Pharmaceutical Research, Vol. 22, No. 5, May 2005 (© 2005)
DOI: 10.1007/s11095-005-2592-9

Research Paper

Human Skin Permeation of Branched-Chain 3-*O*-Alkyl Ester and Carbonate Prodrugs of Naltrexone

Haranath K. Vaddi,[1,2] Mohamed O. Hamad,[1] Jianhong Chen,[1] Stan L. Banks,[1] Peter A. Crooks,[1] and Audra L. Stinchcomb[1,3]

Received November 18, 2004; accepted February 2, 2005

Purpose. Physicochemical characterization and *in vitro* human skin diffusion studies of branched-chain ester and carbonate prodrugs of naltrexone (NTX) were compared and contrasted with straight-chain ester and carbonate NTX prodrugs.
Methods. Human skin permeation rates, thermal parameters, solubilities in mineral oil and buffer, and stabilities in buffer and plasma were determined. Partition coefficients between stratum corneum and vehicle were determined for straight- and branched-chain esters with the same number of carbon atoms.
Results. Branched prodrugs had lower melting points, lower buffer solubilities, and higher mineral oil solubilities than NTX. The transdermal flux values from all of these branched prodrugs were significantly lower than flux values from the straight-chain ester and the methyl carbonate prodrugs. Straight-chain prodrugs had higher partition coefficient values and higher calculated thermodynamic activities than their branched-chain counterparts. The prodrug hydrolysis to NTX in buffer and plasma was slower for prodrugs with increased branching.
Conclusions. Branched-chain prodrugs with bulky moieties had smaller stratum corneum–vehicle partition coefficients and lower thermodynamic activities that resulted in smaller transdermal flux values than straight-chain prodrugs.

KEY WORDS: bioconversion; branched chains; naltrexone; prodrugs; skin permeation.

INTRODUCTION

Prodrugs are chemically modified therapeutic agents that will convert to active drug molecules in biological systems (1). This prodrug strategy can provide various drug delivery advantages, including improved therapeutic efficacy, increased bioavailability, decreased toxicity, decreased gastric irritation, improved organoleptic properties, and others (2–4). Because the human body has an abundance of esterase enzymes, prodrugs with esterase-susceptible linkages can be cleaved by these enzymes to release the parent molecule in tissues and plasma (1). Different prodrug moieties can be attached to the parent drug through various linkages, including ester, carbonate, and carbamate moieties. These linkages are then cleaved at a variety of rates in the body, depending on the chemistry of the molecule (1,2,5). An ideal prodrug should have sufficient chemical stability but readily hydrolyze in a biologic system to release the parent drug for therapeutic effect.

Naltrexone (NTX), an opioid antagonist, is used for alcohol dependence and opioid addiction (6,7). In maintenance therapy for alcohol abstinence, the NTX dose must be given regularly to maintain a minimum effective plasma drug concentration (8,9). Currently, NTX hydrochloride is available as a 50-mg oral tablet (ReVia) in the United States. Standard drug regimens for chronic therapy create noncompliance problems and require high motivation by NTX patients (8,9). Therefore, sustained-release formulations could be extremely useful in maintenance therapy and could result in the improvement of the therapeutic effectiveness of NTX. Additionally, a nonoral delivery route for NTX could reduce oral-NTX associated gastrointestinal side effects, including nausea, abdominal pain, and vomiting (10). NTX bioavailability would also be increased via a delivery route that bypasses first-pass metabolism, as the drug undergoes extensive first-pass metabolism that results in a low oral bioavailability of 5–40% (11).

Drug delivery across the skin to the systemic circulation offers a number of advantages, including controlled drug release rates, avoidance of the first-pass effect, and preclusion of gastrointestinal side effects. Transdermal delivery could be very useful in the hepato-compromised alcoholic and opioid addict patient population, as drug doses may be reduced and the first-pass effect is circumvented. Although transdermal delivery of NTX appears to be clinically justified, NTX does not have suitable physicochemical properties for therapeutic skin absorption rates (12). Penetration enhancers have been used to enhance the permeation of NTX (12), but these chemical enhancers manipulate the skin structure and can cause significant irritation. Prodrugs, with modified physicochemical properties from that of the parent drug, provide

[1] Department of Pharmaceutical Sciences, University of Kentucky College of Pharmacy, Lexington, Kentucky, USA.
[2] Current address: Irma L. Rangel College of Pharmacy, Texas A&M University, Kingsville, Texas, USA.
[3] To whom correspondence should be addressed. (e-mail: astin2@email.uky.edu)

higher skin permeation rates and simultaneously convert into the parent drug. Skin toxicity profiles for topical prodrugs are similar to that of the parent drug when prompt biotransformation occurs. NTX prodrugs have previously been synthesized to increase the oral bioavailability of NTX (3) and to decrease the bitter taste for buccal delivery (4). Prodrugs of NTX can be synthesized by linking the 3-phenolic hydroxyl group of the molecule to various moieties. Previously, straight-chain ester and carbonate prodrugs of NTX were synthesized and evaluated for human skin permeation, and correlations were established for these NTX permeation rates with the extent of prodrug bioconversion and the physicochemical parameters (13,14). Short-chain ester and carbonate prodrugs of NTX exhibited higher skin permeation. In the current study, branched-chain ester and carbonate NTX prodrugs, pivalyl, isovaleryl, 2′-ethylbutyryl, and isobutyryl esters, and isopropyl and tertiarybutyl carbonates were synthesized, and the *in vitro* human skin permeation was compared with that of the straight-chain prodrugs. Prodrug physicochemical properties and plasma half-lives were determined for potential permeation prediction relationships and as crucial parameters in future prodrug optimization for clinical application.

MATERIALS AND METHODS

Materials

NTX base was purchased from Mallinckrodt Inc. (St. Louis, MO, USA). All prodrugs were synthesized from free NTX base. Straight-chain ester prodrugs, 3-*O*-butyryl NTX, 3-*O*-valeryl NTX, 3-*O*-hexanoyl NTX, and 3-*O*-propyloxycarbonyl NTX, were synthesized by previously published methods (13,14). Hanks' balanced salts modified powder, sodium bicarbonate, and light mineral oil were purchased from Sigma Chemical (St. Louis, MO, USA). 4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), gentamicin sulfate, trifluroacetic acid (TFA), triethylamine (TEA), methanol, and acetonitrile (ACN) were obtained from Fisher Scientific (Fairlawn, NJ, USA). 1-Octane sulfonic acid sodium salt was obtained from ChromTech (Apple Valley, MN, USA).

Synthetic Procedure for Branched-Chain Naltrexone Prodrugs

3-O-Pivalyl Naltrexone

A mixture of naltrexone (1.80 g, 5.28 mmol) and TEA (1.90 ml, 13.3 mmol) in 20.0 ml of dry methylene chloride was cooled to 0°C in an ice-bath. To this stirred mixture was added 0.7ml of the pivaloyl chloride (0.7 ml of 99%, 5.63 mmol). The resulting reaction mixture was stirred at room temperature for 15 h and was diluted in methylene chloride (50 ml) and washed with 10% aqueous sodium carbonate (2 × 30 ml) then water (30 ml). The organic solution was separated, dried over anhydrous potassium carbonate, filtered, and reduced to a small volume under reduced pressure. Petroleum ether was added, and the solution stored in the refrigerator (4°C) overnight. The resulting solid that crystallized out was filtered at the pump, washed with chilled pentane, and air-dried to afford a white powder. ^{1}H NMR ($CDCl_3$): δ 0.15 (2H, m), 0.56 (2H, m), 0.88 (1H, m), 1.38 (9H, s), 1.55–1.70 (2H, m), 1.88 (1H, m), 2.15 (1H, m), 2.30 (1H, m), 2.34–2.50 (3H, m), 2.54–2.76 (2H, m), 2.92–3.16 (2H, m), 3.21 (1H, d, J = 5.7 Hz), 4.67 (1H, s), 5.22 (1H, br s), 6.68 (1H, d, J = 8.1 Hz), 6.92 (1H, d, J = 8.1 Hz) ppm. MS (LC-MS electrospray), $M^+ = 426$ *m*/*z*.

3-O-Isovaleryl Naltrexone

Naltrexone (708 mg, 2.08 mmol) was added to isovaleryl chloride (500 mg, 4.15 mmol) and triethylamine (425 mg, 4.2 mmol) in dichloromethane (100 ml). After stirring at ambient temperature overnight, the reaction mixture was diluted with chloroform (100 ml), washed with brine, dried over potassium carbonate, filtered, and the solvent removed on a rotary evaporator to afford the crude product as a brown oil. Purification by silica gel column chromatography (chloroform/methanol, 10/1) afforded 707 mg (yield 80%) of the title compound as a white solid. An analytical sample was obtained by recrystallization from hexanes/ethyl alcohol. ^{1}H NMR ($CDCl_3$) δ 0.10–0.20 (2H, m), 0.50–0.60 (2H, m), 0.85–0.92 (1H, m), 1.05–1.15(6H, m, two CH_3), 1.50–1.65 (2H, m), 1.80–1.95 (1H, m), 2.08–2.18 (1H, m), 2.20–2.33 (2H, m, CH_2CO_2), 2.35–2.42 (1H, m, $(CH_3)_2CH$), 2.45–2.49 (4H, m), 2.55–2.75 (2H, m), 2.95–3.25 (3H, m), 4.68 (1H, s), 5.22 (1H, brs, OH), 6.65 (1H, m), 6.82 (1H, m). HRMS: calculated for $C_{25}H_{31}NO_5$ 425.2203, found 425.2208.

3-O-(2′-Ethylbutyryl) Naltrexone

A stirred mixture of naltrexone (2.00 g, 5.88 mmol) and TEA (1.90 ml, 13.3 mmol) in 20.0 ml of dry methylene chloride was cooled to 0°C in an ice-bath, and 2-ethylbutyryl chloride (0.90 ml of 97%, 6.37 mmol) was added. The reaction mixture was stirred at room temperature for a further 15 h. The resulting reaction mixture was diluted in methylene chloride (50 ml) and washed with 10% aqueous sodium carbonate (2 × 30 ml), then water (30 ml). Then the organic solution was separated and dried over anhydrous potassium carbonate, filtered, and reduced to a small volume under reduced pressure. Petroleum ether was added, and the solution stored in the refrigerator (4°C) overnight. The resulting solid that crystallized out was filtered at the pump, recrystallized from petroleum ether, and air-dried to afford a white powder. ^{1}H NMR ($CDCl_3$): δ 0.15 (2H, m), 0.57 (2H, m), 0.87 (1H, m), 0.9–1.10 (6H, m), 1.50–195 (7H, m), 2.15 (m, 1H), 2.28 (1H, m), 2.34–2.55 (4H, m), 2.56–2.76 (2H, m), 2.92–3.14 (2H, m), 3.21 (1H, d, J = 5.4 Hz), 4.67 (1H, s), 5.20 (1H, br s), 6.66 (1H, d, J = 8.4 Hz), 6.82 (1H, d, J = 8.4 Hz) ppm. MS (LC-MS electrospray), $M^+ = 440$ *m*/*z*.

3-O-Isobutyryl Naltrexone

Naltrexone (533 mg, 1.56 mmol) was added to isobutyryl chloride (332 mg, 3.12 mmol) and triethylamine (316 mg, 3.12 mmol) in dichloromethane (100 ml). After stirring at ambient temperature overnight, the reaction mixture was diluted with chloroform (100 ml), washed with brine, dried over potassium carbonate, filtered, and the solvent removed on a rotary evaporator to afford the crude product as a brown oil. Purification by silica gel column chromatography (chloroform/methanol, 10/1) afforded 545 mg (yield 85%) of the title compound as a white solid. An analytical sample was obtained by

recrystallization from hexanes/ethyl alcohol. ^{1}H NMR ($CDCl_3$) δ 0.08–0.18 (2H, m), 0.48–0.58 (2H, m), 0.8–0.92 (1H, m), 1.15–1.40(7H, m, $(CH_3)_2CH$), 1.50–1.70 (2H, m), 1.80–1.95 (1H, m), 2.05–2.20 (1H, m), 2.25–2.45 (4H, m), 2.55–2.75 (2H, m), 2.80–2.90 (1H, m), 2.95–3.25 (2H, m), 4.70 (1H, s), 5.20 (1H, brs, OH), 6.65 (1H, m), 6.80 (1H, m). HRMS: calculated for $C_{24}H_{29}NO_5$ 411.2040, found 411.2041.

3-O-Isopropyloxycarbonyl Naltrexone

A stirred mixture of naltrexone (300 mg, 0.88 mmol) and TEA (500 μl, 3.5 mmol) in 4.0 ml of dry methylene chloride was cooled to 0°C in an ice-bath, and 1.0 ml of a 1 M chloroformate in toluene (1.0 mmol) was added. The resulting reaction mixture was stirred at room temperature for 3 h and was diluted in methylene chloride (30 ml) and washed with 10% aqueous sodium carbonate (2 × 30 ml) then water (30 ml). The organic solution was separated and dried over anhydrous potassium carbonate, filtered, and reduced to a small volume under reduced pressure. The desired product was precipitated by trituration with excess pentane. The resulting solid was filtered at the pump, washed with pentane, and air-dried to afford a white powder. ^{1}H NMR ($CDCl_3$): δ 0.16 (2H, m), 0.57 (2H, m), 0.88 (1H, m), 1.38 (6H, d, J = 6.3 Hz), 1.55–1.70 (2H, m), 1.89 (1H, m), 2.14 (1H, m), 2.33 (1H, m), 2.38–2.50 (3H, m), 2.56–2.76 (2H, m), 2.95–3.15 (2H, m), 3.21(1H, d, J = 6), 4.72 (1H, s), 4.97 1H, (seven line multiplet, J = 6.3 Hz), 5.22 (1H, br s), 6.68 (1H, d, J = 8.1Hz), 6.92 (1H, d, J = 8.1 Hz) ppm. MS (LC-MS electrospray), M^+ = 428 *m*/*z*.

3-O-Tertiarybutyloxycarbonyl Naltrexone

Triphosgene (4.02 g, 13.42 mmol) was added to *t*-butanol (500 mg, 6.71 mmol) and triethylamine (5.43 g, 53.68 mmol) in dichloromethane (100 ml). The reaction mixture was stirred while cooling with dry ice in acetone. After 2 h, naltrexone (1.15 g, 3.36 mmol) was added, and the stirred reaction mixture was allowed to warm to ambient temperature overnight. The reaction mixture was diluted by chloroform (150 ml), the organic phase was washed with brine, dried over potassium carbonate, filtered, and the solvent removed on a rotary evaporator to afford the crude product as a brown oil. Purification by silica gel column chromatography (chloroform/methanol, 10/1) gave 770 mg (yield 52%) of the title compound as a white solid. ^{1}H NMR ($CDCl_3$) δ 0.10–0.18 (2H, m), 0.50–0.60 (2H, m), 0.8–0.95 (1H, m), 1.15–1.35 (6H, m, two CH_3), 1.55–1.70 (2H, m), 1.82–1.92 (1H, m), 2.10–2.20 (1H, m), 2.25–2.45 (4H, m), 2.55–2.72 (2H, m), 2.95–3.15 (2H, m), 3.15–3.22 (1H, m), 3.30–3.52 (3H, m, CH_3), 4.68 (1H, s), 5.15 (1H, brs, OH), 6.62 (1H, m), 6.82 (1H, m). HRMS: calculated for $C_{25}H_{31}NO_6$ 441.2151, found 441.2316.

Quantitative Analysis

A modified high-pressure liquid chromatography (HPLC) assay from Hussain *et al.* (3) was used for the analysis of NTX and its prodrugs. The HPLC system consisted of a Waters 717 plus autosampler, a Waters 1525 Binary HPLC pump and a Waters 2487 Dual λ Absorbance detector with Waters Breeze software (Waters Corp., Milford, MA, USA). A Brownlee C-18 reversed-phase Spheri-5 μm column (220 × 4.6 mm) with a C-18 reversed phase 7 μm guard column (15 × 3.2 mm) was used with the UV detector set at a wavelength of 215 nm. The mobile phase was 70:30 ACN:0.1% TFA with 0.065% 1-octane sulfonic acid, sodium salt adjusted to pH 3.0 with triethylamine. The flow rate of the mobile phase was 1.5 ml/min and 100 μl of sample was injected onto the column. Diffusion samples were analyzed with a set of standard samples and exhibited excellent linearity over the entire concentration range used in the assays.

The drugs were extracted from the buffer samples by solid phase extraction (30 mg 1cc Oasis HLB, Waters Corp.). Before extracting the aqueous drug samples (5 ml), the cartridge was conditioned with 1 ml of methanol and 1 ml of nanopure water. After sample loading, the cartridge was washed with 1 ml of 5% methanol and the drug was eluted with ACN and analyzed by HPLC.

Differential Scanning Calorimetry of NTX and Prodrugs

Differential scanning calorimetry (DSC) was carried out for NTX and its prodrugs. Heats of fusion and melting points were determined with a TA Instruments 2920 DSC (New Castle, DE, USA). An accurately weighed sample of drug (2–5 mg) was sealed into the aluminum pans and thermograms were recorded at 10°C/min from ambient to 350°C. Measurements were repeated once for a total of two scans on each drug.

Solubility

The solubilities of NTX and its prodrugs in free base form were determined by adding an excess quantity of drug to mineral oil or pH 7.4 HEPES-buffered Hanks' balanced salts solution at 32°C. The drug slurries were equilibrated with shaking for 8 h. The less chemically stable isobutyryl ester buffer solubility was determined after equilibrating excess solid in pre-warmed Hanks' buffer at 32°C for 10 min. The solubilities of NTX and 3-*O*-isopropyloxycarbonyl NTX were also determined under these instantaneous conditions, and it was concluded that the instantaneous solubility values were the same as that of the 8-h equilibration solubilities. After equilibration, samples were withdrawn in a prewarmed glass syringe, filtered through a syringe filter discarding the first 30% of the initial filtrate (Mineral oil: Millex FG-13 filter, Millipore and buffer: nylon filter, Gelman), and measured with respect to volume and extracted with ACN (mineral oil samples) or diluted with Hanks' buffer. The drug from the buffer samples was immediately extracted by solid phase extraction into ACN. All the samples were analyzed by HPLC. The sampling procedure was done in triplicate.

***In Vitro* Skin Diffusion Studies**

Human skin harvested during abdominal reduction surgery was used for the diffusion studies. Skin sections were obtained by using a Padgett dermatome set to 250 μm; these sections were stored at –20°C. Stored skin samples were thawed to room temperature at the time of the experiment. A PermeGear flow-through (In-Line, Riegelsville, PA, USA) diffusion cell system was used for the skin permeation studies. Diffusion cells were kept at 32°C with a circulating water bath. Data was collected by using human skin from a single donor with three cells of NTX and four cells of the prodrug. Because of normal human skin intersubject permeation variability, the prodrug permeation was compared against NTX

permeation within each individual skin sample. The receiver solution was HEPES-buffered Hanks' balanced salts with gentamicin at pH 7.4 set to a flow rate of 1.1 ml/h. An excess quantity (above the saturation solubility) of NTX or prodrug was added to light mineral oil, sonicated for 10 min, and then applied to the skin. An excess quantity of the drug was kept in the donor compartment throughout the diffusion experiment to ensure saturation of the drug in mineral oil 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 in six-hour increments for 48 h. All the samples were stored at 4°C until processed by solid phase extraction. The human tissue use was approved by the University of Kentucky Institutional Review Board.

Cumulative quantity 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 the 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 NTX or prodrug 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.

Chemical Stability of Prodrugs in Buffer

The chemical stability of the prodrugs in Hanks' buffer (pH 7.4) was studied by using subsaturated concentrations of the prodrug. Samples (6 ml) were distributed into sealed vials and placed at 32°C. One vial was removed immediately after starting the experiment and at predetermined intervals for 14 days and stored at –70°C until analysis. Drug was extracted from the buffer using solid phase extraction and the samples were analyzed by HPLC. Percent drug remaining (on a logarithmic scale) was plotted against time to calculate pseudo-first order rate constants and half-lives of the prodrugs.

Plasma Hydrolysis of Prodrugs

Human plasma, which was stored at –20°C, was thawed to room temperature. Known and subsaturated amounts of the prodrug were added to the human plasma, vortexed, and incubated at 37°C. Samples were withdrawn immediately after starting the experiment and at regular intervals for 48 h. A 5-fold volume of ACN was added to each sample to precipitate protein. Each sample was then vortexed and centrifuged at 10,000 × g for 15 min. Supernatant was separated and evaporated under nitrogen at room temperature and the residue was reconstituted with ACN for HPLC analysis. A standard curve was prepared from the drug-spiked plasma samples processed by the same method. Percent drug remaining (on a logarithmic scale) was plotted against time, to calculate pseudo-first order rate constants and half-lives of the prodrugs.

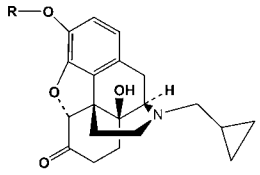
Stratum Corneum/Vehicle Partition Coefficient Studies

Human epidermis with the stratum corneum (SC) side facing up was incubated on filter paper soaked with 0.1% trypsin in 0.5% sodium bicarbonate solution at 37°C for 3 h (15). The SC membrane was separated and dried in a vacuum desiccator. After 24 h, the SC was dipped in acetone for 20 s to remove sebaceous lipids and dried again.

Approximately 5 mg of SC was equilibrated with subsaturated drug solutions in 0.5 g of mineral oil at 32°C for 48 h. An aliquot of the mineral oil solution (10 μl) was withdrawn at the end of the study and was diluted to 1000 μl with acetonitrile. The samples were then analyzed by HPLC. The amount of the drug partitioned into the SC was measured by subtracting the amount present in the mineral oil after equilibration from the initial drug concentration in the mineral oil. The partition coefficient value was expressed as the concentration of prodrug in 1 g of SC to the concentration of prodrug in 1 g of mineral oil.

RESULTS AND DISCUSSION

The branched prodrugs were designed, synthesized, and tested in order to determine physicochemical and permeation differences from straight-chain prodrugs, differences from two prodrug linkages (ester vs. carbonate ester), and differences among this series with varying branching in the prodrug moiety. All prodrugs studied here, except 2′-ethylbutyryl, had single carbons comprising the branched chains. The pivalyl ester and tertiary-butyl carbonate ester (3-*O*-tertiarybutyloxycarbonyl NTX) are structurally identical prodrug moieties, except for the difference in the type of prodrug linkage (Fig. 1). Likewise, the isobutyryl ester and the isopropyl carbonate (3-*O*-isopropyloxycarbonyl NTX) differed only by the type of prodrug linkage to NTX (Fig. 1). Therefore, these two sets of prodrugs provided the opportunity for comparison of just the prodrug linkage effect on physicochemical properties and transdermal flux. The melting points of all the prodrugs were lower than that of NTX, suggesting that the addition of the prodrug moieties to NTX significantly decreased the intermolecular cohesion of the resulting prodrug



DRUG	R-GROUP	MW	MP (°C)
NTX	-H	341	174-176
PIV-ESTER	$-COC(CH_3)_3$	425	158-160
ISOVAL-ESTER	$-COCH_2CH(CH_3)_2$	425	142-145
ETBUT-ESTER	$-COCH(CH_2CH_3)_2$	439	64-67
ISOBUTYRYL-ESTER	$-COCH(CH_3)_2$	411	162-163
ISOPROP- CARBONATE	$-COOCH(CH_3)_2$	427	152-156*
TERT. BUTYL-CARBONATE	$-COOC(CH_3)_3$	441	146-147

* From ref. 14

Fig. 1. Chemical structures, molecular weights, and melting points of NTX and its prodrugs.

Table I. Properties of NTX and its Branched Prodrugs

Drug	Light mineral oil solubility (mM)	Hanks buffer solubility (mM)	Flux from mineral oil (nmol cm^{-2} h^{-1})	Mean permeability coefficient ($K_p \times 10^4$), cm/h	Plasma half-life (h)	Half life in Hanks buffer (h)
NTX	0.24 ± 0.02	5.70 ± 0.60	3.06 ± 1.58	127.5	—	—
PIV-Ester	3.22 ± 0.01	0.12 ± 0.01	1.28 ± 0.49	4.0	9.37	343
Isoval-Ester	1.31 ± 0.07	0.14 ± 0.01	0.85 ± 0.28	6.5	0.54	305
Etbut-ester	5.60 ± 0.25	0.27 ± 0.02	1.97 ± 0.56	3.5	44.70	1136
Isobutyryl-ester	1.17 ± 0.02	0.32 ± 0.00	2.24 ± 0.80	19.1	0.41	54
Isoprop-carbonate	0.87 ± 0.10[a]	0.26 ± 0.02[a]	1.20 ± 0.35[a]	14.0[a]	0.65	315
Tert-Butyl-carbonate	1.57 ± 0.04	1.64 ± 0.00	3.73 ± 0.67	23.8	ST	ST

Flux values are mean of three measurements for NTX and four values for prodrugs with standard deviations.
ST, stable, no significant degradation observed over duration of experiment.
[a] From Ref. 14.

compared to NTX (Fig. 1). The 2′-ethylbutyryl prodrug exhibited the lowest melting point in the series, which corresponded with it having the prodrug moiety with the most carbons in the series. The lower melting points of the prodrugs are consistent with those observed for straight chain ester and carbonate prodrugs of NTX (13,14). Pivalyl and isobutyryl esters exhibited higher melting points than their corresponding carbonate counterparts, indicating that carbonate prodrugs have lower intermolecular cohesion than the ester prodrugs. All the prodrugs had higher solubility than NTX in mineral oil, with the 2′-ethylbutyryl ester having the highest mineral oil solubility, which corresponded with its low melting point (Table I). As expected, the solubilities of the prodrugs were lower than NTX in buffer. The phenolic group of the NTX molecule is important for aqueous solubility through hydrogen bonding, and esterification at this group obviously decreases aqueous solubility.

Figure 2 shows a representative permeation profile used to determine the steady-state flux of the prodrugs and NTX bioconverted from the prodrugs. The NTX flux from the control treatments and the prodrug treatments in each individual skin sample are shown in Fig. 3. The NTX equivalent flux obtained from each of the branched prodrugs was not significantly higher than the NTX control flux (Table I). The 3-*O*-tertiarybutyloxycarbonyl NTX prodrug provided an NTX flux most comparable to that of the NTX control (Fig. 4). This lack of a NTX equivalent flux increase from that of the branched prodrugs did not correlate with the straight-chain ester and methyl carbonate prodrugs, which exhibited significantly higher fluxes than NTX (13,14). The stratum corneum (SC), the uppermost layer of the skin, is the major barrier to drug permeation for most compounds. Lipids in the SC are arranged in bilayers, and lipophilic drugs are presumed to permeate through these lipid bilayers. To investigate the mechanism of the lower permeabilities of the branched prodrugs in the SC, partition coefficient experiments between human SC and the vehicle (mineral oil) were completed for some isomeric straight- and branched-chain ester prodrugs. The *n*-hexyl ester prodrug had a higher partition coefficient value than the branched-chain 2′-ethylbutyryl ester prodrug, with partition coefficient values of 23.14 ± 4.50 and 9.18 ± 1.63, respectively. Similarly, the valeryl ester had a higher partition coefficient than the pivalyl ester prodrug, with partition coefficient values of 60.56 ± 4.95 and 19.12 ± 3.57, respectively. These significantly increased straight-chain prodrug partition coefficients explain the increased human skin

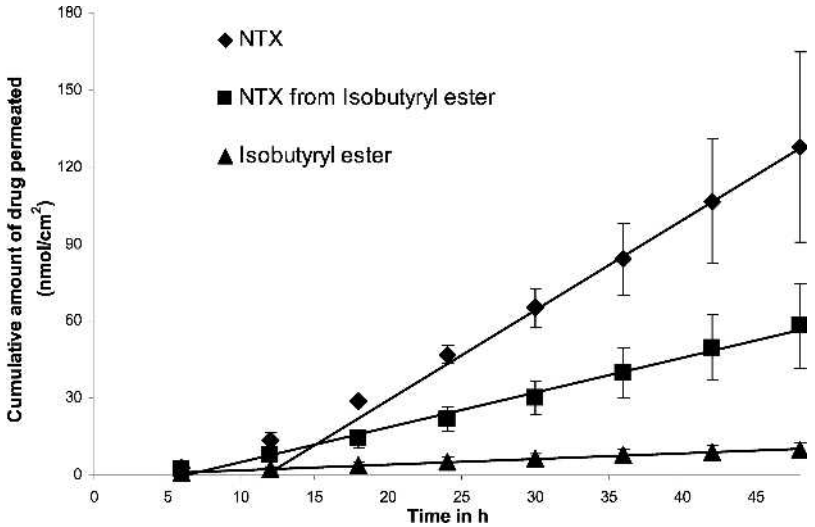


Fig. 2. Representative permeation profiles from saturated solutions for the diffusion from the NTX control, NTX from isobutyryl ester, and intact isobutyryl ester through human skin at 32°C. Data represent mean ± SD (n = 3 for NTX treatment and n = 4 for prodrug treatment).

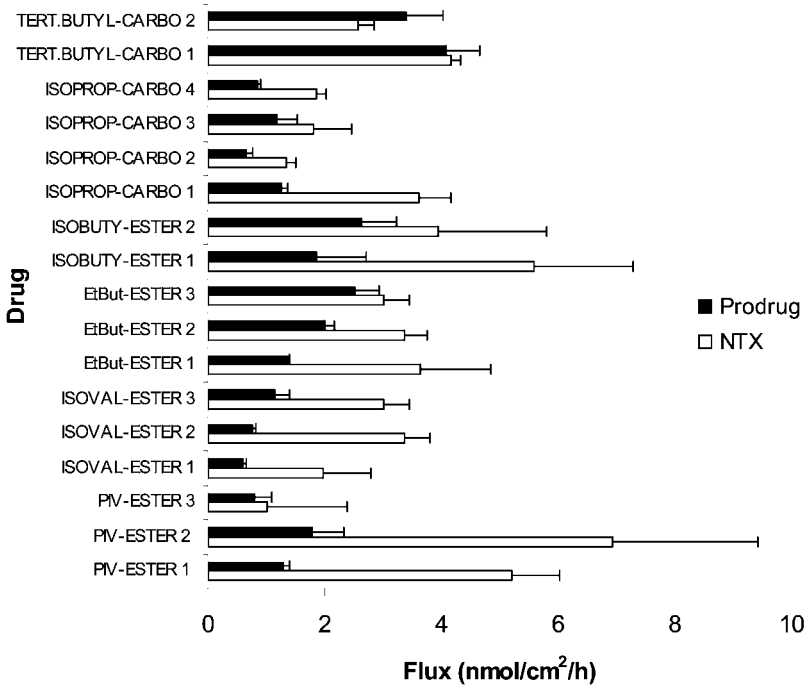


Fig. 3. *In vitro* human skin NTX flux from NTX and NTX equivalent (NTX + prodrug) flux from each prodrug. Data is represented as mean ± SD (n = 3 for NTX treatment and n = 4 for prodrug treatment) using human skin from a single subject.

permeabilities, as compared to branched-chain prodrugs. The higher partition and permeability coefficients of the straight-chain prodrugs in SC also correspond with higher calculated thermodynamic activity coefficients obtained from the DSC thermogram parameters of melting and heats of fusion (Table II). Thermodynamic activity of the drug is an important physicochemical parameter used in predicting solubility in oil, which in turn can help predict permeation through the SC lipids. Stinchcomb *et al.* (16) demonstrated a direct correlation between the thermodynamic activities and hexane solubilities of buprenorphine prodrugs. It is possible that higher thermodynamic activities result in higher partition coefficient values for drugs in the SC. As can be seen in Table II, the valeryl ester had a higher thermodynamic activity than two isomeric branched alkyl esters, that is, pivalyl and isovaleryl. The bulkier of these two branched prodrugs, the pivalyl ester, also had the lowest activity coefficient. Similarly, the *n*-hexyl and *n*-butyl esters had higher thermodynamic activities than their branched counterparts, 2′-ethylbutyryl and isobutyryl, respectively. Additionally, the carbonate esters behaved in an analogous fashion, with the 3-*O*-propyloxycarbonyl NTX having a higher thermodynamic activity than the 3-*O*-isopropyloxycarbonyl NTX. Pillai *et al.* (14) observed the highest prodrug skin permeability with the methyl carbonate prodrug in a series of straight-chain carbonate prodrugs, and this prodrug also had the highest thermodynamic activity value, calculated from DSC thermal parameters.

Although all the branched chain prodrugs had a significantly higher solubility than NTX in oil, similar in magnitude to the oil solubilities of the successful straight-chain prodrugs, this did not result in a higher flux. Both aqueous and oil solubilities have been identified as important parameters in

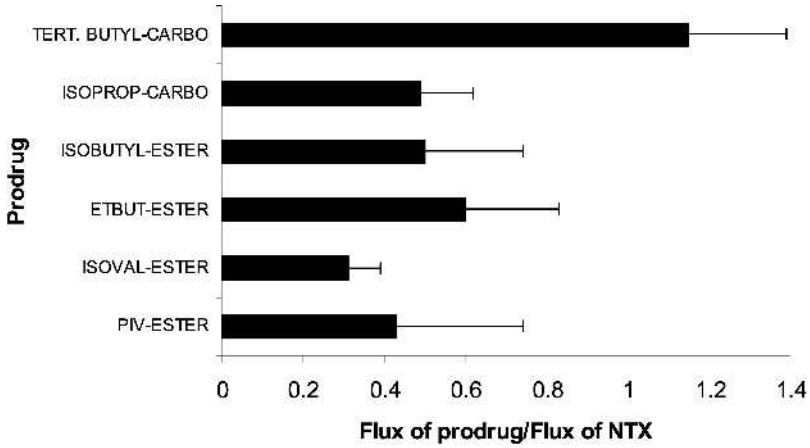


Fig. 4. Mean ratio of NTX equivalent flux from prodrug to NTX control flux ± SD (n = skin from 2–4 subjects, 4 cells each).

Table II. Comparison of Thermal Parameters for Straight Chain and Branched Chain Prodrugs with the Same Number of Carbon Atoms

	Esters							Carbonates	
Thermal parameter	Valeryl	Pivalyl	Isovaleryl	Hexyl	2′-Ethylbutyryl	Butyl	Isobutyryl	Propyl	Isopropyl
Heats of fusion (kJ/mol)	19.62	16.84	14.01	10.64	31.67	20.12	26.81	18.94	24.79
Activity coefficient	0.224	0.116	0.202	0.533	0.137	0.151	0.033	0.196	0.049

Calculated from $\ln a_2 = (-\Delta H_f/RT)(Tf-T/T_f)$; where a_2 is the thermodynamic activity, R is the gas constant, T is room temperature, T_f is fusion temperature, and ΔH_f is the heat of fusion (From J. H. Hildebrand and R. L. Scott. The Solubility of Nonelectrolytes. Reinhold, New York, 1950, Chap. 12).

the design of prodrugs (18,19). Therefore, a modified Potts and Guy equation that incorporates both aqueous and oil solubility terms has been used to predict the flux of prodrugs from lipophilic vehicles (18). Analysis of the fit of data from branched ester and carbonate prodrugs of NTX to the following equation provided an r^2 of 0.92 (MicroMath Scientist).

$$\log J_{\max} = x + y \log S_{oil} + (1 - y)\log S_{aq} - zMW \qquad (2)$$

S_{oil} is the mineral oil solubility, S_{aq} is the Hanks buffer solubility, and MW is the molecular weight of the prodrug. The parameter estimates were $x = 0.51$ (±0.98), $y = 0.46$ (±0.09), and $z = 0.0003$ (±0.0024). The r^2 value indicates a reasonably good correlation, especially considering the small number of data points ($n = 6$) used for the fit. The flux dependency on aqueous solubility is considerably higher than the fit for the straight-chain ester compounds (13), but also somewhat less than the aqueous solubility dependency observed in the straight-chain carbonate series (14). As described by Pillai *et al.* (14), the difference between this group of compounds and the straight-chain carbonate series vs. the successful straight-chain ester compounds was the degree of prodrug bioconversion to NTX that occurred in the skin. In the straight-chain 3-*O*-alkyl ester series, the prodrugs almost completely converted to NTX, and only trace amounts of prodrug were found in the receiver compartment. Here, the branched alkyl esters hydrolyzed to afford NTX in the skin, over a wide percent conversion range of 3–94% (Fig. 5), and a more significant dependence on aqueous solubility was observed in the Roberts and Sloan equation (2). Therefore, prodrugs that undergo rapid bioconversion to NTX should meet less resistance when traversing the viable aqueous tissue of the skin, and be less dependent on the corresponding aqueous solubility that contributes to the rate of that process. However, very skin-stable prodrugs such as tertiary-butyl carbonate esters are extremely dependent on their aqueous solubility for transport across viable tissue into the capillaries. The low molecular weight dependence of this data set is most likely due to the narrow molecular weight range of this group of compounds, as it is well known that molecular weight influences drug flux across the skin.

Skin contains esterase activity and the enzymes involved have been reported to be resistant to the stresses of freezing and storage (19). During skin permeation, it is expected that ester prodrugs are converted to the parent drug by these enzymes. In these diffusion experiments, all branched chain alkyl ester prodrugs were bio-transformed to NTX at various rates (Fig. 5). The highly branched alkyl ester prodrugs, that is, the pivalyl ester and 3-*O*-tertiarybutyloxycarbonyl NTX, and the moderately branched alkyl ester prodrug, 2′-ethylbutyryl ester, hydrolyzed to NTX to a lesser extent than the other prodrugs in these series. The half-lives of the prodrugs in Hanks' buffer indicated that prodrugs containing a higher degree of branching are generally more stable than the other prodrugs in this series (Table I); specifically, the greater the steric hindrance in the vicinity of the carbonyl linkage the greater the stability to hydrolysis by water or esterase enzymes (20,21). Often, prodrugs may not convert completely to the parent drug during skin permeation, and some intact prodrug can appear in the systemic circulation. The plasma hydrolysis rates should indicate the relative prodrug conversion rate to the parent drug in the body. Plasma half-lives of the prodrugs were much shorter than Hanks' buffer half-lives, demonstrating that the prodrugs were enzymatically hydrolyzed by the plasma esterase enzymes. Plasma half-lives of isovaleryl ester, isobutyryl ester, and 3-*O*-isopropyloxycarbonyl NTX prodrugs were less than 1 h, and were also

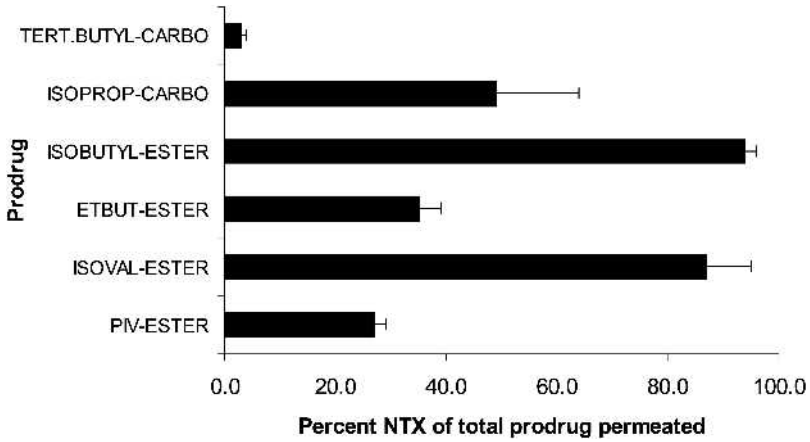


Fig. 5. Percent NTX converted from prodrug during skin permeation ± SD (n = skin from 2–4 subjects, 4 cells each).

more chemically unstable. Prodrugs containing highly and moderately branched groups, that is, pivalyl ester, 2′-ethylbutyryl ester, and 3-*O*-tertiarybutyloxycarbonyl NTX had longer plasma half-lives, which again demonstrated that steric hindrance in the vicinity of the carbonyl linkage decreased the hydrolysis rates (20,21).

In conclusion, the shape (branching) of the side chain used in the design of transdermal prodrugs is important to the success of the compound. These branched-chain prodrugs contained bulky prodrug moieties, which caused decreased thermodynamic activities, decreased SC/vehicle partition coefficients, decreased skin permeability coefficients, and resulted in lower transdermal flux rates. Additionally, the prodrug skin bioconversion rate is intimately involved in the prediction of drug transport rates. A higher degree of branching or bulkier branched groups, although having enhanced chemical stability, did not convert to the parent molecule completely during the skin permeation process and did not enhance the transdermal flux values as previously seen with the corresponding straight-chain 3-*O*-alkyl ester prodrugs.

ACKNOWLEDGMENTS

The authors would like to thank the National Cancer Institute Cooperative Human Tissue Network (CHTN) for supplying the skin. This project was funded by NIH grant R01DA13425.

REFERENCES

1. A. Vigroux, M. Bergon, and C. Zedde. Cyclization-activated prodrugs: N-(substituted 2-hydroxyphenyl and 2-hydroxypropyl)carbamates based on ring-opened derivatives of active benzoxazolones and oxazolidinones as mutual prodrugs of acetaminophen. *J. Med. Chem.* **38**:3983–3994 (1995).
2. P. Vlieghe, F. Bihel, T. Clerc, C. Pannecouque, M. Witvrouw, E. D. Clercq, J. P. Salles, J. C. Chermann, and J. L. Kraus. New 3′-azido-3′-deoxythymidin-5′-yl *O*-(ω-hydroxyalkyl) carbonate prodrugs: synthesis and anti-HIV evaluation. *J. Med. Chem.* **44**: 777–786 (2001).
3. M. A. Hussain, C. A. Koval, M. J. Myers, E. G. Shami, and E. Shefter. Improvement of the oral bioavailability of naltrexone in dogs. *J. Pharm. Sci.* **76**:356–358 (1987).
4. M. A. Hussain, B. J. Aungst, C. A. Koval, and E. Shefter. Improved buccal delivery of opioid analgesics and antagonists with bitterless prodrugs. *Pharm. Res.* **5**:615–618 (1988).
5. R. B. Greenwald, Y. H. Choe, C. D. Conover, K. Shum, D. Wu, and M. Royzen. Drug delivery systems based on trimethyl lock lactonization: poly(ethyleneglycol) prodrugs of amino-containing compounds. *J. Med. Chem.* **43**:475–487 (2000).
6. J. R. Volpicelli, A. I. Alterman, and M. Hayashida. Naltrexone in the treatment of alcohol dependence. *Arch. Gen. Psychiatry* **49**: 876–880 (1992).
7. L. Terenius. Rational treatment of addiction. *Curr. Opin. Chem. Biol.* **2**:541–547 (1998).
8. J. L. Rothenberg, M. A. Sullivan, S. H. Church, A. Seracini, E. Collins, H. D. Kleber, and E. V. Nunes. Behavioral naltrexone therapy: an integrated treatment for opiate dependence. *J. Subst. Abuse Treat.* **23**:351–360 (2002).
9. J. R. Volpicelli, K. C. Rhines, and J. S. Rhines. Naltrexone and alcohol dependence. Role of subject compliance. *Arch. Gen. Psychiatry* **54**:737–743 (1997).
10. H. R. Kranzler, V. M. Lowe, and J. V Kirk. Naltrexone vs nefazodone for treatment of alcohol dependence. A placebo-controlled trial. *Neuropyschopharmacol* **22**:493–503 (2000).
11. P. D. R. Generics. *Medical Economics*, 2nd ed., Medical Economics, Montvale, New Jersey, 1996, pp. 2229–2233.
12. Y. L. Chen. L. L Chun, and D. J. Enscore. Transdermal therapeutic systems for the administration of naloxone, naltrexone, and nalbuphine. Alza U.S. Patent No. 4 573 995. March 4, 1986.
13. A. L. Stinchcomb, P. W. Swaan, O. Ekabo, K. E. Harris, J. Browe, D. C. Hammell, T. A. Cooperman, and M. Pearsall. Straight-chain naltrexone ester prodrugs: diffusion and concurrent biotransformation in human skin. *J. Pharm. Sci.* **91**:2571–2578 (2002).
14. O. Pillai, M. O. Hamad, P. A. Crooks, and A. L. Stinchcomb. Physicochemical evaluation, in vitro human skin diffusion, and concurrent biotransformation of 3-O-alkyl carbonate prodrugs of naltrexone. *Pharm. Res.* **21**:1146–1152 (2004).
15. A. M. Kilgman and E. Christophers. Preparation of isolated sheets of human stratum corneum. *Arch. Dermatol.* **88**:702–705 (1963).
16. A. L. Stinchcomb, R. Dua, A. Paliwal, R. W. Woodard, and G. L. Flynn. A solubility and related physicochemical property comparison of buprenorphine and its 3-alkyl esters. *Pharm. Res.* **10**: 1526–1529 (1995).
17. W. J. Roberts and K. B. Sloan. Prediction of transdermal flux of prodrugs of 5-fluorouracil, theophylline, and 6-mercaptopurine with a series/parallel model. *J. Pharm. Sci.* **89**:1415–1431 (2000).
18. W. J. Roberts and K. B. Sloan. Correlation of aqueous and lipid solubilities with flux of prodrugs of 5-fluorouracil, theophylline and 6-mercaptopurine: A Potts-Guy approach. *J. Pharm. Sci.* **88**: 515–522 (1999).
19. P. G. Hewitt, J. Perkins, and S. A. M. Hotchkiss. Metabolism of fluroxypyr, fluroxypyr methyl ester, and the herbicide fluroxypyr methylheptyl ester. I: during percutaneous absorption through fresh rat and human skin in vitro. *Drug Metab. Dispos.* **28**:748–754 (2000).
20. E. Carvalho, A. P. Francisco, J. Iley, and E. Rosa. Triazene drug metabolites. Part 17: Synthesis and plasma hydrolysis of acyloxymethyl carbamate derivatives of antitumour Triazenes. *Bioorg. Med. Chem.* **8**:1719–1725 (2000).
21. M. Safadi, R. Oliyai, and V. J. Stella. Phophoryloxymethyl carbamates and carbonates-novel water soluble prodrugs for amines and hindered alcohols. *Pharm. Res.* **10**:1350–1355 (1993).