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Original Article

An *In-Vitro* Iontophoretic Permeation Study of Nicotine through Rat Skin

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Abstract

Nicotine transdermal systems are being used as an aid to smoking cessation programs. As the kinetics of nicotine delivery is important in success of a smoking cessation program, rapid and high input of nicotine is required, which is not possible by passive methods and requires enhancement strategies such as iontophoresis. Iontophoretic permeation, of nicotine looks promising, based on published data on human skin. However, to optimize this method, permeation pathways should be known and further parameters have to studied, which are the subject of the present investigation. In this study iontophoretic permeation of nicotine through rat skin was performed and the effects of different variables on this phenomenon were studied. Anodic iontophoresis of nicotine from a solution at pH 2.8, using a 0.5 mA/cm^2 current density resulted in a considerable enhancement (about 3-fold) of nicotine absorption through rat skin. Nicotine concentration and current density showed a directly increasing effect on permeation of the drug, but the effect of concentration was not linear. Pulsatile current delivery was more effective in permeation of nicotine than the continuous method. Anodic iontophoresis was around 2-fold more effective than the cathodic method in increasing the flux. Post iontophoretic permeation studies showed good reversibility of the membrane barrier properties. Results were in good agreement with the reported human data and might be considered as an evidence of the ability of rat skin to model human skin and also the importance of intercellular pathway of the stratum corneum in iontophoretic delivery of nicotine and possibly other drugs. Donor's pH showed no effect on permeation of nicotine under the studied conditions, pH values of <3. Results also showed that the electr-osmotic flow could occur at pH values lower than 4. Finally, this study show that by controlling the effective parameters of iontophoretic delivery, a more effective nicotine transdermal delivery method would achievable.

Keywords: Iontophoresis; Transdermal delivery; Nicotine; Rat skin; Permeation.

Introduction

The insidious effects of smoking cigarettes on human health have led to numerous attempts to promote cessation, including various nicotine replacement therapies. Most recently, several transdermal delivery systems have been successfully marketed through the world. Because of the rapid input and efficient clearance of nicotine, through the lung (1), similar phenomena are needed for delivery of nicotine

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using these systems, and could not be achieved via the above mentioned systems. Nicotine is an ionizable diacidic base with pKa values of 3.04 and 7.84 (2) and a molecular weight of 162.23 Daltons (3) and is, therefore, positively charged at the physiologic pH of the skin (4). Due to its charge and also the nature of skin barrier, nicotine cannot permeate the skin fast enough to simulate its absorption profile through the lungs; a problem which might be solved by some enhancement methods like iontophoresis.

Iontophoresis is a non-invasive physical technique, using a mild electric current to facilitate transdermal delivery of a variety of ionized and non-ionized drugs (5, 6) and has been shown to be very promising in facilitating percutaneous absorption of some bioactive compounds such as insulin and offering an expedient alternative to target drug delivery (7-9).

There are several factors affecting iontophoretic transdermal drug delivery. These factors include physicochemical properties of penetrants, donor and receptor solutions composition, electric current and barrier properties of the membrane of which some are not completely defined and require further studies (10). The magnitude of a compound's potential for iontophoretic delivery is theoretically proportional to its charge (7, 10) and inversely related to its molecular weight (11) and hydrophobicity (12). Donor and receptor solution composition can greatly influence the iontophoretic delivery. Three important factors of drug concentration, solution pH and buffer capacity (concentration) should be considered in formulating a donor solution (7, 10). The relationship between drug concentration and iontophoretic flux is often direct, but influences of donor solution pH and buffer concentration are complex (7, 10). Iontophoretic flux has been shown to be directly related to iontophoretic current intensity. Current can be used continuously or pulsatile. However, the potential current strategy requires further study and may differ based on the peneterant (10-12). Altogether, transdermal absorption of drugs using iontophoresis is a complex phenomenon and influence of each factor on absorption is poorly recognized and would vary depending on the permeant.

Studies on nicotine iontophoresis are limited. Brand and Guy (4) studied different iontophoretic conditions on permeation of nicotine through human cadaver skin to access a rapid absorption of nicotine, similar to nicotine absorption through lungs. The conditions included pH values of 4-9, DC current densities of 0.17 – 0.5 mA/cm^2, nicotine concentrations of 5.25 - 52.5 mg/ml and a time course of 15 – 120 min.

To optimize the iontophoretic permeation of drugs (including nicotine), the iontophoretic permeation pathway should also be known, which has not been well studied as yet. Therefore, we decided to study the above mentioned parameters on permeation of nicotine through different skin models including a hairy skin (rat skin), a hairless membranes (snake skin) and a lamellar liquid crystalline structure and compared the results with the above mentioned human data; of which the rat skin is presented here. Besides this, other factors including charge profile, lower pHs of the receptor solution (below 3, which nicotine carries 2^+ charge) and charge delivery direction will be investigated in this study. Rat skin is a widely used, well-recognized and easily-accessible model in transdermal delivery studies. It has been shown that permeation rate of nicotine through rat and human skin in passive conditions are similar (13). This might show the similarity between passive permeation pathways of two skin models, but cannot be extrapolated to iontophoretic conditions as the pathways might be different.

Experimental

Materials

All materials were used as received. Nicotine was obtained from Merck (Germany). All other solvents and reagents were of analytical grade and obtained from Merck (Germany).

Skin Permeation Studies

Preparation of rat skin

Abdominal skin from 300-350 g weight, 8-10 weeks old male rats was used for this study. Rats were first sacrificed by placing them in a chloroform-saturated chamber. The abdominal hairs were then cut by an electrical hair clipper and

full-thickness skin was separated surgically. The separated skin was cleaned from subcutaneous fat, muscle and vasculature and kept frozen at -20ºC. Frozen skin samples were defrosted at room temperature before use.

General procedure

Permeation studies were performed at 32ºC using home-made Franz-type diffusion cells, with an effective surface area of 1.8 cm^2. Rat skin was sandwiched between two half-cells and equilibrated with donor and receptor phases for 12 h. The contents of receptor and donor chambers were then removed and replaced with 25 ml of receptor solution and 2 ml of donor phase (or its blank solvent), respectively. This point was considered as "time zero". The receptor chambers were stirred at 300 rpm with a Teflon-coated stirring bar during these experiments. The experiments were performed for 4 h under passive conditions.

For iontophoretic studies, two platinum wires, each connected to a 12.6 mm^2 plate, were used as electrodes and inserted into donor and receptor chambers, and powered with an adjustable direct power (DC) sources (Tayf-Asa Co., Iran). Iontophoretic experiments were performed for 6 h of which the first 3 h was active and the second 3 h passive. This passive permeation after cutting the electric current was named the post iontophoretic passive permeation (PIPP). The active part was performed using two different methods of continuous and pulsatile. In pulsatile conditions, a 30/10 min on/off time was used. In all conditions, except in comparative studies of anodic and cathodic iontophoresis, anodic iontophoresis was practiced.

Serial sampling (3 ml) of the receptor phase, with replacement with fresh solution, was performed for 6 h at sampling intervals of 20-30 min. The amount of absorbed drug was measured spectrophotometrically as discussed below.

The cumulative amount of permeated drug was plotted against time and the slope of the linear part of the graph (permeation flux) was measured. Besides passive and iontophoretic (active) experiments, a secondary permeation flux was also calculated in iontophoretic studies. This passive permeation of nicotine after cutting the electric current was named the post-iontophoretic passive permeation (PIPP). Using the Fick's law, permeability coefficient was calculated from the permeation flux (14). Iontophoretic enhancement ratio (IER) was calculated from dividing the active to passive fluxes or permeability coefficients. Ratios of post iontophoretic passive permeation to passive permeation (PIER) were also calculated. Data were analyzed and compared statistically using a two-tailed t-test analysis, assuming that data are distributed normally and populations have equal variances.

Receptor and donor phases

Water or other aqueous systems are usually used as the receptor phase in permeation studies especially for water-soluble compounds such as nicotine, which is freely soluble in water. In our study, 0.1 N HCl solution (pH≈1) and British Pharmacopoeia (15) acetate buffer solution (pH≈2.8) were used as receptor phases (15). Nicotine solutions in acetate buffer or 0.1N HCl were used as the donor phases. Our preliminary studies showed that at high acetate buffer concentrations, iontophoretic permeation of nicotine was very low. This might be due to high interferences between buffer ions and nicotine in the electric field. Using a dilute buffer solution with a concentration of 1/10 of that mentioned in the British Pharmacopeia (15), still at pH of 2.8, the interference was mainly eliminated. This acetate buffer solution was used during this study as donor and receptor phases. pH controls during the experiment and after the last sampling showed sufficient buffer capacity to keep the pH of both donor and receptor phases unchanged during the course of experiments. Low pH values were used for optimum ionization of nicotine and also to provide possibility of comparing the results with those of intercellular lamellar lipid structure of stratum corneum. Nicotine is positively charged under the present conditions used.

Nicotine measurement

Nicotine determination was performed by UV spectrophotometry at 364 nm (λ_{max} of nicotine), using a UV-Visible Double–Beam system

(Shimadzu, Japan). Nicotine concentrations were calculated from the absorption of samples, using a calibration curve constructed from the absorbances of known–concentration solutions. There was no interference with the method and materials released from the skin.

Experimental conditions

The following experiments were designed to examine the effects of concentration, pH, current density and delivery method on iontophoretic and passive permeation of nicotine through rat skin.

Effect of nicotine concentration

Different nicotine concentrations of 5.25, 25 and 52.5 mg/ml in dilute acetate buffer (pH 2.8) were used as donor phases. Nicotine would be ionized and unsaturated under all these conditions. In iontophoretic studies, a pulsatile current of 0.5 mA/cm^2 was used for 3 h followed by another 3 h permeation in the absence of current. In passive conditions, a 4-h study period was used.

Effect of pH donor and receptor phases

Two different pH values of 1 and 2.8 were used as mentioned above. Nicotine concentration in the donor phase was 52.5 mg/ml and all other conditions were as explained in the previous section.

Effect of current density

Two different current densities of 0.17 and 0.5 mA/cm^2 were studied. Dilute acetate buffer containing 52.5 mg/ml nicotine was used as the donor phase. Dilute acetate buffer was used as the receptor phase and also as the donor solution in control studies. All other conditions were as explained in the previous section.

Effect of current delivery method

Continuous and pulsatile current delivery methods were used in a current density of 0.5 mA/cm^2. In continuous method, the power was on continuously for 3 h. But in the pulsatile method, the current was on for 30 min and off for 10 min during a 3 h delivery period. All other conditions were similar to the previous section.

Effect of iontophoresis type (current direction)

In this section a pulsatile current delivery, using the anodic and cathodic types, was practiced. In anodic iontophoresis, the passive electrode (anode) was placed in the donor solution. But in the cathodic iontophoresis, the negative electrode (cathode) was placed in the donor solution. All other conditions were similar to the previous section.

Results and Discussion

Influence of concentration on nicotine permeation

Figure 1 shows sample permeation profile of nicotine in iontophoresis (active and PIPP) (at a constant current of 0.5 mA/cm^2) and passive conditions across rat skin. Permeation data for all concentrations are summarized in Table 1 and enhancement ratios in Table 2.

Flux of nicotine (from a pH 2.8 acetate buffer) increased significantly ($P<0.05$), about 3-folds with concentration, over the range of 5.25-52.5 mg/ml under the iontophoretic condition. However, the relationship between concentration and flux was not linear; i.e. permeability coefficient (Kp) was not constant and changed significantly ($P<0.05$) with concentration in all systems. Results (Table 1) showed that Kp decreases with concentration in all systems. As the donor phases in all concentrations are subsaturated, this might suggest saturation of nicotine in skin channels and pathways or dimmer or trimmer formation of nicotine at higher concentrations, all of which requires further investigations and were out of the

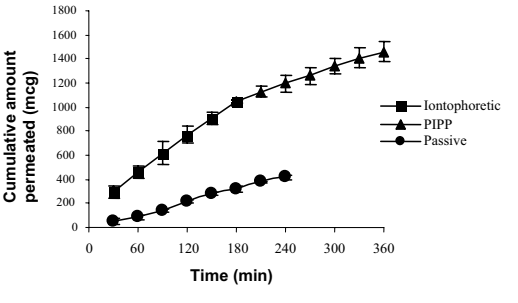

Figure 1. A sample permeation profile showing passive (circles), iontophoretic (square) and post iontophoretic passive permeation (PIPP) (triangles) of nicotine, at a donor concentration of 52.5 mg/ml,through rat skin. Data are mean ± SD, n = 3-4.

Table 1. Effects of concentration on passive, active and post iontophoretic passive (PIPP) permeation of nicotine through rat skin.

Nicotine concentration (mg/ml)	Flux(μg/cm^2/min)			K_p (cm/h)		
	Passive	Active	PIPP	Passive	Active	PIPP
5.25	0.31 ± 0.05	0.88 ± 0.20	0.21 ± 0.04	3.51 ± 0.56	10.10 ± 2.23	2.41 ± 0.42
25	0.75 ± 0.06	2.76 ± 0.10	0.93 ± 0.28	1.79 ± 0.15	6.62 ± 0.24	2.24 ± 0.66
52.5	1.09 ± 0.02	3.31 ± 0.19	1.30 ± 0.27	1.24 ± 0.02	3.78 ± 0.21	1.49 ± 0.31

Data are mean ± SD, n = 3-4.

scope of this investigation. The same trend and phenomenon have been reported for permeation of nicotine through human skin (4), which shows either similarity of permeation pathways in two membranes and/or a phenomenon that is only related to nicotine molecule like dimerization.

Results show that IER and PIER are concentration dependent under the present test conditions (Table 2), but, this correlation was non-linear. As shown in Table 2, IER in all systems and conditions (2-5) is much higher than that of PIER (≈1). Although in concentrations of 25 and 52.5 mg/ml, PIER show values of slightly higher than 1, the differences between corresponding fluxes are not significant ($P<0.05$, n=3-4) and it could be said that rat skin returns to its original barrier properties post iontophoresis. In the case of concentration of 5.25 mg/ml, the enahancement ratio is less than 1, PIER = 0.7, which is still not significant considering the corresponding flux data ($P<0.05$, n=3-4). The trend might reveal that the reversibility might show clear concentration dependency at lower concentrations.

Influence of donor solutions pH

As shown in Table 3, there is no difference between the passive or active flux of nicotine through rat skin at different pH values of 1 and 2.8 ($P<0.05$). this might be due to similar ionic behavior of nicotine and barrier properties of skin in both conditions.

As discussed before, nicotine has two positive charges in both pH values studied. Based on the Henderson-Hasselbalch equation, the ratio of charged nicotine at pH 1 is much higher than pH 2.8. As shown, it seems that permeability of two nicotine types is almost the same under the studied conditions ($P<0.05$, n=3). Similar permeation, despite of charge difference, might indicate similar ionic mobility of the two types or that the charge which is different between molecules is easily covered by counter ions present in the system.

Table 2. Iontophoretic enhancement ratio (IER) and post iontophoretic enhancement ratio (PIER) of nicotine through rat skin at different concentrations. See text for more details.

Nicotine concentration (mg/ml)	IER	PIER
5.25	2.879	0.687
25	3.690	1.250
52.5	3.047	1.199

Influence of current density

Table 4 shows the effects of current density on permeation of nicotine through rat skin. Results (Table 4) show that a 3 times increase in the current density increases the fluxes by about 3 times. These results obey the Nernst law and show that the electrical current does not affect the skin barrier significantly in the range studied. The results are, also, in good agreement with those of human data (4) and might be considered as an evidence for similarity of both skins in iontophoretic permeation. Higher than 0.5 mA/cm^2 current densities, which could damage the skin, are not usual in iontophoretic skin studies.

Influence of current delivery method

Table 5 shows the impact of charge delivery method on nicotine absorption. As shown in Table 5, nicotine flux under pulsatile conditions is significantly ($P<0.05$), about 1.3-fold, more than that of the continuous delivery method. Skin polarizes during iontophoresis and this polarization might reduce permeation of drugs. Application of a direct current for a short period of time followed by a period of zero current is thought to allow the skin to recover and re-polarize, therefore decreasing skin resistance and increasing iontophoretic delivery (10).

Table 3. Effect donor phase pH on nicotine flux (μg/cm^2/min) through rat skin.

pH	Passive	Iontophoretic (active)
1	1.06 ± 0.04	3.38 ± 0.31
2.8	1.09 ± 0.02	3.31± 0.19

Data are mean ± SD, n = 3-4.

Table 4. Effect of current density on nicotine flux across rat skin.

Current density (mA/cm^2)	Flux ($\mu g/cm^2/min$)
0 (Passive)	1.09 ± 0.02
0.17	1.40 ± 0.21
0.5	3.31 ± 0.19

Data are mean ± SD, n = 3-4.

However, as mentioned above, the difference (1.3 times) is minimal and may emphasize the importance of temporary pathways in intercellular lamellar lipids of stratum corneum. The 10 min passive condition in pulsatile current delivery could be an enough time to destroy the temporary pores formed in intercellular lipids resulting current.

Influence of anodic vs cathodic iontophoretic delivery

Table 6 shows that the enhancement observed in rats tested using the anodic and cathodic conditions are about 3.3 and 1.6, respectively. This shows that the iontophoretic enhancement of anodic method is twice that of the cathodic method. This difference shows importance of electro-osmosis flow in iontophoretic transdermal drug delivery of nicotine, as a diacidic positively-charged and low-molecular weight molecule.

Electro-osmotic flow can affect permeation of most drugs through different skin types like rat (16) and human (17) skin and appears in anodic iontophoresis and could enhance positively-charged or non-ionic drugs (16-18). However, it is usually expected at pH values above 4.0 in rat skin (18). The present investigation also shows that the electro-osmotic flow could affect permeation of drugs at lower pH values.

As shown, iontophoresis could increase transdermal absorption of nicotine in rat skin. The delivery profile is sensitive to drug concentration, current density, current delivery method and the iontophoresis type. Pulsatile anodic iontophoresis showed a higher enhancement effect than the other conditions such as the cathodic and/or continuous methods. pH of donor and receptor solutions did not show any significant effect on the iontophoretic delivery of nicotine. It should be noted that under the studied pH range, nicotine carries positive charges. Our studies also show that elecro-osmosis is an important influencing factor at pH values lower than 4. Effect of iontophoresis on barrier performance of skin was reversible. This property permits a precise control of the amount of drug delivered in pulsatile drug delivery systems, if are to be designed.

Table 6. Effect of cathodic vs. anodic current delivery on the permeation of nicotine through rat skin.

Current delivery	Flux ($\mu g/cm^2/min$)
Anodic	3.31± 0.19
Cathodic	1.74 ± 0.25
Passive	1.09 ± 0.02

Data are mean ± SD, n = 3-4.

Table 5. Effect of current delivery method on the permeation of nicotine through rat skin.

Current delivery method	Flux ($\mu g/cm^2/min$)
Continuous	2.81± 0.10
Pulsatile	3.31± 0.19

Data are mean ± SD, n = 3-4.

Similarity of nicotine iontophoretic delivery in rat and human skin is an evidence for modeling abilities of rat skin in human iontophoresis and could show the importance of intercellular pathways of stratum corneum in iontophoretic delivery of drugs. Further studies are in progress in our laboratories regarding the iontophoretic permeation pathways.

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