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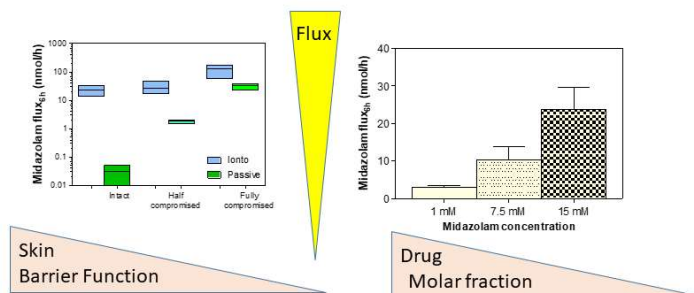
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Iontophoresis of midazolam in paediatrics



Potential of iontophoresis as a drug delivery method for midazolam in pediatrics

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Abstract: Drug delivery to the neonatal and premature pediatric populations is very challenging. This research assessed the potential of delivering midazolam by transdermal iontophoresis as an alternative strategy in pediatric therapy. *In vitro* experiments used intact and tape-stripped porcine skin as models for the skin barrier function of full-term and premature newborns, respectively. Midazolam transdermal transport was significantly enhanced by applying higher currents, increasing the formulation pH, and optimizing the drug's mole fraction in the vehicle. When the skin barrier was decreased to half of its baseline competence, the passive permeation of midazolam increased by approximately 60-fold; and complete stratum corneum removal led to an additional 20-fold enhancement in permeation. Iontophoresis retained control of the drug transport through partially compromised skin. However, a very high passive contribution undermined the iontophoretic control when the barrier was fully compromised. Overall, midazolam delivery could be rate-controlled by iontophoresis in most circumstances, and therapeutically useful fluxes could be achieved.

Keywords: midazolam, pediatric drug delivery, iontophoresis, passive diffusion, transdermal drug delivery, skin barrier function.

1. Introduction

Midazolam, a short acting benzodiazepine, is used in pediatric medicine to treat status epilepticus and febrile convulsions, for conscious sedation for procedures, induction of anesthesia, premedication and in intensive care where continuous sedation is an integral part in patient treatment [1]. The drug is also used in infants and premature neonates and has been administered by various routes, including intravenous, oral, rectal, buccal and nasal.

Midazolam (MW = 325.8 as free base, 362.3 as HCl salt) is a lipophilic ($\log P = 4.33$) weak base ($pK_a = 6.15$) [2,3] (structure shown in Supplementary Material). An extensive first pass metabolism results in low oral bioavailability (15 – 30%) [4]. The drug has a short half-life of ~2 hours (although somewhat longer in neonates) [5] meaning that a sustained therapeutic effect requires frequent dosing. The oral solution has a bitter taste so intravenous administration is the most common approach when treating infants and children. There are, in addition, biopharmaceutical challenges with midazolam, such as its very poor aqueous solubility at $pH > 5$, and its incompatibility with certain drugs (e.g., ranitidine and furosemide) and with parenteral nutrition [6-9]. As a consequence, a separate intravenous line for midazolam is often necessary and this is self-evidently onerous for young pediatric patients. In addition, because of the need for continuous infusion of the drug to ensure consistent sedative effects over relatively long periods, scarring at the injection site is another problem. Rectal or nasal administration have been tried as alternatives to intravenous delivery but not proven useful: rectal administration has resulted in low and inconsistent absorption [4, 10], and the nasal route has been associated with significant mucosal irritation [1, 11].

Iontophoresis expands the benefits of the transdermal route of administration (avoidance of first pass effect, ease of access, and improved patients' convenience) to drugs the physicochemical properties of which make them poor candidates for passive skin delivery (charged and polar drugs for example) [12]. Further, iontophoresis is a controlled drug delivery technique providing transdermal fluxes directly controlled by the intensity of current applied. This allows dose individualization and pulsed delivery profiles. Finally, transdermal iontophoretic devices have been approved for paediatric patients, both for drug delivery (LidoSite®) and non-invasive glucose sampling (GlucoWatch Biographer®) [12-14]. To provide a viable, alternative administration route for midazolam, transdermal iontophoresis must mirror the dosing rates achieved by intravenous infusion. For continuous sedation in paediatric intensive care, the recommended regimens are: (a) premature neonates of gestational age less than 32 weeks, 60 $\mu\text{g}/\text{kg}/\text{h}$ for 24 hours then reduced to 30 $\mu\text{g}/\text{kg}/\text{h}$ to be adjusted according to response for a maximum 4 days; (b) neonates of more than 32 weeks gestational age and infants 1-5 months, 60 $\mu\text{g}/\text{kg}/\text{h}$ adjusted according to response; (c) Older than 6 months, a loading dose of 50 – 200 $\mu\text{g}/\text{kg}$ as a slow intravenous injection if required, followed by continuous infusion of 30 – 120 $\mu\text{g}/\text{kg}/\text{h}$ adjusted according to response [1].

The neonatal skin barrier function is close to that of adults by ~35 weeks of gestation [15]. However, for very premature infants (i.e., less than 34 weeks gestation), the stratum corneum is incompletely formed and barrier function is noticeably impaired [16, 17] as shown clearly by measurements of the rate of transepidermal water loss (TEWL) [18-21]. A lower barrier function is shown by elevated TEWL. As the developing skin barrier matures, TEWL decreases until a constant value is achieved at which point the skin barrier function is fully

established. Among other critical issues, the immature barrier of premature neonates of shorter gestational age is more permeable to drug transport than intact skin, suggesting an opportunity for transdermal delivery [22] (as has been reported previously for theophylline and caffeine [23-24]) and for potential toxicity due to unwanted exposure (hexachlorophene) [22].

Exploring the feasibility of a drug candidate for transdermal delivery faces two particular challenges: (a) excised skin from children (and especially very young infants) is difficult, if not impossible to obtain; and, (b) to assess a drug's skin permeation in the paediatric population demands a spectrum of skin membranes of different levels of barrier function so as to mimic those encountered in premature neonates and full-term infants. The first challenge has been resolved by the use of porcine skin, which is now a well-accepted model for human counterpart [25-27]. The second has been addressed by published research demonstrating that pig skin, from which the SC has been differentially tape-stripped to provide various degrees of barrier compromise (as determined by TEWL measurements), is a useful *in vitro* model for the premature neonatal skin at different levels of maturation [28].

The objective of this study was to investigate, using the porcine skin model, the feasibility of delivering midazolam by transdermal iontophoresis. First, several variables were examined to maximize the iontophoretic electrotransport of midazolam. Second, transdermal delivery of midazolam was assessed across skin barriers from which the SC was stripped to different extents thereby modelling the less resistant skin of premature babies.

2. Materials and Methods

2.1 Chemicals

Midazolam hydrochloride was obtained from Apin Chemicals (Oxford, UK), silver (Ag) wire (99.99%), silver chloride (AgCl, 99.999%), and sodium hydroxide (NaOH) pellets were purchased from Sigma Aldrich (Gillingham, UK). Sodium chloride (NaCl), citric acid monohydrate, trisodium citrate dehydrate, disodium hydrogen phosphate, potassium dihydrogen phosphate, and phosphoric acid (85 %) were obtained from Acros (Geel, Belgium). Acetonitrile and hydrochloric acid (HCl) were provided by Fisher Scientific (Loughborough, UK). All reagents were at least analytical grade and highly purified deionised water (resistivity ≥ 18.2 M Ω .cm, Barnsted Nanopure Diamond™, Dubuque, IA) was used for the preparation of all solutions.

2.2 Skin

Skin preparation has been described elsewhere [26]. Fresh (dorsal) pig skin was acquired from a local abattoir. The tissue was cleaned in cold water and subsequently refrigerated until the following day. The skin was then cut into 20 x 10 cm² pieces and dermatomed (Zimmer™ Electric Dermatome, Dover, Ohio) to a thickness of 750 μ m. The skin samples were individually wrapped in Parafilm™, and frozen (-20°C) until use. Before beginning the permeation experiment, the skin was brought to room temperature over a period of ~30 minutes and excess hair was carefully trimmed. The skin was either used in the permeation study "as-is" or was tape-stripped to create two impaired barriers: either half-

compromised (40 – 60% of SC removed) and fully-compromised (100% of SC removed) barrier.

Tape-stripping procedure [29]: A 10 x 10 cm² area of skin was divided into 3 pieces. The first was not stripped and maintained a fully intact skin barrier; it provided a baseline reading of the TEWL (AquaFlux AF-102, Biox Systems Ltd., London, UK). After stabilization (requiring no more than 3 minutes) these control TEWL values (mean ± SD; n = 6) were 9.0 ± 1.7 g.m⁻².h⁻¹. The second piece of skin was subjected to repeated application-removal of adhesive tapes (2 x 2 cm, Scotch Book Tape, 3M, St. Paul, MN) to progressively remove the SC and produce 100% barrier impairment. A 1.5 x 1.5 cm² template affixed onto the skin ensured that SC was removed from the same location. Periodic measurement of the TEWL allowed the degree of barrier impairment to be quantified and achievement of full barrier impairment was indicated when no further increases in TEWL were observed upon continued tape stripping. The number of tapes required to produce a fully compromised skin ranged from 17 to 25; TEWL across fully-compromised was 153 ± 11 g.m⁻².h⁻¹ SC (n = 11). The third skin sample was again sequentially stripped as before but only until TEWL reached 40 – 60% of that of the fully perturbed barrier. Between 13 and 16 tape-strips were needed to produce half-compromised barriers across which TEWL was 76 ± 7.6 g.m⁻².h⁻¹ (n = 8).

Table 1. Experiments performed to characterize midazolam passive and iontophoretic transdermal delivery across intact and compromised skin.

Variable	Donor			Current Intensity (mA)	n
	Background solution ¹	Midazolam (mM)	pH		
pH	10 mM citrate + 30 mM NaCl	7.5	3	0.36	4
			3.5		3
			4		4
			4.5		3
Current intensity	10 mM citrate + 30 mM NaCl	7.5	3.5	0.10	3
				0.20	3
				0.36	3
Co-ion competition	Water	15.5	3.6	0.36	5
	10 mM citrate				6
	10 mM citrate + 30 mM NaCl	15.0	3.5	0.36	9
	10 mM citrate + 90 mM NaCl				5
Drug concentration	10 mM citrate + 30 mM NaCl	1	3.5	0.36	3
		7.5			3
		15			9
Skin barrier	10 mM citrate + 30 mM NaCl	15	3.5	Passive	3
				0.36	5-8

¹ The citrate buffer comprised 7.4 mM citric acid monohydrate and 2.6 mM trisodium citrate dehydrate. pH changes at the end of all experiments did not exceed ±0.1 in citrate buffer and was no more than ±0.2 in water.

2.3 Iontophoresis set-up

Amber side-by-side two-compartment diffusion cells (active transport area = 0.71 cm²) were used. The experimental details are described elsewhere [29]. The skin separated the two chambers (3 mL) with the epidermal side facing the anode compartment. The solution in the receptor chamber was 154 mM sodium chloride (pH ~ 6) in all cases. For 30 minutes before starting the transport study, both compartments were filled with the drug vehicle to be used (but containing no drug), and with 154 mM sodium chloride, respectively. Both compartments were then emptied and refreshed, respectively, with the donor solution under study (which contained midazolam) and fresh receptor solution; both compartments were magnetically stirred (Multipoint-6 stirrer, Thermo Scientific Variomag, Cole-Parmer, London, UK) at 400 rpm [29]. Direct constant current was applied (KEPCO 1000M, Flushing, NY, USA) for 6 hours to Ag/AgCl electrodes (anode in the donor solution, cathode in the receptor). Hourly samples (1 mL) of the receptor phase were withdrawn and replaced with fresh solution. Sink conditions were maintained in the receptor [30]. Following termination of iontophoresis, both donor and receptor solutions were refreshed (but now with no drug in the 'donor' solution) and the system was left for a further 15 hours at which point the receptor compartment was sampled and subsequently analyzed to determine the amount of midazolam released from the skin [29].

Separate passive diffusion controls were also performed with samples taken every 2 hours for 10 hours and a final sample withdrawn at 21 h. As above, each sample removed was replaced with 1 mL of fresh solution. The experimental conditions investigated are summarized in Table 1.

2.4 Sample analysis

Midazolam was quantified by high-performance liquid chromatography with UV detection (220 nm) (Jasco UK Ltd, Dunmow, UK) including a PU-980 pump with an AS-1595 autosampler, a UV-975 UV-VIS detector, and an Acclaim 120, C18 (150 x 4.6 mm, 5 μm) reversed-phase column (Dionex, UK) thermostatted at 25°C. The mobile phase was 50 mM phosphate buffer (adjusted to pH 2 with phosphoric acid) and acetonitrile (70:30) and was pumped through the system at 1 mL/min [29].

2.5 Data analysis and statistics

Data analysis and interpretation used Graph Pad Prism V.5.00 (Graph Pad Software Inc., CA, USA) [29]. Data are presented as the mean ± standard deviation (SD), unless otherwise stated. Fluxes given are the amounts delivered during a certain time divided by the length of that period. Statistical significance was set at $p < 0.05$. Comparisons between different data sets of data were made using either a two-tailed unpaired t-test (for 2 groups) or a one-way ANOVA (for >2 groups) followed by Tukey's post-test [29].

The transference number (T_{M^+}) of midazolam was computed according to Faraday's law [31]:

$$T_{M^+} = \{J_{\text{total}} \cdot Z \cdot F\} / I \quad \text{Equation (1)}$$

where J_{total} is the total flux observed after 6 h iontophoresis, I is the current intensity applied, F is Faraday's constant, and Z the absolute value of the valence of the drug ion. It was estimated from available literature [31, 33-34] that about 9% of midazolam might have been present as a divalent cation at pH 3.5. For the purposes of this work, however, and the calculation of T_{M^+} using Equation (1), the valence of midazolam has been assumed to be +1.

3. Results

3.1. Passive diffusion experiments across intact skin

The passive flux midazolam from a 15 mM solution of its hydrochloride salt (pH 3.5) through intact pig skin was $0.06 \pm 0.01 \text{ nmol}\cdot\text{h}^{-1}\cdot\text{cm}^{-2}$ after 21 h.

3.2. Iontophoretic experiments across intact skin

3.2.1. Effect of donor pH on the iontophoretic flux of midazolam.

Midazolam aqueous solubility is very low at pH values above 5 but increases significantly at acidic pH where the drug exists predominantly in its ionised form [30]. Iontophoresis experiments were therefore performed with donor solutions having pH values in the range of 3.0 to 4.5 and the results are presented in Figure 1.

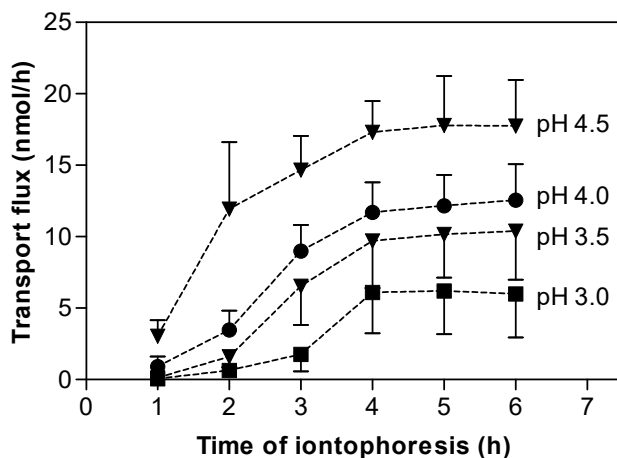


Figure 1: Midazolam iontophoretic transport (mean + or - SD, $n = 3-4$) as a function of donor solution pH. Midazolam was delivered from a 7.5 mM drug solution at a current of 0.36 mA. The flux values over each successive sampling period are plotted as a function of the time at the end of that corresponding sampling interval.

Donor solutions with 7.5 mM midazolam at pH 4.5 were slightly cloudy but no observable precipitation occurred during the experiment. Iontophoretic transport increased with increasing pH; from the pH 4.5 donor solution, $J_{6h} = 18 \pm 3.2 \text{ nmol}\cdot\text{h}^{-1}$, a value significantly higher ($p < 0.05$) than that with the pH 3 donor ($6.0 \pm 3.0 \text{ nmol}\cdot\text{h}^{-1}$). Fluxes from donors at pH 3.5 and 4 fell between these extremes.

3.2.2. Effect of current intensity on the iontophoretic flux of midazolam.

Iontophoresis produced significant elevation of midazolam transport through intact

porcine skin relative to passive diffusion (Figure 2(a)). At the end of a 6-h experiment, in the absence of current, the flux was 14 ± 0.4 pmoles.h⁻¹ compared to values of 2.4 ± 0.64 nmol.h⁻¹, 5.4 ± 0.57 nmol.h⁻¹, and 10.4 ± 3.8 nmol.h⁻¹ at currents of 0.1, 0.2 and 0.36 mA, respectively. Analysis of variance followed by Tukey's post-test confirmed that all these flux values were significantly different from each other ($p < 0.5$). The flux of midazolam, furthermore, was linearly proportional ($r^2 = 0.88$) to the applied current (Figure 2(b)). From the slope of this relationship, it was possible to calculate the drug's transport number (T_{M^+}) for the experimental conditions used as $0.078 \% \pm 9.10^{-3} \% (\pm SE)$.

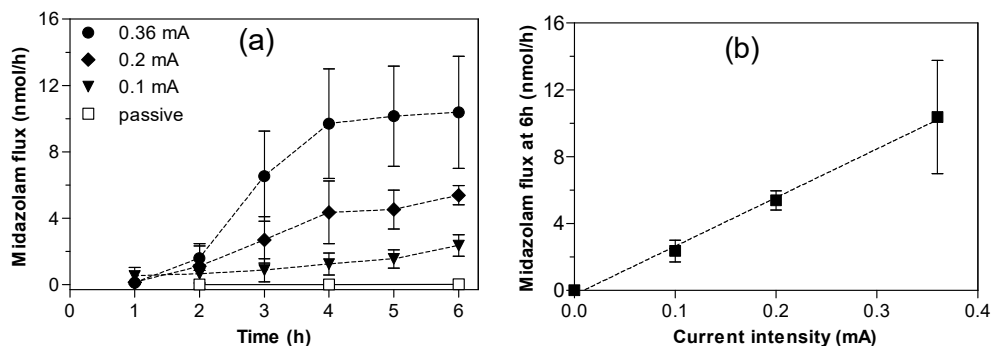


Figure 2: Effect of current intensity on the iontophoretic flux of midazolam. (a) Drug flux (mean \pm SD, $n = 3$) as a function of time and current intensity (passive indicates 0 mA). Fluxes are shown at the end of the corresponding sampling interval. (b) Midazolam flux at 6 hours as a function of applied current. The transference number of midazolam was estimated from the slope of the regression line (J_{6h} (nmoles.h⁻¹) = $29.1 (\pm 3.4) \times$ Intensity (mA) - $0.26 (\pm 0.7)$; $p < 0.0001$, $r^2 = 0.88$). The donor solution in all experiments was 7.5 mM midazolam in 10 mM citrate buffer (pH 3.5) + 30 mM NaCl (37.8 mM sodium competing co-ion).

3.2.3. Effects of co-ion competition and the molar fraction of midazolam.

This series of experiments investigated how the iontophoretic delivery of midazolam depended on: (a) competing co-ion concentration (at fixed drug concentration), and (b) drug concentration (at fixed co-ion concentration). Figure 3 shows that, as expected, midazolam delivery was greatest when no competing co-ions were present ($J_{6h} = 62 \pm 17$ nmol.h⁻¹). In contrast, the smallest drug flux was found ($J_{6h} = 3.0 \pm 0.39$ nmol.h⁻¹) when it was delivered from a 1 mM solution in a buffer containing ~ 37.8 mM Na⁺. There was no significant difference between midazolam iontophoresis from a 15.9 mM solution in water and a 15 mM solution in 10 mM citrate (7.8 mM Na⁺) buffer. However, these fluxes were significantly greater ($p < 0.05$) than those from all the other four vehicles considered (Figure 3). As expected, the iontophoretic transport of midazolam was linearly dependent on the drug concentration (1, 7.5 and 15 mM) in the 10 mM citrate + 30 mM NaCl (37.8 mM Na⁺) buffer (Fig.3). Figure 4 illustrates graphically the linear increase in the deduced transference number of midazolam (T_{M^+}) as a function of the drug's molar fraction in the donor (X_{M^+}). Linear regression of these data ($r^2 = 0.86$) yielded a slope and intercept of $0.48 (\pm 0.03) \times 10^{-2}$ and $0.02 (\pm 0.02) \times 10^{-2}$, respectively.

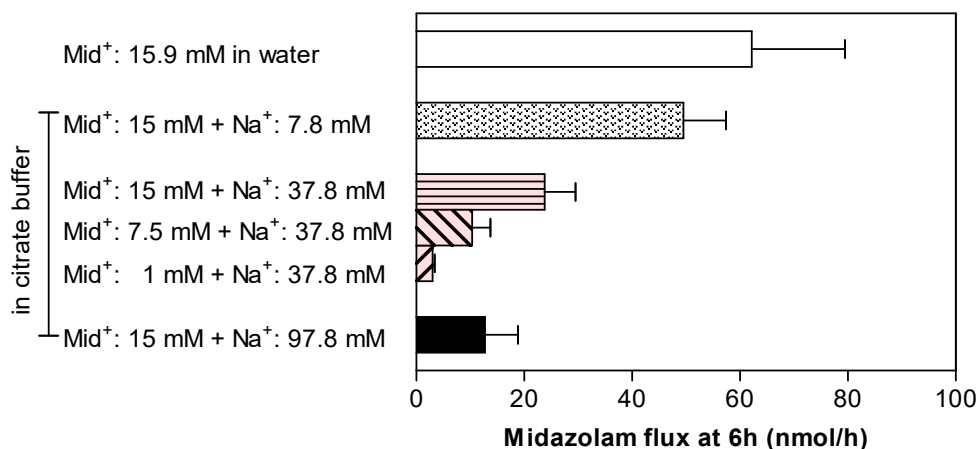


Figure 3: Midazolam fluxes after 6 hours of iontophoresis at 0.36 mA (mean + SD, n = 3-9) as a function of drug and principal competing co-ion (Na⁺) concentrations (see Table 1).

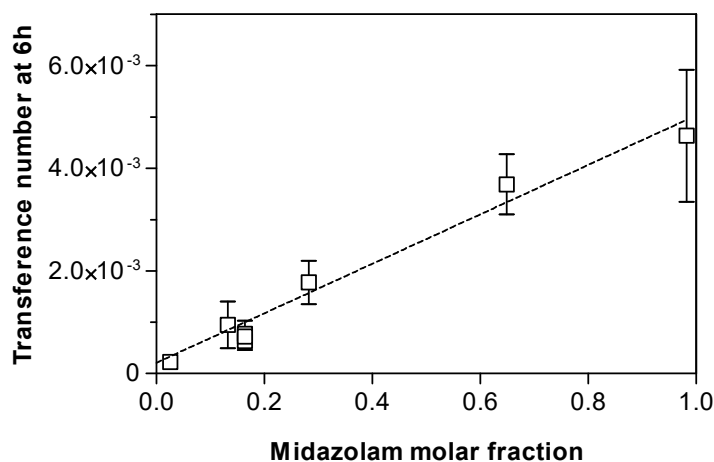


Figure 4: Deduced transference numbers of midazolam (mean ± SD, n = 3-9) at 6 hours of iontophoresis as a function of the drug's molar fraction in the donor vehicle. A simple linear regression of the data is shown:-

3.3. Passive and iontophoretic experiments across compromised skin

Skin barrier impairment led to significant increases in passive fluxes of the drug (Figure 5). After 6 hours exposure, the rate of midazolam transport across intact skin was 0.03 ± 0.01 nmol.h⁻¹, compared to 1.9 ± 0.3 nmol.h⁻¹ and 33 ± 8.4 nmol.h⁻¹ across half-compromised and fully-compromised skin barriers, respectively (i.e., a 1000-fold enhancement in flux when the SC had been completely removed). With iontophoresis at 0.36 mA (Figure 5), the drug fluxes across intact and half-compromised skin barriers were not significantly different (24 ± 5.7 and 27 ± 14 nmol.h⁻¹, respectively). However, the iontophoretic flux was significantly greater (128 ± 46 nmol.h⁻¹) across a fully compromised barrier. Figure 6 illustrates the empirical (and apparently exponential) relationship between the individual passive and iontophoretic fluxes of midazolam and the TEWL values of the corresponding skin samples. Finally, it is worth

noting that iontophoresis delivered significantly more ($p < 0.02$) midazolam than passive diffusion across intact, half- and compromised skin.

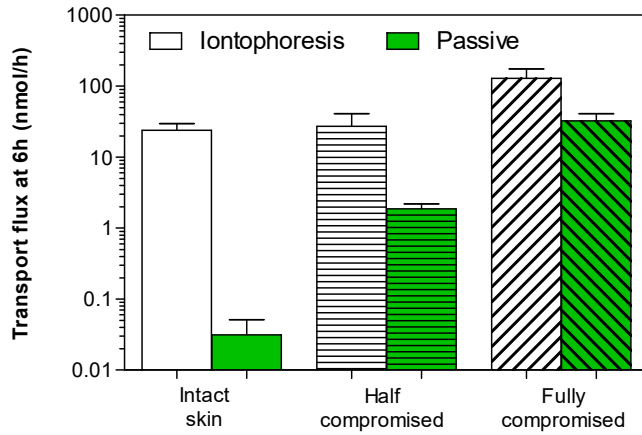


Figure 5: Passive (yellow bars) and iontophoretic (green bars) fluxes at 6 hours of midazolam (mean + SD; n = 3-9) across intact, half-compromised and fully-compromised skin barriers. Note the use of a logarithmic scale for the flux values.

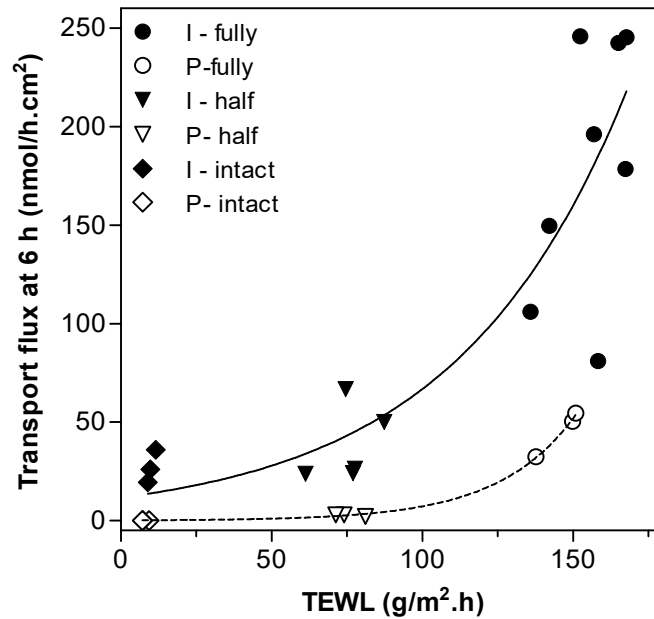


Figure 6: Dependence of individual midazolam fluxes on the TEWL values of the corresponding skin samples. Open symbols represent the passive diffusion (P) data; the filled symbols are the iontophoresis (I) results. Intact, half- and fully-compromised skin barriers are indicated by diamonds, triangles and circles, respectively. The lines through the points are empirical, exponential fits of the data: passive diffusion, $J_{6h} = 0.14 \times \exp(0.04 \times \text{TEWL})$ (n = 9); iontophoresis (n=16, $r^2 = 0.80$: $J_{6h} = 11.6 \times \exp(0.02 \times \text{TEWL})$ (n = 16).

Finally, perturbation of the skin barrier resulted in greater delivery of midazolam to the receptor compartment in the post-iontophoresis period as well (Table 2).

Table 2. Cumulative amount of midazolam (in nanomoles) (a) delivered through intact and compromised skin during 6 hours of iontophoresis at 0.36 mA, and (b) released passively from the skin during 15 h post-iontophoresis (mean \pm SD, n = 3-9).

Experimental period	Intact skin	Half-compromised skin	Fully compromised skin
Iontophoresis for 6 hours	101 \pm 28	91 \pm 57	485 \pm 192
Passively released over 15 h post-iontophoresis	33 \pm 6.1	104 \pm 11	276 \pm 42

4. Discussion

Few studies have characterized the passive transdermal delivery of midazolam. Touitou et al. [34] measured a flux of 9.5 nmol.h⁻¹.cm⁻² following application of midazolam maleate (at 11.3 mM in unbuffered water) to hairless mouse skin *in vitro*. Balaguer-Fernández et al. [35] reported delivery at 3 nmol.h⁻¹.cm⁻² after an 8-hour treatment of excised human skin with midazolam hydrochloride (2.76 mM) from an unbuffered solution (initially at pH 5.5). Direct comparison with the passive flux reported in this study (0.06 \pm 0.01 nmol.h⁻¹.cm⁻²) is difficult because of the different skin models used and the inconsistency in the composition and buffering of the applied formulations. However, in agreement with Balaguer-Fernández et al. [35], iontophoresis was shown to significantly enhance midazolam delivery relative to its passive diffusion.

A first series of iontophoretic experiments investigated how the composition of the donor vehicle (pH, drug concentration and background electrolyte) and the intensity of current applied [31, 36] modified the iontophoretic transport of midazolam. The observed increase in the delivery of the drug with increasing pH requires consideration of a number of factors. First, at the lower end of the pH range, the drug exists in a predominantly positively-charged form, with about 20% having a charge of +2, the remainder +1 [30, 32-33]. These cations are expected to differ in their iontophoretic transport efficiency [37] but exactly to what extent, in a quantitative sense, is unknown. Second, the lower the pH, the greater concentration of H₃O⁺ ions in the donor solution. These highly mobile cations [38], even at sub-millimolar concentrations, can nevertheless compete effectively with the positively-charged drug to carry current across the skin (i.e., decreasing the transference number of midazolam with decreasing pH) [39,40]. Third, porcine skin has been shown to have an isoelectric point of ~4.4 [41]. This means, at pH > 4.4, that the skin supports a net negative charge and is therefore cation-permselective under these conditions. In addition to favouring cation electrotransport, the net negative charge on the membrane means that the imposition of an electric field across the skin induces an electroosmotic flow of solvent in the direction of counter-ion movement, further enhancing the flux of midazolam. In contrast, at pH 3, with the elevation in H₃O⁺ concentration, the skin now assumes a net *positive* charge, becoming *anion-permselective*, and the direction of electroosmotic flow is reversed – all factors now working against the iontophoretic delivery of a cationic drug from the anode.

From the results obtained (Figure 1), it is clear for midazolam that the advantages of a donor solution pH at, or slightly above, the pI of the skin outweigh the greater degree of ionization of the drug at lower pH values; similar observations have been made for other drugs, including apomorphine, rotigotine and 5-OH-DPAT [39,42-43]. That having been said, it must be emphasised that other biopharmaceutical factors also play a role in formulation optimisation. The cloudiness of a 7.5 mM midazolam solution at pH 4.5 suggests that it is at (or very near) saturation and therefore not ideal from a stability point of view. There is a trade-off to be made, therefore, between donor formulation pH and drug solubility (and, perhaps, stability) in terms of developing an effective formulation for iontophoretic delivery. In addition, the relative contributions of electro-migration and electro-osmosis to the total iontophoretic transport must be considered [31, 36, 40]. For example, in an earlier study, a pH 5.5 donor solution of 2.8 mM midazolam was selected to ensure a beneficial electroosmotic contribution and fluxes in the range of 12.2 to 21.3 nmoles.h⁻¹.mA⁻¹ were observed [35]. In this work, the strategy adopted was to optimize electro-migration by increasing the drug concentration and mole fraction: iontophoresis of 7.5 mM midazolam from a donor vehicle at pH 4.5 donor produced a flux of 49 (±8.9) nmoles.h⁻¹.mA⁻¹ flux. Based on these results, all subsequent experiments were performed with pH 3.5 donors as this would ensure complete solubility of midazolam at higher drug concentrations. Because this pH is slightly lower than the range (4.0-5.9) of the “so-called” adult skin pH, its tolerability on premature skin and impact on barrier maturation should be assessed (also bearing in mind that skin acidification is essential for barrier maturation) [44, 45].

The data in Figure 2 re-affirm the value of iontophoresis as a method to control drug delivery and shows that dosing can be sensitively adjusted in a productive fashion by the applied current [31, 36, 46-48]. The potential of personalization of therapy is therefore evident. It is noteworthy, however, that the transference number of the drug deduced from these experiments suggests that only 0.08% of the current is carried across the skin by midazolam cations; the vast majority is transported by H₃O⁺, Na⁺ and Cl⁻ (the latter moving in the cathode-to-anode direction, of course). Despite the very significant enhancement of drug flux achieved by iontophoresis – relative to passive diffusion – the efficiency of the approach is, at best, modest even for a typical small drug such as midazolam.

As previously demonstrated for several drugs [29, 49-52], further manipulation and, eventually, maximization of iontophoretic delivery can be achieved by varying the mole fraction (relative to other cations present) of the active compound in the donor solution. The results in Figures 3 and 4 demonstrate that midazolam transport was directly proportional to its mole fraction in the anodal solution and reached its greatest value when the drug was formulated as an aqueous solution, when a transference number of 0.46% was attained. Molar fraction, rather than nominal concentration, determined the efficiency of midazolam iontophoresis - see, for example, when 7.5 mM/X_{M+} = 0.16 and 15 mM/X_{M+} = 0.13 in Figure 4. This is another important consideration in formulating iontophoretic vehicles. While such an observation conforms nicely with our theoretical understanding, the unbuffered formulation of a drug in water alone may be practically unacceptable for a number of reasons, such as stability, solubility and electrochemistry (e.g., having sufficient Cl⁻ present to satisfy the requirements of the Ag(s) + Cl(aq) → AgCl(s) anode reaction for the duration of treatment at the chosen current intensity).

A further series of experiments, the results of which are presented in Figures 5 and 6 and Table 2, aimed to examine whether iontophoresis might provide a safe and effective approach to deliver midazolam to paediatric patients and, in particular, to premature neonates, the skin barrier function of whom represents a “moving target” after birth [16-24]. To experimentally model completely and partially compromised skin barriers, the stratum corneum (SC) was progressively removed by adhesive tape-stripping and the extent of perturbation was quantified using measurements of transepidermal water loss (TEWL). TEWL can be as high as 60-90 g.m⁻².h⁻¹ in a 25-week gestational age (GA) neonate and decreases exponentially to reach adult values at 34-37 weeks GA [12, 51-52]. Thus, our data with the half-compromised skin is representative of a 25-week GA premature newborn. TEWL matures rapidly with postnatal age [22]; for example, the TEWL in 78 neonates achieved maturation values 2 to 3 weeks after birth and independently of their GA (26-41 weeks). The approach used in our work has been previously described [28, 53-59] and, as before [24, 54, 58, 59], an empirical, exponential relationship between both passive and iontophoretic drug fluxes and the degree of skin barrier competency (assessed by TEWL) was found (Figure 6). The results in Figure 5 show that iontophoresis was clearly controlling drug delivery across intact and half-compromised skin, the most relevant range of barrier competence. Thus, importantly, it was found that iontophoresis can maintain control of delivery at least up to the point where the skin barrier has been reduced to 50% competency. When the SC is completely absent, the iontophoretic control is unable to “keep up with” a large passive diffusion contribution (as indicated by the significantly greater flux observed across a fully compromised barrier). Table 2 also reveals an additional factor to take into consideration. The ‘release’ of midazolam from the differentially compromised skin barriers indicates that this relatively lipophilic drug (log P = 4.3) is able to accumulate in the skin during iontophoresis (an observation possibly linked to the altered direction of conventional electroosmotic flow at lower pH values [60]). As a result, there will be continued delivery of drug occurring after iontophoresis has been terminated, a feature to bear in mind if chronic, repeated dosing is envisaged with rotation of the treated skin sites. Taken together, and from a practical point of view, the results reported – assuming their validation *in vivo* – suggest that iontophoresis of midazolam is perhaps best suited for term and premature neonates with at least a half-competent skin barrier, such that drug delivery is rate-controlled by the current applied (and with due attention to continued input of the drug post-application).

The key question addressed by this aspect of the study, of course, is whether iontophoretic delivery of midazolam may be a viable treatment option for paediatric patients. Assuming that the *in vitro* fluxes measured in this work are duplicated *in vivo*, the target input rates indicated in the British National Formulary for Children are (a) 92-184 nmol h⁻¹ kg⁻¹ for infants born from 32 weeks of gestational age (i.e., approximately 8 weeks premature) to 5 months of age, and (b) 92-368 nmol h⁻¹ kg⁻¹ for children aged from 6 months to 11 years. Based on these numbers, and the availability of an iontophoretic device that delivers the midazolam fluxes achieved across intact and half-compromised skin barriers reported in Figure 5, a simple calculation reveals that an effective treatment should be achievable with simple gel electrodes of a few square centimetres in size per kilogram of infant body weight [12, 31, 61]. For older infants and young children, however, the dimensions of the electrode ‘patches’ becomes progressively impractical with increasing age (and required dose). Assuming the efficacy of iontophoresis was established, safety becomes the next key consideration, i.e., whether the technique will be tolerated by the targeted population. Safety was beyond the

scope of this exploratory *in vitro* study. However, experience with the marketed GlucoWatch Biographer® and LidoSite® devices, and from other reports [12-14], suggest that iontophoresis is similarly well-tolerated by children with mature skin barrier function as it is by adults. To the authors knowledge, there is no published data concerning iontophoresis in the premature population; of course, such studies would be essential to pursue further development of the approach described here.

5. Conclusions

In vitro characterisation of midazolam iontophoresis across the skin has identified the critical factors to optimise for flux maximization and has demonstrated the method's ability to control drug input into a patient when the skin barrier is compromised. Based on these findings, it has then been possible to postulate that an iontophoretic device may be capable of delivering midazolam at therapeutic doses to premature infants and neonates.

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Declarations of Interest: The authors declare no conflicts of interest

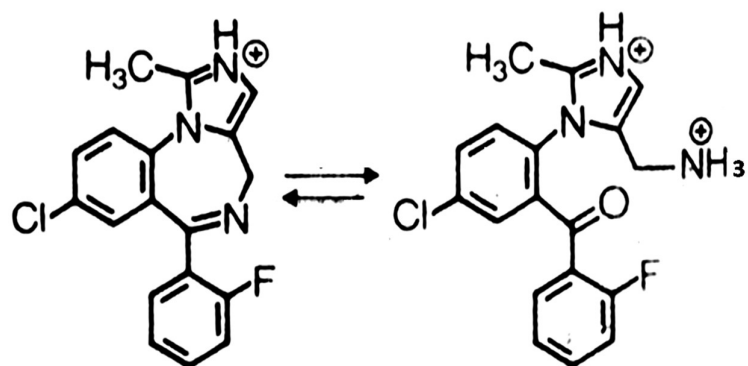
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Supplementary Material



Midazolam exists in a pH-dependant equilibrium with an open-ring structure in acidic conditions (29,30,32,33).