Straight-Chain Naltrexone Ester Prodrugs: Diffusion and Concurrent Esterase Biotransformation in Human Skin

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ABSTRACT: Naltrexone (NTX) is an opioid antagonist used for treatment of narcotic dependence and alcoholism. Transdermal naltrexone delivery is desirable to help improve patient compliance. The purpose of this study was to increase the delivery rate of NTX across human skin by using lipophilic alkyl ester prodrugs. Straight-chain naltrexone-3-alkyl ester prodrugs of 2–7 carbons in chain length were synthesized and evaluated. *In vitro* human skin permeation rates were measured using a flow-through diffusion cell system. The melting points, solubilities, and skin disposition of the drugs were determined. The prodrugs were almost completely hydrolyzed on passing through the skin and appeared as NTX in the receiver compartment. The mean NTX flux from the prodrug-saturated solutions exceeded the flux of NTX base by ~2–7-fold. The amount of drug detected in the skin was significantly greater after treatment with the prodrug solutions compared with treatment with NTX base. The extent of parent drug (NTX) regeneration in the intact skin ranged from 28 to 91%. Higher NTX regeneration percentages in skin appeared to correlate with increased drug delivery rates. Definitively, the highly oil-soluble prodrugs provide a higher NTX flux across human skin *in vitro* and undergo significant metabolic conversion in the skin. J Pharm Sci 91:2571–2578, 2002

Keywords: transdermal; naltrexone; prodrugs; human skin; percutaneous absorption

INTRODUCTION

Naltrexone (NTX), an opioid antagonist, is currently used to help maintain opioid addicts in a drug-free state. Most recently, NTX has been indicated as an adjunct in the treatment of alcohol dependence, as well as reported to reduce alcohol craving in certain alcoholic populations.[1–4] Naltrexone Hydrochloride is currently commercially available in the United States as a 50-mg oral tablet (ReVia™). NTX undergoes extensive first-pass metabolism and has oral bioavailability estimates in the range 5–40%.[5] NTX is also a hepatotoxin that has the capacity to cause dose-related hepatocellular injury. This hepatotoxicity limits dosage increases in those addicts who may benefit from an oral dose of >50 mg/day. Transdermal delivery would circumvent these liver-related problems by allowing lower doses to be used, because first-pass metabolism is bypassed with this route of administration. Certainly, the ability to decrease the drug dosage would benefit the already hepatocompromised alcoholic or narcotic addict. Many of the adverse effects seen with NTX oral therapy (abdominal pain, nausea, and vomiting) may also be reduced by transdermal therapy. Compliance, the major problem with

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addict recovery pharmacological intervention, is likely improved when side effects are reduced and drug doses are adequate to reduce craving.[6] In fact, compliance has been a critical issue in the response of alcoholics to NTX therapy in recent clinical trials.[7,8] The major goal of this research is to enhance the therapeutic value of NTX by reducing side effects and improving addict compliance with transdermal delivery.

NTX itself does not have the essential physicochemical properties that would allow a therapeutic dose of the drug to cross the human skin barrier. We have synthesized prodrugs that increased the NTX maximum flux rate across human skin. Extensive conversion of these prodrugs to NTX occurred in the viable skin layers. The success of these prodrugs has been evaluated in *in vitro* diffusion studies with human skin to determine the level of NTX transdermal delivery enhancement. Most other published transdermal prodrug studies have been done *in vitro* with less than optimum conditions (i.e., cadaver skin, mouse skin, and without viability promoting receiver fluids). In the present study, we have optimized the viability of the *in vitro* experimental conditions.[9] It is critical to use a viable model to assess the performance of a prodrug that undergoes biotransformation in the skin. Without the added mechanism of prodrug biotransformation in the passive diffusion experiment, the true effect of metabolic rates on subsequent active drug (NTX) transdermal delivery rates would be neglected. Several researchers have suggested that prodrug biotransformation rates are critical in predicting active (parent) drug transdermal flux.[10–14]

Prodrug strategies to improve the oral bioavailability of NTX and mask its bitter taste for buccal delivery[15,16] have been reported in the literature, but no information exists about the use of these prodrugs transdermally. The goal of the oral bioavailability study was to block the 3-phenolic hydroxyl group of NTX to prevent conjugation during absorption, as opposed to increasing permeability. Ester prodrugs successfully increase the oral bioavailability and mask the bitter taste of NTX. Some researchers have suggested that a transdermal penetration enhancer formulation with NTX can achieve a desired plasma level in humans.[17] A major advantage of using transdermal prodrugs with prompt biotransformation is that their skin irritation and allergenic potential should only mirror the profile of the active drug, without the possible added toxicities of penetration enhancers.

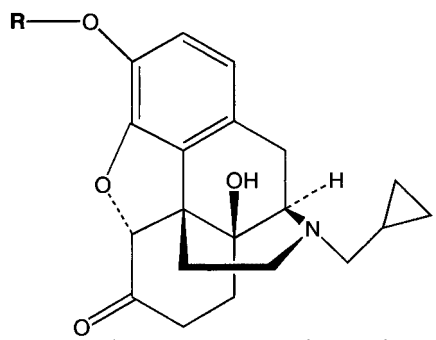
This study includes the synthesis and evaluation of six straight-chain 3-alkyl-ester prodrugs, NTX-3-acetate (ACE-NTX), NTX-3-propionate (PROP-NTX), NTX-3-butyrate (BUT-NTX), NTX-3-valerate (VAL-NTX), NTX-3-hexanoate (HEX-NTX), and NTX-3-heptanoate (HEP-NTX) (Figure 1). The critical information compiled in the preclinical evaluation of these prodrugs consists of melting points, solubilities, and human skin permeation measurements *in vitro*. This study will help us to identify and quantify the critical parameters involved in the optimization of transdermal NTX prodrug flux and concurrent metabolism, in order to improve drug therapy for opioid and alcohol addiction recovery by creating a transdermal dosage form in the future.

MATERIALS AND METHODS

NTX base was purchased from Mallinckrodt Inc., St. Louis, MO. The prodrugs were synthesized directly from NTX base. The melting points of the drugs were measured on a hot-stage melting point apparatus (Fisher-Johns, Pittsburgh, PA). Reagent-grade chemicals were obtained from Sigma Chemical Company (St. Louis, MO).

Quantitative Analysis

The high-pressure liquid chromatography (HPLC) assay was developed from the methods of Hussain



Drug	R-group	MW	MP (°C)
NTX	-H	341	166-169
ACE-NTX	$-COCH_3$	383	114-116
PROP-NTX	$-COCH_2CH_3$	397	147-149
BUT-NTX	$-CO(CH_2)_2CH_3$	411	106-108
VAL-NTX	$-CO(CH_2)_3CH_3$	425	83-85
HEX-NTX	$-CO(CH_2)_4CH_3$	439	62-64
HEP-NTX	$-CO(CH_2)_5CH_3$	453	58-60

Figure 1. Structures, molecular weights (MW), and melting points (MP) of NTX and its ester prodrugs.

et al.[15] The HPLC system consisted of a Waters 717 Autosampler, 501 Pumps, and a 484 Tunable UV Absorbance Detector with Millennium Chromatography Software. A reversed-phase 22-cm Brownlee C-18 Spheri-5 μm column (and guard column) were used with the ultraviolet (UV) detector set at a wavelength of 215 nm. The mobile phase consisted of 0.1% trifluoroacetic acid (adjusted to pH 3 with triethylamine):acetonitrile:sodium heptane sulfonate (300 mL:700 mL:0.3 g) at a flow rate of 1.5 mL/min. One hundred microliters of sample were injected onto the column. Standard curves exhibited excellent linearity over the entire concentration range employed in the assays. The assay sensitivity was 10 ng/mL.

The drugs were isolated from the buffer samples by solid-phase extraction (Oasis HLB, Waters Corp., Milford, MA). The solid-phase extraction cartridge was pretreated with 1 mL of methanol and 1 mL of water before the aqueous drug sample was run through the cartridge. After running the sample through the cartridge, the sample was washed with 1 mL of 5% methanol:water, and eluted with acetonitrile. Sample recovery was 98%.

Prodrug Synthesis

The NTX prodrugs were synthesized according to the method by Hussain and coworkers,[15] with slight modifications. Typically, NTX base (50 mg, 0.13 mmol) was dissolved in 1 mL of methylene chloride and triethylamine (50 μL, 0.7 mmol). The solution was placed in an ice bath and stirred. The acid chloride (0.15 mmol) of the desired prodrug moiety, or a solution thereof in methylene chloride, was added in a dropwise manner. After addition was complete, the reaction mixture was allowed to warm up to room temperature and stirred for an additional 4 h. The progress of the reaction was followed with thin layer chromatography (TLC): small samples (10 μL) of the reaction mixture were spotted on silica gel plates and developed in an *n*-butanol:acetic acid:water (10:1:1) cosolvent system. After drying, the plates were sprayed with 10% H_2SO_4 in ethanol and heated at 100°C for 5 min. NTX and its prodrugs were detected as brown spots with typical R_f values (e.g., NTX = 0.4 and VAL-NTX = 0.6). After completion of the reaction, the methylene chloride solution was washed with 10% aqueous sodium carbonate and then with water, dried over anhydrous sodium sulfate, filtered, and reduced to a small volume on a rotary evaporator. The esters were precipitated by adding excess petroleum ether, and the desired product was filtered and air-dried. Purity was checked by reversed-phase HPLC with UV detection (230 nm) using a water:acetonitrile gradient (0–100% acetonitrile in 20 min) on a C18 analytical column. If the purity was <95%, the compounds were further purified by preparative HPLC. The final products were characterized by ^1H nuclear magnetic resonance spectroscopy (NMR, IBM FTNMR NR/250) and mass spectrometry. ACE-NTX *m/e* was 383.17, PROP-NTX *m/e* was 397.19, BUT-NTX *m/e* was 411.20, VAL-NTX *m/e* was 425.22, HEX-NTX *m/e* was 439.24, and HEP-NTX *m/e* was 453.25.

Solubilities

The solubilities of NTX and its esters were obtained by equilibrating large excesses of each substance with the respective solvent, light mineral oil, or pH 7.4 HEPES-buffered Hank's balanced salts solution at 32°C. To hasten the attainment of equilibrium, each slurry was continuously shaken in a sealed container in a shaking incubation oven. Samples were taken, filtered using a warmed glass syringe and syringe filter (mineral oil:Millex FG-13, Millipore and buffer: glass microfiber, Gelman), measured with respect to volume, and diluted with the appropriate amount of acetonitrile or ethyl acetate. The diluted samples were analyzed by HPLC. The initial 40% of each filtrate was discarded to eliminate the possibility that adsorption of drug on the filter and/or the filtering apparatus might influence the solubility determination. The sampling procedure was repeated at least once for each sample. The equilibration times for all the studies were 8 h.

In Vitro Diffusion Studies

Skin excised during abdominal reduction surgery from 19 patients was used for the permeation studies. Skin samples were sectioned (200 ± 20 μm) from the excised abdominal tissue using a Padgett dermatome; some skin samples were frozen at −80°C and others were used immediately. A PermeGear flow-through (In-Line, Riegelsville, PA) diffusion cell system was used for the permeation studies. Data were gathered from human skin from each individual by using three cells with NTX in the donor compartment and four cells with the prodrug in the donor compartment. The receiver fluid was isotonic phosphate buffer at pH 7.4 in some experiments and, in more recent experiments, it was switched

to a HEPES-buffered Hank's balanced salts solution that has been shown to help preserve tissue viability.[9] The flow rate of the receiver fluid was set at 1.1 mL/h to help maintain sink conditions. The drugs were isolated from the receiver samples by solid-phase extraction and stored at 4°C until HPLC quantitation. The skin surface temperature in the diffusion cells was maintained at 32°C with a circulating water bath. The diffusion experiment was initiated by charging the donor compartment with 0.25 mL of drug suspended (saturated solution) in light mineral oil.

The permeation data were plotted as the cumulative amount of NTX collected in the receiver compartment as a function of time. The flux value for a given run was calculated from Fick's First Law of diffusion:

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

In eq. 1, J_s is the steady-state flux, M is the cumulative amount of intact drug permeating the skin, A is the area of the membrane (0.95 cm^2), K_p is the effective permeability coefficient in cm/h, and ΔC is the concentration difference of drug in the donor and receiver. Because buildup in the receiver cell was kept to a minimum throughout the studies, ΔC is approximated by the donor concentration. When saturated drug solutions are used in the donor compartment, the maximum flux, or J_{max}, can be measured for the compound of interest. For example, the J_{max} values for NTX from a NTX-saturated solution in mineral oil and a NTX-saturated solution in HEPES-buffered Hank's balanced salts are 1.0 ± 0.5 and 1.1 ± 0.4 μg/cm^2/h (standard deviation, $n = 3$), respectively.

Drug disposition in the diffusion cell skin samples was measured at the end of the 48-h experiment. The skin sample was removed from the diffusion cell and rinsed with distilled water to remove the surface formulation. Further removal of the skin surface formulation was accomplished by blotting with a paper towel and stripping with adhesive tape applied to and quickly removed from the surface two times. The treated area of the skin was cut from the skin sample and minced with a scalpel. The wet tissue weight was recorded, and the sample was placed in a vial with 10 mL of acetonitrile. The drug was extracted from the tissue by sonicating the solution for 10 min and shaking the vial overnight. The samples were assayed by HPLC and reported as micromole of drug per gram of wet tissue weight. The mean molar percentage of regenerated NTX to total drug extracted from the skin was calculated for each prodrug.

RESULTS AND DISCUSSION

The melting points of the prodrugs were measured because this physicochemical property is often easily related to the solubilities of the prodrugs.[18] The downward trend in melting points as a function of increasing alkyl chain length can be seen in Figure 1. The prodrug melting points decreased as the alkyl chain was extended; by >100°C in the case of the heptyl ester. Our NTX prodrug melting point data matches those of others who have studied alkyl chain series.[18,19] The fact that the ester prodrugs exhibit lower melting points than NTX base strongly suggests that the added alkyl groups disorder the intracrystalline cohesion of the drug.

The solubilities of NTX and its 3-alkyl-ester prodrugs in free base form are shown in Table 1. As expected, the prodrugs have higher oil (nonpolar) solubilities than the parent drug. The prodrugs exhibit much lower aqueous solubilities than NTX base because of the lack of the free phenolic functional group, a group that increases aqueous solubility through hydrogen bonding. The aqueous solubilities appear to decrease with the increase in alkyl chain length. Other researchers have also observed this relationship.[19]

In almost all cases, the prodrugs were completely hydrolyzed on passing through the skin and appeared as NTX in the receiver compartment. Therefore, plots of the cumulative amounts of NTX permeated through human skin over time from saturated mineral oil solutions of either a prodrug or NTX base were constructed for data analysis. A representative NTX permeation profile is shown in Figure 2. Because human skin exhibits wide intersubject permeation variability, a comparison of the effect of the prodrug versus NTX within each individual skin sample provides valuable information. Trace amounts of straight-chain prodrug detected in the receiver solutions were converted into NTX equivalents and incorporated into the total NTX flux from each prodrug. Steady-state fluxes calculated using the terminal, linear regions of the curves are summarized in Figure 3 and Table 1. The slopes of these regions, equal to J_{max} for a run, were determined using linear regression analysis. In all cases the coefficients of

Table 1. Properties of Naltrexone and its Ester Prodrugs

Drug	Calculated[a] Log P[b]	Light Mineral Oil Solubility at 32°C (S_{oil}), mM	Hank's Buffer Solubility at pH 7.4 and 32°C (S_{aq}), mM	Mean NTX J_{max} from Mineral Oil, nmol/cm^2/h	Permeability Coefficient from Mineral Oil ($K_p \times 10^4$), cm/h
NTX	0.355	0.26 ± 0.01[c]	15.2 ± 1.6[c]	2.5 ± 1.5[c]	95
ACE-NTX	0.361	2.04 ± 0.02	1.93 ± 0.01	15.6 ± 6.3	77
PROP-NTX	0.890	4.41 ± 0.33	1.25 ± 0.04	11.1 ± 3.7	25
BUT-NTX	1.419	4.16 ± 0.09	1.05 ± 0.01	5.6 ± 0.7	13
VAL-NTX	1.948	9.62 ± 0.02	0.59 ± 0.01	6.7 ± 2.8	7
HEX-NTX	2.477	6.95 ± 0.14	0.28 ± 0.01	8.6 ± 2.2	12
HEP-NTX	3.006	7.62 ± 0.40	0.18 ± 0.03	7.1 ± 1.1	9

[a]Calculated from Daylight® 4.51 Software.
[b]Log P = log octanol/water partition coefficient.
[c]Experimental values shown with standard deviations.

determination for the lines were >0.96, and in most cases >0.99. Lag times were determined by extrapolating the steady-state curves (terminal, linear portion) to the X-axis. The lipophilic prodrugs provided a higher flux (J_{max}) of NTX across the skin than NTX base (one-way ANOVA, Tukey post-hoc analysis $p < 0.05$ for all prodrugs except BUT-NTX). ACE-NTX, PROP-NTX, and HEX-NTX had the highest flux of NTX across the skin. Relative permeability coefficients (K_p) were calculated from the mean values of the mineral oil solubilities and J_{max} for each drug (Table 1). These are relative K_p values rather than true permeability coefficients of the prodrugs because bioconversion to NTX occurred rapidly during the diffusion experiments. In 70% of the prodrug experiments, a decreased lag time was observed for the prodrugs versus NTX (seen also in Figure 2).

Drug disposition data from the human skin samples used in the diffusion studies are summarized in Figure 4. A significant extent of parent drug regeneration in the skin was evident in all of the straight-chain prodrug experiments. The mean

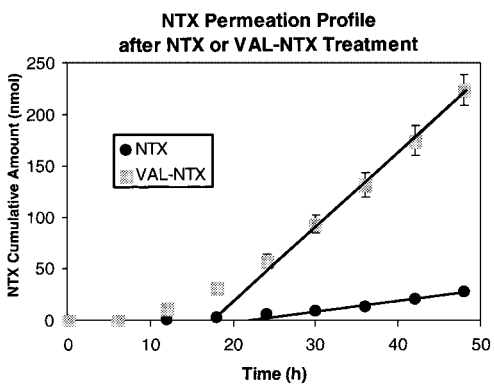


Figure 2. Representative permeation profiles for the diffusion of NTX from saturated solutions through human skin at 32°C. Data represent the mean ± standard deviation of three cells with NTX in the donor compartment and four cells with the VAL-NTX in the donor compartment, using human skin from one individual. The concurrent flux of intact prodrug was negligible.

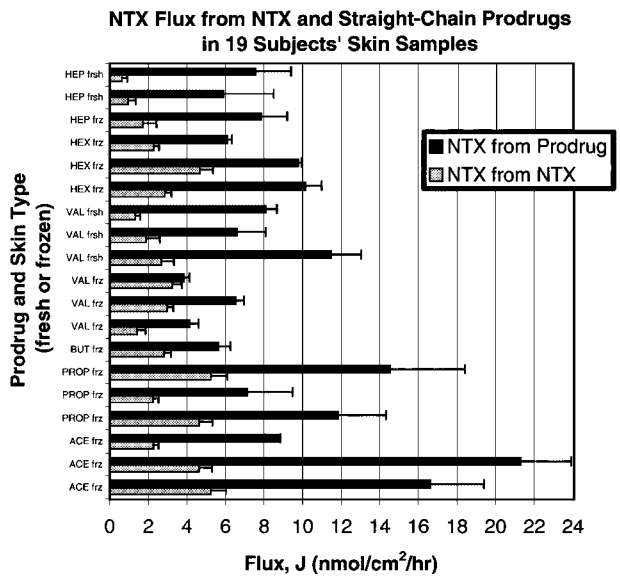


Figure 3. *In vitro* NTX flux from NTX and prodrugs. Data represent the mean ± standard deviation of three cells with NTX in the donor compartment and four cells with the prodrug in the donor compartment, using human skin from one individual. The skin sample storage conditions are shown for each experiment. Previously frozen skin samples (7–12 months) are indicated by frz, and fresh samples are indicated by frsh.

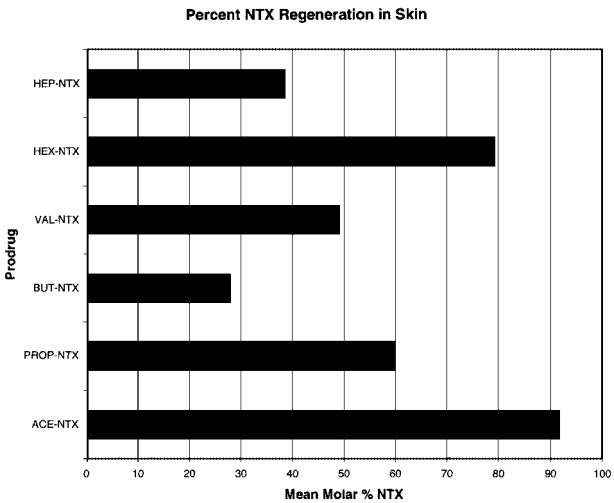


Figure 4. Drug disposition in human skin after a 48-h permeation experiment following application of drugs as saturated solutions in light mineral oil. Data represent the mean molar percentage of regenerated NTX to total drug extracted from the skin.

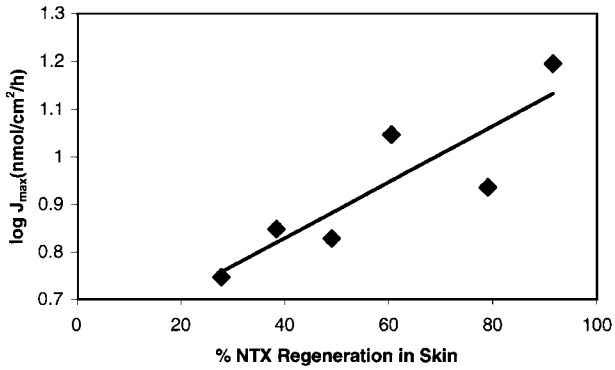


Figure 5. A plot of mean log J_{max} values for each prodrug versus percent NTX regeneration in the skin at the end of the 48-h diffusion experiment.

molar percentage of regenerated NTX to total drug extracted from the skin ranged from 28 to 91%. Skin esterases are quite resistant to the stresses of freezing and storage,[22] which is why we see prodrug conversion in the frozen skin experiments. The rapid enzymatic conversion rate of the prodrugs is a positive attribute for drug delivery because the active drug would be quickly released in the body after crossing the skin. Future experiments using a suitable animal model will enable us to correlate our *in vitro* human skin observations with *in vivo* data.

The increased NTX prodrug oil solubility apparently provided an increase in skin diffusion flux rates (J_{max}) *in vitro*, although there is no clear relationship between the level of oil solubility and the level of flux increase. The amount of total drug detected in the skin was significantly greater (a minimum of twofold) after treatment with the prodrug solutions compared with treatment with NTX base. The relative permeability coefficients decrease with increasing prodrug oil solubility, so the K_p does not seem to be responsible for the increased flux. However, the rate of appearance of parent drug in the receiver compartment appears to be linked to the extent of enzymatic conversion of the prodrugs in the skin (Figure 5, $r^2 = 0.76$). This phenomenon has been seen with hydrocortisone esters studied in hairless mouse skin.[10] We are currently developing diffusion experiments using esterase inhibitors to quantify the effect that metabolism has on flux enhancement.

Roberts and Sloan have suggested that the polar and nonpolar solubilities of topical prodrugs are critical for prediction of the J_{max} from vehicles other than water.[20] These authors used a modification of the Potts and Guy[21] equation to predict the flux of several types of prodrugs across hairless mouse skin. Analysis of the fit of the NTX prodrug data to the following equation, a modified form of the Roberts and Sloan equation, provided an r^2 of 0.96 (MicroMath Scientist software):

$$\log J_{max} = x + y\log S_{oil} + (1-y)\log S_{aq} - z\mathrm{MW} \quad (2)$$

where S_{oil} is the mineral oil solubility, S_{aq} is the Hank's buffer solubility, and MW is the molecular weight of the prodrug. The parameter estimates were $x = 4.36$ (± 4.79), $y = 0.97$ (± 0.40), and $z = 0.010$ (± 0.012). The major difference seen between the fit of the NTX prodrugs and the fit of the Roberts and Sloan prodrugs is that increased aqueous solubility has much less influence on the flux of the NTX prodrugs than on the flux of the Roberts and Sloan prodrugs. It seems logical that aqueous solubilities would be much more critical for the flux prediction of prodrugs that do not undergo rapid metabolic conversion in the skin, where the prodrug dissolution in the aqueous viable tissue could further slow the diffusion and bioconversion to parent drug, a more hydrophilic species that can diffuse through the viable tissue with less resistance than the prodrug in these cases. The NTX prodrugs bioconvert rapidly, and aqueous solubility is not as critical because mostly parent drug is diffusing through the aqueous viable tissue. Another reason for the mismatch between the aqueous solubility dependency of the Roberts and Sloan prodrugs and the

NTX prodrugs could be the use of different membranes. Roberts and Sloan used full-thickness hairless mouse skin, which may have a larger proportion of aqueous tissue than the split-thickness human skin used with the NTX prodrugs.

The straight-chain 3-alkyl-ester prodrugs have served as the first building block in the development of better prodrugs. These studies have taught us that oil solubility and prodrug bioconversion are critical in future prodrug design for further increases in the transdermal NTX delivery rate. Good *in vivo* correlation is always the goal of *in vitro* studies involving percutaneous penetration or any other route of drug delivery. The important step beyond the *in vitro* diffusion studies in this project is *in vivo* correlation for prodrug flux enhancement and metabolic potential as well. Understanding the quantitative structure–permeability relationships for transdermal prodrug flux and concurrent metabolism in humans *in vivo*, and the ability to predict transdermal absorption are the ultimate long-term goals of this project.

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