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International Journal of Pharmaceutics 192 (1999) 159–172

international journal of pharmaceutics

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Transdermal delivery of antiparkinsonian agent, benztropine. I. Effect of vehicles on skin permeation

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Received 30 June 1999; received in revised form 30 August 1999; accepted 31 August 1999

Abstract

The influence of pH and various lipophilic and hydrophilic vehicles on the epidermal permeation of benztropine (BZ) free base and its mesylate salt were studied in vitro using the hairless mouse (HLM) and human cadaver (HC) skin membranes. The pH-partition behavior of BZ base ($pK_a = 10$) was examined using n-octanol and Britton–Robinson buffers over the pH range of 5–12. Unexpectedly, the ionized species of BZ yielded a high partition coefficient ($\log K_{octanol/water} = 2.14$), which was reflected by its relatively high skin permeability ($P = 1.6 \times 10^{-2}$cm h^{-1}). BZ base delivered from a lipophilic vehicle with a solubility parameter range of 7.1–10.3 $(cal\ cm^3)^{1/2}$ exhibited a significantly enhanced rate of permeation as compared to that attained from a hydrophilic vehicle of solubility parameter range between 12.5–23.4 $(cal\ cm^3)^{1/2}$. Among the neat solvents examined, a lipophilic carrier, isopropyl myristate (IPM) provided the most enhancing effect on the permeation of BZ base. In addition, the neat IPM carrier offered the maximum BZ base flux of 150 μg per cm^2 h^{-1} across HC skin, which was approximately 16 times greater than the target delivery rate of BZ from a 10-cm^2 device. In comparison, BZ base exhibited a 2–60 times greater flux than BZ mesylate when delivered from the neat solvents. However, interestingly enough, the binary cosolvents consisting of IPM and short-chain alkanols such as ethanol (EtOH), isopropanol (iPrOH), and tertiary butanol (tBtOH), in particular a 2:8 combination, produced a marked synergistic enhancement of BZ flux from the mesylate salt, whereas a retarding effect was noticed for the permeation of BZ base. The enhancement potency for the BZ mesylate permeation increased linearly with the carbon number of the branched alcohols present in the binary mixtures. A tBtOH-IPM (2:8) combination produced the highest BZ flux from the mesylate salt, i.e., 2016 mg per cm^2 h^{-1}, which was 100-fold greater than from water and 44–540-fold greater than the individual neat solvents, respectively. The observed permeation enhancement of BZ mesylate by the alkanol-IPM mixtures was probably as a result of a combination of decreasing barrier ability of the stratum corneum by the binary vehicles and moderately partitioning BZ mesylate through the viable epidermis/dermis.

Keywords: Transdermal delivery; Benztropine free base and mesylate salt; Enhanced skin permeability; Single and binary enhancers; Isopropyl myristate; Short-chain alkanols

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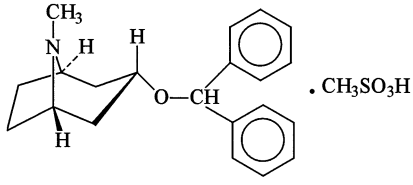
0378-5173/99/$ - see front matter
PII: S0378-5173(99)00305-1

1. Introduction

Parkinson's disease (PD) is a disorder of the extrapyramidal system of the brain involving, in particular, the basal ganglia. The anticholinergic drugs of most value in treating mild and moderate PD are benztropine (BZ) and trihexyphenidyl. BZ mesylate, 3-(diphenylmethoxy)-8-methyl-8-azabicyclo [3,2,1] octane methanesulfonate, is a potent muscarinic receptor antagonist, which is used for the treatment of PD and the extrapyramidal syndrome induced by neuroleptic drugs such as the phenothiazine derivatives (Standaert and Young, 1996).



Benztropine Mesylate

Oral administration, at the usual daily dose of 1–3 mg BID, is currently the principal route of medication for BZ in the treatment of PD, while the injection is especially useful for psychotic patients with acute distonic reactions or other reactions that make oral medication difficult or impossible (Flaherty and Gidal, 1995). The clinical application of BZ, however, is often limited because of its dose-related adverse side effects such as tachycardia, hallucination, paralytic ileus, and urinary retention. More serious side reactions include forgetfulness, sedation, depression, and anxiety. In addition, as with other medicinal agents used in PD, the therapeutic dose should be carefully adjusted and individualized according to age and weight of patients and to the type of parkinsonism being treated (Nelson et al., 1997).

Moreover, BZ is known to undergo extensive metabolism to the N-oxide, N-desmethyl-, 4-hydroxy- and N-desmethyl-4-hydroxy- derivatives following its oral administration (He, 1995). Because of its potential toxicity and metabolism behavior by the oral route, the transdermal delivery of BZ appears as a most attractive alternative route for treating PD and neuroleptic-induced extrapyramidal reactions. It was therefore attempted to develop a device-rate-controlled transdermal delivery system, which provides a convenient administration method for accurate infusion rate and reduction of inter-subject and intra-subject variability.

The first part in this series of investigations intends to report the results of physicochemical characterization and in vitro studies on the transdermal permeation of BZ base and its mesylate salt from various neat and binary vehicles. The enhancement of the skin transport rate of BZ by the use of a lipophilic vehicle such as IPM and its binary cosolvent systems with short-chain alkanols is discussed. Additionally, the comparative transdermal permeabilities of ionized and unionized forms of an azabicyclooctane compound, BZ are also discussed.

2. Materials and methods

2.1. Materials

BZ mesylate was kindly supplied by Merck (Rahway, NJ). Ethanol (EtOH), isopropyl alcohol (iPrOH), tertiary butanol (tBtOH), propylene glycol (PG), polyethylene glycol 400 (PEG 400), mineral oil (MO), and IPM were purchased from Sigma (St. Louis, MO). Silicone medical fluid 360 (SF) was obtained from Dow Corning (Midland, MI). Anhydrous sodium sulfate, potassium hydroxide, and sodium chloride were purchased from Aldrich (St. Louis, MO). Anhydrous phosphoric acid, glacial acetic acid, boric acid, sodium hydroxide, and potassium chloride were obtained from J. T. Baker (South Plainfield, NJ). All other chemicals of reagent grade or better were used as received.

2.2. Preparation of BZ base

BZ base was prepared by adding 2 N KOH to an aqueous solution of BZ mesylate (approximately 0.5 g ml^{-1}) until a pH of 12. The aqueous solution was saturated with NaCl and the free base was extracted into diethyl ether. The ether extracts were dried over anhydrous sodium sulfate

and then evaporated to dryness under reduced pressure using a rotary evaporator. The residue was dried under reduced pressure to the state of viscous colorless oil, which eventually crystallized after storage at −10°C for at least 2 weeks. The product was identified as BZ base by IR, NMR, and GC-MS. High level of purity (>99.8%) was assured via UV spectroscopy, TLC and HPLC with PDA detection, and by the sharpness of endothermic peak in DSC.

2.3. HPLC assay method

Assay of BZ base and mesylate was performed using a Waters HPLC system equipped with a 600E multisolvent delivery system, an 717 Plus autoinjector, a 996 photodiode array detector, and a 2010 Millennium data management system (Waters Corporation, Milford, MA). A reversed phase Novapac C18 column (5 microns; 15 cm × 3.9 mm I.D.; Waters Corporation.), that was maintained at ambient temperature was utilized as the analytical column. Acetonitrile-pH 3.0 triethylamine/phosphoric acid buffer (60:40) combination was used as the mobile phase at a flow rate of 1.0 ml min^{-1}. With an injection volume of 10 μl and detector wavelength set at 259 nm, the chromatographic peak for BZ was detected at retention time of 3.5 min. A linear peak area versus concentration relationship was established with correlation coefficient of >0.999.

2.4. Determination of drug solubility

The solubility studies were conducted by adding excess amounts of BZ base and its mesylate salt into screw-capped vials containing 1–10 ml of aqueous buffers and various pharmaceutical vehicles. The tightly sealed vials were shaken in a water bath set at 37°C until equilibrated. The saturated solutions were then filtered through a pre-warmed 0.45-μm Millipore filters (Millipore, Bedford, MA). The concentrations of BZ in the filtrates were determined by HPLC after appropriate dilution.

2.5. Determination of apparent partition coefficient

The apparent partition coefficients of BZ base and mesylate salt were determined using n-octanol and aqueous buffers with a pH range from 8 to 12. A known weight of the compounds was allowed to partition between equal volumes of n-octanol and the buffer solution placed in a screw-capped test tube. The two-phase systems were equilibrated in a constant-temperature shaker bath maintained at 25°C. Under these conditions, equilibrium was reached within 24 h with no significant changes of drug concentrations in the aqueous and oil phases after shaking for 48 h. After equilibration, the aqueous and oil phases were then separated by centrifugation at 4000 rpm for 10 min. The concentrations of BZ in each phase was determined by a HPLC method. The apparent partition coefficient was the ratio of $C_{octanol}$ and $C_{aqueous}$ buffer.

2.6. Preparation of test solutions

Samples for evaluating the effect of pH on the transdermal transport of BZ were prepared by suspending an excess amount of the drug in Britton–Robinson buffers of various pH (8, 10, or 12) values in a screw-capped vial and shaking the suspension for 24 h in water bath set at 37°C. The suspension was filtered through a 0.45 μm membrane filter and the concentrations of BZ were determined by a HPLC method prior to its use in a permeation experiment. To study further the influence of the pH of the donor solution on the transdermal permeation of BZ, the drug was dissolved in a 20% PEG 400/aqueous buffer solution (pH 8, 10 or 12) to provide a donor drug concentration of 1 mg ml^{-1}.

To investigate the effects of various neat and binary vehicles on the skin permeation of BZ base and its mesylate salt, appropriate amounts of the drugs were dissolved in each test vehicle. In addition, the saturated solutions of BZ base or its mesylate salt in the same solvents were also prepared with the aid of shaking for 24 h at 37°C.

2.7. In vitro skin permeation experiments

The transdermal permeation of BZ base and its mesylate salt was evaluated using abdominal skin of either hairless female mice of the HRS/J strain (6–8 weeks old, Jackson Laboratories, Bar Harbor, ME), or human cadaver (Ohio Valley Tissue and Skin Center, Cincinnati, OH). To obtain the hairless mouse (HLM) skin, the animal was sacrificed just prior to a permeation study by exposure to CO_2, after which, a piece of abdominal skin was surgically removed using a pair of forceps. Human abdominal cadaver skin (thickness of 1.30 ± 0.05 mm) was also used for comparative purposes. Permeation experiments were carried out using a side-by-side horizontal or a vertical Franz diffusion apparatus. A piece of skin was mounted between the two half-cells of the horizontal diffusion system or between the cap and cell body of the Franz diffusion system. The temperature of the cells and their contents was kept at a constant temperature of 37 ± 0.5°C throughout an experiment. In the case of a side-by-side diffusion system, the drug solution (3.5 ml) was placed in the donor compartment, and an equal volume of 20% PEG 400/pH 5.5 buffer solution was then filled into the receptor half-cell. For the vertical system, the drug solution (100 μl) was applied to the outer side of a piece of skin having an exposed area of 0.64 cm^2, and the cell body was filled with the same solution (4.6 ml) used in the horizontal diffusion system. In all the experiments, aliquots (100 μl) of receptor solutions were withdrawn at predetermined times, replaced with an equivalent volume of drug free medium, and assayed for BZ using a HPLC method. Cumulative corrections were made to determine the total amount of BZ permeated at each time interval. The steady-state flux of BZ was determined using the following equation:

$$\frac{1}{A}\left(\frac{d\,M}{d\,t}\right) = J_{ss} = P\Delta C \quad (1)$$

where J_{ss} is the steady-state flux in μg per cm^2 h^{-1}; A is the diffusional area of the skin membrane; d M/d t is the slope of the straight portion of the permeation curve (μg h^{-1}); P is the apparent permeability coefficient in cm h^{-1}; and ΔC is the concentration gradient. Lag time was determined by extrapolating the straight-line portion of the steady-state permeation curve to the time axis. Statistical analyses were performed by Student's *t*-test.

3. Results and discussion

3.1. pH-solubility and pH-partition behavior of BZ

As a baseline study, the pH-solubility and pH-partition profiles of BZ base were determined. BZ is a weak basic compound, whose conjugated acid form has a pK_a of 10 in aqueous solution at 25°C (Albert and Sergeant, 1984). Fig. 1 displays the pH-solubility and pH-partition profiles of BZ base. As expected from the azabicyclooctane functional group, the aqueous solubility decreased appreciably with increasing the pH of aqueous buffer systems over the pH range of 5–12. In contrast, the n-octanol/aqueous buffer partition coefficient increased exponentially over the same pH range. It is generally accepted that the ionized species of weakly acidic and basic drug molecules have a low partition coefficient, and that these electrolytes permeate the skin poorly from aqueous solutions (Scheuplein and Blank, 1971). However, as seen from the pH-partition data presented in Fig. 1, the ionized form of BZ at pH 8 appears to possess an appreciably high partition coefficient value (log *K* 2.14) and thus these charged drug molecules are expected to have a high affinity for lipophilic sites within the skin.

3.2. Effect of pH on the skin permeation of BZ

Several permeation studies were performed to investigate the effect of variation in the pH of aqueous medium, the polarity of the various vehicles and the concentration of drug in each vehicle on the penetration of BZ base and its mesylate salt across HLM and human cadaver (HC) skin membranes. Fig. 2 illustrates how the pH of aqueous buffers affected the skin transport characteristics of the weakly basic drug in a saturated aqueous solution. Also shown in Fig. 2 is the

solubility versus pH profile of BZ base. As the pH of the aqueous solution increased from 8 (at which BZ was predominantly in the ionized form), through 10 (at which 50% of BZ was in its ionized form) to 12 (at which BZ was predominantly in the unionized form), the permeation rate decreased linearly (Fig. 2). The similarities in the trends of both the pH-solubility and pH-permeation profiles suggested that the increase in the transdermal flux of BZ attained at pH 8 may have be as a result of an increase in the solubility of the drug. Since BZ was mainly in ionized form at the pH 8, the results indicate that BZ cations are fairly permeable through the skin. This finding appeared to agree well with the earlier observation that the ionized species of the drug had a relatively high partition coefficient (log K 2.14). A similar result has been reported for the skin per-

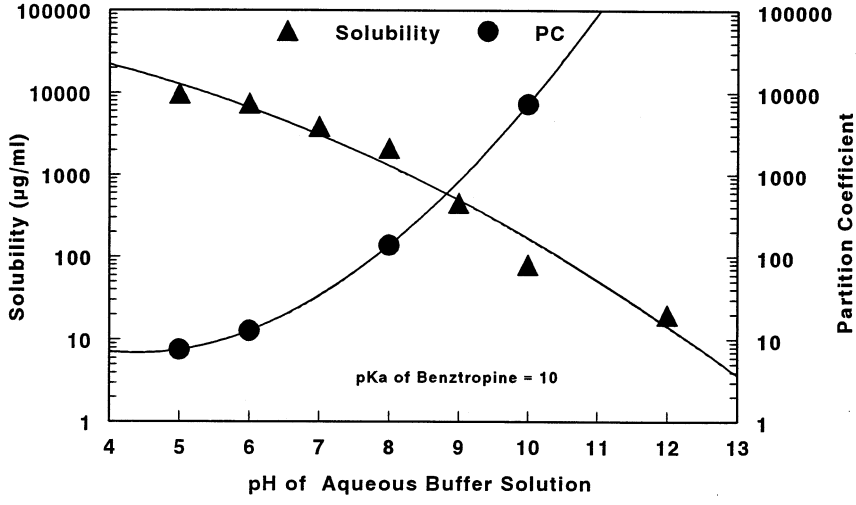


Fig. 1. pH-solubility and pH-partition profiles of benztropine base; solubility and partition coefficient were determined at 37° and 25°C, respectively.

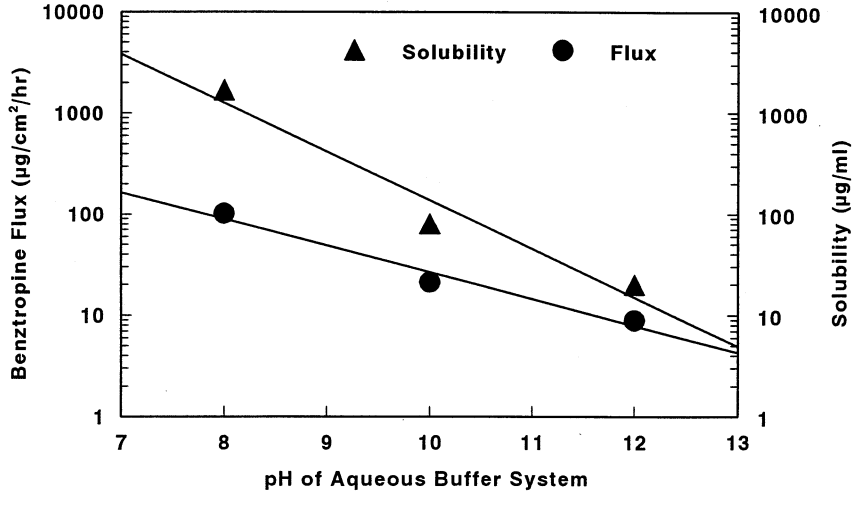


Fig. 2. pH dependence on the aqueous solubility and transdermal flux of benztropine base across hairless mouse skin from a saturated drug solution. Each point represents average of three measurements.

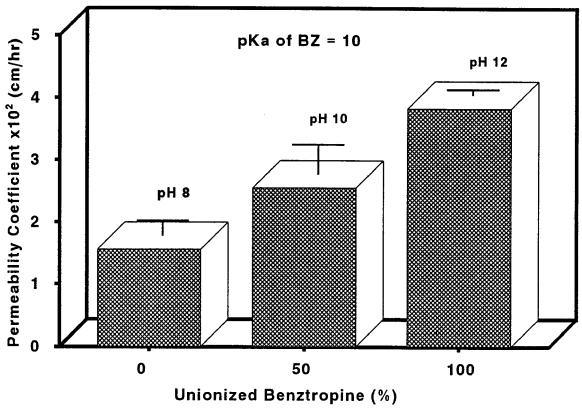


Fig. 3. Effect of ionization on the permeability coefficient of benztropine base across hairless mouse skin from a 1 mg ml^{-1} BZ solution in 20% PEG 400-aueous buffer solutions. Each point represents average of three measurements.

meation of indomethacin, whose anions were found to possess appreciable skin permeability (Chien et al., 1988). To gain a better understanding of the permeability of the ionized and unionized forms of BZ, additional permeation experiments were performed with aqueous solutions of BZ of various pH values and a fixed drug concentration in the donor compartment. For these experiments, sufficient PEG 400 (20%) was added to the aqueous buffer solutions to achieve a sufficiently high concentration of drug in the donor phase, i.e. 1 mg ml^{-1}. The permeation coefficient data obtained in this manner are graphically shown in Fig. 3. The results clearly indicate that the permeability coefficients increased linearly as a function of the concentration of unionized BZ present in an aqueous solution, and that the ionized form of BZ exhibited an unexpectedly high skin permeability ($P = 1.56 \times 10^{-2}$ cm h^{-1}). Therefore, the contribution of the ionized species to the dermal absorption of BZ cannot be considered as negligible as reported for ionized diclofenac molecules (Obata et al., 1993). For comparison, the unionized species of BZ possessed an approximately 2.5-fold greater permeability coefficient relative to the ionized form of the drug.

3.3. Effect of single vehicles on the skin permeation of BZ base

The importance of a vehicle to percutaneous absorption is well documented (Zatz and Sarpotdar, 1987; Rolf, 1988; Ghosh and Banga, 1993; Yamashita et al., 1993). It is also well known that the flux of a drug molecule can be enhanced by chemicals possessing the ability to alter the structure of lipophilic and/or keratinized domains in the stratum corneum (Walters, 1989). These permeation enhancers can be generally classified as polar (hydrophilic) and nonpolar (hydrophobic). In the present study, various hydrophilic and hydrophobic vehicles with Hildebrand solubility parameters ranging from 5.9 to 23.4 $(cal/cc)^{\frac{1}{2}}$ were investigated for their effect on the skin permeation of BZ base. In these experiments, a drug solution or suspension having a concentration of 5 mg ml^{-1} was used as donor phase. The percent saturations of the donor drug solutions varied in the range from 0.01 to 16.3% except for the drug suspension in distilled water. The relevant solubility data and permeation parameters (i.e. lag time, steady state flux, and enhancement factor) are summarized in Table 1. The BZ solubilities in IPM and alkanols were 7825-fold and about 10 000-fold greater than that in water, respectively. BZ solubility in an aprotic polymer vehicle, SF was 306-fold greater than the aqueous solubility. The permeation results clearly show that a lipophilic vehicle with the solubility parameter range of 7.1 and 10.3 $(cal/cc)^{1/2}$ produced 4–10 fold enhancement in BZ flux as compared to the permeability of an aqueous drug solution. On the other hand, SF and iPrOH only doubled the average flux relative to the transport rate from water. The flux of BZ from the three alkanols increased appreciably with increasing carbon number; thus a 2.5-fold increase was observed for every extra methylene group present in the alkanols. Among the lipophilic vehicles examined, IPM exhibited the most enhancing effect on the permeation of BZ, whereas hydrophilic vehicles such as PG and EtOH slowed the rate of permeation below that of a drug solution in water. Several reports in the literature have revealed that the chemical enhancers with solubility parameter

Table 1
Effect of neat vehicles on the transdermal permeation of BZ base across HLM skin at 37°C

Neat vehicle	δ[a] (cal cc^{-1})$^{1/2}$	C_s[b] (mg ml^{-1})	T_{lag}[c] (h)	J_{ss}[c] (μg/cm^2/h)	Average EF for BZ[d]
SF	5.92	30.6	1.6 ± 0.4	47.9 ± 0.1	2.3
MO	7.09	563.7	4.9 ± 0.9	112.9 ± 13.7	5.5
IPM	8.03	782.5	4.3 ± 0.6	215.3 ± 12.5	10.5
tBtOH	10.28	>1000	7.0 ± 2.4	94.5 ± 18.5	4.6
iPrOH	11.24	>1000	9.6 ± 2.0	42.5 ± 7.9	2.1
EtOH	12.55	>1000	3.3 ± 0.3	14.3 ± 0.5	0.7
PG	14.00	435.8	4.9 ± 0.1	5.6 ± 7.1	0.3
H_2O (control)	23.40	0.1	2.0 ± 0.2	20.4 ± 2.4	1.0

[a] Solubility parameters were taken from Vaughan (1985).
[b] Solubilities were determined at 37°C.
[c] Steady-state fluxes and lag times were determined using a 5 mg ml^{-1} drug solution; values are presented as mean ± S.D. ($n = 3$).
[d] Enhancement factor = $(J_{ss})_{vehicle}/(J_{ss})_{control}$.

< 12 (cal/cc)$^{1/2}$ may intervene with lipid components of the skin, but those with solubility parameter > 12 (cal/cc)$^{1/2}$ may selectively partition into the polar components in the skin membrane (Sloan et al., 1986). Another study demonstrated that several fatty acid esters increased the fluidity of the lipid portions of stratum corneum, and thereby enhanced the permeability of drug molecules through the skin layers (Golden et al., 1987). In this context, the permeation data presented in Table 1 imply that BZ molecules transported mainly through the lipoidal pathway of the skin and the lipophilic enhnacers significantly enhanced the permeability of the drug by promoting the fluidity in the lipoidal structure of the skin. Fig. 4 shows how the donor drug concentration level affects the skin permeation of BZ from two lipophilic carriers, i.e. IPM and SF. These data clearly show that the transdermal flux increased linearly with increasing the initial drug concentration from 2 to 10 mg ml^{-1}. The linear relationship observed between the steady state flux and drug concentration indicates that the skin permeation of BZ base was essentially a passive diffusion process. From the linear relationships, the permeability coefficients of BZ from SF and IPM were determined to be 1.3×10^{-2} and 2.9×10^{-2}cm h^{-1}, respectively. These experimentally determined permeability coefficients appeared to be in good agreement with those estimated from the empirical algorithm reported by Flynn and Stewart (1988), in which for compounds with log $K_{o/w}$ values > 2.000, an upper limit permeability coefficient of 1×10^{-2} cm h^{-1} was assigned. BZ base fitting into this partitioning coefficient range (log $K_{octanol/water} = 3.05$) is considered to be sufficiently non-polar for its permeation rate to be controlled with aqueous strata of the epidermis and dermis layers (Flynn, 1983).

3.4. Effect of single vehicles on the skin permeation of BZ mesylate

Fig. 5 shows the flux data obtained from the comparative permeation studies with BZ base and

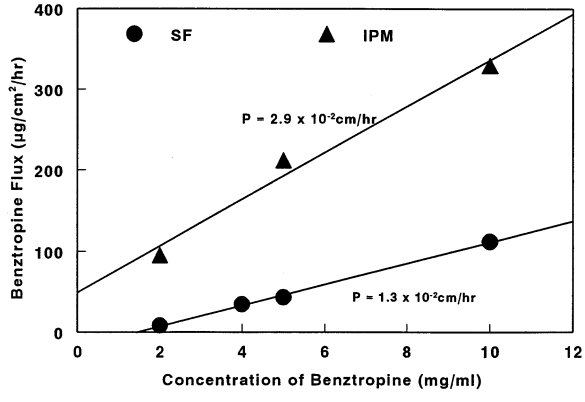


Fig. 4. Effect of drug concentration on the transdermal flux of benztropine base across hairlessmouse skin from isopropyl myristate and silicone fluid. Each point represents average of three measurements.

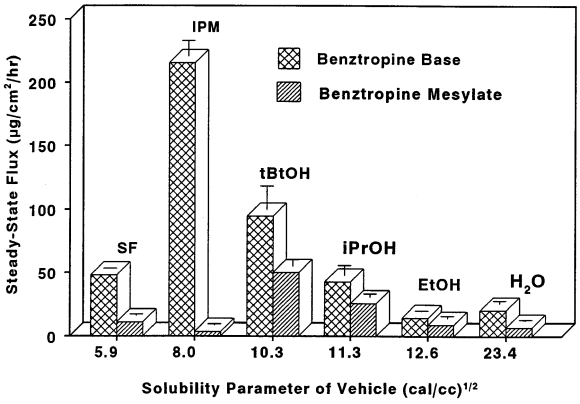


Fig. 5. Steady-state flux of benztropine base and its mesylate salt across hairless mouse skin from various single vehicles (SF, silicone fluid; IPM, isopropyl myristate; tBtOH, tertiary butanol; iPrOH, isopropyl alcohol; EtOH, ethanol; H_2O, water). Each bar represents average of three measurements.

its mesylate salt from various neat vehicles. For these experiments, a 5 mg ml^{-1} drug solution or suspension was used as the donor phase. And thus, the thermodynamic activity varied depending on the solubility of the drugs in the vehicles examined. The percent saturations were determined to be 0.5–16.3% for the free base except for the drug suspension in water and 0.05–5.3% for the mesylate salt except for the drug suspensions in SF and IPM, respectively. As shown in Fig. 5, there is a marked difference in the skin permeability between BZ free base and its mesylate salt when delivered from a lipophilic vehicle such as SF and IPM. The free base produced an approximately 4–60 times greater BZ flux than the mesylate salt. The slow rate of skin transport observed with the mesylate salt from IPM was partially as a result to the extremely low solubility of the mesylate salt in the lipophilic vehicle, i.e. 20 μg ml^{-1}. The permeability difference observed with the free base and mesylate salt diminished appreciably when delivered from relatively polar solvents such as tBtOH, iPrOH and EtOH. In addition, the BZ flux attained with both the free base and mesylate salt increased gradually with increasing carbon number of the alkanols from 2 to 4. The observed modest enhancement of BZ flux from both the free base and meylate salt by the neat alkanols may be explained by the reduction of diffusion barrier via extraction of stratum corneum lipids and proteins by the short chain alkanols, in which the lipid extracting efficiency increased with increasing chain length of alkanols (Goldberg-Cettina et al., 1995).

3.5. Effect of alkanol/IPM binary vehicles on the skin permeation of BZ base and mesylate

It has been reported that binary mixtures of polar and non-polar enhancers offered synergistic enhancement of skin transport of many drug substances and these binary systems also provided reduced skin irritation (Cooper et al., 1985; Catz and Friend, 1990; Pardo et al., 1990; Goto et al., 1993; Goldberg-Cettina et al., 1995). The alkanol/IPM cosolvents were selected to examine the effect of binary composition on the percutaneous penetration of BZ base and mesylate salt, since IPM demonstrated the highest BZ flux enhancement from free base form, and alkanols, in particular tBtOH produced the greatest BZ flux from the mesylate salt. Fig. 6 illustrates the effect of the combinations of IPM and short-chain alkanols (EtOH, iPrOH, and tBtOH) in various proportions on the BZ permeation across the HLM skin. The corresponding values of appropriate solubility of the drugs, lag time, and steady-state flux data are summarized in Table 2. In the permeation experiments, a fixed concentration of the drug solution or suspension, i.e. 5 mg ml^{-1} or equivalent concentration of mesylate salt was utilized as donor phase. Based on the solubilities of the drugs, the percent saturation of the BZ base permeation systems was approximately 0.5%, whereas that of the mesylate donor solutions was in the range of 1.6–100%. Accordingly, the thermodynamic activity of the mesylate salt solutions in the donor phase was higher than the free base permeation systems. The solubility data listed in Table 2 show that the addition of the short chain alkanols to IPM increased appreciably the solubilities of both BZ base as well as its mesylate salt, in which the solubilities of the latter were dominated by the volume fraction of alkanols. The permeation data presented in Table 2 and Fig. 6 clearly indicate that the alkanol-IPM binary cosolvent systems produced a marked enhancement

of BZ flux when delivered the mesylate salt as the permeant. In this case, a substantial synergistic enhancement effect was clearly demonstrated by the binary cosolvent systems. However, in the case of the permeation of free base from the binary mixtures, the transdermal flux was decreased slightly with increasing the volume fraction of alkanols in the binary mixtures. These

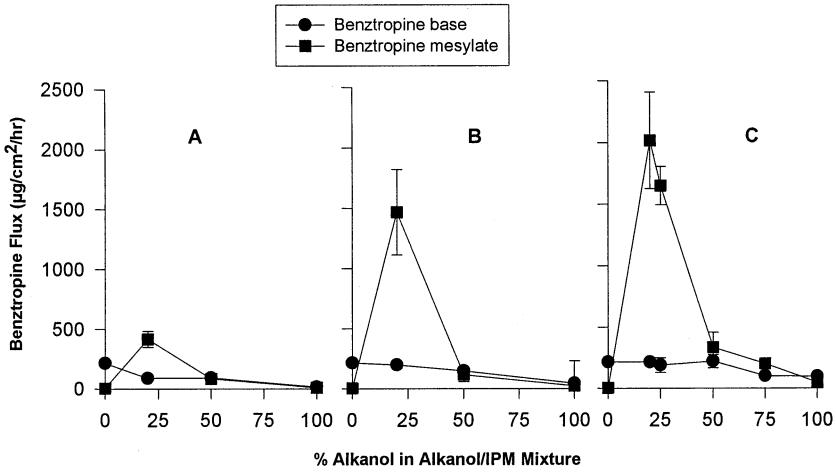


Fig. 6. Steady-state fluxes of benztropine base and mesyalte salt from: (A) EtOH-IPM; (B) iPrOH-IPM; and (C) tBtOH-IPM binary mixtures. Each point represents average of three measurements.

Table 2
Effect of short-chain alkanol/IPM binary vehicles on the transdermal flux of BZ base and its mesylate salt across HLM skin at 37°C

Binary vehicle(v/v)	Benztropine base			Benztropine mesylate		
	C_s[a] (mg/ml)	T_{lag}[b] (h)	J_{ss}[b] (µg/cm²/h)	C_s[a] (mg/ml)	T_{lag}[b] (h)	J_{ss}[b] (µg/cm²/h)
EtOH/IPM						
0/100	782.50	4.3 ± 0.6	215.3 ± 12.5	0.02	7.9 ± 5.8	3.6 ± 0.8
20/80	851.30	5.0 ± 2.5	88.6 ± 9.8	30.53	0.3 ± 0.1	417.2 ± 66.4
50/50	>1000	3.4 ± 0.3	91.8 ± 15.9	219.82	0.6 ± 0.3	84.2 ± 10.8
100/0	>1000	3.3 ± 0.3	14.3 ± 0.5	313.43	2.5 ± 0.1	8.9 ± 1.8
iPrOH/IPM						
0/100	782.50	4.3 ± 0.6	215.3 ± 12.5	0.02	7.9 ± 5.8	3.6 ± 0.8
20/80	>1000	0.9 ± 0.7	198.2 ± 29.1	12.40	0.5 ± 0.3	1467.4 ± 354.3
50/50	>1000	3.1 ± 1.0	147.1 ± 24.8	53.40	3.2 ± 2.7	115.3 ± 56.3
100/0	>1000	9.6 ± 2.0	42.5 ± 7.9	188.15	4.5 ± 0.4	21.4 ± 2.3
tBtOH/IPM						
0/100	782.50	4.3 ± 0.6	215.3 ± 12.5	0.02	7.9 ± 5.8	3.6 ± 0.6
20/80	>1000	2.3 ± 0.2	214.4 ± 36.5	4.38	0.9 ± 0.1	2016.4 ± 391.6
25/75	>1000	2.6 ± 0.2	189.3 ± 60.6	5.01	1.5 ± 0.2	1648.7 ± 155.7
50/50	>1000	1.9 ± 0.4	220.9 ± 54.0	30.50	0.6 ± 0.6	334.3 ± 122.3
75/25	>1000	3.3 ± 0.7	100.2 ± 14.8	49.50	1.8 ± 0.1	200.5 ± 10.7
100/0	>1000	5.9 ± 0.6	95.7 ± 27.1	94.12	5.1 ± 0.5	45.6 ± 8.1

[a] Solubilities were determined at 37°C.
[b] Steady-state fluxes and lag times were determined using a drug solution (5 mg ml^{-1} and equivalent) in each vehicle; values are presented as mean ± S.D. ($n = 3$).

Table 3
Skin permeation rates of BZ base and its mesylate salt across the intact and stripped HLM skin from tBtOH-IPM (2:8) binary cosolvent at 37°C

Drug	Intact skin			Stripped skin		
	T_{lag}[a] (h)	P[b] ($\times 10^2$ cm h)	J_{ss}[a] (μg/cm^2/h)	T_{lag}[a] (h)	P[b] ($\times 10^2$ cm/h)	J_{ss}[a] (μg/cm^2/h)
BZ Base	2.6 ± 0.3[c]	6.4 ± 1.0[d]	159.9 ± 26.7[d]	0.7 ± 0.2[c]	6.5 ± 0.7[d]	162.2 ± 17.8[d]
BZ Mesylate	1.3 ± 0.1[c]	24.9 ± 6.6[d]	622.8 ± 166.9[d]	0.6 ± 0.2[c]	23.0 ± 9.8[d]	574.4 ± 245.1[d]

[a] Steady-state fluxes and lag times were determined using a 2.5 mg ml^{-1} or equivalent drug solution; values are presented as mean ± S.D. ($n = 3$-4).
[b] Apparent permeability coefficients were the steady-state fluxes divided by the initial donor concentration.
[c] Significantly different ($p < 0.05$) in single-tailed unpaired t-test.
[d] Not significantly different ($p > 0.05$) in single-tailed unpaired t-test.

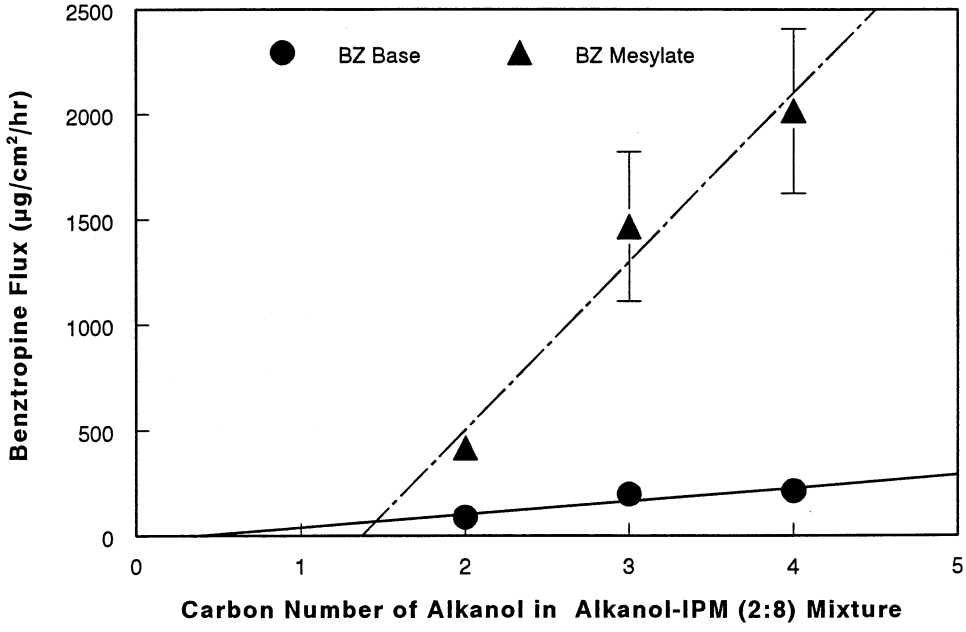


Fig. 7. Effect of carbon number of alkanols present in a 2:8 alkanol-IPM mixtures on the benztropine flux across hairless mouse skin from benztropine base and its mesylate salt solutions. Each point represents average of three measurements.

permeation data imply that the flux of BZ base across the HLM skin from binary cosolvents was not synergistic but additive of the permeability of the drug molecules contributed by each neat solvents present in the binary vehicles in various proportions. Table 3 also presents the lag times observed with the permeation of BZ base and its mesyalte salt from the neat and binary vehicles. In general, a long lag time (2.5–7.9 h) was noticed from both the free base and mesylate salt when delivered from the neat IPM and alkanol vehicles. Such a long lag time was significantly reduced to 0.3–3.3 h from the IPM-alkanol binary vehicles, particularly in the permeation of the mesylate salt. As also shown in Fig. 6. and Table 2, a 2:8 alkanol-IPM combination produced the highest enhancement effect on the transport of BZ mesylate. The enhancement potency increased markedly with increasing carbon number of alkanols present in the IPM binary mixtures. As

shown in Fig. 7, a linear relationship was obtained between the enhancement potency and the carbon number of the alkanols present in the binary cosolvents. In the case of the permeation with the mesylate salt, there was approximately 2.5-fold increase in the permeation enhancement potency per methyl group of the branched alkanols. Among the 2:8 alkanol-IPM binary cosolvent systems examined, the tBtOH combination showed the highest BZ flux, i.e. 2016 μg per cm^2 h^{-1}, from the mesylate salt, which was 100-fold greater than from water and 44–560-fold greater than from the individual neat solvents. However, in the permeation with the free base, there is only a slight increase in the enhancement potency of the binary cosolvent systems as a function of the carbon number of the alkanols (Fig. 7).

3.6. Permeability of BZ base and its mesylate salt through stripped skin

The experimentally observed higher skin permeation rate of BZ mesylate than its free base form was somewhat surprising (Table 2), since it is generally accepted that inorganic and organic salts normally penetrate very poorly into the skin because of their hydrophilic nature (Moriyama, 1960; Malkinson and Rothman, 1963). In an effort to understand the mechanism of the permeation enhancement of the mesylate salt caused by the above binary vehicles, further permeation experiments were performed utilizing the stripped skin. The freshly excised HLM skin was stripped repeatedly (20 times) with cellophane tape (3M's Scotch Brand, 3M Company, Minneapolis, MN) following the technique reported by Goto et al. (1993). In these permeation experiments, a 2:8 tBtOH-IPM binary cosolvent system was employed since this combination produced the highest BZ flux from the mesylate salt among the binary vehicles examined (Table 2). A drug solution with a 2.5 mg ml^{-1} of the free base and equivalent concentration of the mesylate salt was tested as the donor phase. The pertinent skin permeation data obtained with the free base and mesylate salt across the intact and stripped HLM skin are presented in Table 3. The results clearly demonstrate that when the two compounds were delivered from the 2:8 tBtOH-IPM binary mixture, the permeability of BZ across the stripped skin was essentially unchanged as compared with that of the intact skin, although the lag times decreased to an appreciable extent. These results strongly suggest that the binary vehicle seemed to notably or completely reduce the barrier function of the stratum corneum to the BZ permeation. Accordingly, in the time course of permeation process with the BZ base and mesylate salt from the binary vehicle, the epidermis/dermis layer became the major transport rate-limiting component of the skin. In addition, Table 3 show that the apparent permeability coefficient determined with the mesylate salt across the epidermis/dermis layer appeared to be about 3.5 times greater than that of the free base. The relatively lower permeability coefficient value obtained with BZ base can be explained by the difference in the partition behavior of the two compounds. The n-octanol/water partition coefficients determined with the free base and mesylate salt at 37°C were 1117.7 ± 172.1 ($\log K_{octanol/water} = 3.05$) and 0.75 ± 0.01 ($\log K_{octanol/water} = -0.124$), respectively. It is therefore conceivable that BZ base form possessing $\log K_{octanol/water} = 3.05$ was sufficiently non-polar for its permeation rate to be controlled primarily by aqueous strata of the epidermis/dermis layer in the stratum-corneum-disrupted skin. On the other hand, the mesylate salt of BZ having $K_{octanol/water} = 0.75$ was slightly polar to preferentially pass through the aqueous pores of the epidermis/dermis after the drug molecules diffused through the stratum corneum of the skin, which was disrupted by the IPM-alkanol binary vehicle (Flynn and Stewart, 1988). Such a higher permeability of a salt form through the skin has been also reported for diclofenac free acid and its sodium salt with a 20% ethanol-aqueous solution. The ethanolic vehicle decreased the transdermal flux of lipophilic free acid form of the drug, whereas the permeation of diclofenac sodium salt through the ethanol-treated rat skin was significantly increased (Obata et al., 1993).

3.7. Comparative Permeability of BZ base through HLM and HC skin

The abdominal skin freshly excised from hairless mouse has been widely utilized in the permeation experiment because of the following reasons: (i) it has similar barrier property to that of human skin; (ii) the good reproducibility of data generated; (iii) the availability and easy control in its sex, age and skin region; and (iv) the possibility of removing the skin specimen just before an experiment is initiated (Chien, 1987). However, the permeation studies in the literature generally show that the skin of common laboratory animal (mice, rat, rabbit, guinea pig) are more permeable than the skin of human, although, studies by Stoughton (1975) utilizing human and hairless mouse skin showed remarkable similarities in absorption for the skin of the two species for many drug substances. In an effort to find a predictive correlation between the transport rates of BZ molecules through HLM and human skin, the permeability of BZ base through the animal and human skin membranes were measured using neat IPM as the donor vehicle. The HC skin with a thickness of 1.30 ± 0.05 mm was obtained from the abdomen of male Caucasian of 69 years. The first experiment was performed in a horizontal diffusion system using a 5 mg ml^{-1} drug solution as the donor phase. Using a saturated drug solution, additional penetration study was conducted in a vertical Franz diffusion cell with a view to determine the maximum rate of permeation. In this case, the flux of BZ was determined after the application of a finite dose of 70 mg of drug dissolved in 100 μl of IPM to 0.64 cm^2 skin surface. The lag times and steady-state flux data obtained in this manner are presented in Table 4. The results clearly indicate that HLM skin was 7–9 times more permeable for BZ than the skin of human from the both sets of diffusion cell systems. In addition, the lag times observed with the HC skin appeared to be 1.3–2 times longer than that of HLM skin. On the other hand, observed maximum BZ flux across the HC skin was found to be 150 μg per cm^2 hr^{-1}, which was approximately 16 times greater than the target delivery rate, which is required for the effective treatment of PD using a 10 cm^2 transdermal device.

4. Conclusions

The ionized species of an azabicyclooctane compound, BZ, possessed an unexpectedly high partition coefficient, which was reflected in its relatively high skin permeability. BZ base was found to be highly permeable through HLM skin in vitro when delivered from lipophilic solvents such as IPM and MO. BZ base in IPM solution was 7–9 times more permeable across HLM skin than HC skin. From the neat vehicle systems, BZ base exhibited a 2–60 times greater flux across HLM skin than BZ mesylate salt. These differences in permeation data were dependent on the polarity of vehicles examined. The maximum BZ flux (150 μg cm^2 h^{-1}) attained across HC skin from a saturated drug solution in IPM appeared to be approximately 16 times greater than that is

Table 4
Comparative transdermal fluxes of BZ base across HLM and HC skin membranes at 37°C

Diffusion system	Hairless mouse skin		Human cadaver skin		J_{HLM}/J_{HC}
	T (h)	J_{HLM} (μg/cm^2/h)	T_{lag} (h)	J_{HC} (μg/cm^2/h)	
Horizontal system[a]	4.3 ± 0.6	215.3 ± 12.5	5.6 ± 0.3	29.9 ± 4.7	7.2
Vertical system[b]	2.6 ± 0.2	1378.7 ± 158.4	5.3 ± 0.5	149.5 ± 18.2	9.2

[a] Steady-state fluxes were determined using a 5 mg ml^{-1} drug solution in IPM as donor phase; values are presented as mean $\pm$ S.D. ($n = 3$).

[b] Steady-state fluxes were determined using a saturated BZ solution in IPM as donor phase at a dose of 70 mg per 0.64 cm^2 skin surface and expressed as mean $\pm$ S.D. ($n = 3$).

required for effectively treating PD in a 10 cm^2 transdermal device. The binary cosolvent consisting of short-chain alkanol (EtOH, iPrOH, or tBtOH) and IPM, in particular, a 2:8 combination, produced a marked synergistic enhancement of BZ flux when the mesylate salt was used as permeant. In contrast, the addition of alkanols to IPM led to a gradual reduction of the transport rate of BZ base. The observed permeation enhancement of BZ mesylate by the alkanol-IPM mixtures was probably as a result of: (i) effective reduction of diffusional barrier of the stratum corneum by the binary mixtures; (ii) a higher permeability of mesylate salt through the viable epidermis/dermis than free base; and (iii) a higher thermodynamic activity of the mesylate salt than free base in the alkanol-IPM mixtures. Among the binary cosolvents evaluated, a tBtOH-IPM (2:8) combination yielded the highest BZ flux from the mesylate salt, i.e. 2016 μg per cm^2 h^{-1}, which was 100-fold greater than from water and 44–560-fold greater than that obtained from the individual neat solvents. Based on the results of this investigation, a lipophilic vehicle, IPM and its binary cosolvents with short-chain alkanols might form the basis for the development of a novel, device-controlled transdermal therapeutic system for PD utilizing either BZ base or its mesylate salt as the active component.

Acknowledgements

This study was supported by Grants and Research Division of St. John's University and the authors are grateful to Merck and Hoechst Marion Roussel for their support with research compounds and laboratory equipment, respectively.

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