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Electrically-Assisted Skin Delivery of Buspirone Submicron Emulsions

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ABSTRACT

The aim of this study was to demonstrate the potential application of electrically-assisted methods, including iontophoresis and electroporation, on the transdermal delivery of buspirone hydrochloride from a submicron emulsion-based system. The results showed that by applied iontophoresis at 0.5 mA/cm^2 for 3 h or electroporation at 1 pulse, 300 V, 200 ms, 0.5 min spacing for 10 min, increased the cumulative amount of buspirone at 10 h by 2.4 and 2.1 folds, respectively, compared to passive diffusion. The combination of iontophoresis and electroporation did not show synergistic effects. For long-time application of iontophoresis, the enhancement effect decreased in the later stage due to skin polarization. The duty cycle of current applied was found to increase iontophoretic efficiency. When compared to passive diffusion, the lag time of buspirone-loaded submicron emulsion permeated through skin was shortened from 3 h to 0.5 h with the use of both electrically-assisted methods, indicating that more rapid onset of buspirone could be obtained by using electrically-assisted methods.

Key words: buspirone hydrochloride, submicron emulsion, iontophoresis, electroporation

INTRODUCTION

Buspirone hydrochloride is an orally administered anxiolytic drug from the azapirone family of molecules. It is as potent as the benzodiazepines, but does not produce the sedation or motor impairment effect. The usual oral dosage of buspirone is 5 to 20 mg, 3 times/day. The drug is rapidly absorbed after administration, but it undergoes extensive first-pass metabolism. It has a half-life of about 2 to 3 h and a low absolute bioavailability of approximately 4%[(1-3)]. Hence, a transdermal system would provide an alternative way to bypass the disadvantages of the oral route.

However, the most difficult aspect of a transdermal delivery system is to overcome the barrier of stratum corneum against foreign substances. The methods for improving drug permeation through the skin include use drug carriers as vehicle (such as liposome, polymeric nanoparticles and microemulsion), penetration enhancers and physical methods such as microneedle technologies, electrically-assisted methods (iontophoresis and electroporation) and ultrasound[(4-14)]. In order to obtain a synergistic enhancement effect with minimum skin damage and irritation, various combinations of enhancement methods have been tested, including combinations of more than two physical methods, more than two chemical enhancers with different enhancing mechanisms, an electrically-assisted method with chemical enhancers, and electrically-assisted methods with a well-designed drug carrier, and some of them have indeed resulted in improved skin permeation[(8,14-20)].

Our previous study[(21)] showed that the use of a submicron emulsion as a drug carrier could significantly enhance the transdermal effect as compared with the control aqueous group. Unfortunately, the lag time of the buspirone-loaded submicron emulsion permeated through skin was still long (about 3 h). Electrically-assisted methods such as electroporation and iontophoresis have been proven to modify the structure of skin and facilitate drug transportation, particularly for ionic and macromolecular drugs such as peptides and proteins[(15,22,23)]. The electrically-assisted methods were used to shorten the lag time of buspirone-loaded submicron emulsion and further facilitate transdermal delivery in this study.

MATERIALS AND METHODS

I. *Materials*

Buspirone hydrochloride was obtained from Excella

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GmbH (Germany). Sorbitan monolaurate (Span 20, HLB = 8.6) was obtained from Tokyo Chemical Industry (Japan). Polyoxyethylene sorbitan monooleate (Tween 80, HLB = 15) was obtained from Showa Corporation (Japan). Isopropyl myristate (IPM) was obtained from Merck Chemicals (Germany). Ketoprofen was obtained from Sigma-Aldrich (USA). All other chemicals and solvents were of analytical reagent grade.

II. *Preparation of Drug-Loaded Submicron Emulsions*

A mixture surfactant of Span 20 and Tween 80 at a ratio of 2 : 3 was mixed well. The oily and aqueous phases were separately prepared. The oily phase consisted of 0.3 g of IPM and 1.2 g of mixture surfactant, while the aqueous phase consisted of 5.1 g of double-distilled water and 3.4 g of ethanol. The aqueous phase was added to the oily phase, and then shaken by vortex at room temperature. Clear and transparent microemulsions were obtained. Buspirone (0.1 g) was dissolved in the final submicron emulsion formulations.

III. *In Vitro Skin Permeation Study*

The permeability of buspirone-loaded submicron emulsions was determined using a modified Franz glass diffusion cell fitted with the abdominal skin of excised Sprague Dawley rat. The skin was mounted on the receptor compartment with the stratum corneum side facing upwards into the donor compartment and the dermal side facing downwards into the receptor compartment. The donor cell was filled with 1 mL of drug-loaded submicron emulsions and occluded by parafilm. The receptor compartment was filled with 20 mL of 0.1 M phosphate buffer (pH 7.4) and its temperature was maintained at 37 ± 0.5°C by a thermostatic water pump during the experiment. The effective diffusion area was 3.46 cm^2. Five hundred micro-liters of receptor medium was withdrawn at predetermined intervals and replaced immediately with an equal volume of receptor solution to maintain a constant volume. The sample withdrawn from the receptor compartment was then analyzed by high performance liquid chromatography (HPLC). Each data point represented the average of three determinations. The animal experimental protocol was reviewed and approved by the Institutional Animal Care and Use Committee of Kaohsiung Medical University. The committee confirmed that the animal experiment followed the guidelines set forth by the Guide for Laboratory Fact Lines and Care.

IV. *Protocol of Electrically-Assisted Method*

Iontophoresis is the application of a low-level electrical current that facilitates the migration of drug molecules from the transdermal delivery system through the skin membranes. The enhancement effect of iontophoresis is proportional to the current intensity applied and application period. A higher current might damage the skin in the process of iontophoresis. A current intensity of 0.5 mA/cm^2 has been reported in literature to be the maximum acceptable level concerning minimal skin damage and irritation[22]. Therefore, in this study, the current density was set at 0.5 mA/cm^2 to evaluate the enhancement effect of the iontophoresis method on the buspirone-loaded submicron emulsion transdermal delivery. A pair of platinum wires with a diameter of 0.5 mm and an effective length of 1.5 cm was horizontally immersed in the buffer as electrodes, with the anode on the donor site and the cathode on the receptor site. The electrodes were positioned 3 cm apart. They were connected to a direct current power supply (Yokogawa Electrical Co. Model 7651, Japan). The current density was set at 0.5 mA/cm^2. Iontophoresis was applied constantly for 3 h and 10 h.

Electroporation involves the application of high voltage (typically > 100 voltage) and short-duration (μs - ms) pulse to create a new pathways within the membrane bilayers for increasing the permeability. In general, increase the used voltage and application time can increase the enhancement effect of drug transportation and skin damage[13,14]. According to previous study[14], the electroporation protocol was set at 1 pulse (300 V, 200 ms) per 30 s, for 10 min. Voltages were expressed as applied values, instead of transdermal values. The Electroporation (EP) was performed using an exponential decay pulse generator (BTX Co., ECM 630 Electro Cell Manipulator, USA). A pair of platinum wires (0.5 × 1.5 cm^2) was each located 3 cm away from the skin in the diffusion cell. The anode was positioned on the donor site, while the cathode was on the receptor site.

The duty cycle of iontophoresis was as follows: the effect of pulse on/off interval on the iontophoretic flux of buspirone-loaded submicron emulsion was studied at on/off interval ratios varying from 1 : 1, 1 : 2, 2 : 1 and 2 : 2 under a current density of 0.5 mA/cm^2.

The drug transportation of buspirone-loaded submicron emulsions after EP and ITP applied for 10 h were complete at 100.27 ± 2.53% and 97.57 ± 1.22%, respectively, also indicating that the formulation was stable.

V. *HPLC Analysis*

HPLC analysis was carried out using a Hitachi L-7100 series HPLC system. A Merck Lichrocart® C_{18} column (250 × 4.6 mm i.d., particle size 5 μm) was used[4]. The mobile phase was a mixture of 0.01 M ammonium acetate containing 1% triethylamine (adjusted to pH 4 by phosphoric acid) and acetonitrile in the ratio of 50 : 50, at a flow rate of 1.5 mL/min. The UV detection wavelength was set at 245 nm. Ketoprofen (100 μg/mL) was used as internal standard. The limit of quantitation was 5 μg/mL.

VI. *Data Analysis*

The cumulative amount of drug permeation through rat skin was plotted as a function of time. The flux calculated from the linear portion of the plot of cumulative amount versus time ($R^2 > 0.9$) was analyzed. Lag time was defined as the first instance of drug detection. Penetration index (PI) of

Table 1. Permeation parameters of buspirone-loaded submicron emulsion with and without electrically-assisted methods

	Lag time (h)	Q_{3h} (μg/cm^2)	PI $_{Q3h}$	Q_{10h} (μg/cm^2)	PI $_{Q10h}$
Passive diffusion	3.0	50.9 ± 17.6	1.0	413.7 ± 74.5	1.0
ITP for 3 h	0.5	658.2 ± 323.0	12.9	1007.8 ± 492.3	2.4
EP for 10 min	0.5	321.0 ± 36.5	6.3	863.5 ± 38.5	2.1
EP + ITP	0.5	500.3 ± 136.9	9.8	917.2 ± 60.6	2.2

ITP: Iontophoresis at 0.5 mA/cm^2 for 3 h.
EP : Electrophoresis at 1 pulse, 300 V, 200 ms, 0.5 min spacing for 10 min.
EP + ITP: Electrophoresis at 1 pulse, 300 V, 200 ms, 0.5 min spacing for 10 min, and then iontophoresis at 0.5 mA/cm^2 for 3 h.
Q_{3h} : Cumulative amount at 3 h.
Q_{10h} : Cumulative amount at 10 h.
PI (Penetration Index): Cumulative amount of electrically-assisted method/Cumulative amount of passive diffusion.

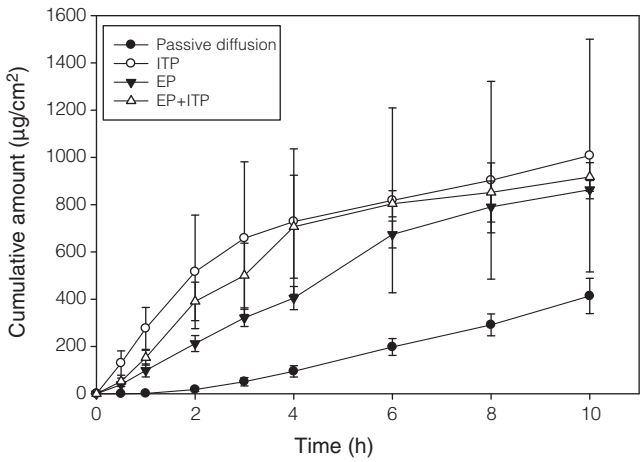

Figure 1. Cumulative amounts of buspirone permeation through rat skin under varying electrically-assisted methods, namely iontophoresis (ITP) at 0.5 mA/cm^2 for 3 h, electroporation (EP) at 1 pulse, 300 V, 200 ms and 0.5 min spacing for 10 min, and combined iontophoresis and electroporation.

the electrically-assisted method was defined as the cumulative amount of passive control.

RESULTS AND DISCUSSION

The plots of cumulative amount versus time for buspirone penetration with and without applying electric current are shown in Figure 1. It can be seen that buspirone penetration was markedly improved by the iontophoresis method, particularly in the first 3 h (during the electric current applying period). The cumulative amounts of buspirone through rat skin by iontophoresis at 3 h and 10 h were 658.2 ± 323.0 and 1007.8 ± 492.3 μg/cm^2 respectively, a 12.9- and 2.4-fold increase as compared with passive diffusion (Table 1), indicating that the contribution of electrophoretic drift enhanced by iontophoresis was significant for buspirone. The possible enhancement mechanisms are (1) electrorepulsion, which is the repulsive force between the applied electric current and entities of the same polarity, (2) increase in skin permeability due to the decrease in skin resistance, and (3) electroosmosis, which is the convective movement of a solvent through a charged "pore" in response to the preferential passage of counter-ions when an electric field is applied[6,7,12]. Furthermore, from Figure 1, it was found that when the current density was switched off after 3 h, the buspirone molecules could continue to permeate across the skin. One explanation is the existence of a drug reservoir within the skin. In the current-off period, the permeating ions continued their release from the skin until the drug reservoir drained off [24]. Another explanation is the alternation of the natural skin. The major route of iontophoretic transport is believed to involve appendageal pores, including sweat ducts and hair follicles. A non-appendageal pore pathway has also been proposed, which probably implies the current flow through "artificial shunts" because of disruption of the organized structure of the stratum corneum[14,18].

Electroporation is the application of high intensity electric field pulses to lipid bilayers of the stratum corneum, followed by the creation of transient aqueous pathways within the membrane bilayers for molecular transport[16,25,26]. As shown in Figure 1 and Table 1, the permeation of buspirone was markedly improved by a 6.3- and 2.1-fold enhancement in cumulative amounts at 3 h and 10 h respectively, relative to the control. It was also found that the penetration rate in the earlier stage (first 6 h) was higher than that in the later stage (6 - 10 h), which indicates that alternation of skin structure by electroporation can be maintained for a period of time and is reversible. The continuous accumulation of buspirone post-electroporation might indicate the formation of a drug reservoir or alternation of skin structure[18,26].

In addition, electroporation is usually coupled with iontophoresis to allow transdermal drug delivery to be further facilitated[6,17]. Hence the effect of the combination of electroporation and iontophoresis on drug permeation was also evaluated in this study. Surprisingly, no synergistic effect was observed (Figure 1). However, the result showed that the iontophoresis method was more efficient than that of the electroporation method for transporting buspirone through skin. The lag time of the permeation of buspirone-loaded submicron emulsion through skin was shortened from 3 h to 0.5 h by using both electrically-assisted methods, compared

to passive diffusion.

The application period of electrical current in iontophoresis was also an important influence on the improvement of drug transport through skin. As shown in Figure 2, the permeation of buspirone submicron emulsions through rat skin was significantly increased by applying iontophoresis at 0.5 mA/cm^2 for 10 h. The cumulative amount at 10 h was 1846.4 ± 525.6 μg/cm^2 for electrical current applied for 10 h, which showed 4.5- and 1.84-fold increases compared with passive diffusion (413.7 ± 74.5 μg/cm^2) and electrical current applied for 3 h (1007.8 ± 492.3 μg/cm^2) respectively, indicating that the enhancement effect of iontophoresis was proportional to the application period. Furthermore, it was found that the permeation rate in the earlier stage was higher than that in the later stage in the long-term electrical current applied (Figure 2), showing that the enhancement effect was decreased by long-term application of electrical current. The phenomenon might be attributed to the stratum corneum of the skin being composed of keratinocytes which contain high intercellular lipids and low content of water, thus being a poor conductor. When continuous electrical current is applied on the skin, this layer of skin may act in resistance and thus result in magnetic polarization currents. This polarization operates against the applied electrical field and greatly decreases the magnitude of effective current across the skin and the efficiency of transdermal delivery of drugs by iontophoresis[9,20,27,28]. In order to avoid the polarization of skin, an intermittent electrical current was used. The effect of on/off interval ratio applied on iontophoresis delivery of buspirone-loaded submicron emulsion is shown in Figure 3. It can be seen that no significant difference in effect was observed in the earlier stage of electrical current applied. However, in the later stage (8 and 10 h) of long-term electrical current applied, the on/off interval ratio of 2 : 2 showed higher cumulative amounts, indicating that the on/off interval ratio could influence the efficiency of iontophoresis[9,20].

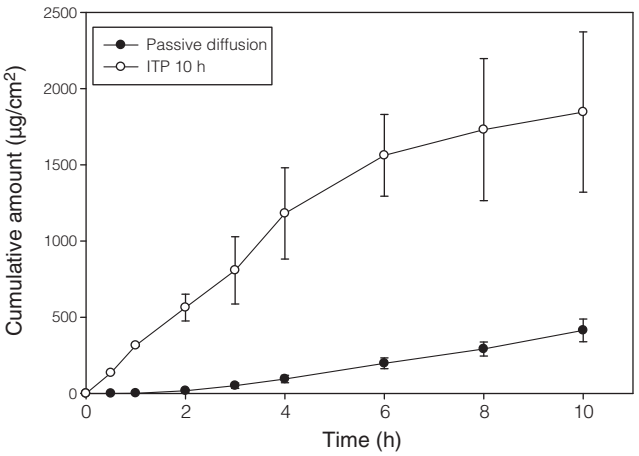

Figure 2. Cumulative amount of buspirone permeation through rat skin after long-term iontophoresis at 0.5 mA/cm^2 for 10 h, compared with passive diffusion.

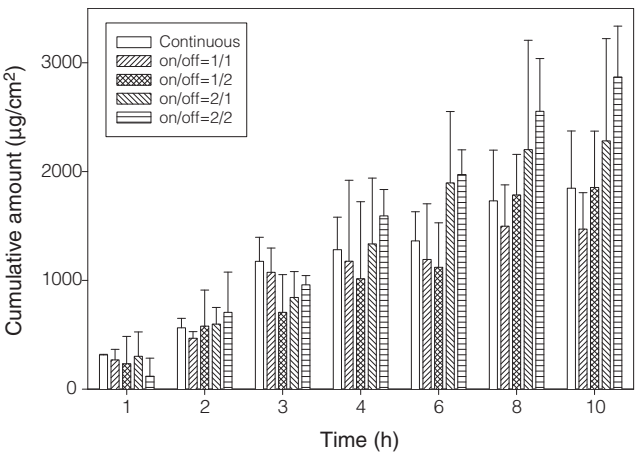

Figure 3. Cumulative amounts of buspirone submicron emulsion permeation through rat skin under continuous and periodic manner iontophoresis.

CONCLUSIONS

The application of electrically-assisted methods could greatly enhance the transdermal permeation of buspirone and shorten the lag time. Iontophoresis at 0.5 mA/cm^2 for 3 h was more efficient than electroporation at 1 pulse, 300 V, 200 ms, 0.5 min spacing for 10 min for the transportation of buspirone through skin. In the iontophoresis method, a suitable on/off interval ratio of the current should be selected to enhance iontophoretic efficiency.

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