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Preservation of skin permeability during in vitro iontophoretic experiments

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Summary

In vitro methodology was developed to investigate the iontophoretic transport of ionic compounds across hairless mouse skin. Improvement of iontophoretic delivery was evaluated by measuring the transport of an ionic model molecule of morphine hydrochloride (MHCl). By using Ag/AgCl electrodes and a simple permeation cell design, it was possible to obtain steady fluxes of MHCl, modulation of the delivery of MHCl with direct current intensity, and stability of pH without the use of buffered solution. Under these simple conditions, an increase in the applied current (from 0.085 to 0.28 mA/cm^2) produced an increase in the observed iontophoresis efficiency. By using a new permeation cell, allowing the use of two disc electrodes, placed on the same side of the skin, it was observed that the electrode localization and geometry of the receptor compartment have an effect on the MHCl fluxes, however, the use of this cell design did not lead to stabilization of the iontophoresis efficiency. The use of pulsed current (1 kHz, on/off ratio 1:1, from 0.085 to 0.56 mA/cm^2) was then investigated. Lower MHCl fluxes were observed compared to the results obtained with constant current, however, an increase in the applied current produced a linear increase in fluxes, leading to stability of iontophoresis efficiency. A parallel study using tritiated water showed that after the application of a 0.28 mA/cm^2 direct current for 5 h, the passive water flux did not return to the initial passive value. With the pulsed current (0.28 mA/cm^2), passive fluxes were the same before and after application of the current for 5 h. This result suggests that the pulsed currents lead to an improvement of the skin state agreement.

Key words: Iontophoresis; Morphine hydrochloride; Tritiated water flux; Ag/AgCl electrode; Iontophoretic permeation cell; pH stability; Direct current; Pulsed current

Introduction

Iontophoresis is a very powerful technique allowing the percutaneous permeation enhancement of ionized drug poorly absorbed by the skin (Tyle, 1986; Banga and Chien, 1988). Nevertheless, because of the multiplicity of parameters (electrical, physico-chemical, cutaneous) occurring during drug permeation through the skin due to the effect of an electrical current, analysis of the iontophoresis mechanisms is very complex. In order to simplify the problem, it is possible to make a distinction between convective and non-convective diffusion:

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Non-convective diffusion results from forces applied directly on the molecules under consideration, and is composed of two effects: a passive diffusional component due to the existence of a thermodynamic activity gradient through the skin, and an electrical component due to electrical forces exerted on the ions in the region where the electrical field is applied (Burnette, 1989).

Convective diffusion reassembles all phenomena where the transport of the molecules is the consequence of solvent displacement induced by an electrical field (Barry and Hope, 1969; Banga and Chien, 1988; Pikal, 1990). The different mechanisms involved in the displacement of solvent are often collectively described by the term iontohydrokinesis (Gangarosa et al., 1980).

Planck (1890) was the first to study theoretically the transport of ions through a membrane in the presence of an electrical field. His description, which considered only non-convective diffusion, was often used for the interpretation of the characteristics of iontophoretic transport, especially by using the Nernst-Planck equation (Planck, 1890), a differential equation which gives the instantaneous value of the flux:

$$J = -D(\mathrm{d}C/\mathrm{d}x) + (DzEC/kT)$$

where D is the diffusion constant, $\mathrm{d}C/\mathrm{d}x$ the concentration gradient in the skin, z the valence of the ion, E the electric field, T the absolute temperature and k the Boltzmann constant ($k = 1.38 \times 10^{23}$ J/K).

In some cases, the principal mechanism responsible for drug displacement is the electrical force. Thus, the diffusional term can be neglected and one solution of the Nernst-Planck equation can be given as follows:

$$J = (D/\mu kT)I$$

where I is the current intensity and μ the mobility of the charge carrier.

This equation demonstrates one of the principal advantages of the iontophoresis; the flux is directly proportional to the current intensity applied. However, this proportionality between flux and current intensity is not always verified with iontophoretic experimental models, particularly with in vitro experimental models where secondary phenomena could modify ions transport mechanisms by iontophoresis.

On the other hand, the enhancement of drug delivery by iontophoresis has limitations as a result of modifications induced by currents in the skin. It is also important to determine iontophoretic conditions which maintain the optimum integrity of the skin.

In this investigation, our first objective was to evaluate an in vitro experimental iontophoretic system which could be a good model prior to in vivo studies and be suitable for examining the mechanisms governing iontophoresis. Furthermore, the effect of pulsed current was evaluated and, in particular, the skin barrier properties were examined under different currents, constant or pulsed, as measured on the basis of passive permeability to tritiated water.

By reference to previous studies (Corish et al., 1989; Maury et al., 1989), morphine hydrochloride (MHCl), a small monovalent cation, was employed as a model of a diffusing solute. It was used to test whether the experimental model was appropriate to obtain results consistent with the theoretical frame of iontophoresis, i.e., whether MHCl flux would remain steady with time and if it could be modulated by the current intensity.

Materials and Methods

Diffusion cells

Static permeation cells of two different kinds were used: the 'one upper compartment' cell (OUC cell) is constitued of one donor compartment and one receptor compartment separated by a skin sample (Fig. 1). The donor compartment is filled with the morphine solution and contains a disc electrode (1.77 cm^2) connected to the positive pole of a current generator. The receptor compartment is filled with an isotonic phosphate buffer (pH 7.4) and contains a wire electrode connected to the negative pole of the generator.

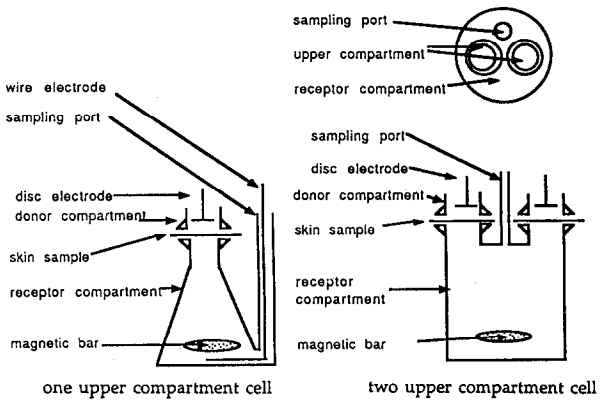

Fig. 1. The two permeation cell designs.

The 'two upper compartment' cell (TUC cell) is constituted of two upper compartments and a lower receptor compartment (Fig. 1). Each upper compartment contains a disc electrode, the compartment filled with the morphine solution is connected to the positive pole and the other upper compartment is filled with isotonic saline solution.

The surfaces of the skin samples and of the disc electrodes are the same with every kind of cell used. The permeation cells were in a thermostated water-bath at 37°C.

Morphine hydrochloride was added to deionized water to provide 10 mg/ml solutions.

Tritiated water (Dositek, France) (400 MBq/ml) was added in deionized water to achieve an activity of approx. 4 μCi/ml.

Skin

Hairless mice were used. For the OUC cell, only abdominal skin was used. For the TUC cell, abdominal skin was placed in the donor compartment and dorsal skin in the cathodic compartment.

Morphine assay: the morphine was assayed with a Waters® HPLC system: an M 501 pump; a Wisp 710 automatic injector; an M 481 UV detector and a μBondapack® column (C18 10 μm × 15 cm).

Operational conditions were as follows: mobile phase, 0.01 M ammonium acetate/acetic acid (pH 4.8) 85%, acetonitrile 15%; flow rate, 1.2 ml/min.

Detection was performed based on a wavelength of 284 nm.

The samples countaining labelled water were placed in a spectrophotometer counter (Beckman LS6000®[3]).

Results and Discussion

Direct current iontophoresis of MHCl with the OUC cell

According to their nature, electrodes can induce electrochemical reactions which are not without consequences on the efficacy of iontophoretic drug delivery. If inert electrodes are used, for example, platinium electrodes, electrons are produced by electrolysis of water which lead to the production at the anode of hydronium ions responsible for pH instability. The protons produced at the anode, or cations resulting from the addition of buffer in order to maintain the pH, behave like competitive ions for the delivery of cations.

To avoid water electrolysis and its consequences, it is possible to use an artifical electrode, such as a silver electrode (Phipps et al., 1989). Its redox potential is lower than that of water, thus the electrode itself produces electrons necessary for transport of electricity.

These kinds of electrodes therefore appear to be appropriate for an experimental model avoiding artefacts due to secondary phemomena. These electrodes were then used in an iontophoretic experiment with a direct current of 0.5 mA (corresponding to a current density of 0.28 mA/cm^2) and MHCl solution (1%). The results of these kinetic experiments are plotted in Fig. 2, which demonstrates that the release of MHCl was stable (duration of experiment 5 h). The flux calculated from the linear regression was $J = 0.50 \pm 0.005$ mg/cm^2 per h. During this experiment, the pH remained stable in both cell compartments; it was also possible to measure a short lag-time, i.e., 0.12 h. The iontophoretic efficiency representing the ratio of the electrical charge quantity transported by the drug to the total electrical transport was 13%.

Although the quantities of MHCl released were considerable (after 5 h of iontophoresis, $\cong$ 50%

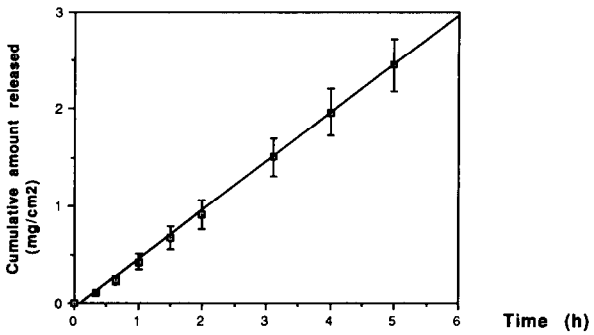

Fig. 2. Kinetics of morphine hydrochloride permeation at 0.5 mA with a one-upper-compartment cell ($n = 6$; error bar = 1 SD).

of the drug present was released), the value of the iontophoretic efficiency indicates that the cation morphine is not the principal charge carrier.

Under the above experimental conditions, the modulation of release by current intensity was investigated. The results illustrated in Fig. 4 (obtained with same skin samples in order to avoid skin heterogeneity) show that the increase in current intensity led to a satisfactory increase in MHCl release. However, inspection of the parameters listed in Table 1 shows that the increase in current intensity led to an abnormal increase in iontophoretic efficiency (total current fraction transported by MHCl ions).

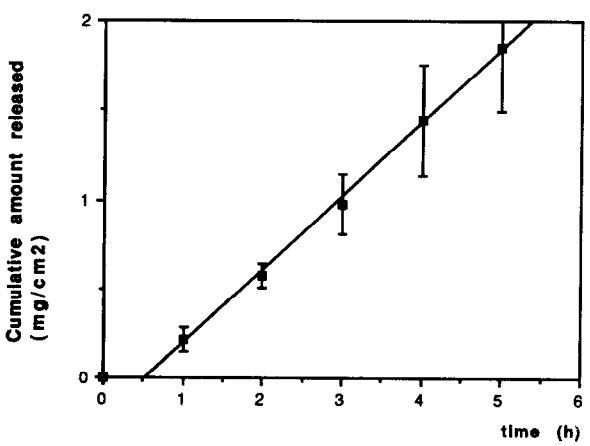

Fig. 3. Kinetics of morphine hydrochloride permeation at 0.5 mA with a two-upper-compartment cell ($n = 6$; error bar = 1 SD).

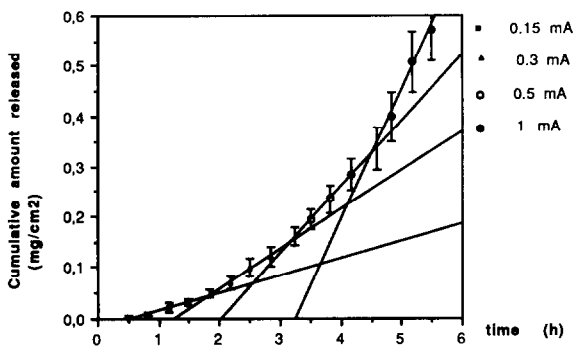

Fig. 4. Kinetics of morphine hydrochloride permeation with increasing intensity of pulsed current in a two-upper-compartment cell ($n = 6$; error bar = 1 SD).

Although the maximal current density (0.28 mA/cm^2) used in this study was lower than the maximal value generally employed in the literature (0.5 mA/cm^2), this increase in iontophoretic efficiency could be indicative of an increase in skin permeability during the experiment. The increase in skin permeability could be due to an alteration, not necessarily irreversible, of the skin barrier properties induced by the current density.

MHCl release with the TUC cell

With the TUC cell, the existence of an electrical wire under the skin (dermal side) is inconsistent with the in vivo conditions for use:

The surface of the wire, used as a cathode, is small compared to the disc electrode, consequently, for the same current intensity, the current density with the wire could be high in comparison to that with the two disc electrodes (even if the wire is far from the skin sample).

TABLE 1

Flux (mean ± SD, n = 6) and iontophoretic efficiency of morphine hydrochloride permeation in one-upper-compartment cell with increasing intensity of direct current

Intensity (mA)	Flux (mg/cm^2 per h)	Iontophoretic efficiency (%)
0.15	0.11 ± 0.01	9
0.3	0.26 ± 0.03	10
0.5	0.51 ± 0.04	13

The fact that the wire is placed under the skin, instead of on the upper side, could artificially lead to an increase in percutaneous delivery.

Thus, in order to conform with the in vivo conditions, we used the TUC cell (Fig. 1) which allows the placement of two circular electrodes on the same side of the skin.

Fig. 3 represents the MHCl release kinetics obtained with the TUC cell, two Ag/AgCl disc electrodes of the same size and a current of 0.28 mA/cm^2. The flux as well as the pH remained steady throughout the experiments (5 h). However, the flux ($J = 0.41 \pm 0.1$ mg/cm^2 per h) was smaller than that determined with the OUC cell ($J = 0.50 \pm 0.05$ mg/cm^2 per h).

Since the quantity of MHCl extracted at the passive electrode was negligible, such differences in flux could be due to an improvement in the state of the skin due to the new position of the electrodes or result from the geometry of the receptor compartment.

In order to differentiate the effects due to electrode modifications (area and localization) from those arising via modification in receptor design, a wire was placed directly in the receptor, through the sampling port, as in the OUC cell experiments.

Comparison of the two cells having the same electrodes localization showed the influence of the design of the receptor compartment, since the flux of 0.50 ± 0.04 mg/cm^2 per h with the OUC cell became 0.69 ± 0.13 mg/cm^2 per h with the TUC cell. Statistical analysis of the results demonstrated a significant difference (Student's t-test with $p = 0.01$)

With the TUC cell, the flux obtained in the case when the cathode was a disc electrode ($J = 0.41 \pm 0.01$ mg/cm^2 per h) was statistically different from that of a wire electrode placed under the skin. ($J = 0.69 \pm 0.13$ mg/cm^2 per h) (Student's t-test $p = 0.01$). These results are listed in Table 2.

On the basis of the data obtained, the shape of the permeation cell, the electrode localization and the electrode area exert an influence on iontophoretic permeation, the TUC cell appearing to be more favorable to drug permeation between the donor and receptor compartments.

TABLE 2

Comparison of morphine hydrochloride fluxes (mg/cm^2 per h; mean ± SD; n = 6) with different cell designs and electrodes localizations

Permeation cell	OUC cell [a]	TUC cell [b]	TUC cell [b]
Cathode localization	receptor	upper compt	receptor
Cathode shape	wire	disc	wire
Flux	0.50 ± 0.04	0.41 ± 0.098	0.69 ± 0.3

[a] One-upper-compartment cell.
[b] Two-upper-compartment cell.

With the TUC cell, the influence of the current intensity on morphine flux is given in Table 3 (with same skin sample). As described above, the iontophoretic efficiency was also increased at 0.28 mA/cm^2, despite the use of two disc electrodes minimizing the effects of a local current density increase. Thus, this increase in skin permeability cannot be due to poor experimental protocol; the two electrodes were identical and both were placed on the same side of the skin.

Pulsed current iontophoresis of MHCl

An increase in iontophoretic efficiency is indicative of an increase in skin permeability reflecting an alteration of the skin related to the current density. Two possibilities could be considered to explain this membrane modification: the first assumption is related to skin lesions occurring by polarization when the intensity or voltage of the applied current reaches high values.

However, a second hypothesis given in literature is related to a mechanism involving ion permeation through the skin:

The diffusion pathways of ions for iontophoresis are different from those for the passive diffu-

TABLE 3

Flux (mean ± SD, n = 6) and iontophoretic efficiency of morphine hydrochloride permeation in two-upper-compartment cell with different intensities of direct current

Intensity	Flux (mg/cm^2 per h)	Efficiency (%)
0.15 mA	0.07 ± 0.02	6
0.3 mA	0.8 ± 0.03	7
0.5 mA	0.42 ± 0.05	11

sion of unionized molecules. The major pathway is through pores (Burnette and Ongpipattanakul, 1988), and pore formation has been suggested as a mechanism which could be current dependent (Sims et al., 1991). Thus, the intrinsic skin permeability could increase with the current intensity without skin alteration.

Some authors have envisaged modulation of the current intensity by the use of non-constant electrical modes (Okabe et al., 1986; Liu et al., 1988). The inactive period would then give the skin the chance to depolarize back to its initial state. If the increase in skin permeability is due to the current intensity, the use of pulsed currents could be valuable in order to preserve the skin characteristics.

In order to consider pulsed currents under conditions which involve limitations in the case of direct currents, experiments were performed at different current densities (from 0.085 to 0.56 mA/cm^2, frequency 1 kHz and on/off ratio 1:1). Each current intensity was applied successively on the same skin sample. The results obtained for direct and pulsed currents are given in Table 4.

Firstly, Fig. 4 shows that pulsed current led to the modulation of MHCl delivery. It is possible to compare fluxes obtained at the same current intensity, for example, the flux determined at 0.085 mA/cm^2 with direct current vs that with pulsed current at a maximal current density of 0.085 mA/cm^2. Furthermore, it is also possible to compare fluxes at the same electrical charge quantity: in this case, since the on/off ratio was 1:1, the result obtained at 0.085 mA/cm^2 with direct current must be compared with pulsed current at a current density of 0.17 mA/cm^2.

In both cases, pulsed current leads to lower MHCl fluxes than direct current. This result is consistent with the data reported by Thysman and Préat (1992) on fentanyl, a drug similar to MHCl. For this molecule, delivery was greater with direct current compared to pulsed current of the same intensity. At the same electrical charge quantity, the difference was not significant at the lowest intensities, however, it became significant ($p = 0.01$) at the highest densities (0.28 mA/cm^2 direct current and 0.56 mA/cm^2 pulsed current). This result was not surprising, since direct current showed an abnormal increase in flux at the highest current density.

The above analysis was confirmed by comparison of the iontophoretic efficiency which was constant for the four current intensities tested with pulsed current; this was not the case for direct current.

TABLE 4

Comparison of morphine hydrochloride fluxes (mg/cm² per h; mean ± SD; n = 6) and iontophoretic efficiency between direct current and pulsed current for different current intensities

	0.15 mA	0.3 mA	0.5 mA	1 mA
Direct current				
J	0.07 ± 0.02	0.18 ± 0.03	0.42 ± 0.05	
Efficiency	6%	7%	11%	
Pulsed current				
J	0.034 ± 0.01	0.08 ± 0.03	0.13 ± 0.02	0.26 ± 0.02
Efficiency	6%	6%	6%	6%

TABLE 5

Tritiated water flux (mean ± SD; n = 6) through skin before, during and after an iontophoresis with direct current (0.5 mA, 5 h)

	Flux ($\mu l/cm^2$ per h)
Passive	2.16 ± 0.5
0.5 mA constant	8.4 ± 3.1
Passive	5.74 ± 2.4

Tritiated water permeation

As the determination of passive skin permeability to tritiated water is a very common technique used to evaluate the barrier properties of the skin, water fluxes for constant or pulsed current were compared.

The results presented in Tables 5 and 6 show water fluxes measured before, during and after the application for 5 h of 0.28 mA/cm^2 direct current or rectangular pulsed current (frequency 1 kHz and on/off ratio 1:1).

Application of the constant current led to an increase in water flux, resulting from convective diffusion under the influence of the electrical

TABLE 6

Tritiated water flux (mean ± SD; n = 6) through skin before, during and after an iontophoresis with pulsed current (maximal intensity 0.5 mA, 5 h, frequency 1 kHz and on / off ratio 1:1)

	Flux (μl/cm^2 per h)
Passive	1.91 ± 0.4
0.5 mA pulsed	3.61 ± 0.2
Passive	1.82 ± 1.0

field (iontohydrokinesis). This increase corresponds to a factor 4.

After 5 h of iontophoresis, the current was stopped, however, the water flux was maintained for at least 3 h. Logically, this flux was smaller than that during current application due to the disappearance of iontohydrokinesis mechanisms, however, during this third period, the flux was significantly greater than in the first passive step. The fact that, after the end of current application, the flux value did not return to its initial value is indicative of some increase in skin permeability due to the current applied. The highest direct current density used in this study (0.28 mA/cm^2) resulted in an increase in passive water flux after 5 h of iontophoresis as compared with the initial passive water flux.

This result is in agreement with the report of Thysman and Préat (1992) where the passive water flux after 6 h of iontophoresis with a direct current of 0.17 mA/cm^2 was considerably increased.

Burnette and Ongpipattanakul (1988) also observed an increase in water passive flux after the application of a direct current of 0.16 mA/cm^2 for a period of 1 h only.

Even if measurement of the passive water permeability shows that direct current does perturb the barrier properties of the skin, then it is important to note that no iontophoretic kinetic process taking place at 0.28 mA/cm^2 during a period of 5 h showed any perturbation of the release (cf. Figs 2 and 3). Therefore, the modifications induced by the currents employed are undoubtedly instantaneous.

Table 6 lists the results obtained with pulsed current. The passive initial flux was comparable to the previous passive flux (Table 5). Thus, further comparisons would not be distorted by differences in intrinsic skin permeability. However, the application of the pulsed current led to a mean flux ($J = 3.61$ μl/cm^2 per h) of approximately one-half of that with direct current ($J = 8.4$ μl/cm^2 per h). This result can be interpreted by simple consideration of the quantity of electricity which was 2-fold that with a pulsed current (on/off ratio 1:1).

After the end of pulsed current application, the flux returned to its initial value. One may therefore conclude that after application, for a duration of 5 h, of a rectangular pulsed current of 1 kHz, on/off ratio 1:1 and maximal intensity 0.5 mA, the initial passive water permeability of the skin was recovered and that this current mode did not lead to modification of the bioelectrical properties of the skin.

Conclusion

By using a simple experimental model, it was verified that the use of Ag/AgCl electrodes yielded stable MHCl fluxes, which were maintained for at least 5 h at the current intensity usually required during iontophoresis experiments. Although the use of a new type of permeation cell (which conforms more closely to the in vivo situation) leads to the better repartitioning of the current density onto the skin, such modifications of the cell geometry and electrodes do not allow the skin permeability to be maintained at the highest current density (0.28 mA/cm^2). This was confirmed by the use of tritiated water: at 0.28 mA/cm^2 constant current, the passive permeability after 5 h of iontophoresis was greater than the initial passive permeability. This phenomenon could be explained by an increase in intrinsic skin permeability with current intensity or by an alteration as a result of skin polarization (which could be both time- and current-intensity-dependent). However, it was found that no increase in MHCl flux could be detected during experiments at 0.28 mA/cm^2 for a period of 5 h. On the other hand, the use of pulsed current led to the skin permeability being maintained from

0.085 to 0.56 mA/cm^2 (frequency 1 kHz; on/off ratio 1:1). Thus, it was hypothesized that pulsed currents lead to better retention of skin integrity. This was confirmed by the use of tritiated water. In fact, with 0.28 mA/cm^2 pulsed current, passive water flux was the same before and after the application of the pulsed current for 5 h. This was not the case with 0.28 mA/cm^2 constant current.

By means of such an iontophoretic in vitro device, it is possible to study under optimal conditions the fundamental mechanisms involved in iontophoresis and to obtain results in agreement with theoretical predictions.

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