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Transdermal delivery of bupropion and its active metabolite, hydroxybupropion: a prodrug strategy as an alternative approach

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Abstract

This investigation includes an evaluation of the percutaneous absorption of bupropion (BUP) and hydroxybupropion (BUPOH) *in vitro* and *in vivo*. In addition, a carbamate prodrug of BUPOH (But-BUPOH) was evaluated *in vitro*. *In vitro* diffusion studies were conducted in a flow-through diffusion cell system. The *in vitro* mean steady-state flux of BUP was significantly higher ($p < 0.001$) compared to BUPOH ($320 \pm 16 \text{ nmol.cm}^{-2}.\text{h}^{-1}$ vs $27 \pm 4 \text{ nmol.cm}^{-2}.\text{h}^{-1}$).

Additionally, a good correlation existed between *in vitro* and *in vivo* results. Mean steady-state plasma concentrations of $442 \pm 32 \text{ ng/mL}$ and $125 \pm 18 \text{ ng/mL}$ were maintained over 48 h after topical application of BUP and BUPOH in hairless guinea pigs *in vivo*, respectively. Although BUP traversed human skin at rates sufficient to achieve required plasma levels, it is chemically unstable and hygroscopic, and unsuitable for transdermal formulation. On the other hand, BUPOH is stable but its transport across skin is much slower. Alternatively, the prodrug But-BUPOH was found to be stable, and also provided a 2.7-fold increase in the transdermal flux of BUPOH across human skin *in vitro*. Thus, But-BUPOH provides a viable option for the transdermal delivery of BUPOH.

Keywords

Bupropion; hydroxybupropion; prodrug; guinea pig skin; human skin; transdermal drug delivery; LC-MS; pharmacokinetics

INTRODUCTION

Bupropion (BUP) is an aminoketone used as an antidepressant and non-nicotine aid to smoking cessation.¹ Its mechanism of action, both as an antidepressant and as an aid to smoking cessation, is thought to involve nicotinic acetylcholine receptors that are linked to dopamine and norepinephrine release.^{2,3} It is proposed that BUP inhibits nicotine-evoked dopamine and norepinephrine overflow from superfused striatal and hippocampal slices, respectively.³ However, in addition to side effects such as nausea and vomiting, there are reported cases of seizures due to BUP overdoses or unintentional exposure.^{4,5} To address the dose-related risk of seizures associated with high peak concentration of the drug following oral administration, BUP HCl is administered in divided doses. In chronic cases, tablets are given three times a day.^{6,7}

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Transdermal delivery of BUP would diminish dose-related side effects, increase bioavailability through reduced metabolism in the gut wall and first pass effect, and allow easier removal of drug input, as well as maintain therapeutic concentrations for long durations with non-invasive zero-order release. Sustained release of drugs delivered *via* the skin would reduce the dosing frequency and eliminate peak plasma levels of the drug, since the skin should provide controlled delivery of drugs at a constant rate for an extended period of time. Additionally, application of a transdermal patch requires less motivation and therefore can be expected to increase patient compliance. Patient compliance is especially problematic among patients suffering from depression and thus, a transdermal patch should certainly be beneficial. Transdermal systems are generally well tolerated and accepted by patients, except for occasional irritation issues in subjects with sensitive skin. Literature reports indicate that BUP is completely absorbed orally, as well as highly skin permeable.⁸⁻⁹ Compared to the hydrochloride salt, BUP free base is more skin permeable and less polar and thus it would be suitable for development into a transdermal dosage form. However, BUP free base is chemically unstable and is also hygroscopic, making it difficult to formulate for delivery through skin. An inclusion complex of BUP with β -cyclodextrin has been used for stabilization of BUP against degradation,¹⁰ but this would not be viable for transdermal delivery due to low permeability characteristics.

BUP is extensively metabolized *in vivo*¹¹ and less than 10% of a BUP dose is excreted unchanged.¹²⁻¹⁴ Metabolic pathways for BUP involve hydroxylation of the tertiary-butyl group almost exclusively by CYP2B6 enzymes to give hydroxybupropion (BUPOH). BUP can also undergo reduction of the carbonyl group to the alcohol moiety of either threo-BUPOH or erythro-BUPOH.¹³⁻¹⁴ BUPOH, a major metabolite in humans, has been reported to have a similar potency to BUP.¹⁵⁻¹⁷ BUP metabolites have been reported to accumulate in plasma and achieve levels from 10 to 100 times greater than that of the administered agent.¹⁸⁻¹⁹ It has been suggested that the pharmacologic activity of BUP might be due to, or receive significant contributions from one or more BUP metabolites.¹¹⁻¹⁶ In some of the assays, there was evidence suggesting that BUPOH was apparently more "antidepressant" than BUP.¹⁶ In contrast to BUP, BUPOH is more chemically stable. However, the presence of a hydroxyl functionality in BUPOH retards its diffusion across skin. One of the strategies used to enhance skin permeation is the prodrug approach, which involves the chemical modification of a therapeutic agent for the purpose of improving physicochemical, biopharmaceutical, and drug delivery properties. Ideally, an inactive pro-moiety is covalently attached to the active parent molecule through various linkages including ester, carbonate, and carbamate moieties, and the resulting prodrug is converted to the parent drug once it crosses the dermal barrier, depending on the structure of the prodrug molecule.²⁰⁻²¹ The purpose of the present study was to evaluate permeation of BUP and BUPOH across human skin *in vitro* and in guinea pig skin *in vivo*. Additionally, a carbamate prodrug of BUPOH, But-BUPOH (Fig. 1), was synthesized and evaluated for transdermal delivery across human skin *in vitro*.

EXPERIMENTAL

Materials and methods

Methanol, ammonium formate, and acetonitrile (HPLC grade) were obtained from Fisher Scientific (Fairlawn, NJ). WIN 55, 212-2 mesylate salt, n-butyl isocyanate, and sodium sulphate were obtained from Sigma (St. Louis, MO). BIO-PSA[®] 7-4602 silicone adhesive was a gift from Dow Corning Corp. (Midland, MI). MEDIFILM[®] 437 rate controlling membrane and MEDIFLEX[®] 1502 (backing membrane; pigmented metalized polyester) were gifts from Mylan Technologies, Inc. (St. Albans, VT). SCOTCHPAK[™] 9742, a fluoropolymer release liner, was a gift from 3M[™] Drug Delivery Systems (St. Paul, MN).

Water was purified by a Barnstead NANOpure[®] Diamond[™] Ultrapure filtration system (Barnstead International, Dubuque, IA).

Synthetic procedures

The detailed synthetic procedures for the preparation of BUP (C₁₃H₁₈ClNO, MW = 239), and BUPOH (C₁₃H₁₈ClNO₂, MW = 255) have been reported elsewhere.^{22,23} Synthesis of the carbamate prodrug of BUPOH, But-BUPOH (Fig.1), is as described below. BUPOH (0.50g, 1.96 mmol) was dissolved in dry dichloromethane under a nitrogen atmosphere; triethylamine (0.197g, 1.96 mmol) was added, and the mixture stirred at room temperature. After 30 min n-butyl isocyanate (0.194g, 1.96 mmol) was added drop-wise over 10 min and the reaction mixture was stirred overnight. After the reaction was complete, as indicated by TLC, the reaction mixture washed with water and the organic layer dried over anhydrous sodium sulfate. The organic phase was concentrated *in vacuo* and purified by column chromatography on silica gel (dichloromethane/methanol 95:5) to afford 500 mg (72%) of the n-butylcarbamate ester of N-[2-(1-hydroxy-2-methyl)propyl]2-amino-3-chlorophenylpropan-1-one as an oily compound. ¹H NMR (CDCl₃) δ 0.64-0.69(t,3H), 1.02-1.050(m,2H), 1.23-1.26(m,2H), 1.416(s,6H), 2.080(s,3H), 3.20(m,1H), 3.38-3.43(t, 2H), 3.84(s,2H), 7.15-7.28(m,4H). ¹³C NMR (CDCl₃) δ 14.16, 14.26, 20.44, 26.12, 31.89, 42.28, 62.25, 71.82, 118.01, 122.21, 129.04, 129.16, 130.41, 130.97, 131.80, 134.99, 154.0. MS: (30 eV): MS (LC-MS electrospray), M⁺ = 355 m/z, (M⁺ - H₂O) = 337 m/z, (M⁺ - NH₂(CH₂)₃CH₃) = 265 m/z.

In vitro studies across human skin

Human skin from abdominoplasty surgery was obtained from the National Cancer Institute's Cooperative Human Tissue Network (CHTN). The use of human tissue was approved by the University of Kentucky Institutional Review Board. The samples were dermatomed immediately using a Padgett[®] dermatome set to 250 μm; the sections were stored at -20°C. A PermeGear flow-through (In-Line, Hellertown, PA,) diffusion cell system was used for the skin permeation studies. Skin surface temperature was maintained at 32°C with a circulating water bath. For But-BUPOH, data was collected by using human skin from a single donor with three cells of BUPOH (control) and four cells of But-BUPOH in each diffusion experiment. Because of normal human skin inter-subject permeation variability, the prodrug was compared against BUPOH permeation within each individual skin sample. These studies were repeated three times with human skin from different donors. The receiver solution was HEPES-buffered Hanks' balanced salt solution pH 7.4 set at a flow rate of 1.1 mL/h. These diffusion study conditions were chosen in order to maintain tissue viability according to previous studies by Collier et al.²⁴ A saturated solution of each of the drugs in mineral oil was applied to the skin in order to maintain a maximum and constant thermodynamic activity of the drug. Each cell was charged with 0.25 mL of the respective drug solution. Samples were collected at 6 h intervals for 48 h. Two-hundred and fifty μL aliquots of the diffusion samples were evaporated under nitrogen, reconstituted with 1 mL of acetonitrile, and analyzed by high pressure liquid chromatography (HPLC) for drug content.

The cumulative amount of drug collected in the receiver compartment was plotted as a function of time. The flux value for a given experiment was obtained from the slope of the steady-state portion of the cumulative amount of drug permeated *vs* time plot. Apparent permeability coefficient values were computed from Fick's First Law of diffusion:

$$\frac{1}{A} \left(\frac{dM}{dt} \right) = J_s = K_p \Delta C \quad (1)$$

In Eq. (1), J_s is the steady-state flux, M is the cumulative amount of drug permeating skin, A is the area of the skin (0.95 cm^2), K_p is the effective permeability coefficient in cm/h , and ΔC is the difference in concentrations of the drug solutions between the donor and receiver compartments. Sink conditions were maintained in the receiver throughout the experiment, so ΔC was approximated by the drug concentration (solubility in this case) in the donor compartment.

Transepidermal water loss (TEWL) readings were used to determine the barrier integrity of the skin before and after treatment with the drug solutions. The typical basal values of TEWL in adults with healthy skin range from $5\text{-}10 \text{ g}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$ ²⁵. However, if the skin is damaged, there will be significant water loss and TEWL values would be expected to be much higher.

Solubilities

Solubilities of BUP, BUPOH, and But-BUPOH were determined by adding an excess quantity of drug to mineral oil or pre-warmed HEPES-buffered Hanks' salt solution pH 7.4 at 32°C , with equilibration while shaking in a water bath at 60 rpm for 48 h. Samples were drawn into a pre-warmed glass syringe, filtered through a syringe filter (Mineral oil: Millex FG-13, Millipore, Billerica, MA, and buffer: nylon filter, Gelman, East Hills, NY), measured with respect to volume, and diluted with an appropriate volume of acetonitrile or buffer. Aliquots of the drug solutions from the buffer samples were evaporated under nitrogen, reconstituted with acetonitrile, and vortexed. The drug in mineral oil was extracted using acetonitrile. The sampling procedure was done in triplicate, discarding the first 40% of the initial filtrate. All samples were analyzed by HPLC.

Chemical stability in buffer, pH 7.4

The chemical stability of the drugs in isotonic phosphate buffer, pH 7.4, was studied by using sub-saturated concentrations of the drugs. Samples were distributed into vials, then sealed, and equilibrated at 32°C . One vial was removed immediately after starting the experiment and at predetermined time intervals for 14 d, and stored at -70°C until analysis. Two-hundred and fifty μL of sample solution was diluted with acetonitrile and analyzed by HPLC. Concentration vs time curves for the disappearance of the drugs were fitted to a first-order reaction mathematical model using nonlinear least-squares regression analysis (SCIENTIST[®], Micromath Inc., Salt Lake City, UT) to obtain estimates for the apparent first-order rate constants and half-lives.

Fabrication of transdermal patches of BUP and BUPOH

BUP and BUPOH HCl salts were converted into their free base forms for transdermal delivery. Drug reservoir formulations of BUP base (300 mg/mL) and BUPOH base (30 mg/mL) were prepared in mineral oil, vortexed, and sonicated for 5 min. All solutions were saturated and contained excess solid. The transdermal patches were fabricated by sandwiching the drug reservoir between a drug-impermeable backing laminate (MEDIFLEX[®] 1502) and a polyurethane membrane. A release slip composed of SCOTCHPAK[™] 9742 was used to leave a small opening into the reservoir of the empty device. The membrane/adhesive laminate was heat-sealed to the metallized polyester backing membrane. The slip was removed to form a small port and the drug solution ($500 \mu\text{L}$) was injected into the reservoir. After injecting the drug solution into the reservoir, the port was heat-sealed.

Animal studies

All animal studies were approved by the University of Kentucky IACUC. Male and female Hairless IAF and Hartley guinea pigs (Charles River) weighing 335-490 g were used for these studies. Prior to surgery, the animals were treated with glycopyrrolate and buprenorphine (to induce analgesia), and then ketamine (100 mg/kg, i.p.) and xylazine (8 mg/kg, i.m.) were used for anesthetic purposes. Catheters were surgically implanted into the jugular vein. A baseline “blank” blood sample was drawn from each animal immediately before drug treatment. For i.v. bolus experiments, a dose of 1 mg/kg for both BUP and BUPOH were infused over a period of 30 s. For transdermal delivery, the developed TTS patches (two patches, 14.5 cm² diffusional area) were applied to the dorsal region of the hairless guinea pigs. Blood samples were obtained for 48 h while patches were on the animal, and for another 48 h after patches were removed. Blood samples were obtained for 72 h following intravenous administration. Blood samples were immediately centrifuged at 10,000 × g for 3 min, plasma separated, and stored at -70°C until analysis by liquid chromatography-mass spectroscopy (LC-MS).

Calibration standards and quality control samples

Individual standard stock solutions of BUP (1 mg/mL) and BUPOH (1 mg/mL) were prepared in acetonitrile. Working calibration standards at concentrations of 0.5 -500 ng/mL in plasma were prepared fresh daily. Six levels of quality control (QC) samples at 5, 20, 50, 100, 200, and 400 ng/mL, were prepared in plasma for the determination of intra-day and inter-day accuracy and precision. A stock solution of WIN 55, 212-2 mesylate salt (1 mg/mL) was prepared in acetonitrile, and a 500 ng/mL internal standard (IS) working solution was prepared from the stock solution using acetonitrile as the diluent.

Extraction procedure

Ninety-five µL of drug free guinea pig plasma were spiked with 5 µL of working solutions of BUP, BUPOH, and IS to yield final respective concentrations in the range of 0.5-500 ng/mL. QC samples were prepared in a similar manner. All QC samples and standards were diluted with nanopure water to 1 mL, vortexed for 30 s, and isolated from plasma by solid phase extraction (Oasis MCX[®], Waters Corp., Milford, MA). Solid phase extraction cartridges were pretreated with 1 mL of methanol followed by 1 mL of water before the diluted samples were loaded. After running the samples through the cartridges, cartridges were washed twice with 1 mL of 4% formic acid in water followed by 1 mL of acetonitrile. Samples were eluted with 1 mL of 60% methanol with 2% ammonium hydroxide in water, evaporated under nitrogen, and reconstituted with 100 µL of water: acetonitrile (1:1, v/v).

Quantitative analysis

HPLC conditions—The HPLC system consisted of a Waters (Milford, MA) 717 Plus autosampler, 1525 binary pumps, and a 2487 dual wavelength UV absorbance detector with Breeze[™] software. A Brownlee C₁₈ reversed-phase Spheri-5-µm column (220 × 4.6 mm) with a C₁₈ reversed-phase 7-µm guard column (15 × 3.2 mm) was used with the UV/VIS Detector set at a wavelength of 215 nm. The mobile phase consisted of methanol:25 mM phosphate buffer adjusted with TEA to pH 3.0 (82:18, v/v). The flow rate of the mobile phase was 1.5 mL/min with 100 µL sample injections. Retention times were found to be 3.66, 5.77, and 6.96 min for BUPOH, But-BUPOH, and BUP, respectively. Standards were analyzed with each set of diffusion samples. Standard calibration plots showed excellent linearity over the concentration range (50-2000 ng/mL) employed for the assays. All standard calibration plots had a correlation coefficient (*r*²) value of at least 0.99. The assay sensitivity was at least 20 ng/mL or better for all three drugs.

LC-MS conditions—Chromatography was performed on a Waters Symmetry[®] C₁₈ (2.1 mm × 150 mm, 5 μm) column at 35°C with a mobile phase consisting of acetonitrile:10 mM ammonium formate (32:68, v/v) at a flow rate of 0.25 mL/min. A Waters Symmetry[®] C₁₈ (2.1 mm × 10 mm, 3.5 μm) guard column was also used. The LC-MS system consisted of a Waters Alliance 2695 HPLC pump (Waters, Milford, MA), a Waters Alliance 2695 autosampler, and Micromass ZQ detector (Waters, Milford, MA) using electrospray ionization (ESI) for ion production. Selected ion monitoring (SIM) was performed in the positive mode for BUP *m/z* 184 [240>>184], BUPOH *m/z* 238 [256>>238], and WIN 55,212-2 mesylate salt *m/z* 235 (dwell time 0.30s). Capillary voltage was 4.5kV and cone voltage was 30 V. The source block and desolvation temperatures were 120 and 250°C, respectively. Nitrogen was used as nebulization and drying gas at flow rates of 50 and 450 L/h, respectively. The retention times for BUP, BUPOH, and WIN 55, 212-2 were 8.40 ± 0.11, 3.85 ± 0.05, and 6.02 ± 0.09 min, respectively. Calibration graphs were constructed using a linear regression of the ratio of the drug peak area to internal standard peak area vs nominal drug concentrations.

LC-MS method validation—The method was validated for precision, accuracy, calibration plot, and reproducibility over a concentration range of 0.5 – 500 ng/mL using six calibration standards each containing the two analytes of interest and three replicated QC samples at each concentration level in three separate runs. The matrix effect and possibility for ionization suppression or enhancement for both analytes were investigated. This was accomplished by extracting “blank” normal plasma with the solid phase extraction procedure described above, reconstituting extracted sample with water:acetonitrile (1:1, v/v), analyzing, and comparing the peak areas of the analytes with that of analytes in water:acetonitrile (1:1, v/v). Extraction recoveries of the analytes were calculated by comparing the peak area ratios for each compound to that of the corresponding internal standard in samples that had been spiked with both analytes of interest prior to extraction with samples to which both analytes had been added post-extraction. The extraction recoveries of BUP and BUPOH in QC samples were also performed to prove consistency across the complete dynamic range.

Pharmacokinetic analysis

The pharmacokinetic analysis of BUP and BUPOH plasma concentrations vs time profiles after intravenous bolus administration was carried out by fitting the data to a two compartmental model (WinNonlin Professional, version 4.0, Pharsight Corporation, Mountain View, CA) with the following exponential expression:

$$C(t) = Ae^{-\alpha t} + Be^{-\beta t} \quad (1)$$

where C(t) is the drug plasma concentration at time t, A and B are preexponential constants, α and β are the distribution and elimination rate constants, respectively and t is time. Pharmacokinetic parameters, such as terminal elimination half-life, *t*_{1/2(β)}, distribution half-life, *t*_{1/2(α)}, area under the curve from 0 to infinity, AUC_{0-∞}, and total body clearance (CL) were estimated using the WinNonlin software. Peak plasma concentrations (C_{max}) after intravenous administration were used to calculate the initial volume of distribution as given by the following equation:

$$V_d = \frac{\text{dose}}{C_{\max}} \quad (2)$$

where V_d is the initial volume of distribution and C_{max} is the maximum drug plasma concentration.

Transdermal delivery data were analyzed using a non-compartmental model to determine peak concentration (C_{max}), lag time to steady-state concentration (t_{lag}), and area under curve from 0 to 48 h, AUC_{0-48} . The steady state plasma concentrations of BUP and BUPOH after application of patches were calculated by using the equation:

$$C_{ss} = \frac{AUC_{0-t}}{time} \quad (3)$$

The predicted steady-state plasma concentrations of BUP and BUPOH in the guinea pigs following the application of the transdermal patches were calculated from the *in vitro* steady-state flux as given by the equation below:

$$C_{ss} = \frac{J_{ss}A}{CL} \quad (4)$$

where C_{SS} is the predicted steady-state plasma concentration (ng/mL); J_{SS} is the steady-state flux obtained from *in vitro* experiments; A is the area of the applied patch ($\sim 14.5 \text{ cm}^2$); CL is the total body clearance. The statistical significance of the *in vitro* and *in vivo* results was computed with a one-way ANOVA followed by Tukey's post-hoc analysis using SigmaStat (SPSS, Inc., Richmond, CA). Data is represented as mean \pm standard deviation (SD).

RESULTS AND DISCUSSION

One of the prerequisites of any drug development program, including drugs targeted for transdermal delivery, is that each drug must exhibit sufficient stability in a desired medium to be formulated into a dosage form as well as maintain its integrity during storage or under experimental conditions in receiver fluid. Results of the stability studies of BUP, BUPOH, and But-BUPOH in buffer, pH 7.4, are as shown in Table 1. BUP was shown to be unstable and degraded to unknown products which have HPLC retention times different from either BUP or its metabolite, BUPOH. Conversely, there was no significant ($p > 0.05$) degradation observed in both BUPOH and But-BUPOH solutions over a 14 d period.

Figure 2 shows a representative permeation profile used to calculate the steady-state flux values of BUP, BUPOH, and But-BUPOH. But-BUPOH partly hydrolyzed on passing through skin and appeared in the receiver solution as a mixture of BUPOH and intact prodrug, But-BUPOH. Molar equivalents of BUPOH were used to calculate the total transdermal flux of the prodrug. When cumulative BUPOH equivalents were compared after treatment with But-BUPOH, approximately 86% of the total flux was from intact prodrug and 14% was BUPOH originating from the skin hydrolysis of the carbamate prodrug. The mean flux values of the drugs are shown in Table 1. Results from the present study showed that steady-state flux, cumulative amount permeated through skin in 48 h, and skin drug disposition were significantly higher ($p < 0.001$) for BUP compared to BUPOH. The flux was about 12.5-fold greater for BUP compared to BUPOH. The high transdermal flux of BUP through skin could be attributed to its higher solubility in both aqueous and oil media as shown in Table 1. The impact of solubility of any drug on its diffusion across skin is well understood.²⁶⁻²⁹ A drug with high oil solubility partitions well into the lipids of the stratum corneum and this is supported by the high calculated log partition coefficient (clog P) values of BUP as well as the high drug concentration in skin after a 48h diffusion study (Fig. 3). Additionally, BUP diffusion across the aqueous medium of the epidermis and dermis meets

less resistance due to its adequate aqueous solubility. Other than degradation problems, it is clearly evident that the transdermal flux of BUP ($\sim 320 \text{ nmol}\cdot\text{cm}^{-2}\cdot\text{h}^{-1}$) would meet the required minimum therapeutic delivery as calculated from the current available oral dosing. If 300 mg/day of BUP is given with an oral bioavailability of 5-20%, the daily dose range is 63-250 μmol . Therefore, a therapeutic delivery rate of BUP from a 25- cm^2 patch is in the range of 105-416 $\text{nmol}\cdot\text{cm}^{-2}\cdot\text{h}^{-1}$ and would not need further enhancement or optimization. In addition to its lower water and oil solubility (as compared to BUP), permeation of BUPOH was significantly decreased, largely due to its capacity to hydrogen bond with ceramides, a major constituent of the lipids of the stratum corneum. Studies have shown that hydrogen bonding has a retarding effect on the permeability of a drug.^{30,31} Results from these previous studies showed that the presence of one H-bonding group brings about a dramatic reduction in diffusivity (as much as a ten-fold decrease), and the presence of two or three H-bonding groups causes further reductions, although additional groups have no effect on the minimal diffusivity value due to the saturable number of H-bonding groups.³⁰ In an attempt to minimize the retarding BUPOH permeation properties, the But-BUPOH prodrug was designed and its transdermal delivery was compared to BUPOH. Results revealed that the BUPOH flux value was about 2.7-fold greater after treatment with the prodrug, as compared to treatment with BUPOH. However, the transdermal flux of BUPOH after treatment with the prodrug did not reach the high flux values as seen with BUP. The polarity and H-bonding capability of the carbamate group could be the reason. Another reason attributed to the lower flux from the prodrug is the increase in molecular weight of the prodrug as compared to BUP. Nevertheless, with slight optimization of the patch formulation, But-BUPOH would be more chemically stable than BUP and meet the required delivery rate for therapeutic efficacy.

The lag times were significantly longer ($p < 0.05$) for BUPOH as compared to BUP. This seems plausible, since the lag time is determined by the diffusivity, often estimated by the diffusion coefficient, and is indicative of how fast a drug traverses the skin. The presence of the hydroxyl group and consequent H-bonding leads to a decreased diffusivity of BUPOH as compared to BUP. A lower diffusion coefficient means that a drug takes a much longer time crossing the different layers of the skin before finally appearing in the receiver compartment and thus, exhibits a longer lag time. As shown in Table 1, the lag time of BUPOH was decreased on treatment with the prodrug. In all donor skin samples used, no significant ($p > 0.05$) TEWL was observed before and after the completion of the 48h diffusion studies, indicating that no damage to the stratum corneum lipids or proteins had occurred as a result of the drug or vehicle treatments.

The LC-MS assay described here had to be developed for the simultaneous quantification of BUP and its active metabolite, BUPOH. The assay is highly sensitive and specific as compared to the previously reported HPLC methods.^{19,32-35} Zhang et al reported an HPLC method utilizing an octadecylsilane column to quantify BUP in plasma samples, however no attempt was made to quantify BUPOH.³² Another HPLC method developed by Loboz et al to separate BUP and its metabolite, BUPOH reported the LOQ for BUP and BUPOH as 1 ng/mL and 10 ng/mL, respectively.³³ Therefore, monitoring the metabolite with this previously published assay would provide less sensitivity than the assay developed for this study.⁹ Worrall et al reported in their studies that higher levels of BUPOH were associated with poor therapeutic efficacy whilst lower levels achieved the desired response; thus, precise monitoring of the metabolite may be crucial with BUP therapy.³⁶ As such, a more sensitive method was needed to monitor both BUP and BUPOH. Additionally, HPLC analysis as well as reported LC-MS-MS methods required at least 500 μL or more of plasma, and this would not be feasible in small animal models such as guinea pigs, where the volume of an individual plasma sample is low ($< 200 \mu\text{L}$) in serial pharmacokinetic sampling protocols.

Method development began with the selection of HPLC and MS conditions. A mobile phase of 10 mM ammonium formate:acetonitrile (32:68, v/v) at a flow rate of 0.25 mL/min provided desirable chromatographic conditions for separation and ionization of BUP, BUPOH, and IS. ESI was conducted in the positive ion mode since BUP and BUPOH contain a secondary amine group that may be protonated in solution. Major product ions m/z 184 [240 \gg 184] and m/z 238 [256 \gg 238] were observed in BUP and BUPOH, consistent with the loss of the C_4H_9 moiety and H_2O , respectively. SIM for the IS was performed at m/z 235. Both analytes of interest and the IS were well resolved and free of interference from endogenous compounds in plasma. Additional peaks were only observed at 2.3-2.5 min, and these were well separated from the drug peaks. The coefficients of variation (CVs, %) of the mean peak areas of BUP and BUPOH at any given concentration were small (<10%), strongly indicating little or no difference in ionization efficiency. The calibration plots of BUP and BUPOH were linear over the range from 1.25-500 ng/mL with correlation coefficients above 0.99. These results demonstrate accuracy, reproducibility, and good fit to the non-weighted regression lines. The mean (n=3) calibration plots for BUP and BUPOH were $y = 0.0224x + 0.0436$, $R^2 = 0.9969$ and $y = 0.0189x + 0.0633$, $R^2 = 0.9984$, respectively, where y and x are the peak area ratios of analyte to IS and concentration (ng/mL) of analytes, respectively.

The mean recoveries of BUP, BUPOH, and IS carried out in triplicate in the concentration range of 1.25-500 ng/mL were 93.9% (% CV 4.4), 94.5% (% CV 3.4), and 99.3% (% CV 1.1), respectively. Table 2 lists absolute recoveries of BUP and BUPOH. Similar consistencies were observed in extraction recovery values of QC samples, and were between 89.6% and 97.3% for BUP and between 84.2% and 102.3% for BUPOH. The coefficients of variation were small (< 7.0 %) indicating that the extracts were “clean” with no co-eluting compounds and no significant matrix effect on ionization. The lower limits of quantification, LLOQ, defined as the lowest concentrations of BUP and BUPOH which can still be determined with acceptable accuracy within 80-120% and precision of 20% were found to be 1.25 ng/mL and 1 ng/mL for BUP and BUPOH, respectively. Results from intra-day and inter-day validation assays indicated that the accuracy of the assay was > 84.4% and the %CV did not exceed 7.9%.

For the purpose of comparing *in vitro* and *in vivo* transdermal permeation of BUP and BUPOH, one prerequisite was to establish the basic pharmacokinetic parameters of the drugs after intravenous administration. The clearance value after intravenous administration is needed in order to calculate the *in vivo* flux value across the skin from the steady-state plasma concentration value. The described method was used for the determination of pharmacokinetic parameters of BUP and BUPOH following intravenous and transdermal administration of each drug in guinea pigs. Figure 4 and Figure 5 show plasma concentration profiles of observed and predicted concentrations of BUP and BUPOH following intravenous administration of 1 mg/kg of the respective drug. The plasma concentration profiles of BUP and BUPOH followed a two compartmental model. The observed plasma concentrations of both drugs were in good agreement (correlation > 0.99) with the predicted plasma concentrations obtained after intravenous administration. The pharmacokinetic parameters are shown in Table 3. The maximum plasma concentrations of BUP and BUPOH were 315 ± 56 ng/mL and 303 ± 16 ng/mL, respectively. There was no significant difference ($p > 0.05$) between the mean steady-state volumes of distribution of BUP and BUPOH (11.2 ± 3.3 L/kg vs 8.4 ± 4.1 L/kg). Similarly, there was no significant difference ($p > 0.05$) between the systemic clearance and terminal elimination half-lives for BUP and BUPOH, suggesting that they were eliminated at almost the same rate.

The plasma profiles following the application of BUP and BUPOH transdermal patches are shown in Figure 6 and Figure 7, respectively. The pharmacokinetic parameters, including

C_{max} , T_{max} , C_{SS} , AUC_{0-48} , and T_{lag} are given in Table 4. As observed in the *in vitro* experiments, BUP maintained a significantly higher ($p < 0.05$) mean steady-state plasma concentration than BUPOH (442 ± 31 ng/mL vs 125 ± 18 ng/mL), indicating that BUP permeated the skin at a much higher rate than BUPOH. Similarly, BUP had a shorter lag time compared to BUPOH. However, the difference in the mean steady-state plasma concentrations is much less *in vivo* than predicted from *in vitro* data (i.e., four-fold vs ten-fold). This is mainly due to extensive metabolism of BUP into BUPOH *in vivo*. The mean steady-state plasma concentrations were maintained throughout the 48h of patch application, and declined rapidly after the removal of the patches, indicating no substantial skin reservoir effect. *In vivo* studies with the carbamate prodrug could not be carried out because of the insufficient amount of the drug. Further optimization of the prodrug design *in vitro* will be completed prior to prodrug *in vivo* experiments and synthesis of a larger amount of prodrug. The summary of the current work suggested that carbamate prodrugs may be promising drug candidates.

A good *in vitro/in vivo* correlation is always desirable in studies involving any route of drug administration, including transdermal drug delivery. Thus, attempts were therefore made to determine whether there was any correlation between *in vitro* and *in vivo* studies of these drugs. In this study, *in vitro/in vivo* correlations were based on mean steady-state plasma concentrations, considered as one of the important pharmacokinetic parameters in evaluating the potential of a transdermal candidate. In the current study, the predicted steady-state plasma concentrations of BUP and BUPOH were 1000 ng/mL and 169 ng/mL after topical application of BUP and BUPOH, respectively (Table 3). Predicted plasma levels of BUP are significantly ($p < 0.05$) higher than the observed steady-state plasma levels, with the predicted value being slightly more than two-fold the observed values. But taking into consideration the extensive metabolism as indicated by the high levels of the metabolite (Fig. 6), the predicted plasma concentration provides a good estimation of the anticipated steady-state plasma concentrations of drug and metabolite combined. Additionally, taking into consideration experimental errors in pharmacokinetic parameters, such as systemic clearance as well as the permeation parameters, no significant ($p > 0.05$) difference was found between the predicted values and the observed experimental values of BUP and BUPOH.

CONCLUSION

The purpose of this investigation was to evaluate the *in vitro* and *in vivo* percutaneous absorption of BUP and BUPOH. Additionally, *in vitro* permeation studies of a carbamate prodrug of BUPOH, But-BUPOH, were conducted across human skin in an effort to address the potential transdermal drug delivery problems of BUP and BUPOH. Although, *in vitro* and *in vivo* transdermal studies showed that BUP had sufficient flux to meet the required therapeutic plasma levels, it was shown to be chemically unstable. In contrast, BUPOH was stable but its transdermal permeation was significantly limited. The carbamate prodrug of BUPOH, But-BUPOH, was chemically stable and also provided a 2.7-fold increase in the transdermal flux of BUPOH across human skin *in vitro*. A good correlation existed between *in vitro* and *in vivo* BUP and BUPOH results. These results show that optimization of the BUPOH chemical structure for percutaneous permeation enhancement utilizing But-BUPOH provides a viable option for the transdermal delivery of BUPOH.

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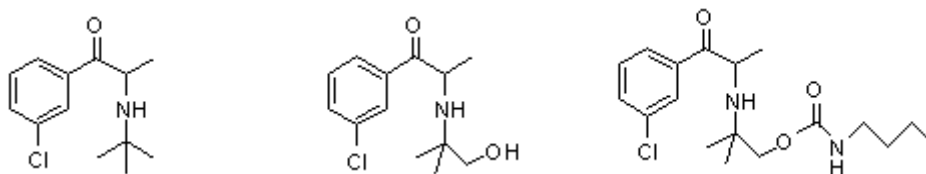


Figure 1.
Chemical structures of BUP, BUPOH, and But-BUPOH, the carbamate prodrug of BUPOH.

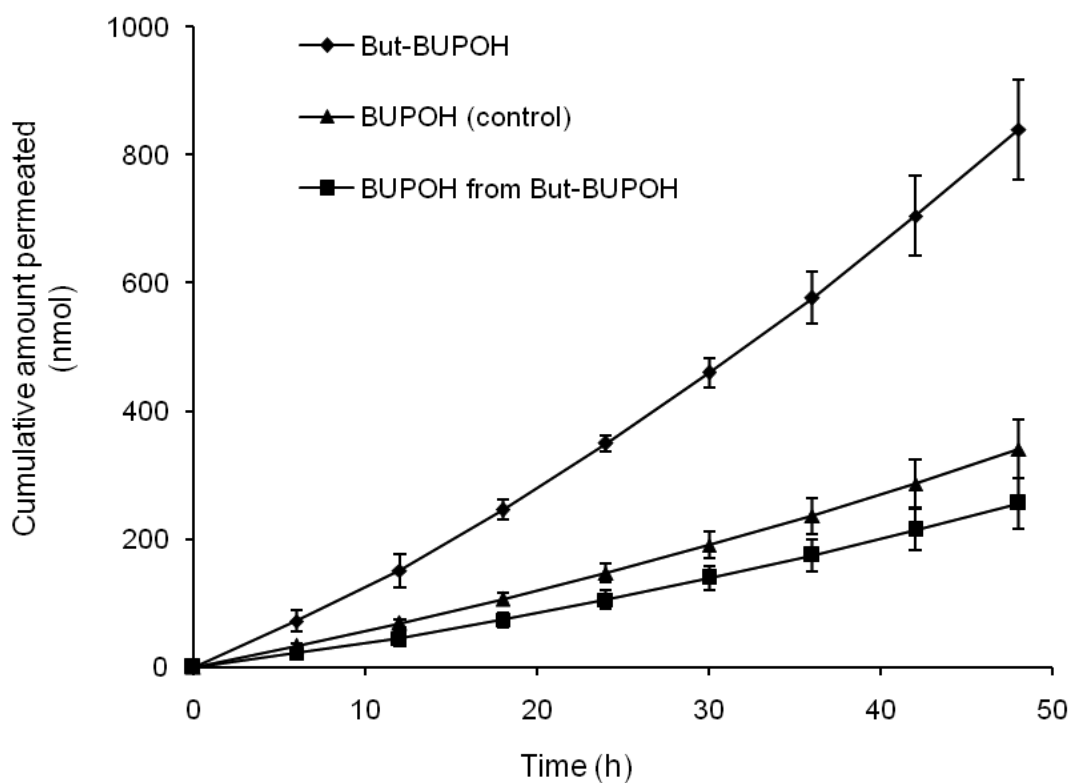


Figure 2. Representative permeation profile from saturated solutions of the carbamate prodrug, But-BUPOH, and BUPOH (control) through human skin in vitro at 32°C. Data is represented as mean \pm SD (n = 4 for But-BUPOH treatment and n = 3 for BUPOH treatment).

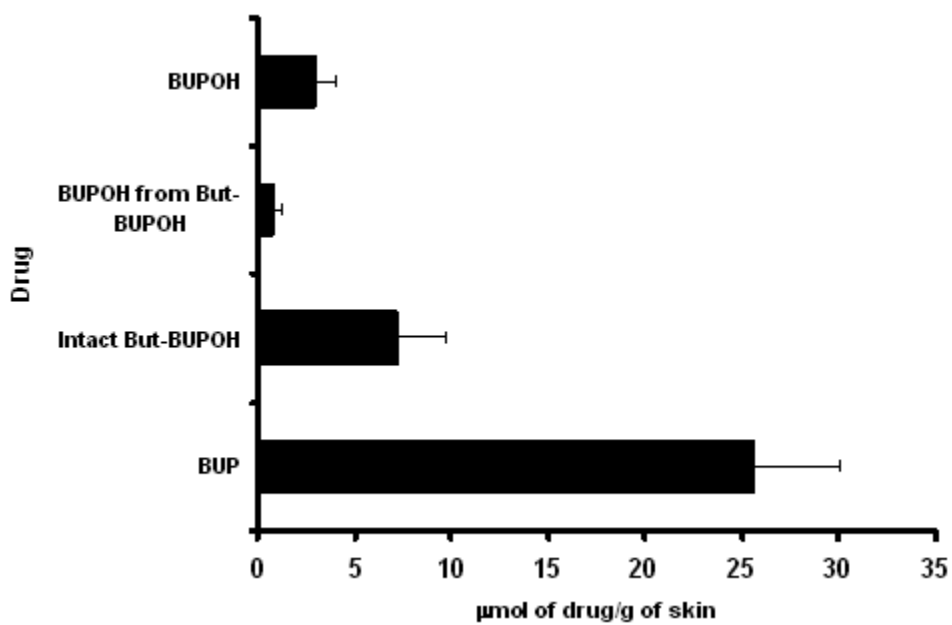


Figure 3. Drug concentrations of BUP, BUPOH, and But-BUPOH in the skin after a 48 h diffusion study following topical delivery of the drugs as saturated solutions. Data is represented as mean \pm SD.

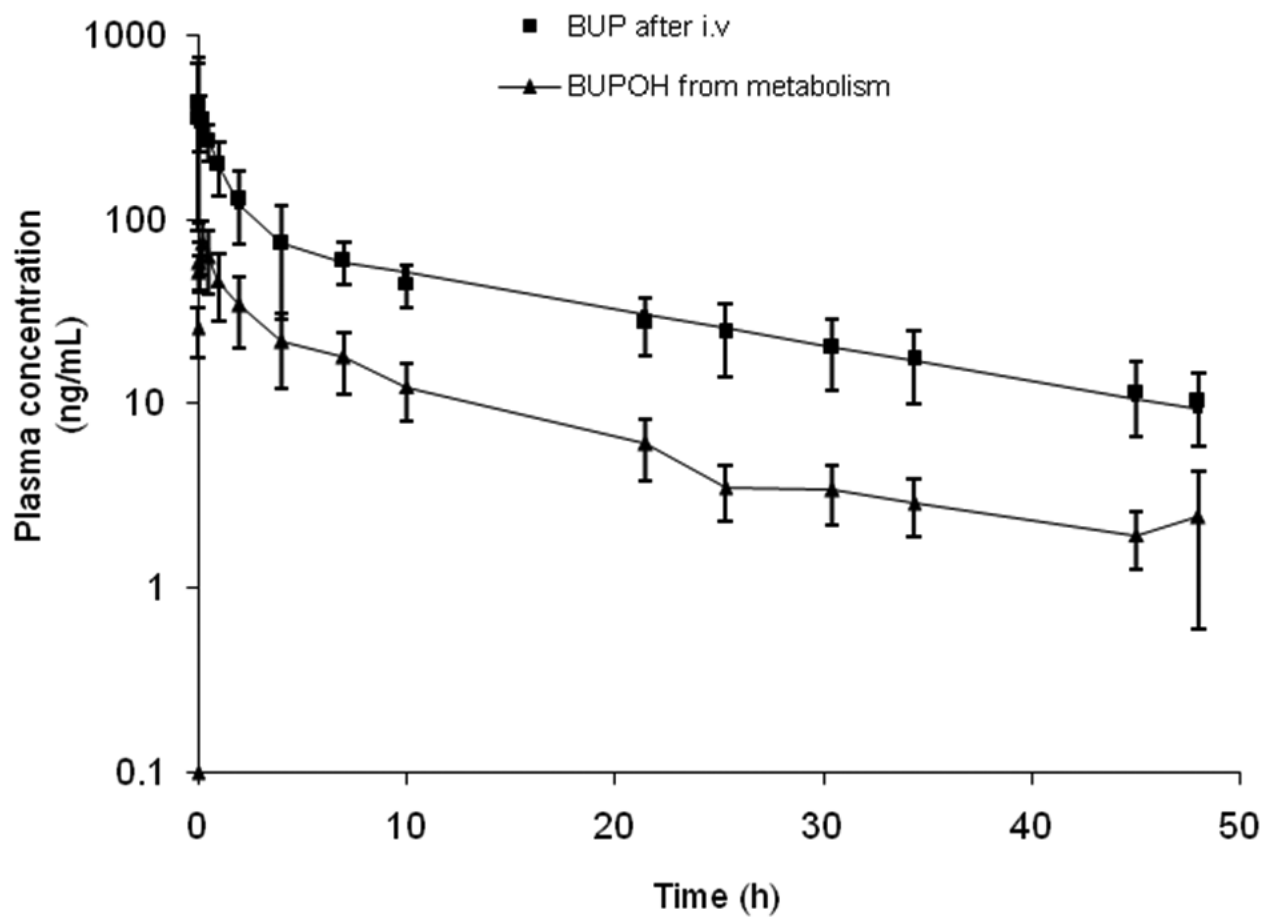


Figure 4. Mean (\pm SD) plasma profiles of BUP and BUPOH after intravenous administration of BUP HCl (1 mg/kg) in guinea pigs (n = 3).

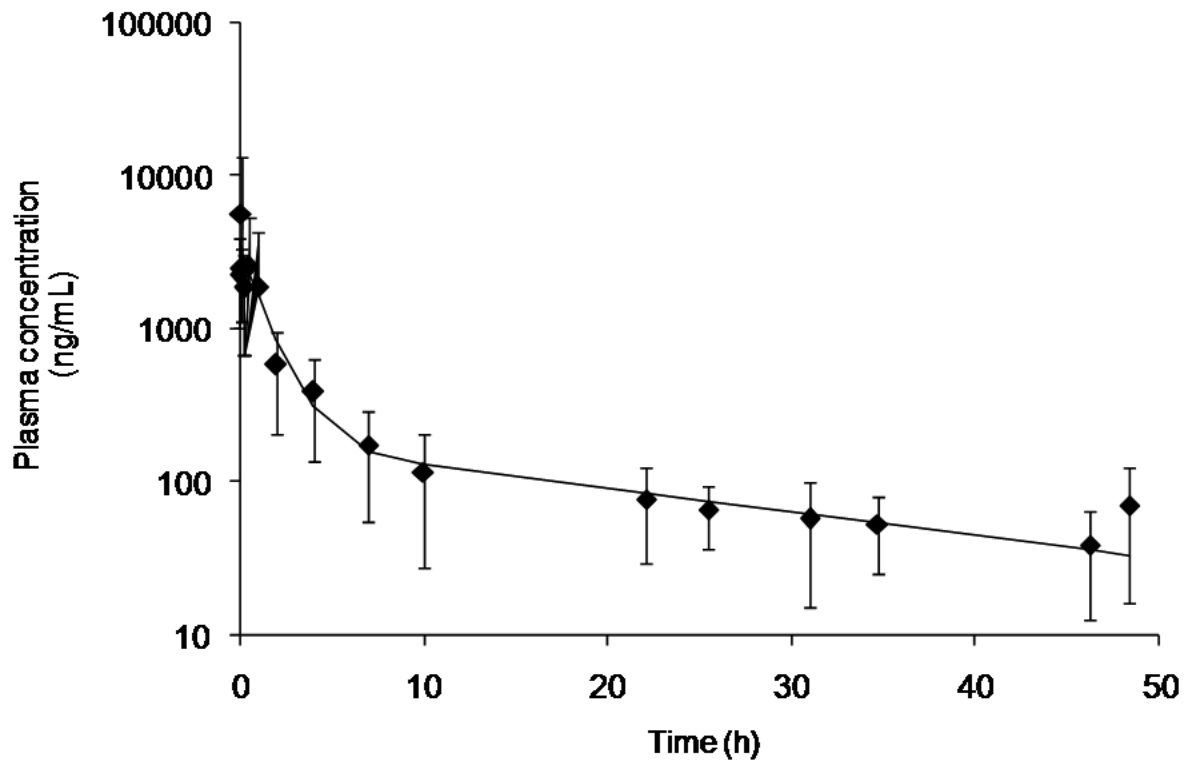


Figure 5. Mean (\pm SD) plasma profiles of BUPOH after intravenous administration of BUPOH (1 mg/kg) in guinea pigs (n = 3).

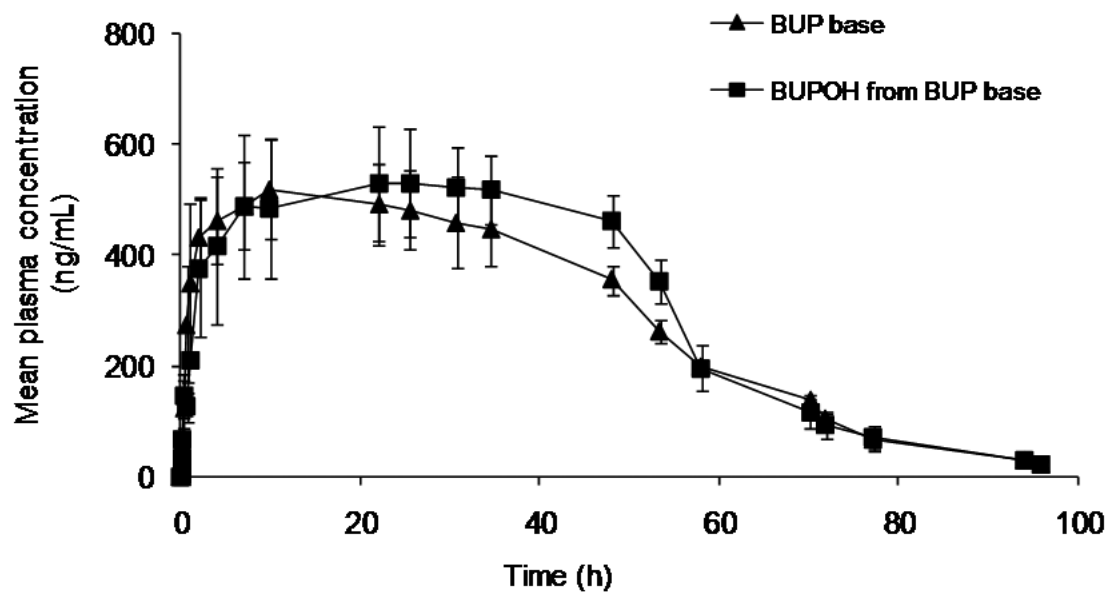


Figure 6. Mean (\pm SD) plasma profiles of BUP and BUPOH after transdermal patch application of BUP free base in guinea pigs ($n = 6$).

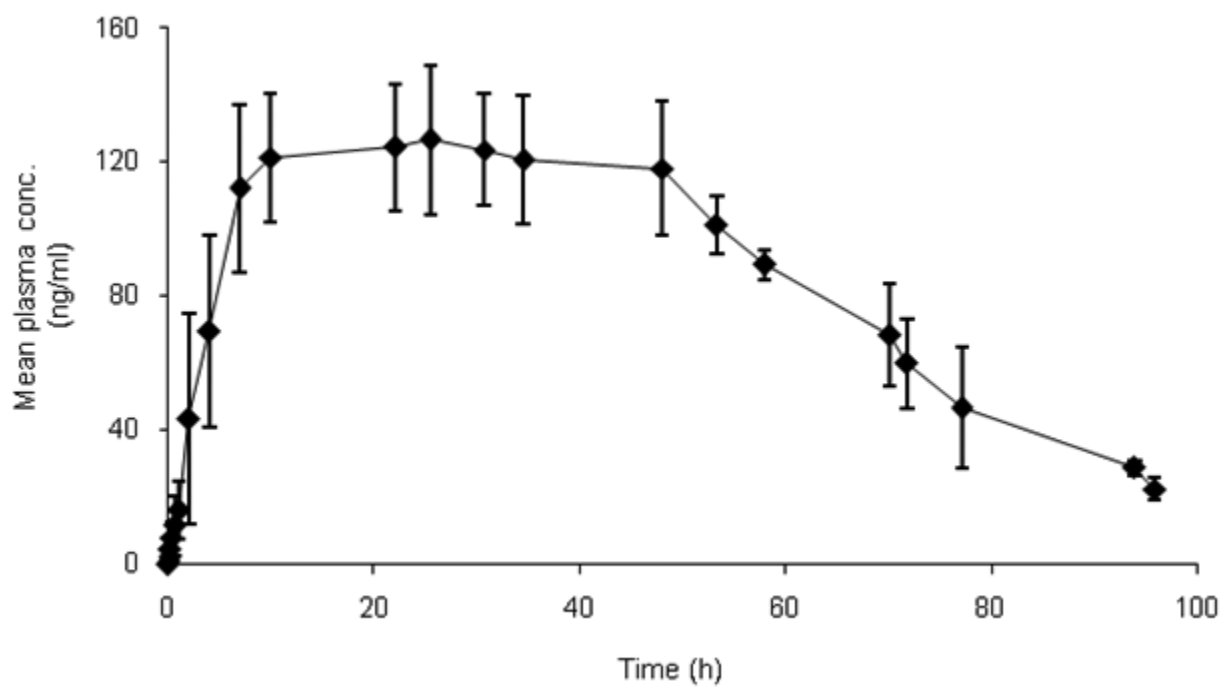


Figure 7. Mean (\pm SD) plasma profiles of BUPOH after transdermal patch application of BUPOH free base (n = 6).

Table 1
Physicochemical properties and permeation parameters of BUP, BUPOH, and But-BUPOH

Drug	Mineral oil solubility (mM)	Aqueous solubility (mM)	α clog P	Half life in buffer, pH 7.4, (days)	Flux from mineral oil (mmol cm ⁻² h ⁻¹)	Lag time (h)	Mean permeability coefficient, (K _p × 10 ³), cm/h
BUP	54.2 ± 3.6	51.1 ± 0.6	3.43	9.9	316.2 ± 15.6	1.3 ± 0.3	5.8 ± 0.1
BUPOH	3.9 ± 0.3	5.2 ± 0.7	2.87	stable	27.1 ± 4.2	14.2 ± 1.5	6.8 ± 0.8
But-BUPOH	16.4 ± 4.9	<i>b</i> ND	<i>b</i> ND	stable	72.5 ± 7.6	8.3 ± 2.4	4.4 ± 1.5

^aDetermined from Daylight® 4.51 software

^bNot determined.

Table 2

Recovery data for QC samples of BUP and BUPOH (n = 3)

BUP		BUPOH			
Concentration (ng/mL)	Recovery (%)	%CV^a	Concentration (ng/mL)	Recovery (%)	a%CV
5	92.4	2.6	5	102.3	3.5
20	97.3	5.2	20	94.2	3.0
50	89.6	4.1	50	84.2	4.8
100	94.4	2.8	100	101.5	3.1
200	94.6	5.1	200	91.4	2.6
400	95.0	6.7	400	93.6	3.6

^a%CV = coefficient of variation

Table 3

Pharmacokinetic parameters of BUP and BUPOH after intravenous (1 mg/kg) doses in guinea pigs (n = 3)

Parameter	BUP	BUPOH
	Mean \pm S.D	Mean \pm S.D
C _{max} (ng/mL)	315 \pm 56	303 \pm 16
AUC (ng/mL)h	2343 \pm 623	5222 \pm 1342
AUMC (ng/mL)h ²	58,229 \pm 1760	209,164 \pm 4076
CL (L/h)	1.1 \pm 0.4	0.6 \pm 0.2
V _{ss} (L/kg)	11.2 \pm 3.3	8.4 \pm 4.1
α (1/h)	0.40 \pm 0.10	0.45 \pm 0.03
β (1/h)	0.03 \pm 0.01	0.15 \pm 0.10
t _{1/2(α)}	1.9 \pm 1.7	2.2 \pm 0.2
t _{1/2(β)}	26.6 \pm 3.4	29.1 \pm 6.1
Initial V _d	3.2 \pm 0.5	3.3 \pm 0.2
MRT (h)	24.9 \pm 4.6	41.3 \pm 9.8

Table 4

Pharmacokinetic parameters of BUP and BUPOH after transdermal patch application in guinea pigs (n = 6)

Parameter	BUP	BUPOH
AUC ₀₋₄₈ (ng/mL*h)	21,773 ± 7286	5419 ± 904
C _{max} (ng/mL)	531 ± 196	127 ± 22
T _{max} (h)	20.3 ± 9.9	27.2 ± 3.0
Observed C _{SS} (ng/mL)	442 ± 31	125 ± 18
Predicted C _{SS} (ng/mL)	1000	169
T _{lag} (h)	3.5 ± 3.5	17.0 ± 8.7