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ENHANCED TRANSPORT OF SALBUTAMOL SULPHATE THROUGH RAT SKIN VIA LONTOPHORESIS

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The effects of various factors like pH of the donor solution, current density and permeation enhancers on the passive and iontophoretic transport of salbutamol sulphate (SS) have been studied through rat skin. The transport of the drug was greater during iontophoresis in comparison to passive diffusion at all pHs studied. Maximum steady state flux (26.38 $\mu g/cm^2/hr$) was found at pH 11 during cathodal iontophoresis. However, the fraction change in flux was maximum at pH 7.4 during anodal iontophoresis. It was observed that an increase in the current density resulted in the increase of both cathodal and anodal iontophoretic flux. Permeation enhancer, dimethylsulphoxide (DMSO), increased the permeability coefficient of SS across the rat skin. One of the most important applications of this study is the possibility of enhancing and controlling the transdermal delivery of salbutamol sulphate by iontophoresis.

Keywords: Iontophoresis; Flux; pH; Current density; Permeation enhancers

Introduction

Iontophoresis is a process which causes an increased penetration of ionized substances into or through a tissue by the application of an electric field (1). It is being increasingly investigated as a technique for enhancing the penetration of ionic drugs through the skin as the transport of ionic and polar solutes are not favoured by passive diffusion (2, 3). Iontophoresis has been used with success for local (4, 7) as well as systemic delivery of drugs (8, 10). It appears to be a safe technique for transdermal delivery of solutes (11,12).

The factors affecting iontophoretic transport of solutes across the skin have been already discussed by several workers (13, 18). However, to ascertain the iontophoretic delivery, a particular compound has to be investigated individually as drugs differ in their physico-chemical characteristics.

Therefore, the purpose of this study was to investigate the effect of pH. current density and premeation enhancers on the transport of Salbutamol Sulphate, a highly selective β agonist.

Salbutamol sulphate undergoes extensive first pass metabolism and only 50% of the oral dose is systematically available. It has a short elimination $t_{1/2}$ of about 5 hrs. Its oral use is declining because of higher risk of cardiovascular side effects. This drug even worsen the bronchial hyperactivrty by repeated use (19). It has a pKa values of 9.3 (amino group) and 10.3 (phenolic group) which permits protonation and deprotonation in the pH range of 7.4 to 11.0. It is also freely soluble in water (20). The above attributes make SS a suitable candidate for transdermal delivery by iontophoresis.

Materials and Methods

Materials

Salbutamol sulphate was supplied by Cipla Ltd., Bombay. Other chemicals were trisodium citrate (Glaxo Laboratories Ltd., Bombay), gtycine (Glaxo Laboratories Ltd., Bombay), disodium hydrogen phosphate (E Merck Pvt. Ltd. Bombay), potassium dihydrogen orthophosphate (Central Drug House Pvt. Ltd., New Delhi) and dimethyl sulphoxide (Ranbaxy Laboratories Ltd., S.A.S. Nagar, Punjab).

Methods

Fabrication of constant current source: This was fabricated by University Science Instrumentation Center. Banaras Hindu University, Varanasi, India. 9V DC could supply the constant current in the range 0-10 mA at the load resistance limited maximum up to 800 ohm. The current was displayed on analog mili ampere meter already fitted in the device.

Preparation of buffer solutions: The donor solution was prepared by mixing equal proportions of 30 mM of trisodium citrate, disodium phosphate and glycine. The pH of the buffer was adjusted to different pHs (7.4, 9.4,10.3 and 11) by adding 1 M sodium hydroxide or 1 M hydrochloric acid. The receiver fluid was 0.2 M phosphate buffer (pH 7.4) (15).

Preparation of epidermis: Male albino rats (8-12 week old) with an average body weight of 200-500 g were euthanatized by ether (I.P.) in a closed chamber (21). Hairs from the abdominal area were gently trimmed and then abdominal skin was removed. The excess of the adipose tissue was removed by gentle scrapping before using the whole thickness skin. The skin pieces were soaked in the receptor buffer solution for approximately one hour prior to placing in between the cells.

In-vitro studies: The method for *in-vitro* transport studies was similar to one described in our previous works (13,15). The donor and receiver compartments contained 5 ml of SS solution in the donor buffer of the desired pH and phosphate buffer (pH 7.4), respectively. The surface area of the epidermis exposed to the solution was 2.855 cm^2.

Platinum electrodes and the constant current source were used for iontophoresis. Anodal iontophoresis was carried out by inserting anode in the donor compartment and cathode in the receiver compartment. Cathodal iontophoresis was done by reversing the polarity.

The changes in pH during iontophoresis were monitored and corrected by the addition of microliter amounts of 1M NaQH or 1M HCl solutions. By this technique the pH was kept within ±0.2 units of the desired pH as determined at the end of the experiment.

Samples of 0.5 ml were withdrawn at regular intervals from the receiver compartment and the same volume was replaced by the receiver fluid. Samples (0.1ml) were taken from the donor compartment before and after the experiment to make sure that the decrease in donor concentration did not exceed 10% in order to maintain receiver drug concentration at a level less than 10% of donor concentration (3). The samples were diluted appropriately and the absorbances were measured at 276 nm spectrophotometricalty (Shimadzu spectrophotometer). The experiments were carried out in triplicate for each condition studied.

Data analysis: The SS concentration was corrected for sampling effects according to the equation described by Hayton and Chen (22).

The cumulative amount of SS transported per unit surface area was plotted against time, and the slope of the linear portion of the plot was estimated as the steady-state flux (J_{SS}). The permeability coefficient (Kp) was calculated as: $K_P = J_{SS}/C_V$ where. Cv is the total donor concentration of SS.

Statistical comparisons were made with Student's t-test (one-tail). The level of significance was taken as $p<0.05$.

Results and Discussion

Tablet 1 depicts the effect of pH on the steady-state flux, fraction ionized and fraction change in flux during passive diffusion and iontophoresis, Iontophoretic flux (both anodal and cathodal) was greater than passive at all the pHs studied. Anodal flux was significantly greater ($p<0.05$) at pH 7.4 and 9.3 and lower ($p>0.05$) at pH 10.3 and 11.0 than cathodal flux. The maximum flux (26.68 $\mu g/cm^2/hr$) was observed at pH 11.0 during cathodal iontophoresis. The highest fraction change in flux was observed at pH 7.4 during anodal iontophoresis where 99% of SS existed as cationic form.

It is evident from Fig.1 that increase in current density from 0.1-0.4 mA/cm^2 increased the flux nearly 12 and 6 times than passive flux during anodal and cathodal iontophoresis, respectively. A linear relationship between the flux and the current density was observed during anodal ($r=0.99$. at pH 7.4) and cathodal ($r=0.94$, at pH 11) iontophoresis. However, the increase in cathodal iontophoretic flux with current density was not significant ($p>0.05$) except at $0.4 mA/cm^2$.

Table 1. Effect of pHs on the steady state flux, fraction ionized and fraction change in the flux of Salbutamol Sulphate.

Ph	Steady State Flux (Jss) ($\mu g/cm^2/hr$)			Fraction ionized[a]	Fraction Change in J_{ss}[b]	
	Passive	Anodal	Cathodal		Anodal	Cathodal
7.4	2.26	25.2	3.5	0.99	0.91	0.35
9.3	8.94	19.95	11.97	0.50	0.55	0.25
10.3	9.6	14.31	23.2	0.50	0.33	0.58
11.0	6.69	7.4	26.28	0.99	0.096	0.74

a. Fraction of salbutamol sulphate ionized has been calculated by the Henderson-Hasselbalch equation.

b. Fraction change in flux = $\frac{\text{Iontophoretic flux} - \text{Passive flux}}{\text{Iontophoretic flux}}$

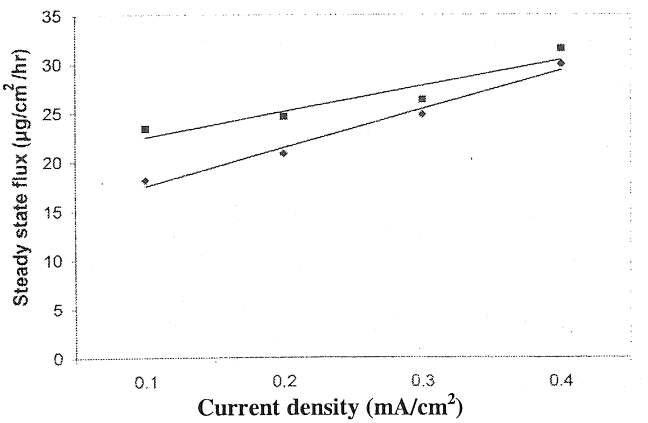

Fig. 1. Effect of current density on the steady state flux of salbutamol sulphate.

◆ Anodal, pH 7.4 ■ Cathodal, pH 11

The increase in the concentration of permeation enhancer DMSO significantly increased ($p<0.05$) the permeability coefficient of SS during passive diffusion and iontophoresis (Table 2). Iontophoresis coupled with DMSO significantly increased ($p<0.05$) the permeability coefficient of SS in comparison to the passive diffusion with DMSO. DMSO enhanced the permeability coefficient (E_2) by 4, 6 and 7 folds during passive diffusion and 14,17,20 folds during iontophoresis (E_1) at concentrations of 15, 25 and 35% respectively.

In the passive diffusion studies, the increase in the flux with increase in the pH from 7.4 to 10.3 and then decrease of flux at pH 11 were in accordance with pH-partition hypothesis according to which the transport of the unionized moeity of the drug was favoured through lipophilic biologic membrane (23,24). Although the ionization at pH 7.4 and 11 were equal the passive flux at pH 11.0 was greater than at pH 7.4. The water diffusion through excised hairless mouse skin has been consistent in the pH range 4 to 10. However, an increase in water diffusion (25) and decrease in the skin impedance (26) was noted at pH>10. The greater flux of SS at pH 11 can also be attributed to lesser impedance of the epidermis at this pH.

Table 2. Effect of different concentrations of DMSO on the permeability coefficient and enhancement factor of SS through rat skin during passive diffusion and iontophoresis [current density (0.3 mA/cm^2); drug concentration (8 mg/ml); pH 7.4].

Concentration of DMSO (%w/v)	**Permeability coefficient (cm/h) (cm/h) x 10^{-2}**		**Enhancement factor**	
	Passive	**Iontophoresis anodal**	**E_1**	**E_2**
0	2.82	3.15	-	-
15	12.25	45.06	14.30	4.34
25	17.75	55.92	17.75	6.29
35	21.50	64.32	20.41	7.62

$E_1 = P_{ie}/P_i$, $E_2 = P_{pe}/P_p$

Where,

P_{ie} = Permeability coefficient during iontophoresis with penetration enhancer

P_{pe} = Permeability coefficient during passive diffusion with penetration enhancer

P_i = Permeability coefficient during iontophoresis without penetration enhancer

P_p = Permeability coefficient during passive diffusion without penetration enhancer

The decrease of anodal flux and increase of cathodal flux with on increase in pH can be attributed to two different pKa values 9.3 (amino group) and 10.3 (phenolic group) of the drug. At pH 7.4 and 11.0, most of the drug (99%) was ionized and existed in cationic and anionic form, respectively. At pH 9.3 and 10.3, it exists in unionized as well as ionized form. Thus, with on increase in pH from 7.4 to 11.0, the cationic fraction decreases and anionic fraction increases. The transport of cations is favoured during anodal and that of anions during cathodal iontophoresis. This may be the possible explanation for higher fraction

change in flux during anodal iontophores at pH 7.4 and during cathodal iontophoresis at pH11.0. However, the maximum fraction change in flux (0.91) was at pH 7.4 during anodal iontophoresis. This may be due to electrical as well as convective solvent flow effects. The convective solvent flow was in the direction of flow of current and was facilitated to a greater extent during anodal than cathodal iontophoresis (17.29).

The linear relationship between flux and current density was in accordance with our earlier results (13.15) and can well be validated with work of Burnette and Marrero (17).

DMSO has increased the permeability coefficient of the SS during both passive diffusion and iontophoresis. DMSO is strongly hygroscopic and its presence in the stratum corneum greatly increases the hydration of the tissue (27). and thereby its permeability. DMSO can increase the permeability coefficient by reducing the resistance of both intercellular and transcellular routes. The transcellular route provides the main pathway for polar drugs and intercellular route is more significant for non-polar drugs during percutaneous absorption (28). At pH 7.4, the drug was in ionized form and it may be possible that transcellular route was predominant in enhancing the flux of the drug under the influence of different concentrations of DMSO used. Iontophoresis with DMSO leads to creasing of the epidermal surface of the human skin which increases the surface area of the skin. This creasing may provide more channels to increase the transport of the drug (14). This may have possibly occurred in case of rat skin also and resulted in greater permeability coefficient of SS during iontophoresis in conjuction with DMSO.

In conclusion, iontophoresis has enhanced the transport of SS through rat skin. The resulting flux was found to be proportional to the applied current density. The combination of iontophoresis with permeation enhancer is beneficial and may obviate the need of higher current strength for the transport of drug. These findings suggest that SS can also be delivered across the human skin at a controlled rate by iontophoresis.

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