Bioconversion of Naltrexone and Its 3-O-Alkyl-Ester Prodrugs in a Human Skin Equivalent

DANA C. HAMMELL,[1] ELZBIETA I. STOLARCZYK,[1] MITCH KLAUSNER,[2] MOHAMED O. HAMAD,[1] PETER A. CROOKS,[1] AUDRA L. STINCHCOMB[1]

[1]Department of Pharmaceutical Sciences, College of Pharmacy, University of Kentucky, Lexington, Kentucky 40536-0082

[2]MatTek Corporation, Ashland, Massachusetts 01721

ABSTRACT: The purpose of this study was to compare the percutaneous absorption and bioconversion of naltrexone (NTX), naltrexone-3-O-valerate (VAL), and naltrexone-3-O-(2′-ethylbutyrate) (ETBUT) in a human skin equivalent model (EpiDerm^{TM}) and in fresh human skin *in vitro*. NTX diffusion and metabolism to 6-β-naltrexol (NTXol) were quantitated and compared in the EpiDerm^{TM} and in excised fresh human skin. VAL and ETBUT diffusion and bioconversion studies were also completed in EpiDerm^{TM}. Naltrexone bioconverted to levels of $3 \pm 2\%$ NTXol in the EpiDerm^{TM} and $1 \pm 0.5\%$ in fresh human skin. VAL hydrolyzed rapidly in the EpiDerm^{TM} and mainly ($93 \pm 4\%$) NTX was found in the receiver compartment, similar to human skin. More intact ETBUT permeated the EpiDerm^{TM} tissue compared to VAL, and only $15 \pm 11\%$ NTX was found in the receiver. Significantly higher fluxes of NTX and the prodrugs were observed with the EpiDerm^{TM} compared to human skin. A similar flux enhancement level was observed for VAL, compared to NTX base, in the EpiDerm^{TM} and the human skin. Metabolically active human epidermal models like EpiDerm^{TM} are useful as an alternative experimental system to human skin for prediction of topical/transdermal drug/prodrug bioconversion. © 2005 Wiley-Liss, Inc. and the American Pharmacists Association J Pharm Sci 94:828–836, 2005

Keywords: naltrexone; transdermal drug delivery; prodrugs; percutaneous absorption; *in vitro* models; tissue engineering; EpiDerm^{TM}

INTRODUCTION

Transdermal naltrexone delivery may help improve patient compliance in the treatment of narcotic dependence and alcoholism.[1–3] Transdermal patches are a much less invasive drug treatment option than the subcutaneous NTX implants that are currently under investigation. Motivated addicts could easily use once-a-week patch treatments at home, without the physician visits that would be required with implants or depot injections. The percutaneous penetration rate of NTX is subtherapeutic; however, straight-chain 3-O-alkyl-ester prodrugs of NTX have been shown to improve the percutaneous absorption rate of NTX across human skin *in vitro*.[4] Fresh human surgical waste skin provides a good estimate of clinical drug percutaneous absorption and biotransformation. However, human skin equivalent models can also be useful tools for assessing epidermal drug metabolism and topical/transdermal prodrug metabolic conversion.[5]

The main purpose of this study was to evaluate the transdermal naltrexone prodrugs' bioconversion rates in a human epidermis model, and compare these results with human surgical waste skin data. A branched-chain (naltrexone-3-O-(2′-ethylbutyrate) or ETBUT) and a straight-chain (naltrexone-3-O-valerate or VAL) prodrug were evaluated, along with naltrexone base (NTX). A branched-chain prodrug was originally designed

to improve the chemical stability of the ester linkage. However, these physicochemical property changes in this ETBUT prodrug resulted in a decreased transdermal NTX flux rate across human surgical waste skin *in vitro*, compared to the other successful straight-chain 3-O-alkyl-ester prodrugs.[4] The branched-chain and straight-chain prodrugs were purposefully compared in this study to see if similar results could be obtained in a human skin equivalent for drugs with significantly different metabolic stabilities. Prodrug bioconversion to NTX, and NTX conversion to 6-β-naltrexol (NTXol) were measured in the human skin equivalents. NTXol is naltrexone's major active metabolite formed after systemic administration in humans, and is believed to be critical to the pharmacologic effect of the drug.[6−9] Tissue homogenate bioconversion and chemical hydrolysis studies were used to assess the degree of NTX and prodrug metabolic stability.

Three slightly different models of the human skin equivalent were used, including EpiDerm[TM] 606, 606X, and 200. The EpiDerm[TM] 200 is available in smaller sized wells (8 mm internal diameter) than the larger EpiDerm[TM] 606 and 606X (22 mm diameter). EpiDerm[TM] 200 and 606 are identical tissues, differing only in the size of the well in which they are cultured. This tissue is made up of normal human epidermal keratinocytes (NHEK) derived from neonatal-foreskin tissue. EpiDerm[TM] 606X differs slightly from 606 in stratum corneum lipid profile, thereby providing slightly different barrier characteristics. Published reports of prodrug bioconversion studies in commercial human skin equivalent models like EpiDerm[TM] appeared in the literature as early as 1994.[10] These human skin equivalent models are becoming more popular for investigating substrate bioconversion that results in therapeutic and toxic effects, especially in studies examining enzyme induction and inhibition, carcinogenesis, irritation, cytotoxicity, and inflammation. Corrections for permeability differences in skin equivalent models can be made and incorporated into predictive models of transdermal ester-prodrug/drug diffusion and bioconversion.

MATERIALS AND METHODS

Chemicals

NTX base was purchased from Mallinckrodt Inc (St. Louis, MO). 6-β-Naltrexol hydrochloride (NTXol) was a gift from the National Institute on Drug Abuse research resources drug supply system (NIDA, Research Triangle Park, NC). VAL and ETBUT were synthesized from the NTX free base as previously described.[4] Hanks' balanced salts modified powder, sodium bicarbonate, and light mineral oil were purchased from Sigma Chemical (St. Louis, MO). 4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), gentamicin sulfate, trifluroacetic acid (TFA), triethylamine (TEA), methanol, ammonium acetate, ammonium hydroxide, and acetonitrile (ACN) were obtained from Fisher Scientific (Fairlawn, NJ).

Quantitative Analysis

The HPLC assay was developed from the methods of Hussain et al.[11] The EpiDerm[TM] EPI-606 VAL samples were analyzed using a Waters 717 Plus Autosampler, 1525 Binary Pump, 1500 Column Heater, and a 2487 2-channel UV/VIS Detector with Breeze Software. 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 comprised of acetonitrile (ACN):0.1% trifluoroacetic acid (TFA) (85:15). The 0.1% TFA buffer was adjusted with triethylamine (TEA) to a pH of 3.0. The flow rate of the mobile phase was 1.5 mL/min with 100-μL injections of the samples. Standards were analyzed with each set of diffusion samples, and the linear regression of sample concentration versus peak area exhibited excellent linearity ($R^2 \geq 0.99$). The sensitivity of the assay was 250 ng/mL for VAL and 250 ng/mL for NTX.

The EpiDerm[TM] EPI-606X ETBUT samples were analyzed using a Perkin-Elmer Series 200 Autosampler, Pump, Column Oven, and a 785A UV/VIS Detector with Turbochrom Professional Version 4.1 Software. 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 comprised of ACN:0.1% TFA (85:15). The 0.1% TFA was adjusted with TEA to a pH of 3.0. The flow rate of the mobile phase was 1.5 mL/min with 100-μL sample injections. Standards were analyzed with each set of diffusion samples and exhibited excellent linearity over the entire concentration range employed in the assays. The

sensitivity of this assay was 50 ng/mL for ETBUT and NTX.

The NTX/NTXol samples were analyzed using an Alliance Waters 2690 Separations Module, Micromass ZQ detector, and a 996 Photodiode Array Detector with MassLynx 3.5 Software. Electrospray ionization (ESI) was done in the positive mode. A Symmetry® C-18 reversed-phase 5-μm column (2.1 × 150 mm) with a Symmetry® Sentry C-18 reversed-phase 3.5-μm guard column (2.1 × 10 mm) was used. The mobile phase was comprised of ACN w/5% 2 mM ammonium acetate and 2 mM ammonium acetate w/5% ACN and used at a gradient flow. This gradient flow rate was set at 0.25 mL/min with 20-μL injections of the samples. Standards were analyzed with each set of diffusion samples and exhibited excellent linearity. The sensitivity of the assay was 50 ng/mL and 5 ng/mL for NTX and NTXol, respectively.

In Vitro Diffusion Studies

Skin harvested during abdominal reduction surgery was used for the diffusion studies. Skin tissues were excised by using a Padgett dermatome set at 250 μm. A PermeGear flow-through (In-Line, Riegelsville, PA) diffusion cell system was used for the diffusion studies. Data was collected utilizing three different specimens of human tissue from different donors. Four diffusion cells were used for each NTX treatment on each specimen of skin. A receiver solution of HEPES-buffered Hanks' balanced salts solution at pH 7.4 with gentamicin was collected from the diffusion cells at a flow rate of 1.1 mL/h. A 0.25-mL aliquot of NTX-saturated solution in light mineral oil (0.24 mM)[12] was applied to each piece of skin in the diffusion cells. Samples were collected in 6-h increments for 48 h to obtain steady state, and the diffusion cells were kept at 32°C with a circulating water bath. After each 6-h sample was obtained, it was stored at 4°C until solid phase extraction was used to concentrate the sample.

EpiDerm™ (EPI-606 and EPI-606X, MatTek Corporation, Ashland, MA) was used for the human skin equivalent diffusion studies. Twenty-two millimeter diameter pieces of the EpiDerm™ tissue were removed from the cell culture inserts using forceps and the tissues were mounted into a PermeGear flow-through (In-Line) diffusion cell system. Data was collected utilizing three different lot numbers of tissue for the EPI-606 and EPI-606X. For each EPI-606 tissue shipment, three

cells were used for NTX and three more cells for VAL treatments. For the EPI-606X tissue, two cells were used for NTX with the first shipment. For the second shipment, four cells were used for NTX and four additional cells for ETBUT treatments. For the third shipment, four cells were used for NTX. A receiver solution of HEPES-buffered Hanks' balanced salts solution at pH 7.4 with gentamicin was collected at a flow rate of 1.1 mL/h. Saturated drug solutions in light mineral oil (VAL 9.62 mM[4] and ETBUT 5.60 mM) were applied to the skin in the cells. Each cell received 0.20–0.25 mL of the respective drug solution. Samples were collected in 6-h increments for 24 h, and a circulating water bath maintained the diffusion cells at 32°C. After each 6-h sample was obtained, it was stored at 4°C until solid phase extraction was completed.

The concentration of NTX, VAL, and ETBUT in each piece of tissue was determined after completion of the diffusion experiment. Each piece of tissue was rinsed with filtered water and blotted with a paper towel. The tissue was tape stripped twice to remove surface drug. The area of skin that was in contact with the drug solution was cut out, chopped into tiny pieces, and placed in a preweighed vial. The vial was then weighed to calculate the mass of the skin. For the NTX diffusion cells, 10 mL of ACN:filtered water (75:25) was added to the tissue-containing vial and parafilm was used to seal the lid. For the VAL and ETBUT diffusion cells, 10 mL of ACN was added to the vial and parafilm was also used to seal the lid. The vials were vortexed, sonicated for 10 min, and placed in a shaker overnight. The following day, a 1 mL sample was obtained from the vials and placed in HPLC vials for analysis. The mean molar percentage of regenerated NTX to total drug extracted (or mean molar percentage of NTXol to total drug extracted) from the skin was calculated for each drug treatment.

Sample Processing

VAL and ETBUT samples collected from the fraction collector were processed through solid-phase extraction cartridges (30 mg 1 cc Oasis HLB, Waters Corp, Milford, MA). The cartridges were preconditioned with 1 mL of methanol and then 1 mL of filtered water. Five milliliters of each sample was run through each cartridge. The sample-loaded cartridges were then washed with 1 mL of 5% methanol in filtered water. Samples were eluted with enough acetonitrile to obtain a

sample with a final volume of 1 mL, and this eluant was transferred into autosampler vials for chromatographic analysis.

NTX samples collected from the fraction collector were processed through solid-phase extraction cartridges (30 mg 1 cc Oasis HLB, Waters Corp.). The cartridges were preconditioned with 2 mL of methanol, 1 mL of filtered water, followed by 500 μL of 1 M ammonium hydroxide. Five milliliters of each sample containing 15 μL of ammonium acetate were vortexed and then extracted through the cartridges. The drug-loaded cartridges were then washed with 1 mL of 5% methanol in filtered water, and then 1 mL of filtered water. Samples were eluted with 2 mL of 2-propanol followed by 1 mL of ACN. Samples were evaporated under nitrogen and reconstituted with 500 μL of ACN. Reconstituted samples were placed in autosampler vials containing low-volume inserts for LC/MS analysis.

In Vitro Homogenate Metabolism Studies

EpiDerm^{TM} (EPI-200, MatTek Corporation, Ashland, MA) was used for the homogenate metabolism studies. EPI-100-ASY assay medium was heated to 37°C in a water bath, and 0.3 mL was transferred to each well in a sterile 24-well plate. Tissue inserts were placed in the wells, covered, and incubated at 37°C for 1 h in 5% CO_2. Following incubation, tissue was removed from each insert using forceps and rinsed with 750 μL of Dulbecco's Phosphate Buffer Solution without $MgCl_2$ and $CaCl_2$. Tissue was placed in a 5-mL ground glass homogenizer containing 1 mL of chilled HEPES-buffered Hanks' balanced salts solution pH 7.4 with gentamicin. After all the tissue from the cells was added and homogenized, an additional 3 mL of HEPES-buffered Hanks' balanced salts solution with gentamicin was added. An aliquot of 200 μL was obtained from the homogenate and frozen for the protein assay. The remaining homogenate was either frozen or used for a drug incubation experiment.

Homogenate was vortexed and filtered through a Spectra/Mesh polypropylene filter (1 mm mesh opening, 1020 μm thickness, Spectrum Laboratories, Inc, Rancho Dominguez, CA). Homogenate was then divided into vials and placed in a 37°C shaking water bath. Homogenate aliquots were spiked with the respective drug, vortexed, and a zero time point sample was obtained. Samples were obtained at several time points for VAL, ETBUT, and NTX incubations. The homogenate

samples were added to microcentrifuge tubes containing 450 μL of ACN and 50 μL of ethyl acetate, vortexed, and centrifuged for 20 min at 10,000 × g in the Micro 13 microcentrifuge (Fisher Scientific, Fairlawn, NJ). Supernatant was removed, transferred to 3-mL culture tubes, and evaporated under nitrogen in a 37°C water bath. Samples were reconstituted with 350 μL of ACN, vortexed, and sonicated for 10 min. Samples were transferred into microcentrifuge tubes and spun for 5 min at 10,000 × g. Finally, the extracted supernatant was placed into autosampler vials containing low-volume inserts for chromatographic analysis.

For chemical hydrolysis comparison, 1 mL of HEPES-buffered Hanks' balanced salts solution with gentamicin was placed into a 15-mL conical tube, placed in a 37°C shaking water bath, and spiked with the respective drug. Initial time zero samples were obtained and additional samples were obtained over a 1-week time period. For drug extraction, the samples were added to microcentrifuge tubes containing 450 μL of ACN and 50 μL of ethyl acetate, vortexed, and centrifuged for 20 min at 10,000 × g. Supernatant was removed, transferred to 3-mL culture tubes, and evaporated under nitrogen in a 37°C water bath. Samples were reconstituted with 350 μL of ACN, vortexed, and sonicated for 10 min. Samples were placed into autosampler vials containing low-volume inserts for chromatographic analysis.

Protein Assay

Bovine albumin was dissolved in HEPES-buffered Hanks' balanced salts solution with gentamicin to give a 1-mg/mL solution for the preparation of dilutions (0–40 μg/mL) for a calibration curve (Bio-Rad Protein Assay, Hercules, CA). HEPES-buffered Hanks' balanced salts solution with gentamicin was added to the homogenate (60-fold dilution). A flat-bottomed 96-well plate was loaded with 160 μL of standard or sample in triplicate. Dye reagent concentrate (40 μL) was added to the wells and the plate was incubated at room temperature for 5 min, shaken, and read on the SpectraMax 190 (Molecular Devices Corp, Sunnyvale, CA) at an absorbance of 595 nm. Results were reported in mg/mL of total protein.
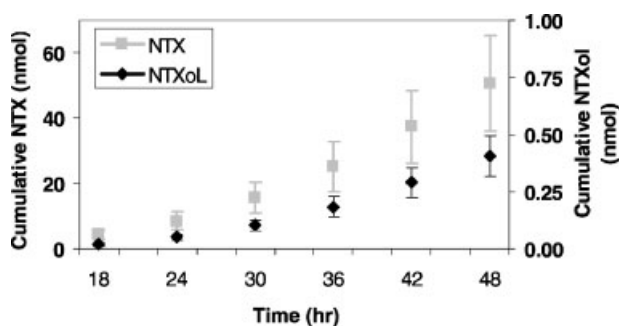
RESULTS AND DISCUSSION

The diffusion data were plotted as the cumulative quantity of drug collected in the receiver compart-

ment as a function of time. The flux value for a given experiment was computed from Fick's First Law of diffusion:
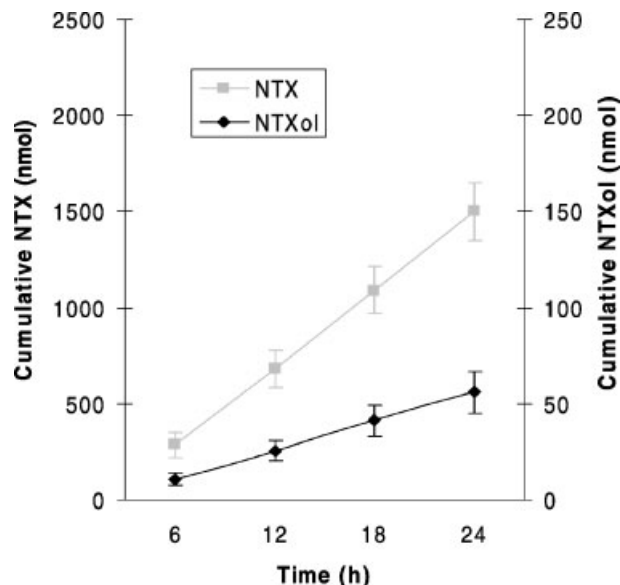
$$\frac{1}{A}\left(\frac{dM}{dt}\right) = J_s = K_p \Delta C \qquad (1)$$

In eq. 1, J_s is the steady-state flux, M is the total quantity of the drug permeated in time t, A is the area of tissue in the diffusion cell (0.95 cm^2), K_p is the effective permeability coefficient in cm/h, and ΔC is the difference in concentration of drug in the donor and receiver. Sink conditions were maintained throughout the experiments.

Naltrexol formation from NTX in human skin has not been reported previously in the literature; however, it is well known that 6-β-naltrexol is the major metabolite formed after oral NTX dosing in humans. NTX metabolized to $1 \pm 0.5\%$ (0.41 ± 0.20 nmol) NTXol in the fresh human surgical waste skin (Fig. 1) and to $3 \pm 2\%$ (28–55 nmol) NTXol in the intact EpiDerm^{TM} human skin equivalent (Figs. 2 and 3). These NTXol percentages of the cumulative NTX that appeared in the receiver compartments are not significantly different, and are reasonably comparable with the extent of epidermal metabolism of other topically applied drugs.[13] NTXol formation percentages in human skin and EpiDerm^{TM} disposition studies were also comparable (Table 2). Although the mean NTX flux across the EpiDerm^{TM} tissue (Table 1) is approximately 30-fold (19–38-fold range) higher than that across excised human surgical waste skin (2.5 ± 1.5 nmol/cm^2/h),[4] the percentage of NTXol formed from the NTX diffused is consistent. This same 30-fold increased mean flux in the EpiDerm^{TM} 606 (Table 1) versus that in

Figure 2. Permeation profile of NTX and NTXol formed from NTX in EpiDerm^{TM} 606. Three diffusion cells were used with each shipment. Points represent mean $\pm$ standard error ($n = 9$ cells).

human surgical waste skin (6.7 ± 2.8 nmol/cm^2/h NTX + VAL)[4] is also seen with the VAL prodrug. Constant steady-state flux rates of NTXol were observed throughout the 48-h human skin experiments and the 24-h EpiDerm^{TM} experiments. The two different EpiDerm^{TM} model types, 606 and 606X, provided different mean flux values for NTX, indicating the necessity of staying with one model or the other for rank order permeation comparisons of different drugs. The percent NTXol formation from NTX in human skin appeared to match the EpiDerm^{TM} 606X (Fig. 4) value more
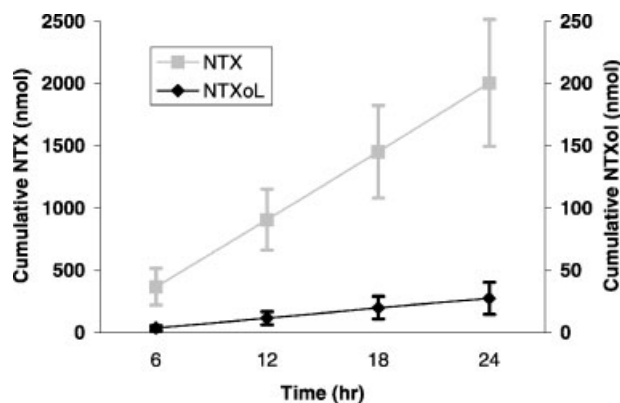

Figure 1. Permeation profile of NTX and NTXol formed from NTX in fresh human tissue. Three pieces of human tissue from three different donors were used. Points represent mean $\pm$ standard error (NTX, $n = 10$ cells and NTXol, $n = 7$ cells).

Figure 3. Permeation profile of NTX and NTXol formed from NTX in EpiDerm^{TM} 606X. Two different shipments were used. Points represent mean $\pm$ standard error ($n = 4$ cells).

Table 1. Tissue Permeation Data

Drug Treatment	EpiDerm^{TM}	Drug Measured	Mean Flux, J (nmol/cm^2/h) $\pm$ Standard Deviation
NTX[a]	606	NTX	48.6 ± 25.2
		NTXol	2.8 ± 1.3
NTX[b]	606X	NTX	96.3 ± 51.5
		NTXol	1.4 ± 0.7
VAL[a]	606	VAL	16.2 ± 18.2
NTX-O-CO(CH$_2$)$_3$CH$_3$		NTX	185.6 ± 81.8
		NTX + VAL	201.8 ± 90.4
ETBUT[c]	606X	ETBUT	25.2 ± 4.6
NTX-O-COCH(CH$_2$CH$_3$)$_2$		NTX	4.4 ± 3.2
		NTX + ETBUT	29.6 ± 4.8
	Human Skin		
NTX		NTX	1.9 ± 1.8
		NTXol	0.02 ± 0.01
VAL[d]		NTX + VAL	6.7 ± 2.8
ETBUT		NTX + ETBUT	1.97 ± 0.56

[a]$n = 9$ cells.
[b]$n = 4$ cells.
[c]$n = 3$ cells.
[d]Data from ref. 4.

closely than the EpiDerm^{TM} 606 value (Table 1). The 606X membrane model stratum corneum has a slightly different biochemical makeup than the 606 model, but still has the same bilayer structure consisting of intact stratum corneum and viable epidermal layer pictured in the Figure 4 micrograph. Either model seems acceptable for diffusion experiments, but care must be taken when comparing data from one model to another.

The mean steady-state flux values from the prodrugs studied in the EpiDerm^{TM} tissue are described in Table 1. Although the flux from these prodrugs was higher than experiments done with human skin,[4] the percent bioconversion to NTX in the tissue was comparable to the previous human

Table 2. Skin Disposition Percentages from Extracted Tissue Samples at the End of the Diffusion Experiments

Drug Treatment	Tissue	% Drug of Total Extracted Drug $\pm$ Standard Deviation
		NTXol
NTX	EpiDerm	0.32 ± 0.25
NTX	Human	0.20 ± 0.20
		NTX
VAL	EpiDerm	37 ± 30
VAL	Human	49 ± 47
ETBUT	EpiDerm	13 ± 18
ETBUT	Human	26 ± 7

skin diffusion studies. VAL hydrolyzed quickly in the skin equivalent, and mainly ($93 \pm 4\%$) NTX was found in the receiver compartment. The VAL prodrug increased the total NTX equivalent delivery rate across the EpiDerm^{TM} by a factor of fourfold greater than the delivery rate from NTX base, which is similar to the flux enhancement of threefold seen in the human skin experiments.[4] More intact ETBUT permeated the EpiDerm^{TM} tissue, and only $15 \pm 11\%$ NTX was found in the receiver. This is comparable to what has been seen in human skin ($35 \pm 4\%$ NTX, unpublished data). Tissue disposition studies also showed comparable NTX formation percentages from the prodrugs in human skin and EpiDerm^{TM} (Table 2). The ETBUT prodrug provided a NTX equivalent flux that is less than that from NTX base, which has also been seen in human skin *in vitro* (1.97 ± 0.56 nmol/cm^2/h) and in hairless guinea pigs *in vivo* (unpublished data). Branched prodrugs may have decreased stratum corneum/vehicle partition coefficients and corresponding decreased permeability coefficients due to the molecular chain ordering (bulk) of the prodrug moiety.[14]
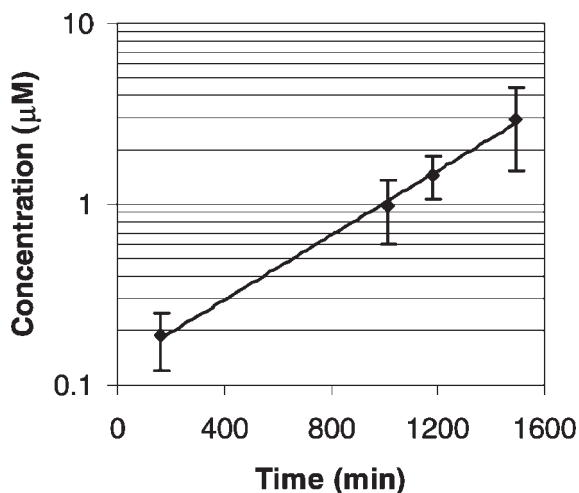
Figure 5 shows the formation of NTXol from NTX in an EpiDerm^{TM} 200 homogenate experiment. This homogenate metabolite formation corroborated the NTXol formation seen in the intact tissue experiments. To ascertain that this conversion was metabolic and not chemical hydrolysis
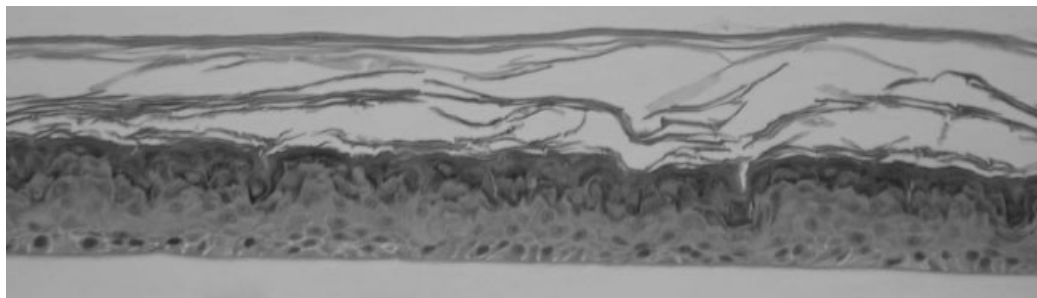
Figure 4. H&E stained cross-section of EpiDerm[TM] 606X tissue ($8\times$ objective).

alone, a corresponding buffer stability study was done at the same temperature and NTX concentration. No detectable levels of NTXol were found over a 1-week incubation of the chemical stability solution, proving that the NTXol formation was from metabolism of NTX by the epidermis.
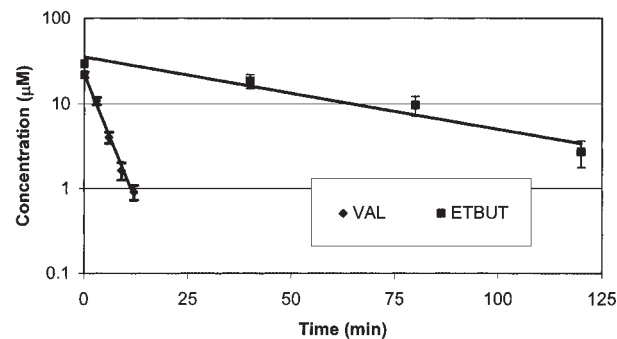
Figure 6 shows examples of the EpiDerm[TM] 200 homogenate study profiles with VAL and ETBUT. Profiles from homogenates containing identical protein concentrations were compared to each other. Protein concentrations ranged from 0.29–1.08 mg/mL of total protein in the homogenate samples. The prodrugs hydrolyzed to form NTX. As expected, the bulkier branched ester ETBUT hydrolyzed 14-fold slower than VAL in the enzyme-rich homogenate, due to steric hindrance of the former prodrug to ester hydrolysis (Fig. 7). The half-life for ETBUT was 36 min and the half-life for the rapidly hydrolyzable VAL was 3 min. There was no significant difference between the ester hydrolysis of the prodrugs in fresh versus previously frozen homogenate. The chemi-

cal hydrolysis of these prodrugs in the buffer at the same incubation temperature was studied for 1 week (Fig. 8). Chemical hydrolysis to NTX occurred slowly. VAL underwent chemical hydrolysis at a rate 1.6-fold faster than ETBUT. Approximate chemical hydrolysis half-lives for these prodrugs can be estimated at 62 h for VAL and 99 h for ETBUT. This chemical hydrolysis is not a major portion of the hydrolysis during the diffusion studies, as samples are obtained every 6 h and refrigerated immediately to slow chemical breakdown in the buffer receiver media.

Lamb et al. studied prodrug hydrolysis differences in a human surgical waste skin homogenate and in an intact human LDE Testskin bioequivalent model.[10] The metabolic activity in the Testskin model was one-fifth that of the surgical waste skin homogenate, but this is not unexpected when comparing intact tissue with homogenate. Homogenization of tissue exposes enzymes that may not be as readily available to the drug during transit through intact membranes. Prodrug and drug diffusion in intact tissue provides a rate-limiting step to bioconversion. Furthermore, it is also possible that intact skin equivalents, with drug


Figure 5. Formation of NTXol from NTX in EpiDerm[TM] 200 homogenate. Points represent mean $\pm$ standard deviation ($n = 2$ different shipments of tissue).

Figure 6. Bioconversion of VAL and ETBUT in MatTek EpiDerm[TM] 200 homogenate. Points represent mean $\pm$ standard deviation ($n = 4$ different shipments of tissue).

VAL
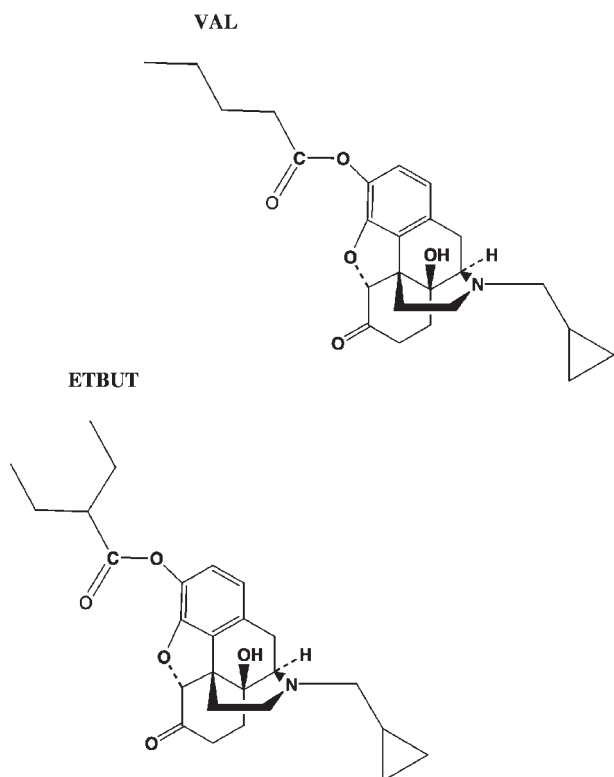
ETBUT

Figure 7. Chemical structures of VAL and ETBUT.

diffusion rates that often exceed diffusion rates in human surgical waste tissue, may also exhibit metabolism rates closer to saturation levels than what is seen with the surgical specimens. The exact metabolic activity was not compared in intact versus homogenized tissue for the NTX prodrugs, but rather drug and metabolite formation were compared in intact human and human equivalent tissues. One result that can be compared with the Lamb et al. study is the 93% and
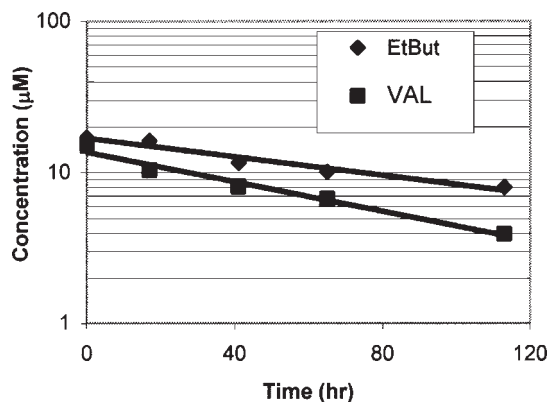
Figure 8. Chemical stability of VAL and ETBUT in Hanks' buffer at 37°C.

15% NTX formation after EpiDerm[TM] permeation from VAL and ETBUT, respectively, versus the ~100% and 35% NTX formation in human tissue. So with this comparison only, it would appear that human skin equivalents may have a decreased esterase metabolic activity compared to human skin, but these two sets of data are not significantly different based on the variability in metabolism that is observed in human and human-derived tissue experiments.

The purpose of this study was to assess the utility of using the EpiDerm[TM] skin equivalent model for evaluation of transdermal prodrugs and epidermal metabolism. Upon occasion, human surgical waste tissue, although the ideal biomembrane for *in vitro* transdermal studies, is difficult to obtain in large quantities, and arrives sporadically to the laboratory. To have an experimental tissue alternative, the EpiDerm[TM] model was contrasted and compared to previous human skin data. Although the flux values obtained in this skin equivalent tissue were significantly higher than those obtained in human skin, predictive and comparable data were still obtained. The percent formation of the important NTXol metabolite was similar in EpiDerm[TM] and human tissue. The flux enhancement data from the prodrugs, compared to NTX, was predictive of the enhancement seen in human skin diffusion studies. VAL has been identified as a successful prodrug in human skin *in vitro*,[4] as well as in the in the EpiDerm[TM] experiments. Conversely, ETBUT was not a successful prodrug under either of these experimental scenarios. Similar prodrug bioconversion profiles were observed in the intact EpiDerm[TM] and human skin experiments. EpiDerm[TM] homogenate drug bioconversion experiments seem to be useful for screening of metabolite formation and comparison of relative rates of bioconversion. In conclusion, metabolically active human epidermal models can be predictive tools used for topical/transdermal drug/prodrug bioconversion and transport studies. However, care should be taken not to overestimate drug transport rates and metabolite formation from these skin equivalent models.

ACKNOWLEDGMENTS

Corporation for their generous donation of Epi-Derm^{TM} for these studies.

REFERENCES

1. Carreno JE, Alvarez CE, San Narciso GI, Bascaran MT, Diaz M, Bobes J. 2003. Maintenance treatment with depot opioid antagonists in subcutaneous implants: An alternative in the treatment of opioid dependence. Addict Biol 8:429–438.

2. Volpicelli JR, Rhines KC, Rhines JS. 1997. Naltrexone and alcohol dependence. Role of subject compliance. Arch Gen Psychiatry 54:737–743.

3. Litten R, Allen J. 1998. Advances in the development of medications for alcoholism. Psychopharmacology (Berl) 139:20–33.

4. Stinchcomb AL, Swaan PW, Ekabo O, Harris KK, Browe J, Hammell DC, Cooperman TA, Pearsall M. 2002. Straight-chain naltrexone ester prodrugs: Diffusion and concurrent esterase biotransformation in human skin. J Pharm Sci 91:2571–2578.

5. Stinchcomb AL. 2003. Xenobiotic bioconversion in human epidermis models. Pharm Res 20:1113–1118.

6. McCaul ME, Wand GS, Rohde C, Lee SM. 2000. Serum 6β-naltrexol levels are related to alcohol responses in heavy drinkers. Alcohol Clin Exp Res 24:1385–1391.

7. Rukstalis MR, Stromberg MF, O'Brien CP, Volpicelli JR. 2000. 6-Beta-naltrexol reduces alcohol consumption in rats. Alcohol Clin Exp Res 24:1593–1596.

8. Porter SJ, Somogyi AA, White JM. 2000. Kinetics and inhibition of the formation of 6beta-naltrexol from naltrexone in human liver cytosol. Br J Clin Pharmacol 50:465–471.

9. Wang D, Raehal KM, Bilsky EJ, Sadee W. 2001. Inverse agonists and neutral antagonists at mu opioid receptor (MOR): Possible role of basal receptor signaling in narcotic dependence. J Neurochem 77:1590–1600.

10. Lamb KA, Denyer SP, Sanderson FD, Shaw PN. 1994. The metabolism of a series of ester pro-drugs by NCTC 2544 cells, skin homogenate and LDE testskin. J Pharm Pharmacol 46:965–973.

11. Hussain MA, Koval CA, Myers MJ, Shami EG, Shefter E. 1987. Improvement of the oral bioavailability of naltrexone in dogs: A prodrug approach. J Pharm Sci 76:356–358.

12. Pillai O, Hamad MO, Crooks PA, Stinchcomb AL. 2004. Physicochemical evaluation, *in vitro* human skin diffusion and concurrent biotransformation of 3-O-alkyl carbonate prodrugs of naltrexone. Pharmaceut Res 21:1146–1152.

13. Bickers DR, Mukhtar H. 1991. Dermal metabolism of drugs and carcinogens. In: Scott RC, editor. Predict. Percutaneous Penetration. London: IBC Tech Serv., pp 248–258.

14. Xiang TX, Anderson BA. 1998. Influence of chain ordering on the selectivity of dipalmitoylphosphatidylcholine bilayer membranes for permeant size and shape. Biophys J 75:2658–2671.