

Enhanced iontophoretic delivery of buspirone hydrochloride across human skin using chemical enhancers

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Abstract

Buspirone hydrochloride (BH) is a structurally and pharmacologically unique anxiolytic that is used to treat a variety of different anxiety conditions. The marketed product is named BuSpar®. The *in vitro* iontophoretic delivery of BH through human skin was investigated in order to evaluate the feasibility of delivering a therapeutic dose of BH by this route. We also examined the influence of co-formulations of chemical enhancers (Azone®, oleic acid, menthone, cineole, and terpineol) on BH permeation, both without iontophoresis and with iontophoresis—to look for possible synergistic effects. By applying iontophoresis at 0.5 mA/cm², it was possible to achieve a BH steady state flux of approximately 350 µg/cm² h, which would be therapeutically effective if clinically duplicated. Importantly, 24 h of iontophoresis at 0.5 mA/cm² did not affect skin morphology and after the current was switched off, the skin's permeability to BH rapidly reverted to its pre-iontophoretic level. Without iontophoreis, BH transdermal flux was significantly enhanced by the application of 2.5% (v/v) concentrations of Azone®, oleic acid, or menthone but not cineole or terpineol. Furthermore, this paper identified a synergistic transport enhancement effect developing when very low current (0.025 mA/cm²) iontophoresis was applied in conjunction with Azone® treatment.

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1. Introduction

Buspirone hydrochloride (BH) is an orally ingested anxiolytic that is structurally and pharmacologically different from all other anxiolytics, including barbiturates and benzodiazepines. Uniquely amongst anxiolytics, BH does not exhibit anticonvulsant or muscle relaxant properties, does not affect psychomotor function and does not induce physical dependence

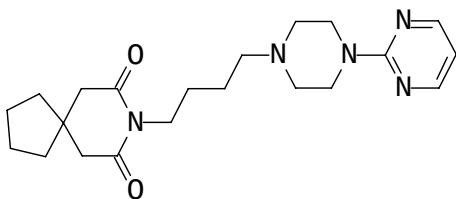
or sedation. The drug is used to treat generalized anxiety disorder, anxiety caused by alcohol craving or smoking cessation, as well as attention-deficit/hyperactivity disorder (ADHD) in children. Since 1984, children and adolescents suffering from anxiety disorders have been treated with BH (Balon, 1994) and clinical trials have demonstrated the superiority of this therapy over other alternative treatments (Ratey et al., 1989; Realmuto et al., 1989). Fig. 1 presents the chemical structure of buspirone.

Although BH is rapidly absorbed from the gastro-intestinal tract, it is subjected to extensive first pass metabolism. For instance, the mean elimination half life of unchanged buspirone after a single 10–40 mg oral dose is merely 2–3 h (Dollery, 1999).

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Molecular weight	422 Da
Octanol-water partition coefficient	Log P = 1.6
Aqueous Solubility	312mg/ml
Melting Point	202 °C
pKa	7.2

Fig. 1. Chemical structure and physico-chemical properties of buspirone.

Therefore, current BH treatment generally involves taking three daily oral doses of between 5 and 20 mg each. Due to the chronic nature of the therapy required, a decrease in the number of daily doses would be desirable, as it would greatly enhance patient compliance. Alternatively, the use of an effective transdermal buspirone delivery device would also be expected to facilitate therapy success. The transdermal route might also allow administration of lower doses through avoidance of first-pass metabolism and provide sustained and constant plasma concentration levels. However, due to the hydrophilic properties of buspirone, the molecule does not readily permeate human skin and, so far, no transdermal system is commercially available.

It is known that the co-application of an electric current can greatly enhance the transdermal penetration of many compounds—a process termed iontophoresis (Guy et al., 2000; Guy et al., 2001; Junginger, 2002; Kanikkannan, 2002). The principal advantage of this technique is that the flux can be accurately controlled and manipulated by varying the externally applied current. We have already shown that iontophoresis constitutes a feasible means for enhancing the transdermal delivery of buspirone in an *in vitro* hairless mouse model (Al Khalili et al., 2003). The aim of the current study was to extend this application to human skin. We also report the influence of chemical enhancers on BH penetration,

both as an alternative to, as well as in combination with iontophoresis. Five different compounds were investigated, including Azone[®] and oleic acid, representing two commonly-employed enhancers (Moser et al., 2001). The other three accelerants were terpenes. These naturally occurring volatile oils exhibit relatively high enhancement ability, low cutaneous irritancy, and have been given the designation of generally recognized as safe (GRAS) by the FDA (Godwin and Michniak, 1999; El-Kattan et al., 2001). We selected three chemically-distinct monocyclic terpenes that are also structural isomers: cineole (an ether), terpineol (an alcohol), and menthone (a ketone). We used a software program to determine the partition coefficients of these terpenes. We employed scanning electron microscopy in order to view skin morphology after both passive and iontophoretic delivery of BH.

2. Materials and methods

2.1. Chemicals

BH, oleic acid, terpineol, cineole, triethylamine, and phosphoric acid were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Menthone was obtained from Acros Organics (Geel, Belgium). Phosphate buffered saline tablets were bought from Fluka (Buchs, Switzerland). HPLC-grade solvents (water, methanol, and acetonitrile) were purchased from Pharmco (Brookfield, CT). Azone[®] was a gift from Dr. Das Bolikal of the Department of Chemistry, Rutgers University (Piscataway, NJ). Silver wire (0.5 mm diameter, 99.9%) and silver chloride 99% were obtained from Aldrich Chemical Co. (Milwaukee, MI).

2.2. Human skin

The National Disease Research Interchange (NDRI) provided sheets of whole human skin, derived from the abdominal regions of female Caucasian cadavers. Before delivery, the tissue was sectioned by dermatome into 400 μ m-thick samples. The sectioned skin was stored at -70°C for a period of up to 1 year. Previous work has shown that the permeability of human cadaver skin is largely unaffected after prolonged freezing for up to 15 months (Harrison et al., 1984). Just before each experiment, the skins were

allowed to thaw to room temperature and then used immediately as barrier membranes.

2.3. Preparation of electrodes

Iontophoresis was performed using silver/silver chloride electrodes. Silver chloride electrodes were prepared as follows. Silver wires (0.5 mm diameter) were connected to the anode of a 12 V source and immersed in 0.1 M hydrochloric acid. Silver chloride powder was melted in a basin and picked up by another silver wire. This silver wire was connected to the negative pole of the power source at one end and immersed in the hydrochloric acid solution at the other end. A gray silver chloride layer was gradually coated on the anodal silver wires and after 24 h these wires were ready for use as cathodal electrodes in iontophoresis experiments.

2.4. Transport studies

Whole human skin sections were mounted in modified Franz diffusion cells (PermeGear, Riegelsville, PA), exhibiting a diffusion-available surface area of 0.64 cm² and a receptor compartment volume of 5.1 ml. The receptor compartments were filled with 0.155 M phosphate buffered saline (pH 7.4), which was stirred at 600 rpm. The fluid in each receptor compartment was maintained at 37 ± 0.5 °C by the use of a thermostatic water pump (Haake DC10, Karlsruhe, Germany) that circulated water through the jacket surrounding each main chamber. Under these conditions, the temperature at the skin surface was 32 ± 0.5 °C. The skin sections were initially left in the Franz cells for 2 h in order to facilitate hydration of the membranes. After this period, 1 ml of the appropriate formulation (either aqueous solution or 50:50 v/v ethanol/water) of BH (either 2 or 3% w/v) was deposited on to the surface of each skin sample. For chemical enhancer studies, these formulations always contained 2% (w/v) BH and a 2.5% (v/v) concentration of the appropriate enhancer (either Azone[®], oleic acid, terpineol, cineole, or menthone) dissolved in 50:50 (v/v) ethanol/water. For the iontophoresis experiments, a silver wire representing the anode was immersed in the donor compartment, while a silver chloride-coated cathode was inserted into the receptor compartment. Both electrodes were connected to an

A360D constant current stimulus isolator (World Precision Instruments Inc., Sarasota, FL). Direct current was used throughout at a selected current density value (0.025, 0.1, 0.25, or 0.5 mA/cm²). At the beginning, middle, and end of each experiment, a milliammeter (Radioshack, Fort Worth, TX) was momentarily incorporated into the circuit in order to verify current density levels through the iontophoretic system. Identical experiments using vehicle alone but no iontophoresis (0 mA/cm²) were also performed. In all experiments, the donor compartment of each Franz cell was covered with a taught layer of Parafilm[®]. At selected time points (either 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 24 h; or 14, 16, 18, 20, 22, 24 h), 300 µl samples were withdrawn from the receptor compartment and these were stored at –20 °C prior to HPLC analysis. Withdrawn samples were immediately replaced by an equivalent volume of blank buffer solution. Each permeation experiment was conducted in triplicate or quadruplicate.

2.5. Quantification of buspirone hydrochloride

Analysis of samples was performed on a HPLC system (Hewlett Packard 1100) incorporating a degasser (Model G1322A), an autosampler (Model G1313A), a quaternary pump (Model G1311A), and a diode array (Model G1315A). BH was quantified according to a method adapted from [Cardoso and Schapoval \(1998\)](#). Samples were eluted on a C₁₈-Microsorb[®] column (Varian; 15 cm × 4.6 mm; 5 µm) using a mobile phase consisting of methanol:acetonitrile:water:triethylamine (in percent) 40:10:50:1 with the pH adjusted to 2.7 using phosphoric acid. The elution parameters were a flow rate of 1 ml/min and an injection volume of 25 µl. The detection wavelength was 240 nm. Calibration of the system was achieved by using 10 µg/ml solutions of BH as an external standard. The analytical parameters for this assay were as follows: retention time was 3.2 min, limit of detection was 0.5 µg/ml, and reproducibility relative standard deviation was 2%. The drug concentration values were corrected for progressive dilution using the equation:

$$M_t(n) = V_r \cdot C_n + V_s \cdot \sum C_m \quad (1)$$

where $M_t(n)$ is the current cumulative mass of drug transported across the membrane at time t , C_n represents the current concentration in the receiver medium

and $\sum C_m$ denotes the summed total of the previous measured concentrations [$m = 1$ to $(n - 1)$]; V_r is the volume of the receiver medium and V_s corresponds to the volume of the sample removed for analysis. Statistical analysis of the data was performed by employing t tests, with the significance level set at 0.05.

2.6. Scanning electron microscopy (SEM)

Human skin samples were mounted in Franz diffusion cells, allowed to hydrate for 2 h and subsequently treated with 1 ml solutions of aqueous ethanol (50% v/v) containing 2% (w/v) BH. The skins were left in the Franz cells for 24 h while exposed to either passive diffusion conditions or DC iontophoresis (0.5 mA/cm^2), as described in Section 2.4. At 24 h, the skin sections were removed from the diffusion cells and fixed in 5% aqueous glutaraldehyde solution for 1.5 h at room temperature. The samples were then immersed overnight in water at 4°C . The fixed skin sections were progressively dehydrated by immersion for 2 h at a time in aqueous ethanol solutions, exhibiting serially increasing ethanol concentrations. This process was completed when the samples were immersed twice in absolute ethanol for 2 h each time. The skin samples were critically dried in a critical point drier (Model 020, Balzers, Lichtenstein) and subsequently coated with a gold/palladium mixture on a sputter coater (Model SPD004, Balzers, Lichtenstein). Specimens were then observed under a scanning electron microscope (Model 1830I, Amray Inc., Bedford, MA) and photographed.

2.7. Partition coefficient determinations

The octanol–water partition coefficients of the terpenes were determined by QSAR calculations performed by appropriate IBM-compatible software—ACD/log P, Version 1 (Advanced Chemistry Inc., Ont., Canada).

3. Results

3.1. Influence of current density

Fig. 2 illustrates the buspirone penetration-time profiles associated with a range of iontophoretic cur-

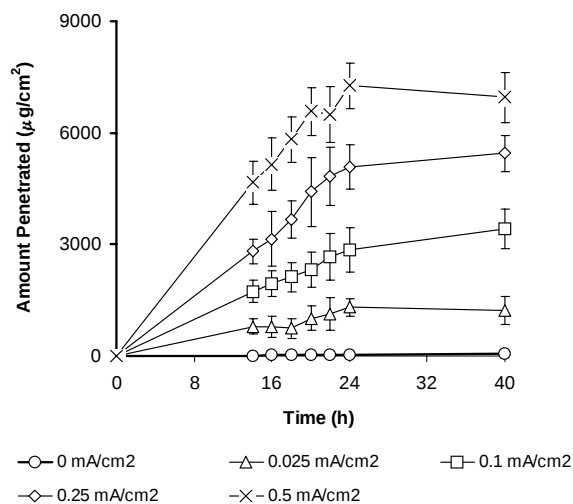


Fig. 2. Iontophoresis of buspirone hydrochloride over 0–24 h; the amount of drug ($\mu\text{g/cm}^2$) penetrated against time and the influence of current density ($n \geq 3$, error bars represent S.D. values).

rent densities ($0\text{--}0.5 \text{ mA/cm}^2$). In these experiments, iontophoresis was applied between 0 and 24 h at which point the current was switched off but the permeation experiment was allowed to proceed until 40 h. The topical formulation consisted of 2% (w/v) buspirone dissolved in 50% (v/v) aqueous ethanol. It can be seen that in the absence of electric current, buspirone flux through human skin was negligible. As the current density was progressively increased, the flux also increased. Interestingly, for all investigated current densities, as soon as the current was switched off at 24 h, buspirone flux decreased fairly quickly such that the amounts of drug in the receiver compartment at 40 h was similar to or perhaps slightly greater than the amounts at 24 h. This suggests that buspirone does not form a reservoir in human skin. The pattern also indicates that the iontophoretic permeabilization of the skin is reversible, even following a 24-h application of a 0.5 mA/cm^2 current.

Fig. 3 shows the same data in the form of a plot of buspirone steady state flux (14–24 h) as a function of current density. It can be discerned that the relationship between flux and current density is linear. A direct proportionality between current density and ion flux is a general characteristic of iontophoresis, predicted by the Nernst–Planck equation (Guy et al., 2000).

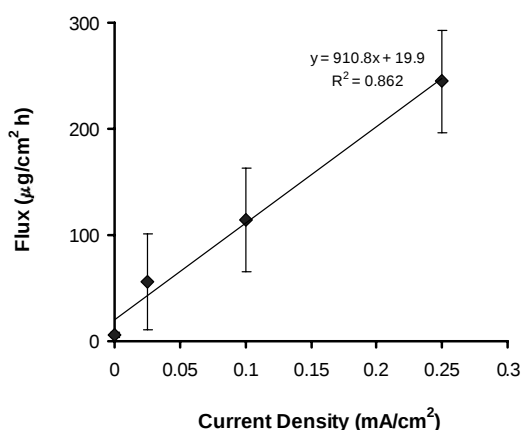


Fig. 3. Iontophoretic flux of buspirone hydrochloride as a function of current density ($n \geq 3$, error bars represent S.D. values).

3.2. Influence of vehicle and BH concentration

In order to determine the role of donor phase composition on the system, four separate iontophoretic experiments were performed representing two different buspirone concentrations (2% w/v and 3% w/v), each using two different vehicles (water and 50% v/v aqueous ethanol). Current density was fixed at 0.5 mA/cm² throughout. Table 1 presents the steady state flux data for this study. It can be seen that in terms of iontophoretic flux, there was no significant difference between each of the four formulations.

Regarding the concentration parameter, while in some cases the iontophoretic flux of a permeant increases in proportion to its topical concentration, other systems have exhibited little or no change in fluxes over a broad concentration range (Bellantone et al., 1986; Miller and Smith, 1989; Wearley et al., 1989; Padmanabhan et al., 1990). The discrepancy can be

Table 1
The influence of donor phase composition on buspirone iontophoretic flux (current density at 0.5 mA/cm²)

BH concentration (% w/v)	Vehicle composition	
	Water	50% (v/v) aqueous ethanol
2	341 ± 18	351 ± 8
3	357 ± 17	363 ± 39

Values represent mean flux ± standard deviation, expressed as µg/cm² h.

explained by the fact that at high drug concentrations, charged skin pores become saturated with ions such that further elevations in drug concentration do not yield increased flux (Lauger, 1973; Sage, 1995; Badkar and Banga, 2002). This is clearly the case with BH at donor phase concentrations above 2% (w/v).

The use of 50% aqueous ethanol in the formulation did not affect buspirone iontophoresis in comparison to a purely aqueous vehicle. Both vehicles have a similar pH of 5.6 for ethanol:water and 5.1 for water. A significant enhancement in iontophoretic drug flux has not been reported in the literature with ethanol. It is possible that solubility of BH in water is high enough that there was sufficient ionic drug available for transport in the 50% ethanol/water formulation.

3.3. Influence of current application mode

The aim of this section of the study was to investigate the effect of repeatedly switching current on and off on BH transdermal delivery. This was done in order to prevent the skin from developing a polarization potential, which reduces the efficiency of iontophoresis (Lashmar and Manger, 1994). The protocol involved having the selected application mode (e.g. 2-h on/2-h off, 2-h on/2-h off, etc.) applied between 0 and 12 h at which point the current was permanently switched off but permeation was allowed to proceed until 24 h. When switched on, the current density was always 0.5 mA/cm². The donor phase consisted of 2% (w/v) BH dissolved in water. As can be seen from Fig. 4, in the absence of electric current, buspirone flux through human skin was negligible. As the percentage of “current-on” time was progressively augmented, drug flux also increased. It is noteworthy that when the current was continuous, BH flux was constant. However, as the power was switched off for progressively greater periods, the flux rate became progressively more variable. This effect was particularly prominent for the 2-h on/2-h off application mode. This paradigm indicates that BH does not form an appreciable reservoir in human skin. Significantly, for all application modes, as soon as iontophoresis was terminated at 12 h, buspirone flux declined fairly rapidly such that the amounts of drug in the receiver compartment at 24 h was just slightly greater than the amounts at 12 h. Again, this suggests that following iontophoresis, the skin recovers its barrier properties to buspirone.

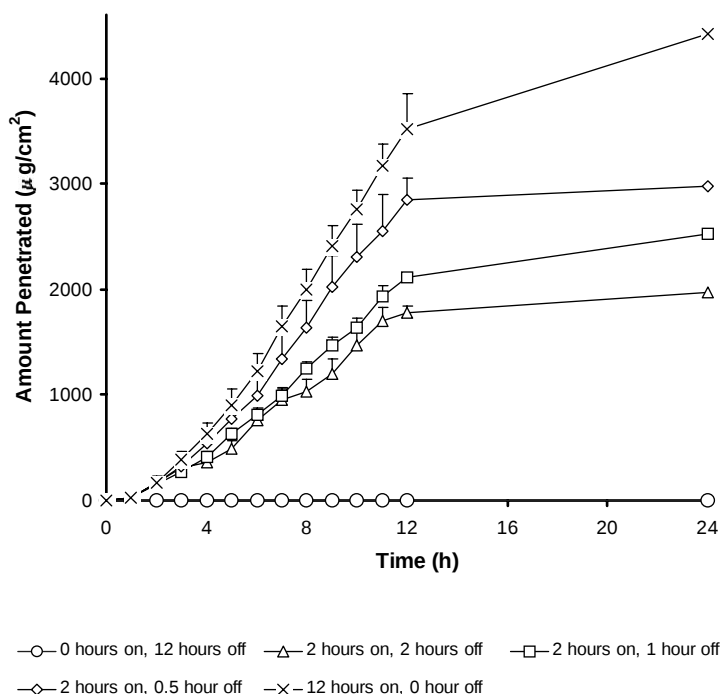


Fig. 4. Iontophoresis of buspirone hydrochloride over 0–12 h; the influence of current application mode ($n \geq 3$, error bars represent S.D. values).

Fig. 5 presents the same 0–12 h data in the form of a graph of BH mean flux versus cumulative “current-on” time. There is clearly a linear relationship ($r^2 = 0.968$) between the two parameters as would be expected for

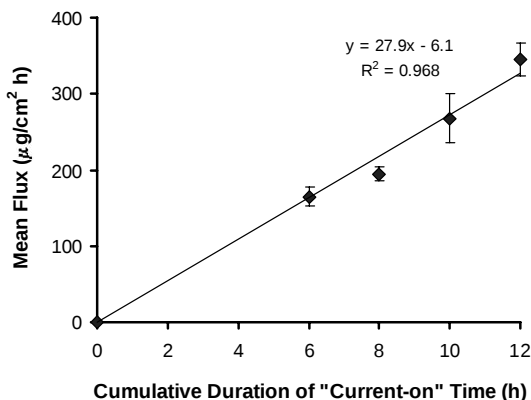


Fig. 5. Mean flux ($\mu\text{g}/\text{cm}^2 \text{ h}$) of buspirone hydrochloride plotted against cumulative current on-time duration (h) ($n \geq 3$, error bars represent S.D. values).

iontophoretic systems in which the solute does not form a skin reservoir and the skin regains its original permeability once the current is switched off.

3.4. Histological analysis

Figs. 6 and 7, respectively, present S.E.M. surface views and transverse sectional views of human skin samples that were exposed to either buspirone passive delivery or buspirone iontophoresis (24 h at $0.5 \text{ mA}/\text{cm}^2$). Visual analysis of these images, as well as other similar S.E.M. images from different portions of the treated skin samples, revealed that 24 h of iontophoresis did not noticeably change the morphology of whole human skin.

3.5. Influence of chemical enhancers alone

The objective of this set of studies was to assess the effects of chemical enhancer co-application on BH penetration through human skin. No iontophoresis was undertaken. Fig. 8 depicts the BH permeation

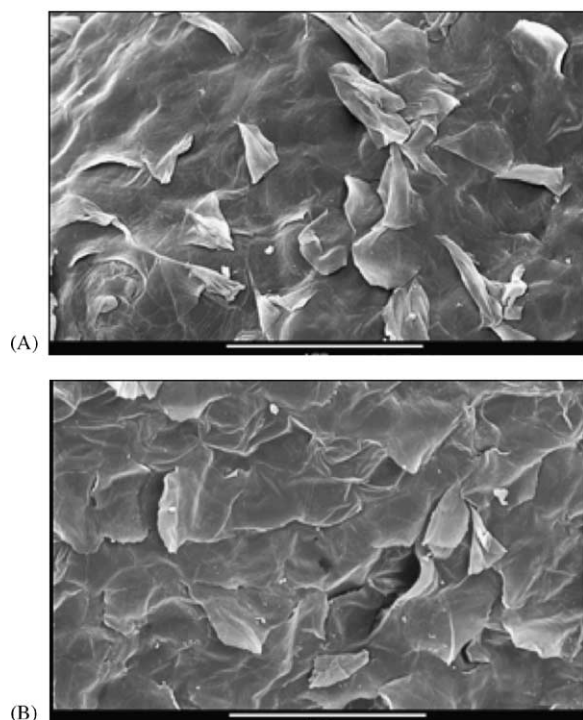


Fig. 6. Representative surface view of human skin (original magnification: 500 $\times$). (A) Following passive buspirone treatment for 24 h. Scale bar = 100 μ m. (B) Following buspirone iontophoresis (0.5 mA/cm²) for 24 h. Scale bar = 100 μ m.

profiles associated with the application of Azone, oleic acid, and menthone while Table 2 lists the BH steady state flux data (14–24 h) for all the enhancer treatments. It can be seen that treatment with Azone[®], oleic acid, and menthone yielded approximately 27-, 40-, and 45-fold respective increases in BH flux. Intriguingly, in contrast to menthone, the application of cineole or terpineol produced only negligible or no BH transport enhancement. All three monocyclic terpenes are structural isomers, as well as liquids at room temperature exhibiting similar densities ($\sim$ 0.9 g/ml). Hence, at 2.5% (v/v), all three compounds were present at almost equal molarity within the donor phase. Furthermore, the three terpenes exhibit very similar log octanol–water partition coefficients (menthone = 2.63 ± 0.25 ; cineole = 2.82 ± 0.25 ; and terpineol = 2.73 ± 0.28). The distinctiveness of each terpene lies mostly in its chemical class where terpineol is an alcohol, cineole is an ether, and menthone is a ketone. Our data therefore suggest that for

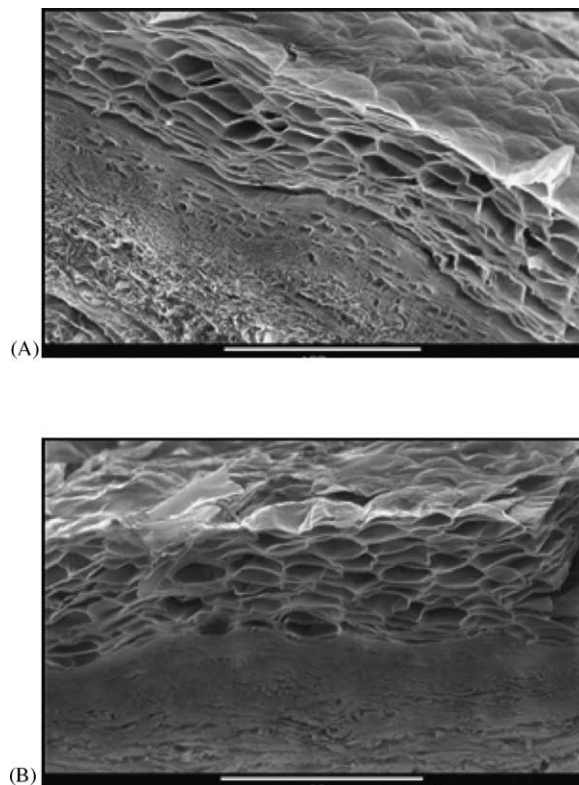


Fig. 7. Representative cross-sectional view of human skin (original magnification: 500 $\times$). (A) Following passive buspirone treatment for 24 h. Scale bar = 100 μ m. (B) Following buspirone iontophoresis (0.5 mA/cm²) for 24 h. Scale bar = 100 μ m.

Table 2

The influence of different treatments on BH flux through human skin $n \geq 3$

Treatment	Mean steady state flux ($\pm$ S.D.) μ g/cm ² h	ER
Control	1.9 ($\pm$ 0.6)	1.0
0.025	56.1 ($\pm$ 44.9)	29.5
Azone	51.9 ($\pm$ 10.4)	27.3
Azone + 0.025	166.1 ($\pm$ 19.4)	87.4
Oleic acid	77.5 ($\pm$ 11.7)	40.8
Oleic acid + 0.025	134.0 ($\pm$ 25.4)	70.5
Menthone	85.6 ($\pm$ 18.1)	45.0
Menthone + 0.025	65.6 ($\pm$ 23.8)	34.5
Cineole	4.3 ($\pm$ 2.2)	2.2
Cineole + 0.025	23.5 ($\pm$ 4.9)	12.3
Terpineol	1.5 ($\pm$ 0.2)	0.8
Terpineol + 0.025	29.2 ($\pm$ 18.2)	15.4

ER (enhancement ratio) = treatment flux/control flux; 0.025 represents iontophoresis at 0.025 mA/cm².

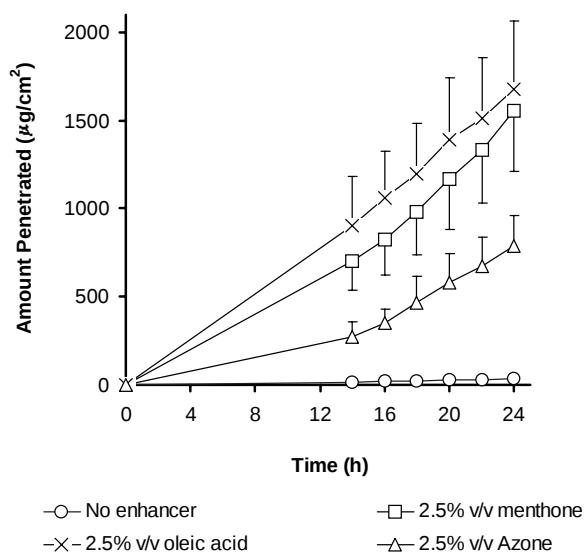


Fig. 8. The influence of chemical enhancers on buspirone hydrochloride penetration through human skin ($n \geq 3$, error bars represent S.D. values).

monocyclic terpenes, ketones are more effective enhancers than ethers or alcohols.

3.6. Influence of iontophoresis with chemical enhancers

Fig. 9 compares the BH penetration-time profiles associated with the application of Azone[®], low-intensity iontophoresis (0.025 mA/cm^2), and Azone[®] used in conjunction with low intensity iontophoresis. The combination approach clearly produced greater BH transport than either treatment applied separately. Moreover, from Table 2, it can be determined that $\text{ER (Azone} + 0.025) > \text{ER (Azone)} + \text{ER (0.025)}$ which means that Azone[®] and iontophoresis acted synergistically to enhance BH transport through human skin. Synergism generally indicates that the two modalities act by using the same pathway or mechanism. Table 2 also lists the results of experiments involving oleic acid where calculations showed that $\text{ER (oleic acid} + 0.025) \approx \text{ER (oleic acid)} + \text{ER (0.025)}$. This type of relationship indicates oleic acid and

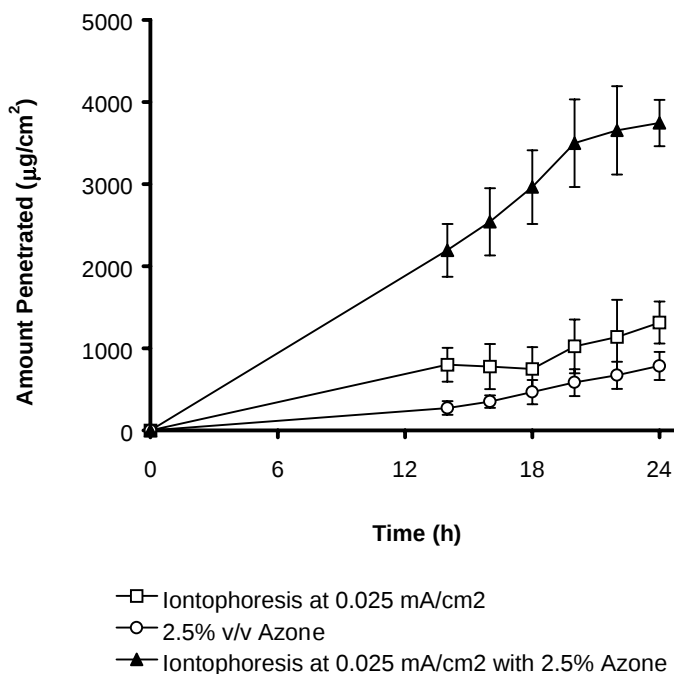


Fig. 9. The effect of Azone[®] and iontophoresis on buspirone hydrochloride penetration through human skin ($n \geq 3$, error bars represent S.D. values).

iontophoresis enhance BH transport via two separate mechanisms/routes. In contrast, the enhancement ratio values shows that for all three terpene compounds, $ER(\text{terpene} + 0.025) < ER(\text{terpene}) + ER(0.025)$. This indicates that the two modalities may affect the same mechanism but act in antagonism to each other, thereby suppressing each other's activity.

4. Discussion

The present study demonstrated that whereas BH penetrated passively through human skin at a negligible rate, use of iontophoresis could dramatically enhance the transdermal delivery of this anxiolytic drug. Furthermore, as in most iontophoretic systems, there was a linear relationship between current density and drug flux. At the maximal investigated current density of 0.5 mA/cm^2 , the observed flux was $350 \mu\text{g/cm}^2 \text{ h}$. This means a 5 cm^2 patch would deliver BH at a rate of 1.75 mg per hour or 42 mg in 24 h . This is sufficient to achieve serum concentrations of BH that would be therapeutically efficacious for most chronic conditions (Ratey et al., 1989). In other examples of iontophoretic delivery (Inada et al., 1994; Kim et al., 1993; Turner et al., 1997), it was demonstrated that when the power was switched off, it took time for the skin to recover its barrier properties to the test permeant. However, when the power was switched off in our studies, transdermal BH flux returned relatively rapidly to its pre-iontophoresis level. This suggests that stratum corneum perturbations induced by iontophoresis were readily reversible. Crucially, visual analysis of scanning electron microphotographs indicated that a 24 h application of a 0.5 mA/cm^2 current did not affect the skin architecture. This compares well with several other studies that have shown that iontophoretic current densities up to this value are generally well tolerated in humans (Ledger, 1992; Singh et al., 1998). Of course, scanning electron microscopy represents a relatively elementary tool for analyzing meaningful skin barrier alterations. More refined techniques include freeze fracture electron microscopy and X-ray diffraction (Craane-van Hinsberg et al., 1997). Other biophysical techniques such as ATR-FTIR spectroscopy, DSC or transepidermal water loss (Jadoul et al., 1999) could be undertaken to examine this issue in greater detail.

In this study, we employed direct current iontophoresis. One of the limitations of this type of power source is that it causes the skin to develop a polarization potential, which reduces the efficiency of iontophoresis (Lashmar and Manger, 1994). Many approaches have been employed to counteract skin polarization. These include using pulsed direct current (Kanebako et al., 2002), alternating current (Li et al., 1999), or on/off current application modes (Fang et al., 1996). From a power consumption point of view, the use of on/off application modes represents the easiest way to counteract skin polarization (Sage, 1995). We deduced that since BH did not form a reservoir in human skin, the use of an on/off application mode appreciably reduced drug flux and was not appropriate for BH delivery.

Investigations with chemical enhancers only (i.e. no iontophoresis) indicated that skin treatments with Azone[®], oleic acid, and menthone significantly enhanced BH flux although not to the extent induced by iontophoresis at 0.5 mA/cm^2 . In contrast to menthone, the presence of cineole or terpineol in the donor phase did not markedly affect BH penetration. As previously explained, this suggests that for monocyclic terpenes, ketones are more effective enhancers than alcohols or ethers. This is to some extent mirrored in the literature reports where the superior accelerant activity of menthone over cineole was reported for diclofenac sodium flux through rat skin (Arellano et al., 1996) as well as for piroxicam penetration through porcine skin (Doliwa et al., 2001). The greater effectiveness of ketones may be attributable to the fact that minor variations in accelerant-lipid bilayer interactions at the molecular level can have a great impact on the extent of skin permeabilization achieved (Barry, 2001). However, another group has reported that all three terpenes enhanced imipramine flux through rat skin to a similar extent (Jain et al., 2002). Clearly, more research is required in order to firmly establish whether the greater activity of monocyclic terpene ketones in human skin is a general principle.

One possible concern is that even at current densities around 0.5 mA/cm^2 , most of the current may be penetrating through appendages such as the hair follicles. Thus, the actual current density in the follicle may be high enough to damage growing hair (Barry, 2001). One way of overcoming this problem is to use low current density iontophoresis in

conjunction with chemical enhancement. We combined very low current density (0.025 mA/cm^2) iontophoresis with Azone[®] application and determined that the two modalities synergistically enhanced BH transport. Ganga et al. (1996) documented a similar phenomenon in that the combination of Azone[®] and iontophoresis synergistically promoted in vitro metoprolol tartrate delivery through human skin. This type of synergism may be due to Azone[®] molecules perturbing the lipid bilayers of the stratum corneum, thus reducing the skin barrier's size-selectivity and electrical impedance, leading to an increased rate of drug transport (Mitrugotri, 2000). This was confirmed in vivo in human volunteers, in that the application of Azone[®] with iontophoresis reduced skin impedance to a greater extent than that produced by iontophoresis alone (Kalia and Guy, 1997). In our studies, the Azone[®]-current density combination we examined still produced less BH transport than that produced by iontophoresis alone at the maximal current density (0.5 mA/cm^2). However, this aspect should be further explored by varying the current densities and Azone[®] concentrations. An advantage of applying iontophoresis together with Azone[®] is that the patch or device required to deliver the combination is unlikely to be significantly more complex than that necessary for iontophoresis alone.

Our study also showed that oleic acid enhanced BH delivery in an additive manner with iontophoresis. This type of additive effect between iontophoresis and oleic acid has been documented for the delivery of luteinizing hormone releasing hormone through human epidermal membrane (Smyth et al., 2002). It is known that oleic acid interacts with the intercellular lipids of the stratum corneum in a slightly different fashion than that of Azone[®] in that it undergoes phase separation (Barry, 2001). Such differences could explain the different interactions of the two enhancers with iontophoretic current. Of less interest for therapeutic application was the fact that the combination of terpenes and current application antagonized BH delivery relative to the sum of enhancements produced by each modality alone. This type of paradigm may be due to the enhancer's presence in the tissue somehow limiting the penetration of the drug (Doliwa et al., 2001).

In conclusion, the results from this study indicate that by applying DC iontophoresis at 0.5 mA/cm^2 it is possible to deliver BH through human skin at a rate

that would be therapeutically efficacious if clinically replicated. Much lower currents could potentially be used if synergism between Azone[®] and iontophoresis is exploited. Clearly, more work is required in order to optimize the identified synergistic effect and to further characterize the biophysical interactions occurring in skin during BH iontophoresis.

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