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Iontophoretic Transdermal Delivery of Buspirone Hydrochloride in Hairless Mouse Skin

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ABSTRACT

The transdermal delivery of buspirone hydrochloride across hairless mouse skin and the combined effect of iontophoresis and terpene enhancers were evaluated in vitro using Franz diffusion cells. Iontophoretic delivery was optimized by evaluating the effect of drug concentration, current density, and pH of the vehicle solution. Increasing the current density from 0.05 to 0.1 mA/cm^2 resulted in doubling of the iontophoretic flux of buspirone hydrochloride, while increasing drug concentration from 1% to 2% had no effect on flux. Using phosphate buffer to adjust the pH of the drug solution decreased the buspirone hydrochloride iontophoretic flux relative to water solutions. Incorporating buspirone hydrochloride into ethanol:water (50:50 vol/vol) based gel formulations using carboxymethylcellulose and hydroxypropylmethylcellulose had no effect on iontophoretic delivery. Incorporation of three terpene enhancers (menthol, cineole, and terpineol) into the gel resulted in a synergistic effect when combined with iontophoresis. Menthol was the most active enhancer, and when combined with iontophoresis it was possible to deliver 10 mg/cm^2/day of buspirone hydrochloride.

KEYWORDS: iontophoresis, terpene, buspirone hydrochloride, gel, transdermal

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INTRODUCTION

Buspirone hydrochloride (BH) is an orally administered anxiolytic that is structurally and pharmacologically distinct from all other anxiolytics, including benzodiazepines and barbiturates. It differs from other anxiolytics in that it does not possess anticonvulsant or muscle relaxant properties, does not impair psychomotor function, and does not cause sedation or physical dependence. BH is used to treat generalized anxiety disorder and anxiety caused by alcohol craving or smoking cessation, as well as attention deficit hyperactivity disorder in children. Since 1984, BH has been used in children and adolescents with anxiety disorders, and researchers have reported significant improvement with its use.[1] The reported side effects were very mild, and many clinical trials have shown the superiority of BH over other traditional stimulant-based treatments.[2,3]

Although BH is rapidly absorbed in humans, it undergoes extensive first-pass metabolism such that the unchanged compound accounts for only 1% of the radioactivity in the plasma after oral administration of a 20-mg single dose of BH. Moreover, the average elimination half-life of unchanged BH after single doses of 10 to 40 mg is about 2 to 3 hours.[4] Consequently, treatment with BH typically requires 3 daily doses of between 5 and 20 mg each. Because of the chronic nature of the treatment, a reduction in the number of daily doses would be advantageous, as it should radically improve patient compliance. Thus, an effective transdermal buspirone delivery system would be expected to have a great impact on therapy success.

Unfortunately, because of the low permeability of the skin, only a few drugs are good candidates for transdermal delivery. Ions permeate the skin at a much lower rate relative to neutral compounds. Techniques such as ion pairing,[5] chemical enhancers,[6] and electric current[7] were successfully used to enhance ionic drug permeation. Iontophoresis involves the use of low-density electric current (approximately 0.5 mA/cm^2) to drive charged molecules across the skin. The current cycle is constructed by

placing an electrode in a drug-containing compartment in contact with skin; a ground electrode is placed on the body to complete the circuit.[8,9] Drug transport across the skin is enhanced by 3 mechanisms: charged species are driven primarily by electric repulsion from the driving electrode, the flow of electric current may increase the permeability of skin, and electroosmosis may affect uncharged molecules. Efficiency of transport depends mainly on polarity, valency, and mobility of the charged species as well as on the electrical duty cycles and formulation components.[10]

The purpose of this study was to evaluate the potential transdermal delivery of BH using iontophoresis and to evaluate the parameters affecting the efficacy of the enhancement technique. Moreover, the study involved the possibility of using terpene enhancers to potentiate the effect of iontophoresis.

MATERIALS AND METHODS

Chemicals

Hydroxypropylmethylcellulose (HPMC) (viscosity of 2% aqueous solution at 20°C: 80-120 cps), carboxymethylcellulose (CMC) (medium viscosity, viscosity of 2% aqueous solution at 20°C: 400-800 cps), cineole, terpineol, (-)menthol, BH, triethylamine, and phosphoric acid were purchased from Sigma (St Louis, MO). High-performance liquid chromatography (HPLC) solvents (water, methanol, and acetonitrile) were purchased from Pharmco (Brookfield, CT). Silver wire (0.5-mm diameter, 99.9%) and silver chloride 99% were obtained from Aldrich Chemical Co (Milwaukee, WI).

Preparation of Skin Membranes

Eight-week-old male hairless mice (Charles River Laboratories, Wilmington, MA) were euthanized by carbon dioxide asphyxiation. Then the skins were excised and kept at -70°C until used. Immediately before the experiment, the skins were taken out and left to thaw at room temperature. The dorsal side of mouse skins was cleaned of adhering fat deposits and then cut into small pieces. These pieces were carefully mounted on top of the diffusion cells and left to hydrate for 1 hour before the application of the BH formulation.

Preparation of Electrodes

Iontophoresis experiments were conducted using silver/silver chloride electrodes. The silver chloride electrodes were prepared as follows: silver wires (0.5-mm diameter) were immersed in 0.1N HCl solution and connected to the anode of an electric current source (12 V). Silver chloride powder was melted in a basin and picked up by another silver wire, which was connected to the negative pole of the current source. A gray silver chloride layer was gradually coated on the anodal silver wires, and after 24 hours these wires were ready for use as iontophoresis cathodal electrodes.

Preparation of Gels

HPMC and CMC were prepared in an ethanol:water (50:50 vol/vol) base to produce gels of equivalent viscosity (HPMC at 3% and CMC at 1%). Polymer powders were allowed to swell in the ethanol:water base for 24 hours and then were mixed using VirtiShear kinetic mixer Model 225326 (Virtis Company Inc, Gardener, NY) until a homogeneous clear gel was obtained. Gels were then stored for at least 48 hours prior to the experiment. Gel viscosity was measured using cylinder-type Brookfield viscometer model LVF (Stoughton, MA). Viscosity was recorded after 30 seconds. These gel bases were used to incorporate BH and terpene enhancers.

Transdermal Permeation Experiments

Skins were mounted on top of modified Franz diffusion cells (PermeGear, Riegelsville, PA) with a surface area available for diffusion of 0.64 cm^2 and a 5.1-mL receptor compartment volume.

The receptor compartments of the diffusion cells were filled with phosphate-buffered saline (pH = 7.4) containing 0.1% vol/vol of 36% aqueous formaldehyde as preservative and were stirred at 600 rpm. The receptor fluids were thermostated at 37 ± 0.5°C. Under these conditions the temperature at the skin surface was 32 ± 0.5°C. Hairless mouse skins were left for 1 hour to hydrate before the start of the experiment. After this hydration period, 1 mL of drug solution or gel was applied on top of each skin. Then the donor compartments of the diffusion cells were covered with a triple layer of Parafilm® (American National Can, Menasha, WI).

For iontophoresis experiments, a silver wire representing the anode was placed in the donor compartment, and a silver chloride cathode was placed in the receptor compartment. Both electrodes were attached to an A360D constant current power supply (World Precision Instruments Inc, Sarasota, FL).

At predetermined times (1, 2, 3, 4, 5, 6, 10, 12, and 24 hours), 300-µL samples were withdrawn from the recep-

tor compartment and were immediately replaced by the same volume of the buffer solution. These samples were accounted for in the calculation of the corrected BH concentration in the samples for the subsequent calculation of the diffusion parameters. All samples were kept at -70°C prior to HPLC analysis. All experiments were conducted for 24 hours.

The iontophoresis experiments were conducted according to the general procedure described above. The following factors affecting BH iontophoretic delivery were studied.

BH Concentration and Composition of the Donor Vehicle

Two concentrations of BH were used, 1% and 2%. The concentration effect was studied using water and ethanol:water (50:50 vol/vol) solutions.

Current Density

Two current densities were used, 0.05 and 0.1 mA/cm^2. Direct current was used throughout. The current effect was studied using BH water solutions at 1% and 2%.

pH

Iontophoretic delivery of BH was conducted at 3 pH values—4.2, 6, and 8—with a final buffer concentration of 0.03M using a phosphate buffer and an ethanol:phosphate buffer (50:50 vol/vol) solution.

Iontophoresis Using Gel Formulations

BH was dissolved in HPMC and CMC gels prepared as described under "Preparation of gels" to give a final concentration of 2% wt/wt of BH. Iontophoretic experiments were conducted as described above: 1 mL of gel formulations was applied on top of each skin and all experiments were conducted over 24 hours.

Enhancement of BH Permeation Using Terpene Enhancers

The effect of three terpene enhancers (menthol, cineole, and terpineol) was studied using ethanol:water solutions and gel formulations. Gels (prepared as described above) and ethanol:water (50:50 vol/vol) solutions containing 2% enhancers and 2% BH were applied on top of the skins. Experiments were conducted according to the protocol described above.

The combined effect of enhancers and iontophoresis was studied using gel formulations that contain 2% BH and 2% terpene enhancers. In this set of experiments, 1 mL of gel formulations was applied on each skin, and iontophoretic delivery was conducted at a current density of 0.1 mA/cm^2.

HPLC Analysis of BH

Analysis of samples was performed on a Hewlett Packard 1100 with a degasser (Model G1322A), an autosampler (Model G1313A), a quaternary pump (Model G1311A), and a diode array (Model G1315A, Hewlett Packard, Waldbronn, Germany). BH was eluted according to a method adapted from Cardoso and Schapoval[11] on C18-Microsorb-MV column (15 cm x 4.6 mm; 5 μm) from Varian Instruments (Walnut Creek, CA) using a mobile phase consisting of methanol:acetonitrile:water:triethylamine 40%:10%:50%:1%. The pH was adjusted to 2.7 using phosphoric acid. The flow rate was 1 mL/min. BH was detected at 240 nm, and calibration of BH in the samples was determined using an external standard technique.

The analytical parameters for this assay were as follows: retention time 3.2 minutes, limit of detection 0.5 μg/mL, Reproducibility Relative Standard Deviation SD 2%. Statistical analysis of the data was done using t tests with the number of samples (n) taken into account and the significance level set at 0.05.

RESULTS

Effect of Current Density, Concentration, and Vehicle Composition

As shown in **Figure 1**, the passive diffusion using water solutions resulted in flux and Q_{24} values of 2.39 ± 0.91 μg/(h.cm^2) and 49.9 ± 16.9 μg/cm^2, respectively. However, the use of the ethanol:water solution resulted in a comparable flux and Q_{24} of 1.71 ± 0.31 μg/(h.cm^2) and 39.4 ± 5.56 μg/cm^2, respectively. The water solution had a pH of 5.1, while the ethanol:water solution had a pH of 5.6. The passive diffusion of BH was inadequate in pro-

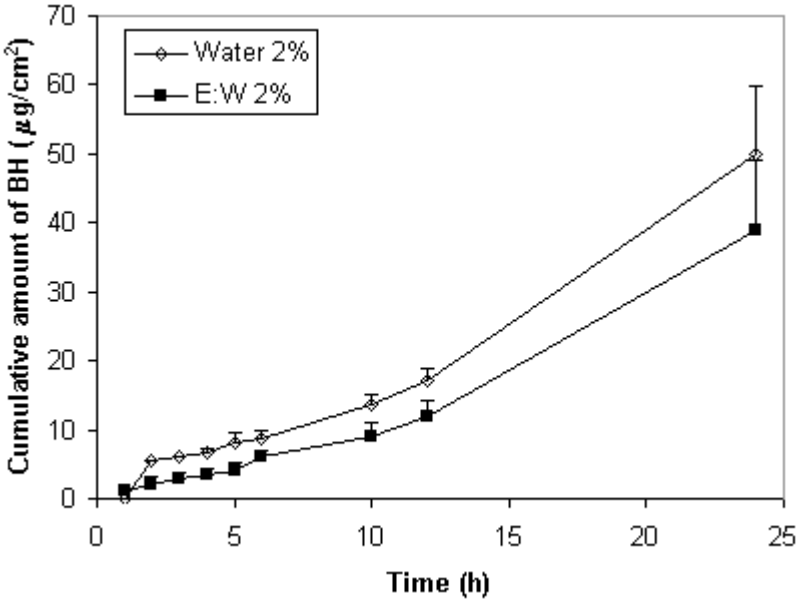

Figure 1. Cumulative amounts of BH permeated through hairless mouse skin upon the application of 1 mL of 2% water and ethanol:water (E:W) solution (n = 4).

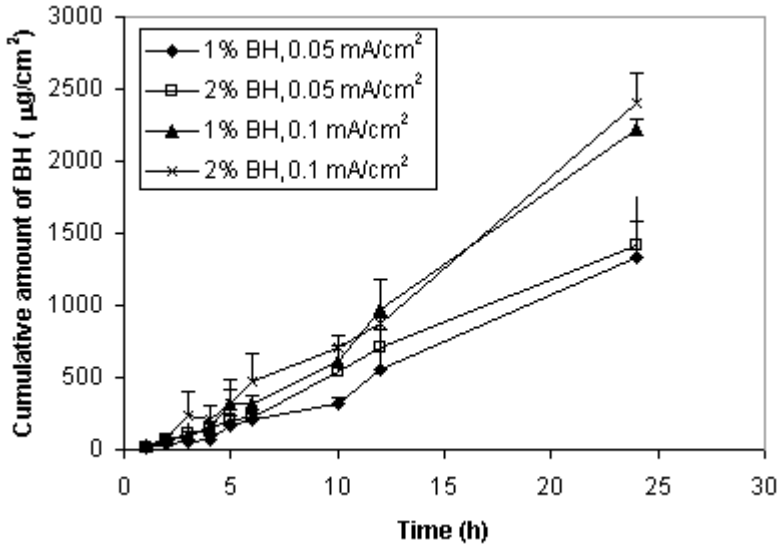

Figure 2. Cumulative amounts of BH permeated through hairless mouse skin using water solutions containing 1% and 2% BH at current densities 0.05 and 0.1 mA/cm^2 (n = 4).

viding the needed amount of drug (15-30 mg/day), so enhancement of BH through the skin became necessary.

To optimize the delivery method, parameters affecting the iontophoretic delivery such as current density, drug concentration, and vehicle composition were evaluated using hairless mouse skin. As seen in **Figure 2**, a change in BH concentration from 1% to 2% did not affect the iontophoretic delivery of BH using current densities of 0.05 and 0.1 mA/cm^2. Q_{24} values using 1% and 2% solutions were 1332.1 ± 258.0 and 1422.4 ± 336.8 µg/cm^2, respectively, at 0.05 mA/cm^2; and 2288.2 ± 67.9 and 2404.5 ± 204.6 µg/cm^2, respectively, at 0.1 mA/cm^2.

On the other hand, increasing current density from 0.05 to 0.1 mA/cm^2 resulted in an increased BH flux by 2-fold at 1% and 2% concentration: from 62.65 ± 3.94 to 95.97 ± 6.91 and from 59.29 ± 8.16 to 113.09 ± 30.99 µg/(h.cm^2), respectively.

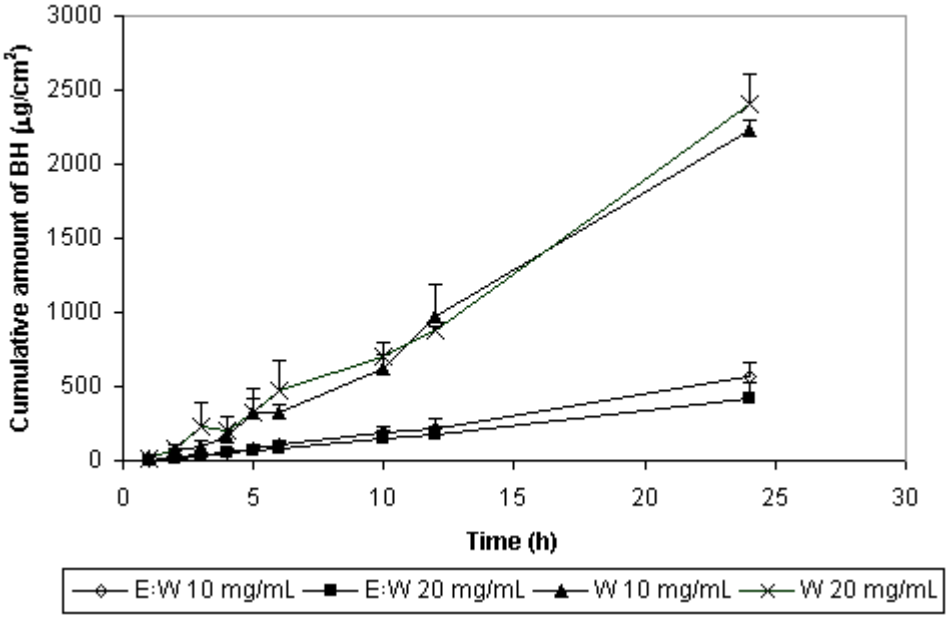

Figure 3. Cumulative amounts of BH permeated through hairless mouse skin using water and ethanol:water (E:W) solutions containing 1% and 2% BH at current density 0.1 mA/cm^2 (n = 4).

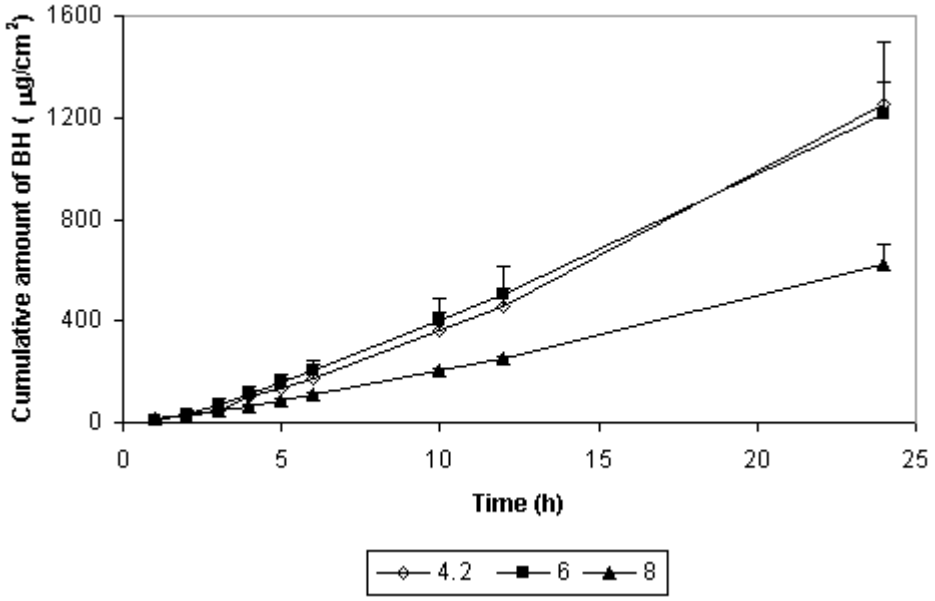

Figure 4. Cumulative amounts of BH permeated through hairless mouse skin using phosphate buffered solutions containing 2% BH at current density 0.1 mA/cm^2 as a function of solution pH (n = 4).

Ethanol is a vehicle widely used in pharmaceutical preparations. It was incorporated in the drug vehicle as 50:50 (vol/vol) to serve as a cosolvent to solubilize permeation enhancers and enhance transdermal drug delivery. Ethanol had no significant effect on the passive permeation of BH. However, as seen in **Figure 3**, under iontophoretic conditions, ethanol reduced the flux of BH by 5-fold compared to the water solution.

Effect of Donor Vehicle pH

The use of buffered solutions is necessary to control the ionization of the permeant. In this study, the iontophoretic delivery of BH was evaluated at three pH values—4.2, 6, and 8—using phosphate and ethanol:phosphate solutions. As seen in **Figure 4**, the permeation of BH from phosphate solutions buffered at pH 8 was reduced as compared to that of pH 4.2 and 6 and resulted in flux

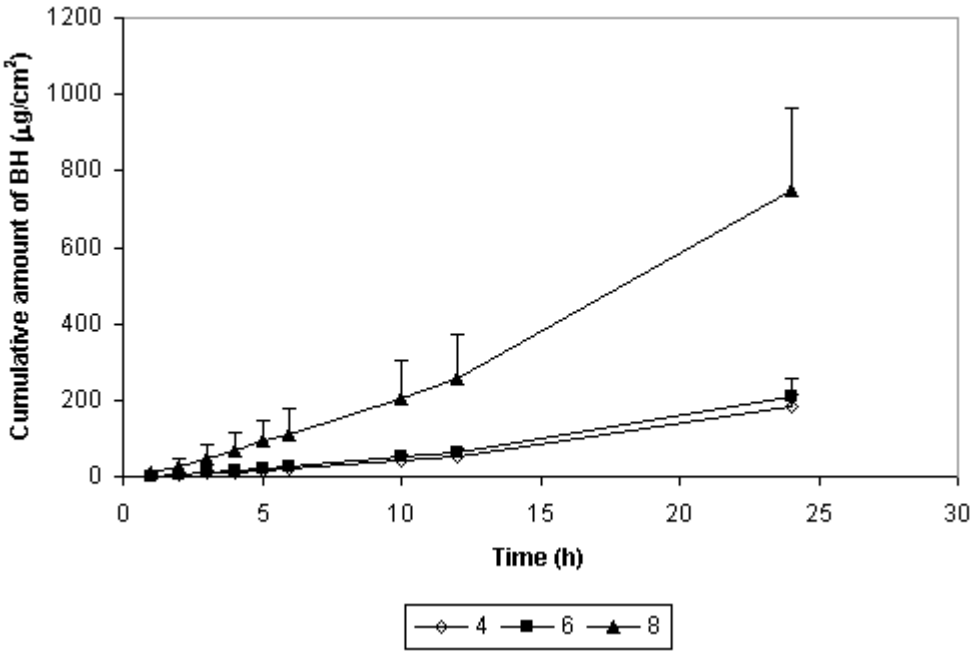

Figure 5. Cumulative amounts of BH permeated through hairless mouse skin using ethanol:phosphate buffered solutions (50:50 vol/vol) containing 2% BH at current density 0.1 mA/cm ^{2} as a function of solution pH (n = 4).

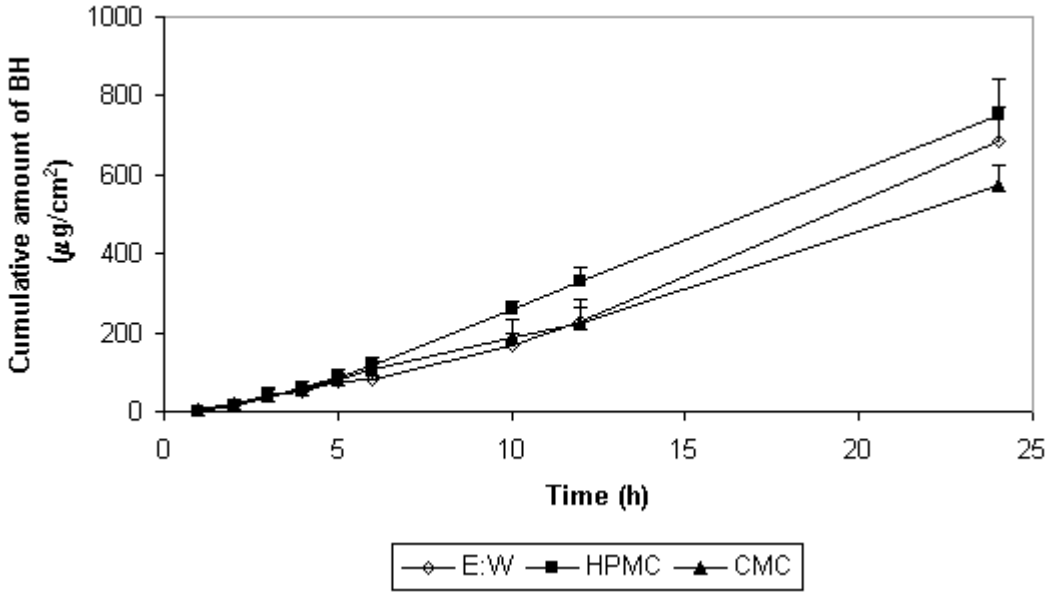

Figure 6. Cumulative amounts of BH permeated through hairless mouse skin using ethanol:water (E:W) (50:50 vol/vol) solution, HPMC gel, and CMC gel containing 2% BH at current density 0.1 mA/cm^2 (n = 4).

values of 28.90 ± 5.13, 61.12 ± 2.14, and 56.24 ± 14.65 µg/(h.cm^2), respectively. However, as can be seen by comparing **Figure 5** and **Figure 6**, ethanol:phosphate (50:50 vol/vol) solutions buffered at pH 8 resulted in no significant effect on the permeation of BH relative to ethanol:water solutions: 31.42 ± 7.30 and 26.49 ± 3.45 µg/(h.cm^2), respectively (see **Figure 5**). Moreover, ethanol:phosphate solutions buffered at pH 4.2 and 6 further reduced the flux of BH: 9.39 ± 1.28 and 10.67 ± 2.57 µg/(h.cm^2), respectively, relative to the ethanol:water solutions.

Delivery of BH from Gel Formulation Using Combination of Iontophoresis and Permeation Enhancers

HPMC gels were prepared at 3% wt/wt while CMC gels were prepared at 1% wt/wt. To potentiate the effect of iontophoresis, terpene enhancers were added to the drug vehicle at a 2% concentration. The cumulative amount of BH permeated across hairless mouse skin using iontophoresis at a current density of 0.1 mA/cm^2 from HPMC and CMC gels was compared to that of the ethanol:water solution. The permeation profile of BH from the gel formulations showed no significant difference from that of the ethanol:water solution (**Figure 6**). The BH flux from HPMC was 55.59 ± 11.18 μg/(h.cm^2), from the ethanol:water solution was 31.42 ± 7.30 μg/ (h.cm^2), and from CMC was 27.19 ± 4.15 μg/(h.cm^2).

Terpene enhancers were incorporated to enhance the effect of iontophoresis and to decrease the load of each enhancement technique. **Figure 7** and **Figure 8** show the effect of terpene enhancers on the flux of BH upon the application of 1 mL gel/cell. Terpene enhancers were superior to iontophoresis. In general, terpene enhancers increased the flux of BH by more than 200-fold relative to a 15-fold increase using iontophoresis alone. However, compared with cineole and terpineol, menthol showed the highest activity. Menthol, cineole, and terpineol increased flux values of BH by 300-, 148-, and 235-fold using HPMC gels and 204-, 140-, and 198-fold using CMC compared to that of the control (HPMC and CMC gels), respectively. However, the use of terpene enhancers in combination with iontophoresis resulted in a synergistic effect on the flux of BH from CMC gel. BH flux increased from 27.19 ± 4.15 and 347.10 ± 14.96 when iontophoresis or menthol were used alone to 546.84 ± 40.54 μg/(h.cm^2) when both were combined. This is a synergistic effect. In contrast, the flux of BH from HPMC gels was increased from 55.59 ± 11.18 and 523.10 ± 80.34 using iontophoresis and menthol alone to 637.81 ± 92.88 μg/ (h.cm^2) when menthol was combined with iontophoresis. This marginal increase is statistically insignificant and indicates that no synergy occurred under these conditions.

DISCUSSION

The passive diffusion of BH using pure water solutions resulted in flux and Q_{24} values of 2.39 ± 0.91 μg/(h.cm^2) and 49.9 ± 16.98 μg/cm^2, respectively. This passive delivery of BH was inadequate in providing the clinically required amounts of drug (15-30 mg/day). Since human skin is less permeable than hairless mouse skin, it is apparent that it is necessary to significantly enhance BH transdermal delivery.

Iontophoresis is potentially effective in enhancing the permeation of most ionic compounds. In this study, we examined iontophoretic delivery using water and water:ethanol solutions containing 1% and 2% BH. We used 2 current densities, 0.05 and 0.1 mA/cm^2. As seen in **Figure 2**, BH concentration had no effect on the iontophoretic delivery of BH through hairless mouse skin using current densities of 0.05 and 0.1 mA/cm^2. Some researchers have shown that the flux of the ionic permeant increases proportionally to its concentration in the donor vehicle. However, others have shown little or no change in flux over a broad concentration range for a number of solutes.[12-15] The rate of ion transport through pores that have no fixed charges is generally proportional to the ion concentration according to Fick's law. However, with a charged pore the ion flow may be diffusion limited, and so the membrane (pore) conductivity reaches a limiting value at high concentrations when the pores become saturated.[16-18]

On the other hand, increasing current density from 0.05 to 0.1 mA/cm^2 resulted in an almost doubling of BH flux at 1% and 2% concentration: from 62.65 + 3.94 to 95.97 + 6.91 and from 59.29 + 8.16 to 113.09 + 30.99 μg/(h.cm^2). The current represents the driving force for the movement of ionic species across the skin. A direct proportionality between current density and the flux of a permeating ion was shown by several researchers for both positively and negatively charged ions.[15]

While the use of ethanol:water BH solutions had no significant effect on the passive permeation of BH, it resulted in reduced flux of BH by 5-fold compared to water solution of the same concentration and current density (**Figure 3**). It is likely that the addition of ethanol reduced vehicle conductivity and consequently reduced the amount of current carried by the BH ions. Moreover, the addition of ethanol is thought to inhibit the ionization of BH and thereby decreases its specific conductance. Inhibition of BH ionization decreases the concentration of the BH cation that can carry the current and hence decreases its flux. It is thought that the inadequate conductance in the ethanol-containing donor solution increases Ag^+ ion production by electrochemical oxidation at the anode. These new cations compete with the BH cations, thus reducing their flux.[19]

Above the pH value of 4 (the isoelectric point of the skin) the skin has an average negative charge. The higher the pH, the more the negative charges per pore, and so a higher flux is expected for cationic species. However, depending on the ionization behavior of the permeant, increasing the pH of the donor vehicle might inversely

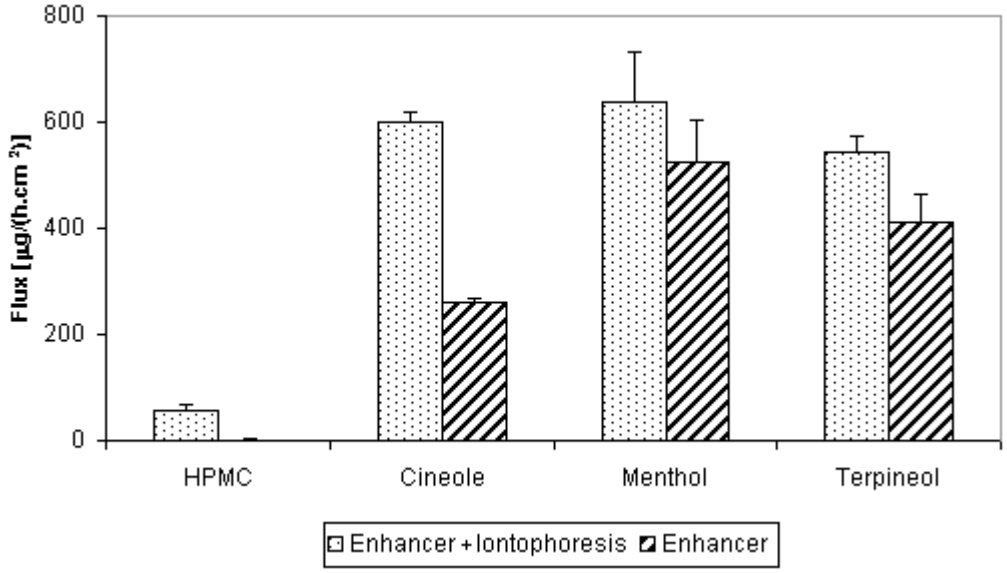

Figure 7. Effect of combining terpene enhancers with iontophoresis on the flux of BH from HPMC gels (n = 4).

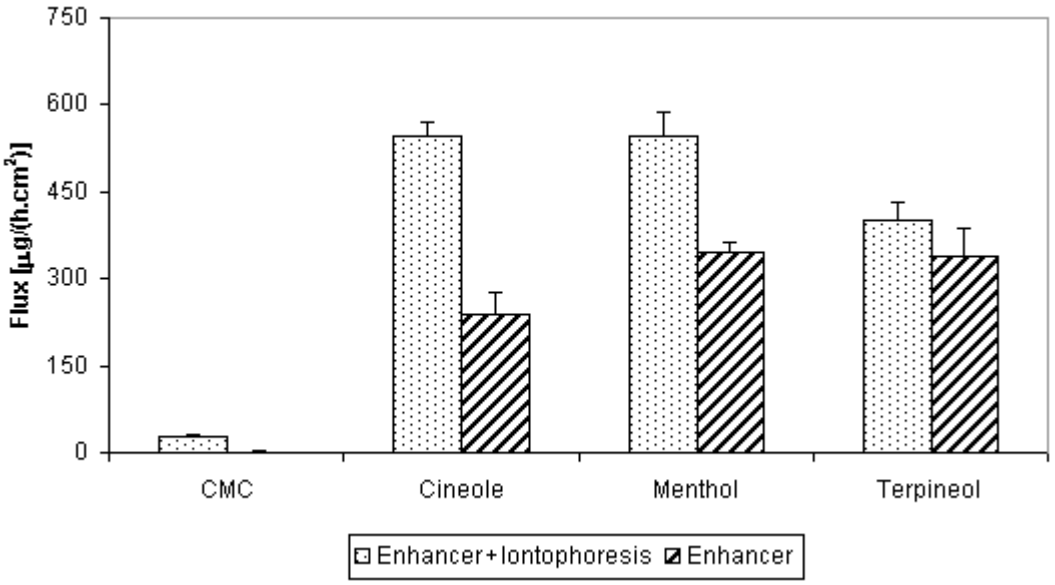

Figure 8. Effect of combining terpene enhancers with iontophoresis on the flux of BH from CMC gels (n = 4).

affect its flux. For example, increasing pH in the donor vehicle inhibits ionization of basic compounds, and so this will retard their permeation. On the other hand, acidic compound permeation is expected to be enhanced as a result of increased ionization upon increasing the pH of the vehicle. In practice, there will be a need for balanced pH values that ensure complete ionization of drug species in the vehicle and the ionization of the skin, ensure the stability of the drug, and minimize skin irritation. In this study, it was expected that increasing the net negative charge of the skin by increasing the vehicle pH would increase the iontophoretic flux of BH (cationic drug). However, BH has a pKa of 6.25. In solutions buffered at pH 8, ionization of BH was inhibited, which resulted in formation of the unionized base, which was precipitated out of the solution (the base form is sparingly soluble in water). Moreover, the addition of buffer to the BH solution decreased its flux as compared to the deionized water solution (**Figure 4**). One group studied the importance of choosing the buffer components on the flux of the ionic permeant.[20] It was shown that higher fluxes of salicylic acid and phenylethylamine were obtained using zwitterionic amino acid buffers such as tricine and 4-(2-Hydroxyethyl)-1-piperazineethane-sulfonic acid (HEPES), which have relatively low specific conduc-

tance and low ionic mobilities, as compared to Tris-HCl and phosphate buffer, which have highly mobile ions that will compete to a higher degree with the drug ion.

On the other hand, ethanol:phosphate (50:50 vol/vol) solutions at pH 8 resulted in a higher flux of BH relative to those of pH 4.2 and 6 (**Figure 5**), because of the presence of ethanol, which is thought to prevent the precipitation of the base form of BH.

Iontophoresis enhances drug delivery across the skin by electrorepulsion and electroosmosis.[21] At pH values higher than 4, the skin is negatively charged and cation permselective. Thus, current passage causes a net convective solvent flow from the anode to the cathode, facilitating cation transport, inhibiting anion transport, and enabling the enhanced transport of neutral polar solutes.[21,22] The relative importance of electrorepulsion and electroosmosis depends on the physicochemical and electrical characteristics of the membrane and of the permeant. The skin's negative charge can be reduced, neutralized, or even reversed by the iontophoresis of certain cationic, lipophilic species.[23,24] The avid association of these compounds with the fixed negatively charged sites in the skin significantly attenuates electroosmotic flow across the skin. The negatively charged skin at pH 8 is permselective to the highly mobile K+ ions over the less mobile negative phosphate ion. This permselectivity creates a net movement of the solvent from the cathode to the anode, facilitating the movement of both the ionized and the un-ionized neutral forms of BH. This electroosmotic transport of BH is thought to compensate for the inhibition of the ionization of BH at pH 8 and upon the addition of ethanol.

In terms of clinical use, the iontophoretic patch or semisolid dosage form will most likely incorporate a polymeric delivery system. These systems can control the drug release rate over a long period of time, and the manufacturing process is straightforward.[25,26] Moreover, because of their high water content, polymeric gels exhibit a high electrical conductivity, making them the vehicles of choice for electrically assisted transdermal drug delivery. Many researchers have reported successful iontophoretic delivery of various drug molecules from gel bases.[27-30]

In this study, 2 types of polymer gels were prepared using HPMC and CMC at proximate viscosity in order to eliminate the viscosity effect on drug delivery. At higher viscosities there is decreased drug release from gel bases,[31] decreased vehicular conductivity, and reduced iontophoretic drug transport.[32] Higher polymer concentrations are thought to decrease the proportionality of water in the vehicle, resulting in a decrease in the conductivity of the formulation.[33] The permeation profile of BH from HPMC and CMC gels was not significantly different from that of the ethanol:water vehicle—26.49 ± 3.45 μg/(h.cm^2)—(**Figure 6**). Several researchers showed that diffusion of low-molecular weight substances was not different from that of water solutions.[34] However, BH flux from CMC was significantly lower than that from HPMC gel. CMC may interfere with iontophoresis because of the competition of its counterion (Na^+) with buspirone. Moreover, the anionic nature of the polymer may reduce the mobility of the cationic drug. It has been shown that using ionizable polymers should be avoided if possible in iontophoretic drug delivery. For example, cationic and anionic polymers were shown to reduce the iontophoretic delivery of sodium nonivamide acetate from hydrogels.[30,35]

Transdermal systems may require a large application area to be therapeutically effective, and this may decrease patient compliance. One way to reduce device size is to use a combination of permeation techniques such as iontophoresis and chemical enhancers. Moreover, the combination of enhancers and iontophoresis would moderate the iontophoretic regimen required to achieve the target flux and thus improve the tolerability of the skin. Enhancers such as Azone, fatty acids, and terpenes were shown to achieve a synergistic effect with iontophoresis.[27,36,37] Terpenes are naturally occurring volatile oils that exhibit good toxicological profiles, high percutaneous enhancement abilities, and low cutaneous irritancy at low concentrations (1%-5%).[38-40]

As seen in **Figure 7** and **8**, terpene enhancers were superior to iontophoresis in enhancing the transdermal permeation of BH. In general, they enhanced the flux of BH by more than 200-fold relative to a 15-fold increase using iontophoresis alone. Menthol showed the highest activity compared to cineole and terpineol. The three enhancers increased BH flux values by 300-, 148-, and 235-fold using HPMC gels and 204-, 140-, and 198-fold using CMC compared to that of the control (HPMC and CMC gels), respectively. It has been shown that the activity of terpene enhancers depends upon the physicochemical properties of the drug as well as the properties of the enhancer itself.[38,41] Polar terpenes were shown to be more effective in enhancing the permeation of polar drugs, while nonpolar terpenes were more effective in enhancing the permeation of lipophilic drugs. Indomethacin (lipophilic) drug absorption has been shown to be markedly enhanced by the addition of hydrophobic cyclic monoterpenes, while hydrophilic terpenes showed minor effects.[42] In contrast, under similar conditions, alcohol terpene (menthol) was found to be the most effective in enhancing the permeation of diclofenac sodium (hydro-

philic).[38] Moreover, Arellano et al[43] showed that alcohol terpenes were the most effective accelerants for this nonsteroidal anti-inflammatory drug, followed by ketones and oxides.

Addition of chemical enhancers is one way to potentiate the effectiveness of iontophoresis. Chemical enhancers that disrupt intercellular lipid organization can have a synergistic effect on iontophoresis, and terpenes belong to this category. **Figure 7** and **Figure 8** show the effect of the combination of terpenes and iontophoresis on the permeation of BH. Under most conditions, combination treatment synergistically increased the flux and Q_{24} of BH relative to iontophoresis or enhancers alone. The combination of menthol and iontophoresis showed the highest activity relative to the other enhancer-iontophoresis combinations. In CMC gel, BH flux increased from 27.19 ± 4.15 and 347.10 ± 14.96 when iontophoresis or menthol was used alone to 546.84 ± 40.54 $\mu g/(h.cm^2)$ when they were combined.

In conclusion, terpenes were more effective than iontophoresis alone in enhancing BH transdermal delivery across hairless mouse skin. However, the combination of terpenes and iontophoresis generally resulted in a synergistic increase in BH flux. Moreover, menthol was the most effective enhancer relative to cineole and terpineol where the combination of iontophoresis with menthol delivered 10 mg/cm^2 / day from HPMC gel. At this delivery rate, it is possible to easily achieve a daily dosage of BH of 50 mg/day with a $5cm^2$ patch on the skin. Although the daily dose of BH is much lower than this, at this point this high delivery rate may be justified, given that human skin is known to be several times less permeable than hairless mouse skin.

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