Physicochemical Evaluation, *in Vitro* Human Skin Diffusion, and Concurrent Biotransformation of 3-O-Alkyl Carbonate Prodrugs of Naltrexone

Omathanu Pillai,[1] Mohamed O. Hamad,[1] Peter A. Crooks,[1] and Audra L. Stinchcomb[1,2]

Received February 10, 2004; accepted March 28, 2004

Purpose. The purpose of this study was to evaluate the physicochemical properties and *in vitro* human skin diffusion of the 3-O-alkyl carbonate prodrugs of naltrexone (NTX).

Methods. Melting points and heats of fusion (ΔH_f) were determined using differential scanning calorimetry. *In vitro* human skin permeation rates of NTX and its prodrugs were measured using a flow-through diffusion cell system. Drug disposition in the skin was quantified at the end of the diffusion experiment. The solubilities of the drugs were determined in mineral oil and isotonic buffer. Partitioning of the prodrugs from vehicle to skin was determined using isolated sheets of human stratum corneum (SC).

Results. All the prodrugs hydrolyzed to NTX on passing through the skin, and the methyl NTX-3-O-carbonate (ME-NTX) provided the highest NTX flux, apparent permeability coefficient (K_p), and calculated relative thermodynamic activity from the melting point and ΔH_f. The ME-NTX SC/vehicle partition coefficient was the highest of the prodrug series, although similar to the NTX SC/vehicle partition coefficient value. The shortest chain prodrugs underwent the highest extent of bioconversion to NTX upon passing through the skin.

Conclusions. Within this 3-O-alkyl carbonate prodrug series, the shortest chain prodrug was the most skin-permeable compound with the highest partition coefficient and a significant extent of bioconversion.

KEY WORDS: biotransformation; human skin; naltrexone; prodrugs; transdermal drug delivery.

INTRODUCTION

Naltrexone (NTX) is an opioid antagonist used in the treatment of alcohol dependence and opioid addiction (1,2). Currently, NTX hydrochloride is available as a 50 mg oral tablet (ReVia) in the United States. NTX undergoes extensive first-pass metabolism and has oral bioavailability estimates ranging from 5% to 40% (3). Oral NTX therapy is associated with a number of gastrointestinal adverse effects (abdominal pain, nausea and vomiting), thus limiting its clinical utility (4). Consequently, the main disappointment in NTX maintenance therapy has been the poor long-term compliance with therapy, and NTX is the drug of choice for only very highly motivated patients (2,5,6). To address this issue, several depot injections of NTX are under clinical investigation (5,7), but this relatively invasive dosage form would require a visit to a health care professional for treatment.

Alternatively, transdermal NTX delivery would avoid first-pass metabolism in addition to diminishing the gastrointestinal side effects of NTX. The application of a transdermal patch requires less patient motivation (as opposed to surgical implantation or injection) and hence can be expected to improve compliance with NTX therapy. Furthermore, transdermal delivery also provides flexibility in terms of dose (different patch sizes) and also allows a total dose reduction in drug that should lower the hepatotoxicity risk of NTX. This would prove beneficial to the already hepato-compromised alcohol and opoid addicts.

NTX *per se* does not have the ideal physicochemical properties (8) to provide a transdermal delivery rate for a therapeutic dose of the drug. If a 50–100 mg/day dose of NTX is given with an oral bioavailability range 5–40%, the daily dose range is 7–118 μmol. Therefore, a therapeutic delivery rate of NTX from a 25-cm^2 patch is in the range 11–197 $nmol \cdot cm^{-2} \cdot h^{-1}$. The use of permeation enhancers has been investigated with the intention of reaching therapeutic NTX levels in humans with a transdermal patch (9). However, the issue of skin irritation and safety of some penetration enhancers would make this previous approach less desirable than a prodrug approach. The physicochemical properties of NTX are being optimized in the current studies by the design of prodrugs with higher permeation and prompt biotransformation in the skin. These prodrugs are primarily bioconverted in the skin by general esterase enzyme activity, but prodrug passing through the skin intact can also undergo bioconversion in the plasma, liver, and other organs. Hussain *et al.* (10,11) have described various prodrugs of NTX to improve oral bioavailability and to mask the bitter taste of NTX for buccal delivery. Earlier studies from Stinchcomb *et al.* (12) showed that straight-chain ester prodrugs of NTX increased the transdermal delivery rates of NTX across human skin. Here, the prodrug synthesis, physicochemical evaluation, *in vitro* diffusion and biotransformation across human skin of a homologous series of 3-O-alkyl carbonate prodrugs of NTX are described. As shown in Fig. 1, a series of alkyl carbonate prodrugs was synthesized, namely methyl NTX-3-O-carbonate (ME-NTX), ethyl NTX-3-O-carbonate (ETH-NTX), *n*-propyl NTX-3-O-carbonate (PROP-NTX), isopropyl NTX-3-O-carbonate (ISOPROP-NTX), *n*-butyl NTX-3-O-carbonate (BUT-NTX), and amyl NTX-3-O-carbonate (PENT-NTX). The overall objective was to identify and quantify the important prodrug physicochemical properties that may be responsible for changes in NTX transdermal delivery rates; namely, melting point, lipophilicity, solubility, and biotransformation. The study of this series of NTX prodrugs is part of the therapeutic goal to develop a successful transdermal dosage form of NTX. Data from this study will help to identify the critical physicochemical parameters that can be used to create a quantitative structure permeability relationship for the future design of transdermal prodrugs for other poorly skin permeable drugs.

MATERIALS AND METHODS

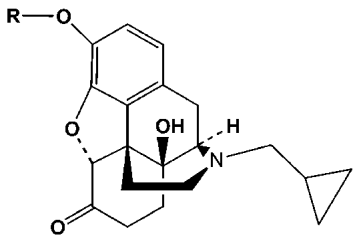
Materials

NTX base was purchased from Mallinckrodt Inc (St. Louis, MO, USA). Carbonate prodrugs were synthesized

[1] Division of Pharmaceutical Sciences, University of Kentucky College of Pharmacy, Lexington, Kentucky 40536, USA.

[2] To whom correspondence should be addressed. (e-mail: astin2@email.uky.edu)

0724-8741/04/0700-1146/0 © 2004 Plenum Publishing Corporation



DRUG	R-GROUP	MW	MP (^{O}C)	Calculated Log P[b]
NTX	-H	341	174-176	0.36
ME-NTX	$-COOCH_3$	399	120-121	0.14
ETH-NTX	$-COOCH_2CH_3$	413	130-132	0.67
PROP-NTX	$-COO(CH_2)_2CH_3$	427	105-107	1.20
ISOPROP-NTX	$-COOCH(CH_3)_2$	427	152-156	0.98
BUT-NTX	$COO(CH_2)_3CH_3$	441	ND[a]	1.73
PENT-NTX	$COO(CH_2)_4CH_3$	455	ND[a]	2.26

The melting points were determined from DSC experiments and are represented as a range from two measurements

[a] Melting point was not determined as they were oils

[b] Determined from Daylight ® 4.51 software

Fig. 1. Chemical structure, molecular weight, melting point, and lipophilicity of NTX and its carbonate prodrugs.

from the NTX base. 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), 1-octane sulfonic acid sodium salt, methanol, and acetonitrile (ACN) were obtained from Fisher Scientific (Fairlawn, NJ, USA).

General Synthetic Procedure for the Synthesis of Naltrexone 3-O-Carbonate Prodrugs

A mixture of naltrexone (120 mg, 0.35 mmol) and triethylamine (50 μl, 0.35 mmol) in 10 ml of dry methylene chloride was cooled to 0°C in an ice-bath. To this stirred mixture was added the appropriate alkylchloroformate (0.40 mmol). After stirring for 10 h, the resulting reaction mixture was diluted with methylene chloride (5 ml) and organic liquors washed with 10% w/v aqueous sodium carbonate (2 × 20 ml), then water (20 ml). Then, the organic layer was separated, dried over anhydrous potassium carbonate, 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 and air-dried to afford a white powder. The naltrexone carbonate prodrugs were characterized by ^{1}H-NMR spectroscopy and liquid chromatography-mass spectroscopy.

Methyl Naltrexone-3-O-Carbonate

NMR ($CDCl_3$): δ 6.92 (d, 1H, aromatic, J = 8.4 Hz), 6.68 (d, 1H, aromatic, J = 8.4 Hz), 4.22 (q, 2H, OCH_2, J = 7.2 Hz), 1.38 (t, 3H, CH_3, J = 7.2 Hz) ppm. MS (LC-MS electrospray), M^+ = 400 m/z. MP 120–121°C.

Ethyl Naltrexone-3-O-Carbonate

NMR ($CDCl_3$): δ 6.91 (d, 1H, aromatic, J = 8.4 Hz), 6.68 (d, 1H, aromatic, J = 8.4 Hz), 3.91 (q, 3H, CH_3) ppm. MS (LC-MS electrospray), M^+ = 414 m/z. MP 130–132°C.

Isopropyl Naltrexone-3-O-Carbonate

NMR ($CDCl_3$): δ 6.92 (d, 1H, aromatic, J = 8.1 Hz), 6.67 (d, 1H, aromatic, J = 8.1 Hz), 4.97 (seven line multiplet, 1H, OCH, J = 6.3 Hz), 1.37 (d, 6H, $C(CH_3)$, J = 6.0 Hz) ppm. MS (LC-MS electrospray), M^+ = 428 m/z. MP 152–156°C.

n-Propyl Naltrexone-3-O-Carbonate

NMR ($CDCl_3$): δ 6.92 (d, 1H, aromatic, J = 8.1 Hz), 6.68 (d, 1H, aromatic, J = 8.1 Hz), 4.21 (d of t, 2H, OCH_2, J_t = 6.3 Hz, J_d = 2.6 Hz), 1.77 (m, 2H, CH_2, J = 7.2 Hz), 1.00 (t, 3H, CH_3, J = 7.5Hz) ppm. MS (LC-MS electrospray), M^+ = 428 m/z. MP 105–107°C.

n-Butyl Naltrexone-3-O-Carbonate

NMR ($CDCl_3$): δ 6.92 (d, 1H, aromatic, J = 8.4 Hz), 6.68 (d, 1H, aromatic, J = 8.4 Hz), 4.26 (m, 2H, OCH_2), 1.73 (m, 2H, CH_2, J = 7.5 Hz), 1.45 (six line multiplet, 2H, CH_2, J = 7.6 Hz), 0.96 (t, 3H, CH_3) ppm. MS (LC-MS electrospray), M^+ = 442 m/z. Not crystalline at room temperature.

Amyl Naltrexone-3-O-Carbonate

NMR ($CDCl_3$): δ 6.91 (d, 1H, aromatic, J = 6.0 Hz), 6.69 (d, 2H, aromatic, J = 6.0 Hz), 4.24 (m, 2H, OCH_2), 1.724 (m, 2H, CH_2), 1.38 (m, 2H, CH_2), 1.366 (m, 2H, CH_2), 0.92 (t, 3H, CH_3) ppm. MS (LC-MS electrospray), M^+ = 456 m/z. Not crystalline at room temperature.

Quantitative Analysis

A modified high-pressure liquid chromatography (HPLC) assay from Hussain *et al.* (10) was used for the analysis of NTX and its prodrugs. The HPLC system consisted of a Waters (Milford, MA, USA) 717 Autosampler, 1525 Pumps, and a 2487 dual wavelength UV absorbance detector with Millennium Chromatography software (Milford, MA, USA). A Brownlee Valueline 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/VIS detector set at a wavelength of 215 nm. The mobile phase was composed of 80:20 ACN:0.1% TFA with 0.65 g/L 1-octane sulfonic acid, sodium salt (adjusted to pH 3.0 with triethylamine). The flow rate of the mobile phase was adjusted to 1.5 ml/min and 100 μl of sample was injected onto the column. Standards were analyzed with each set of diffusion samples and exhibited excellent linearity over the entire concentration range used in the assays. The sensitivity of the assay was 10 ng/ml and 50 ng/ml for NTX and the prodrugs, respectively.

The drugs were extracted from the buffer samples by solid phase extraction (30 mg 1cc Oasis HLB, Waters Corp.). Before loading the aqueous diffusion samples (5 ml), the cartridge was conditioned with 1 ml of methanol and 1 ml of water. After loading the sample, the cartridge was washed with 1 ml of 5% methanol:water, and the drug was eluted with acetonitrile. Sample recoveries were >80% for NTX and the prodrugs.

Differential Scanning Calorimetry of NTX and Prodrugs

Differential scanning calorimetry (DSC) was carried out for NTX and the solid samples of prodrugs after recrystallization with acetone. The heats of fusion, ΔH_f, and melting points were determined with a TA Instruments 2920 DSC (New Castle, DE). An accurately weighed sample of drug (2–5 mg) was placed into the aluminum pan, and the heating curves were recorded at 10°C/min from ambient to 350°C. Measurements were repeated once for a total of two scans on NTX and the prodrugs.

Solubility

The solubilities of NTX and its carbonate prodrugs were determined by adding excess quantity of drug to each respective solvent (mineral oil or prewarmed pH 7.4 HEPES-buffered Hanks' balanced salts solution) at 32°C, with equilibration while shaking in a water bath at 60 rpm. Samples were drawn into a prewarmed glass syringe, filtered through a syringe filter (Mineral oil: Millex FG-13 filter, Millipore, Billerica, MA, USA, and buffer: nylon filter, Gelman, East Hills, NY, USA), and measured with respect to volume and diluted with the appropriate volume of acetonitrile or buffer. The drug from the buffer samples was immediately extracted by solid phase extraction, and the drug from mineral oil was extracted using acetonitrile. The sampling procedure was done in triplicate, discarding the first 40% of the initial filtrate. All the samples were analyzed by HPLC. Equilibration time for the solubility studies was 48 h in mineral oil. For buffer solubilities, samples were taken after 15 min of sonication (to minimize the hydrolysis of prodrugs). No significant difference was found between 15 min sonication solubility measurements and equilibrium solubility measurements taken after 8 and 48 h for the most stable prodrug.

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 either stored at −20°C or used immediately. 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 for each prodrug. The studies were repeated at least once with human skin from a different donor. The receiver solution was HEPES-buffered Hanks' balanced salts with gentamicin at a pH of 7.4 and a flow rate of 1.1 ml/h. These diffusion study conditions were chosen in order to optimize tissue viability according to previous studies by Collier *et al.* (20). A saturated NTX or prodrug solution in light mineral oil was applied to the skin, and each cell was charged with 0.25 ml of the respective drug solution. Samples were collected in 6-h 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.

The cumulative quantity of NTX equivalents collected in the receiver compartment was plotted as a function of time. The flux value for a given experiment was obtained from the slope (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 in the donor and receiver solutions. Sink conditions were maintained in the receiver throughout the experiment, so ΔC was approximated by the drug concentration in the donor compartment. All the skin permeation parameters were tested for statistical significance ($p < 0.05$) by one-way ANOVA and Tukey post hoc analysis (SigmaStat).

Drug disposition in the skin samples was measured at the end of the 48-h experiment. The skin tissue was rinsed with filtered water and blotted with a paper towel. To remove the drug formulation adhering to the surface, the skin was tape stripped twice using Scotch tape. The area of skin in contact with the drug was cut out, minced with a scalpel, and placed in a preweighed vial. Drug was extracted from the skin by equilibrating with 10 ml of acetonitrile at 32°C with shaking (60 rpm) in a water bath overnight. Samples were analyzed by HPLC to determine NTX and prodrug content in micromole of drug per gram of wet tissue weight.

Stratum Corneum/Vehicle Partition Coefficient Studies

Human epidermis with the stratum corneum (SC) side facing up was incubated on filter papers soaked with 0.1% trypsin in 0.5% sodium bicarbonate solution in a Petri dish at 37°C for 3 h (13). The SC membrane was separated and

washed briefly with hexane to remove superficial lipids, rinsed with isotonic saline, and dried in a vacuum desiccator for 2 days.

Approximately 5 mg of SC was equilibrated with subsaturated NTX or prodrug 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 SC/vehicle partition coefficient was calculated by a method reported previously (14). 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 NTX or prodrug in 1 g of SC divided by the concentration of NTX or prodrug in 1 g of mineral oil.

Stability of Prodrugs in Buffer

The stability of the prodrugs in Hanks' (pH 7.4) buffer was studied by using a subsaturated concentration of the prodrug. Samples (6 ml) were distributed into screw-capped vials and placed at three different temperatures (4°C, room temperature, and 32°C). One vial was removed every day for 7 days. 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 the rate constant and half-life of the prodrugs.

RESULTS

Physicochemical Properties of NTX Base and Its Carbonate Prodrugs

The melting points of all the prodrugs were less than the parent NTX, and the longer alkyl-chain derivatives ($>C_3$) were oils (Fig. 1). The decrease in the melting point reflects the disorder of the intramolecular cohesion caused by the addition of the added alkyl groups. Heats of fusion (ΔH_f), which are a measure of the crystal lattice energy, had an inverse relationship to the skin permeability of the prodrugs. The calculated thermodynamic activity, a parameter encompassing the melting point and the heat of fusion, was highest with ME-NTX and lowest with ISOPROP-NTX (Table I).

Table I shows the aqueous solubility and oil solubility of NTX and the carbonate prodrugs. As anticipated, the prodrugs have higher oil solubility than NTX, whereas the aqueous solubility decreased with increasing alkyl chain length. ME-NTX is the most aqueous soluble prodrug and BUT-NTX is the most oil-soluble prodrug ($p < 0.05$). The aqueous stability (pH 7.4 buffer) is dependent on the alkyl chain length. ME-NTX and ISOPROP-NTX are the most chemically labile and stable prodrugs, respectively (Table I). However, the half-lives of all the prodrugs in the buffer at 32°C were significantly longer than the duration (48 h) of the skin diffusion experiments, ranging from 73 to 315 h. Because all the diffusion samples were refrigerated within 12 h of collection, this means the most significant chemical degradation that occurred after passage through the skin was 11%.

In Vitro Skin Diffusion and Concurrent Biotransformation of the NTX Prodrugs

Figure 2 shows a representative profile used to calculate the steady-state flux of the prodrugs and NTX released from the prodrugs. All prodrugs hydrolyzed on passing through the skin and appeared as a combination of NTX and intact prodrug in the receiver solution. Each prodrug was tested in at least two different skin donor samples using NTX as a control for each set. The mean flux of NTX from each prodrug and the mean NTX control flux from each group of individual experiments are shown in Fig. 3. As shown in Table I, the flux of NTX from ME-NTX was higher than all other prodrugs, and it was the only prodrug in the group that provided a higher flux than NTX base ($p < 0.05$). There was no significant difference in the flux of ISOPROP-NTX when it was delivered either from saturated mineral oil vehicle or saturated aqueous vehicle (Hanks' buffer, pH 7.4). Other prodrugs were not tested in aqueous vehicle, as they were relatively less stable in Hanks' buffer as compared to ISOPROP-NTX. The ratio of prodrug flux to total drug flux (NTX + prodrug) (Fig. 4) was larger for the longer chain prodrugs, and the highest percent of NTX permeated from ME-NTX and ETH-NTX, as compared to the other prodrugs in the

Table I. Properties of NTX and Its Alkyl Carbonate Prodrugs

Drug	Light mineral oil solubility (mM)*	Hanks' buffer solubility (mM)*	NTX flux from mineral oil ($nmol \cdot cm^{-2} \cdot h^{-1}$)	Mean permeability coefficient ($K_p \times 10^3$) (cm/h)	Thermodynamic activity†	Stability rate constant ($k \times 10^3$) (h^{-1}) in Hanks' buffer‡
NTX	0.24 ± 0.02	5.6 ± 0.40	2.9 ± 0.74	12.1	0.14	—
ME-NTX	0.33 ± 0.02	3.0 ± 0.06	6.9 ± 0.50	20.9	0.38	9.5
ETH-NTX	0.59 ± 0.02	0.63 ± 0.04	3.4 ± 0.17	5.8	0.16	4.8
PROP-NTX	1.8 ± 0.11	0.98 ± 0.03	1.1 ± 0.16	0.60	0.20	5.0
ISOPROP-NTX	0.87 ± 0.10	0.26 ± 0.02	1.2 ± 0.35	1.4	0.05	2.2
BUT-NTX	7.1 ± 0.46	0.63 ± 0.07	3.6 ± 0.51	0.51	NA	5.4
PENT-NTX	3.4 ± 0.12	0.17 ± 0.01	2.6 ± 0.10	0.77	NA	4.6

NA, not available.

* Solubility studies were conducted at 32°C.

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

‡ Stability studies were conducted at 32°C for 7 days.

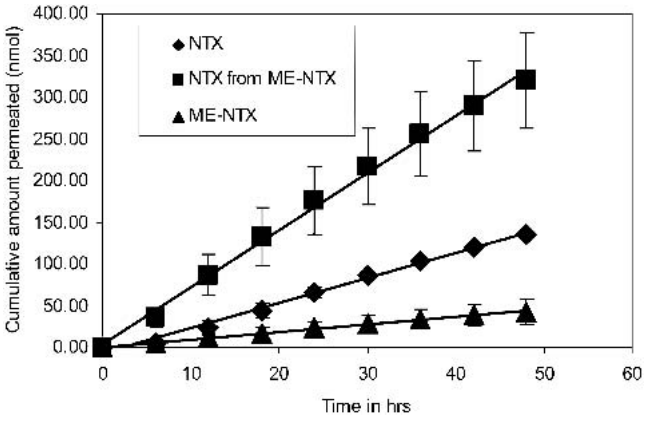


Fig. 2. Representative permeation profile for the diffusion of NTX, NTX from ME-NTX, and ME-NTX from saturated solutions through human skin at 32°C. Data represents mean ± standard deviation (n = 4 for prodrug and n = 3 for NTX).

series. Drug disposition studies showed (Fig. 5) that the prodrugs regenerated to greater than 50% NTX in the skin, with the exceptions of PROP-NTX and ISOPROP-NTX. ME-NTX had the highest drug concentration in the skin as compared to the other prodrugs. This observation was also substantiated by the higher SC partition coefficient ($p < 0.05$) of ME-NTX from mineral oil, as opposed to the other prodrugs (Fig. 6).

DISCUSSION

Although the melting points of all the prodrugs were less than NTX, there was no definitive downward trend with the increase in the alkyl chain length, unlike that observed with the straight chain esters of NTX (12). On the other hand, the ΔH_f values showed a linear inverse relationship with the permeability coefficient for the straight chain alkyl carbonate prodrugs. Therefore, it is conceivable that ΔH_f measurement can serve as a useful guide to identify promising straight chain prodrugs. Stinchcomb *et al.* (15) have shown a direct correlation between thermodynamic activity and hexane solubility of

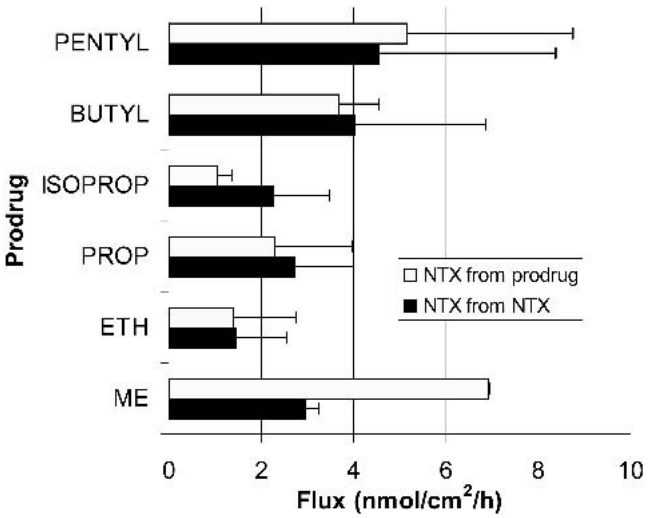


Fig. 3. *In vitro* NTX flux from NTX and its prodrugs. Data is represented as mean ± standard deviation (n ≥ 2 pieces of skin from different subjects).

prodrugs. In this study, we did not find any direct correlation between mineral oil solubility and thermodynamic activity, but ME-NTX, which exhibited the highest calculated thermodynamic activity (and lowest ΔH_f), was the most promising prodrug. The high flux achieved with ME-NTX can be attributed to the increased partitioning of the prodrug from the vehicle to the skin, which also resulted in a high apparent permeability coefficient.

The higher oil solubility did not result in a higher flux of the prodrugs, but ME-NTX, which had a higher aqueous solubility than the other prodrugs (but not higher than NTX), showed a higher flux among the prodrugs. Apart from oil solubility, aqueous solubility has been identified to be an important criterion in the design of prodrugs by a few researchers (16). In this regard, Beall and Sloan (17) have reported that the more water soluble alkyl carbonate prodrugs of 5-fluorouracil (5-FU) effectively delivered 5-FU across the skin. A modified Potts and Guy equation that incorporates an aqueous solubility term has been used to predict the flux of prodrug from lipophilic vehicle (18). Analysis of the fit of NTX carbonate prodrugs to the following equation provided an r^2 of 0.75 (Micromath, Scientist Software).

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

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 $x = -2.85$ (±4.25), $y = 0.11$ (±0.39), and $z = -0.008$ (±0.103). The r^2 was low compared to the fit of the Roberts and Sloan prodrugs, but seems to be a reasonably good correlation, considering the smaller number of data (n = 6) used for the fit. It appears from the estimates of the parameters in this equation that aqueous solubility plays an important role in the flux of this series of prodrugs. Earlier studies from Stinchcomb *et al.* (12) with simple NTX straight-chain esters showed that aqueous solubility did not play as significant a role as seen with this carbonate series. The main difference between this series of prodrugs and the alkyl ester series of prodrugs is the difference in metabolic conversion of the prodrugs in the skin. In the alkyl ester series, the drugs were almost completely converted to NTX in the skin, and only trace amounts of intact prodrug were seen in the receiver compartments. In this carbonate series, we see 11–51% intact prodrug in the receiver compartments. Additionally, skin disposition studies for the alkyl ester series showed much higher levels of regenerated NTX in the skin than seen with these carbonate prodrugs. Therefore, as implied in the Stinchcomb *et al.* (12) publication, the importance of the physicochemical parameters on the influence of the success of prodrug flux is highly dependent on the lability of the prodrugs to bioconversion in the skin. Prodrugs that undergo fast bioconversion are not as rate-limited by their transport through the viable aqueous tissue of the skin, because they have primarily bioconverted to their generally more hydrophilic parent forms. Prodrugs that undergo slower bioconversion to the parent compound will show a more significant dependence on water solubility because they must traverse the aqueous tissue in a generally less water soluble form than their parent structure. Alternatively, the dependence on water solubility could actually be a coincidental correlation that merely reflects the relative lability to bioconversion. In other words, the more water soluble compounds are generally the

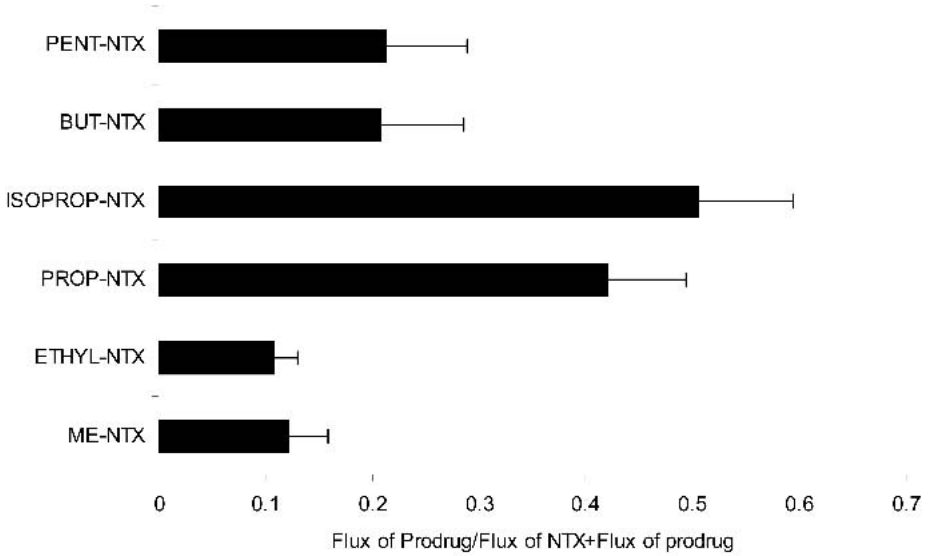


Fig. 4. Ratio of prodrug flux to total drug flux (NTX + prodrug). The values were calculated from the flux of prodrug and the NTX flux from prodrug.

shorter chain prodrugs that also happen to undergo faster bioconversion rates in the skin. Nonetheless, the water solubility may be an easier property to measure than tissue bioconversion rates, so the water solubility could be an indirect predictor of transport rates.

Relative rates of diffusion and biotransformation determine the ability of a prodrug to produce increased flux over the parent drug. Recently, Boderke *et al.* (19) have modeled the diffusion and concurrent metabolism of prodrugs in skin. The parameters that determine the residence time of the prodrug in skin have been identified as the thickness of the membrane (L) and the diffusion coefficient (D), and the metabolic half-life of the prodrug is determined by the concentration of prodrug in the donor solution (C_d), partitioning of the prodrug from the vehicle to the skin (P), and the maximum metabolic rate (V_{max}). All these terms are related by a dimensionless coefficient as given in the following equation:

$$2\alpha = \frac{L^2/D}{[C_d P]/[2V_{max}]} \tag{3}$$

Based on this equation, ME-NTX is expected to have a relatively higher biotransformation rate compared to its residence time in the skin. Hence, a smaller amount of prodrug is detected in the receiver medium (Fig. 5). On the other hand, in the case of PROP-NTX, the residence time is less than the biotransformation rate. This is evident from the lesser NTX disposition in the skin and a higher proportion of prodrug in the receiver medium for PROP-NTX, as compared to ME-NTX (Figs. 5 and 6). Although the percent NTX regenerated in the skin for BUT-NTX and for PENT-NTX are comparable to ME-NTX, the low partitioning of the former two prodrugs do not favor the attainment of a high concentration of the prodrug in the skin.

In conclusion, the physicochemical parameters aid in op-

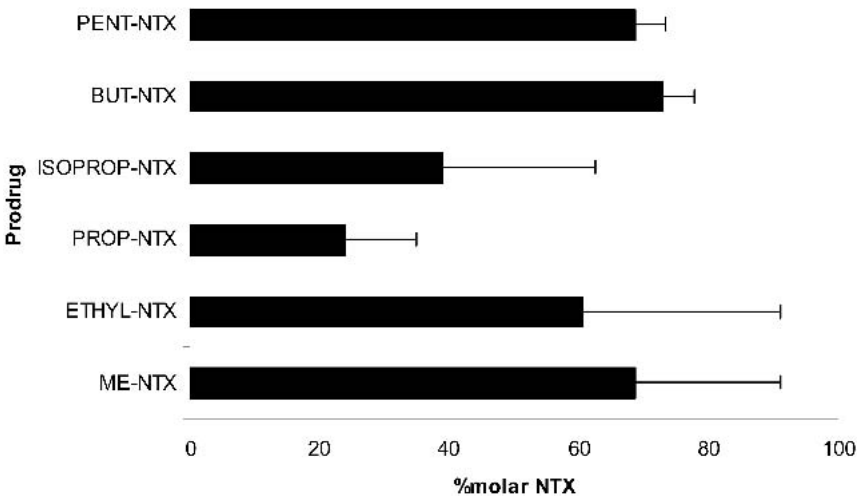


Fig. 5. Drug disposition in human skin after a 48-h permeation experiment following application of saturated solutions in mineral oil. Data represents the mean (n = 4) molar percentage of regenerated NTX to total drug extracted from the skin.

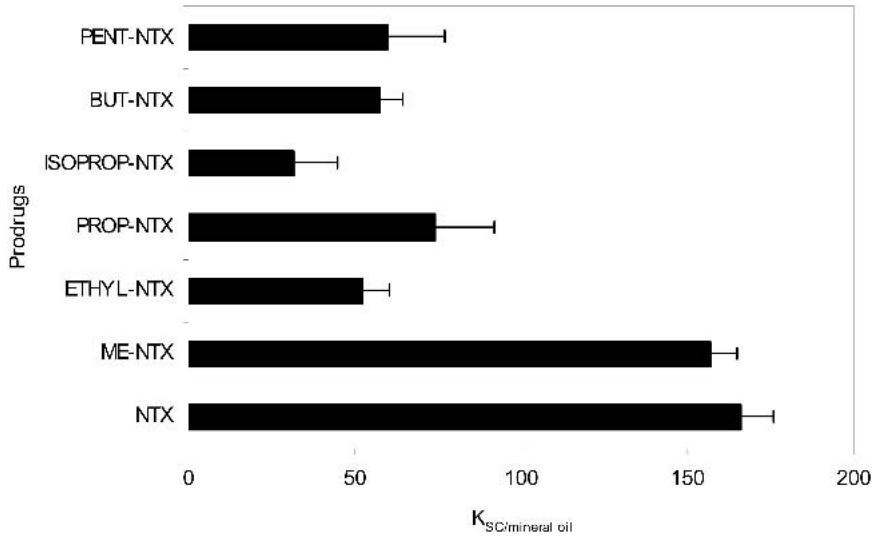


Fig. 6. SC/mineral oil partition coefficient ($K_{SC/mineral\ oil}$) of prodrugs after 48-h equilbration at 32°C. Data is represented as mean ± standard deviation (n = 3).

timizing the design of NTX prodrugs. Partitioning of prodrug from the vehicle to the skin plays an important role in the extent of the appearance of NTX in the receiver medium, as does the bioconversion rate of the prodrug. The flux from the most permeable ME-NTX prodrug did not meet the minimum necessary therapeutic delivery rate of 11 $nmol \cdot cm^{-2} \cdot h^{-1}$, but the required 60% increase in the flux may be possible with further formulation efforts. Results from this study have provided valuable information for optimization of the relative oil and aqueous solubilities of the prodrugs, based on their relative skin bioconversion rates, to achieve a higher flux of NTX for therapeutic treatment.

ACKNOWLEDGMENTS

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

REFERENCES

1. J. R. Volpicelli, A. I. Alterman, and M. Hayashida. Naltrexone in the treatment of alcohol dependence. *Arch. Gen. Psychiatry* **49**: 876–880 (1992).
2. L. Terenius. Rational treatment of addiction. *Curr. Opin. Chem. Biol.* **2**:541–547 (1998).
3. *PDR Generics*, 2nd Ed., Medical Economics, Montvale, New Jersey, 1996, pp. 2229–2233.
4. H. R. Kranzler, V. M. Lowe, and J. V Kirk. Naltrexone vs nefazodone for treatment of alcohol dependence. A placebo-controlled trial. *Neuropyschopharmacology* **22**:493–503 (2000).
5. 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. Substance Abuse Treat.* **23**:351–360 (2002).
6. 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).
7. L. L Gooberman. Pharmaceutical delivery device and method of preparation therefor. U.S. Patent No. 6,203,813 (March 20, 2001).
8. A. Naik, Y. N. Kalia, and R. H. Guy. Transdermal drug delivery: overcoming the skin's barrier function. *Pharm. Sci. Technol. Today* **3**:318–326 (2000).
9. Y. L. Chen, L. L Chun, and D. J. Enscore. Transdermal therapeutic systems for the administration of naloxone, naltrexone, and nalbuphine. U.S. Patent No. 4,573,995 (March 4, 1986).
10. M. A. Hussain, C. A. Koval, M. J. Myers, E. G. Shami, and E. Shefter. Improvement of the oral bioavailability of naltrexone in dogs: A prodrug approach. *J. Pharm. Sci.* **76**:356–358 (1987).
11. M. A. Hussain, B. J. Aungst, C. A. Koval, and E. Shefter. Improved buccal delivery of opoid analgesics and antagonists with bitterless prodrugs. *Pharm. Res.* **5**:615–618 (1988).
12. 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).
13. A. M. Kligman and E. Christophers. Preparation of isolated sheets of human stratum corneum. *Arch. Dermatol.* **88**:702–705 (1963).
14. C. Surber, K. P. Wilhelm, M. Hori, H. I. Maibach, and R. H. Guy. Optimization of topical therapy: partitioning of drugs into stratum corneum. *Pharm. Res.* **7**:1320–1324 (1990).
15. 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).
16. 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).
17. H. D. Beall and K. B. Sloan. Transdermal delivery of 5-fluorouracil (5-FU) by 1-alkylcarbonyl- 5-FU prodrugs. *Int. J. Pharm.* **129**:203–210 (1996).
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. Boderke, K. Schittkowsi, M. Wolf, and H. P. Merkle. Modeling of diffusion and concurrent metabolism in cutaneous tissue. *J. Theor. Biol.* **204**:393–407 (2000).
20. S. W. Collier, N. M. Sheikh, A. Sakr, J. L. Lichtin, R. F. Stewart, and R. L. Bronaugh. Maintenance of skin viability during in vitro percutaneous absorption/metabolism studies. *Toxicol. Appl. Pharmacol.* **99**:522–533 (1989).