effect of nail thickness on the fluxes, and our data (Table 1) seem to agree with previous findings.[6–9] For example, set II included the anodal delivery experiment at pH 7.4, which was performed with the thickest group of nails (270 ± 10 μm) and provided the highest measured flux, as compared to sets I (220 ± 20 μm) and III (230 ± 10 μm), which were similar in thickness but produced different mannitol fluxes.

It could be hypothesised that nail permeability is modified by their use and exposure to external factors. For example, the thumb and the forefinger are under continuous stress during daily life activities and nail tips have no mechanisms for 'recovery' so this stress could have a permanent effect on their permeability. In addition, transonychial water loss is typically higher for the fingernails from the left hand[19] and it has been shown that nails grow faster in the dominant hand.[20] It is therefore possible that the nail plates from different fingers or hands will differ in their permeability. Thus, it is interesting to compare the results obtained with nail tips originating from different fingers (Figure 6). Set II used only nails from the middle finger and provided results consistently higher than set III, which used nails from the little fingers. However, the results obtained with nail tips sourced from the same finger of different volunteers were not more reproducible than those from different fingers, as shown by sets I and II at pH 4, which use nails that were all from the middle finger (F3), and by sets I and III at pH 7.4, which used nails from the little finger (F5). There are not sufficient data at this time to conclude an effect of finger and hand on the nails' permeability. This should be ideally confirmed in the future. Unfortunately, nails are very difficult to collect, which challenges the development of systematic studies.

It has been suggested that nail hydration has an important effect on nail permeability and that nail swelling is a requirement for enhanced drug penetration in passive conditions.[7,21,22] However, all the nails were similarly hydrated in the studies presented here, ruling out hydration as the main cause for the variability observed. Nail hydration was for a much longer period in previous studies,[6–9] which probably modified the nails' electrical resistance with respect to this work.[19,23] However, it is unclear how hydration and experiment length might modify mannitol and glucose electrotransport. The short hydration time (10–15 min) used in this work was based on previous studies on nail hydration,[24] on in-vivo studies[19] and because it represents a closer approach to a practical in-vivo application.

Some drugs, such as salicylic acid, griseofulvin, terbinafine or itraconazole, bind extensively to keratin.[9,25,26] Drug binding can considerably delay transport across the membrane and could cause variability if it differs among individual nails. Thus, a final experiment was performed to examine whether mannitol binds to nails and if its binding could be a factor contributing to variability. This possibility was rejected as mannitol was easily released from the nail clippings and showed no evidence of accumulation.

Due to the internail variability observed, the enhancement factor observed with respect to passive conditions is extremely dependent on the pair of experiments considered (Figure 6), an observation that could partially explain the contradictory observations previously reported by different groups. Hao *et al*.[6–8] used the same nails for passive, anodal and cathodal sequential delivery and observed a very moderate enhancement and Murthy performed independent experiments with different nails.[9] Our data also highlight the importance of reporting either fluxes or cumulative delivery. For example, the amount of mannitol delivered across the nails 13LF5, 7LF3 and 2LF3 increased from 0.45, 12.3 and 20.7 nmol to 0.89, 24.0 and 44.7 nmol, respectively (sets II–III, anodal delivery pH 4 and 7.4). Had this data been reported as an enhancement factor, which by chance is very similar for the three nails (1.97, 1.95 and 2.16), key information about the absolute amount delivered and its associated variability would have been lost.

Ultimately, it remains to establish if the results reported here with nail clippings are predictive of the in-vivo situation. Sodium iontophoretic fluxes across the nail showed modest variability both *in vitro*[27] and *in vivo*.[19] However, sodium transport is mostly due to electrorepulsion and not to electroosmosis. Future studies are required to establish the magnitude and variability of the electroosmotic contribution during iontophoresis across the nail plate *in vivo*. This is fundamental; if the findings reported here are representative of the in-vivo situation it will be extremely difficult to predict transungual fluxes for a drug that is only or primarily transported by electroosmosis. Furthermore, failure to ensure an enhanced, reproducible and well-controlled drug input would make it difficult to justify the choice of an iontophoretic nail drug delivery system with respect to a simple and more economic passive diffusion device.

Conclusions

The electroosmotic flux of mannitol resulting from the application of iontophoresis to human nail tips was studied. Mannitol transport was not hindered by keratin binding. An important internail variability was observed, which overwhelmed the effect of current application, polarity of delivery and pH on mannitol delivery. The results from experiments minimising internail variability by keeping the same nail clippings along the different stages (pH and polarity) supported a finding that the nail tips are negatively charged at physiological pH. However, the fluxes of mannitol measured during these experiments remained very dissimilar, suggesting that the electroosmotic transport of drugs across the nail may not be easily predictable.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

Funding

This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

Acknowledgements

We thank the Department of Pharmacy and Pharmacology for a studentship for J. Dutet. We thank our colleagues in the Skin and Nail Bioscience group, especially Prof. R.H. Guy, for helpful discussions.

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