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Microneedle-assisted percutaneous delivery of naltrexone hydrochloride in Yucatan minipig: in vitro-in vivo correlation

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Abstract

Although microneedle-assisted transdermal drug delivery has been the subject of multiple scientific investigations, very few attempts have been made to quantitatively relate *in vitro* and *in vivo* permeation. The case of naltrexone hydrochloride is not an exception. In the present study a pharmacokinetic profile obtained following a “poke and patch” microneedle application method in Yucatan minipig is reported. The profile demonstrates rapid achievement of maximum naltrexone hydrochloride plasma concentration followed by a relatively abrupt concentration decline. No steady-state was achieved *in vivo*. In an attempt to correlate the present *in vivo* findings with formerly published *in vitro* steady-state permeation data, a diffusion-compartmental mathematical model was developed. The model incorporates two parallel permeation pathways, barrier thickness-dependent diffusional resistance, microchannel closure kinetics, and a pharmacokinetic module. The regression analysis of the pharmacokinetic data demonstrated good agreement with independently calculated microchannel closure rate and *in vitro* permeation data. Interestingly, full-thickness rather than split-thickness skin employed in *in vitro* diffusion experiments provided the best correlation with *in vivo* data. Data analysis carried out with the model presented herein provides new mechanistic insight and permits predictions with respect to pharmacokinetics coupled with altered microchannel closure rates.

Keywords

Naltrexone; microneedles; permeation; pharmacokinetics; mathematical model; IVIVC

Introduction

Naltrexone is a potent, competitive opioid receptor antagonist used to treat opioid and alcohol addiction¹. Multiple reports concerning naltrexone delivery via the transdermal route

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Disclosure

The content of the present work is largely based on data included in Mikolaj Milewski's PhD dissertation “Microneedle-assisted transdermal delivery of naltrexone hydrochloride: in vitro permeation and in vivo pharmacokinetics” at University of Kentucky 2011.

Conflict of interest disclosure

Audra Stinchcomb, Stan Banks, and Dana Hammell are significant shareholders in AllTranz Inc, a transdermal specialty pharmaceutical company developing microneedle-related products.

have been generated by Stinchcomb et al.^{2–13}. There is continuing interest in exploring the ability of microneedles (MN), a novel skin permeation enhancement method, to substantially increase the rate of percutaneous transport of naltrexone, as well as other drugs. The ultimate goal of these studies is to develop a multiple-day naltrexone transdermal drug delivery system. Such a system could become a viable alternative to the currently available oral and injectable dosage forms associated with drawbacks, such as poor patient compliance and severe local injection site reaction, respectively^{14, 15}. Former studies demonstrated that although naltrexone and its active metabolite (6 β -naltrexol) do not permeate through skin at therapeutically-relevant rates^{4, 9}, the use of a “poke (press) and patch” MN application method¹⁶ proved to significantly improve *in vitro* transport^{17, 18}. In a broader context, a myriad of studies demonstrated great effectiveness of MN for increasing *in vitro* percutaneous flux of various drugs^{17, 19–30}. On the other hand, the number of literature reports exploring *in vivo* drug delivery with a “poke and patch” method is much more limited. The few examples include: naltrexol base and hydrochloride delivery in hairless guinea pigs¹⁸, naltrexone hydrochloride delivery in hairless guinea pigs³¹, naltrexone hydrochloride delivery in humans³², ketoprofen delivery in rats³³, nicardipine hydrochloride delivery in hairless rats³⁴, and L-carnitine delivery in rats³⁵. The outcomes of the above studies demonstrated marked differences in the shape of pharmacokinetic (PK) profiles and effectiveness of MN skin treatment. Additionally, the closure process of microchannels formed as a result of the skin MN-treatment was a topic of several literature reports^{36–40}. The conclusions with respect to the timeframe of microchannel closure varied according to experimental conditions and methods used for making the assessment. Very few qualitative^{32, 34} and quantitative⁴¹ attempts have been made to relate *in vitro* and *in vivo* permeation. The present work focuses on the establishment of a diffusion-compartmental model-based quantitative *in vitro-in vivo* correlation (IVIVC) for a “poke and patch” method.

The purpose of the present work was to establish an IVIVC for MN-assisted transport of naltrexone hydrochloride. It was achieved via 1) leveraging formerly published *in vitro* permeation data from NTX•HCl³⁰, 2) evaluation of NTX•HCl “poke and patch” pharmacokinetics in Yucatan minipig, 3) development of a functional mathematical model describing: *in vivo* drug transport across MN-treated skin, its body disposition, and elimination. Former research had provided a framework for analysis of *in vitro* MN-enhanced transport of NTX•HCl, which was now expanded upon to accommodate the rate of microchannel closure and drug pharmacokinetics.

Experimental Section

Chemicals

NTX•HCl was purchased from Mallinckrodt (St. Louis, MO, USA). Water was purified using NANOpure Diamond™, Barnstead water filtration system. 1,2-propanediol, Hanks' balanced salts modified powder, and sodium bicarbonate were purchased from Sigma-Aldrich (St. Louis, MO). 4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), gentamicin sulfate, trifluoroacetic acid (TFA), triethylamine (TEA), 1-octane sulfonic acid sodium salt, and acetonitrile (ACN) were obtained from Fisher Scientific (Fairlawn, NJ). Reagent-grade chemicals were obtained from Sigma Chemical Company (St. Louis, MO).

In vitro diffusion study – estimation of naltrexone diffusivity in dermis

NTX•HCl was added to water to obtain the concentration of 110 mg/ml. The solutions were placed in glass vials, closed tightly with a plastic cap, sonicated for 15 min and left in the shaking water bath at 32°C (skin surface temperature) overnight before use to ensure all of the drug was dissolved.

Full-thickness Yucatan minipig skin was harvested from the dorsal region of a euthanized 6-month-old animal. Animal studies were approved by the University of Kentucky IACUC. Subcutaneous fat from skin samples was removed with a scalpel and sparse hair clipped. Skin samples were immediately placed in a plastic bag and frozen (-20°C) until use. Before each permeation study, skin samples were allowed to thaw for approximately 30 min. Skin was dermatomed by removing whole epidermis and varying thickness of dermis so that a range of dermis thicknesses was obtained from 0.8 – 2.2 mm. Dermis thickness was evaluated with calipers. A PermeGear flow-through diffusion cell system (In-Line, Hellertown, PA) was used for the *in vitro* diffusion studies. Isotonic HEPES-buffered Hanks' balanced salts solution (pH 7.4) was used as the receiver solution. The flow rate of the receiver fluid was set at 1.5 ml/h to maintain sink conditions. Skin samples in the diffusion cells were kept at 32°C using a circulating water bath. The diffusion experiments were initiated by charging the donor compartment with 0.7 ml of the donor solution. Samples were collected from the receiver compartment in 6 h increments over 48 h. All samples were stored at 4°C until HPLC analysis. The permeation data were plotted as the cumulative amount of NTX collected in the receiver compartment as a function of time. Fourteen diffusions cells were employed ($n = 14$).

Intravenous bolus injection preparation

NTX•HCl was dissolved in sterile 0.9% saline to obtain a drug concentration of 40 mg/ml. Solution for intravenous (IV) injection was filtered through a sterile $0.22\ \mu\text{m}$ filter into a sterile scintillation vial under a laminar biosafety hood. Syringes were prefilled to deliver a desired dose: 1 mg/kg, 9 mg/kg or 10 mg/kg.

Transdermal formulation and protective patch preparation for *in vivo* studies

Commercially available AquaSite[®] (Derma Sciences, Princeton, NJ) hydrogel sheets were cut into circular discs, 4 cm in diameter. Next, a solution of benzyl alcohol 1%, ethanol 5%, propylene glycol 10% and water for injection 84% (v/v) were prepared and NTX•HCl was dissolved to obtain a concentration of 110 mg/ml. A four-fold excess of NTX•HCl solution, with respect to the weight of the hydrogel, was equilibrated with the discs for 48 h. The equilibrium NTX•HCl concentration in the hydrogel discs was determined by HPLC to be 90 mg/g. Next, the transdermal formulation was autoclaved at high pressure saturated steam at 121°C for 15 min (verified for chemical stability by HPLC).

The protective patches (4 cm internal diameter) were prepared by sandwiching a rubber ring to create a reservoir between a drug-impermeable backing membrane and an adhesive around the edge of a rubber spacer.

Animal studies

Male and female Yucatan minipigs (Sinclair Bio-Resources; Columbia, MO) weighing approximately 40 kg were used for *in vivo* studies. These animals underwent surgical procedure at Sinclair Bio-Resources to cannulate the jugular veins and implant Vascular Access Ports (VAPs) (Access Technologies; Skokie, IL). All animal studies were in accordance with institutional guidelines approved by the University of Kentucky IACUC.

Intravenous bolus injections—NTX•HCl bolus was administered via VAP through a controlled infusion over 3.5–5 min. Three independent experiments ($n=3$) were carried out with three different NTX•HCl doses of 1 mg/kg, 9 mg/kg or 10 mg/kg. Blood samples were collected prior to drug treatment (baseline). After dosing, the VAP was flushed with sterile heparinized saline. Blood samples (2 ml) were drawn from the VAP at scheduled time intervals for 55 h after the intravenous dose. The blood samples were immediately centrifuged at $10,000\times g$ for 3 min; plasma was separated and stored at -70°C until analysis.

Transdermal PK studies—4 sites (each 4 cm in diameter) were designated on the dorsal region of Yucatan minipig and each treated with MN to obtain 400 microchannels (4 sites times 400 microchannels equals 1600 microchannels in total per minipig). Stainless steel MN (750 μm -long) arrays employed herein were obtained from Dr. Prausnitz's laboratory (details can be found in reference ⁴⁰ and under "treatment E" in Table 1). NTX•HCl hydrogels were applied to the sites. Sterile and drug-loaded hydrogel discs were applied on the MN-treated skin areas and covered with protective patches and Bioclusive* transparent dressing to prevent dislocation throughout the duration of the experiment. Additionally, a jacket was placed on the minipig to protect the patches. Three independent experiments (n=3) were carried out. Blood samples were collected prior to drug treatment (baseline). After application of patches, blood samples were obtained over 72 h followed by patch removal and blood sampling for another 24 h. The blood samples were immediately centrifuged at 10,000xg for 3 min. Plasma was separated and stored at -70°C until analysis.

Quantitative analysis - HPLC

Quantitative analysis of NTX concentrations from the *in vitro* study was carried out by HPLC using a modification of the assay described by Hussain et al. ⁴². The HPLC system consisted of a Waters 717 plus autosampler, a Waters 600 quaternary pump, and a Waters 2487 dual wavelength absorbance detector with Waters EmpowerTM software. A Brownlee (Wellesley, MA, USA) C-8 reversed phase Spheri-5 μm column (220 $\times$ 4.6 mm) with a C-8 reversed phase guard column of the same type (15 $\times$ 3.2 mm) by Perkin Elmer[®] was used with the UV detector set at a wavelength of 215 nm or 278 nm. The mobile phase consisted of 70:30 (v/v) ACN:(0.1% TFA with 0.065% 1-octane sulfonic acid sodium salt, adjusted to pH 3.0 with TEA aqueous phase). Samples were run at a flow rate of 1.5 ml/min with a run time of 4 min. The injection volume used was 100 μl . The retention time of NTX was 2.1 ± 0.1 min. Samples were analyzed within a linear range of the NTX standard curve from 100 – 10,000 ng/ml. The standard solutions exhibited excellent linearity over the entire concentration range employed in the assays.

Quantitative analysis – LC-MS/MS

Plasma samples obtained from *in vivo* Yucatan minipig studies were quantified by a LC-MS/MS method.

Calibration standards—Stock solutions of NTX•HCl and 6- β -naltrexol hydrochloride NTXol•HCl in acetonitrile were prepared at 100 ng/ml concentration. Working solutions were prepared by serial dilution of the stock solutions. Calibration standards of NTX•HCl and NTXol•HCl were prepared daily at concentrations 1, 2, 3, 5, and 10 ng/ml in Yucatan minipig plasma by spiking Yucatan pig plasma with 20 μl of NTX•HCl and NTXol•HCl working solutions at adequate concentrations.

Sample extraction procedure—All standards and samples of volume 200 μl were extracted with 1 ml of acetonitrile:ethyl acetate (1:1, v/v), resulting in protein precipitation. The mixture was vortexed for 15 s and centrifuged at 12,000xg for 20 min. The supernatant was transferred to a glass tube and evaporated under nitrogen. Then, the residue was reconstituted in 100 μl acetonitrile, sonicated for 10 min and vortexed for 15 s. Samples were transferred into low volume inserts into HPLC vials and injected onto the LC-MS/MS system.

LC-MS/MS conditions—Waters Atlantis HILIC Silica 5 μm , 2.1 $\times$ 150 was used as the chromatographic column with a mobile phase consisting of methanol with 0.1% acetic acid:buffer 95:05 (v/v). The aqueous buffer consisted of 20 mM ammonium acetate and 5% methanol. The injection volume was 40 μl . Liquid chromatography was carried out in the

isocratic mode at the flow rate of 0.5 ml/min with a total run time of 4 min. The retention times for NTX and NTXol were as follows: 2.2 and 2.1 min, respectively. The LC-MS/MS system consisted of a HPLC Waters Alliance 2695 Separations Module, Waters Micromass[®] Quattro Micro[™] API Tandem Mass Spectrometer and MassLynx Chromatography software with Waters QuanLynx (V. 4.1) analysis software. Positive mode atmospheric pressure chemical ionization was used for detection of both compounds (APCI +). Multiple reaction monitoring (MRM) was carried out with the following parent to daughter ion transitions for NTX and NTXol: m/z 341.8→323.8, m/z 343.8→325.8, respectively. The corona voltage was 3.5 μ A, cone voltage 25 V, extractor 2 V, RF lens 0.3 V, source temp 130 °C, APCI probe temperature 575 °C. The collision gas was 20 eV. Nitrogen gas was used as a nebulization and drying gas at flow rates of 50 and 350 l/h, respectively. Calibration plots were prepared by the linear regression analysis of the standard peak areas versus nominal drug concentration.

Lower quantification limits (LLOQ): based on the coefficient of variation (CV) < 20 % criterion the following limits of quantification were estimated: NTX 0.5 ng/ml, NTXol 0.5 ng/ml.

Statistical analysis

The statistical analysis of data (95% confidence intervals determination) was carried out with the use of Scientist[®] software (Micromath).

Model development

In order to quantitatively analyze formerly published *in vitro* data³⁰ and currently collected “poke and patch” *in vivo* data, a combined diffusion-compartmental mathematical model was developed. The essential elements incorporated into the model are as follows: recognition of two parallel pathways for drug permeation following MN skin treatment (intact skin pathway and microchannel pathway), the barrier thickness-dependent diffusional resistance of the microchannel pathway, direct relationship between electrical admittance of the microchannels and their permeability to hydrophilic and ionized drugs, and a pharmacokinetic three-compartmental model for description of NTX disposition and elimination from the body. A schematic diagram of the model is shown in Figure 1.

The model can be divided into 3 modules.

Module 1 – Skin diffusion

One-dimensional diffusion model accounts for diffusion of drug molecules from a formulation, across skin, to a certain hypothetical skin depth where an absolute clearance exists (sink conditions). The drug concentration on the skin surface is assumed to remain constant at all times, which is typically true for skin-controlled diffusion where flux is not sufficiently high to deplete drug from the formulation over the study duration. In the present study it was confirmed experimentally by observing no change in NTX•HCl concentration in formulation from the beginning to the end of the *in vivo* study (data not shown). For untreated skin only, one permeation pathway – the intact skin pathway – is recognized. The barrier nature of the intact skin pathway is well-approximated by the barrier properties of the stratum corneum alone. This was experimentally confirmed *in vitro* by comparing the rate of NTX•HCl permeation through untreated skin against skin devoid of stratum corneum, which resulted in a several hundred-fold increase in transport rate (data not shown). After MN-treatment, another permeation pathway – the microchannel pathway – is formed. The barrier nature of this pathway is bi-layered, the first layer corresponds directly to the microchannel and the second to the viable tissue (dermis) between microchannel and hypothetical skin

depth at which an absolute clearance exists (sink conditions). However, since naltrexone diffusivity in the dermis is substantially lower than in the microchannel interior (as shown experimentally in the present work, see “Estimation of naltrexone diffusion coefficient in the dermis” section), it is assumed that all diffusional resistance will come primarily from the viable tissue. Thus, a mono-layered membrane model is used for simplicity. Fractional skin surface area is assigned to each pathway which describes relative contribution of individual pathway flux to the total flux through MN-treated skin. At any time the total flux, J_{TOT} , equals:

$$J_{TOT} = f_{ISP} \cdot J_{ISP} + f_{MCP} \cdot J_{MCP} \quad (1)$$

where f_{ISP} is the fractional skin surface area of intact skin pathway, J_{ISP} is the flux through intact skin pathway, f_{MCP} is the fractional skin surface area of microchannel pathway, and J_{MCP} is the flux through microchannel pathway. Assuming a homogenous nature of skin layers: stratum corneum and viable tissue, the cumulative amount-time profiles can be derived by solving Fick's second law of diffusion:

$$\frac{\partial C_m}{\partial x} = D_m \cdot \frac{\partial C_m}{\partial x^2} \quad (2)$$

where C_m is drug concentration in the membrane, D_m is drug diffusion coefficient in the membrane, and x is spatial coordinate. After imposing the following initial conditions:

$$C_m(x, 0) = 0 \quad (3)$$

and boundary conditions:

$$C_m(0, t) = C_v \quad (4)$$

$$C_m(h_m, t) = 0 \quad (5)$$

it was shown⁴³ that the solution in terms of cumulative amount of drug exiting the membrane is:

$$Q(t) = -D_m \cdot A \cdot \int_0^t \left. \frac{\partial C_m}{\partial x} \right|_{x=h_m} dt = A \cdot C_v \cdot h_m \left[\frac{t}{t_d} - \frac{1}{6} - \frac{2}{\pi^2} \sum_{n=1}^{\infty} \frac{(-1)^n}{n^2} \cdot e^{\left(-\frac{t}{t_d} \cdot \pi^2 \cdot n^2\right)} \right] \quad (6)$$

where the diffusion time, t_d is:

$$t_d = \frac{h_m^2}{D_m} \quad (7)$$

Hence, for intact skin pathway (stratum corneum as a membrane) the flux per unit area as a function of time is given by:

$$J_{ISP} = -D_{SC} \cdot \left(\frac{dC_{SC}}{dx} \right)_{x=h_{SC}} = \frac{D_{SC}}{h_{SC}} \cdot C_v \left[1 + 2 \sum_{n=1}^{\infty} (-1)^n \cdot e^{\left(-\frac{t}{t_d} \cdot \pi^2 \cdot n^2\right)} \right] \quad (8)$$

and for microchannel pathway (dermis as a membrane) the analogous solution is:

$$J_{MCP} = -D_{VT} \cdot \left(\frac{dC_{VT}}{dx} \right)_{x=h_{VT'}} = \frac{D_{VT}}{h_{VT'}} \cdot C_v \left[1 + 2 \sum_{n=1}^{\infty} (-1)^n \cdot e^{\left(-\frac{t}{t_d} \cdot \pi^2 \cdot n^2\right)} \right] \quad (9)$$

The above solutions are assumed to adequately describe drug transport through MN-treated skin under *in vitro* conditions.

Two additional major factors which affect drug transport *in vivo* exist: the dermal capillary clearance and the microchannel closure process. In the current work the presence of a dermal capillary clearance *in vivo* is ignored and skin is regarded as an unperfused flap of a hypothetical thickness at which an absolute clearance exists. Hence, it is essentially modeled in the same way as the skin in *in vitro* experiments. Although not very realistic, this approach was chosen on purpose so that the IVIVC can be established in terms of the “effective” *in vivo* skin thickness. The second factor - the presence of a microchannel closure process - and its inclusion in the model is described in the following section.

Module 2 – Microchannel closure

Upon formation of microchannels in the skin *in vivo*, the tissue repair process initiates. The fundamental assumption about *in vitro* and *in vivo* microchannel pathway permeability included in this module is the following: *in vitro* microchannel pathway permeability is a constant and remains high indefinitely, while *in vivo* microchannel pathway permeability decreases as a function of time. Multiple studies demonstrated that following *in vitro* MN treatment of skin, the permeability of the microchannel pathway remains constant as manifested by achievement and maintenance of steady-state flux^{17, 27, 30}. For simplicity, the permeability of the microchannel pathway can be interpreted in terms of the fractional skin surface area of microchannels that remains open. Such treatment is an obvious generalization, as behind the decreasing permeability of the microchannel pathway *in vivo* lies a cascade of biological processes related to wound healing, which ultimately leads to the complete repair of the injured skin^{44–46}. These processes will affect both the drug diffusivity in the microchannel pathway and skin surface opening of microchannels. However, for the sake of the drug delivery discussion, it is a compelling simplification. Hence, the equation that can adequately describe the total flux *in vivo* is as follows:

$$J_{TOT,C} = f_{ISP} \cdot J_{ISP} + f_{OMF} \cdot f_{MCP} \cdot J_{MCP} \quad (10)$$

where $J_{TOT,C}$ is total flux corrected for microchannel closure, f_{OMF} stands for open microchannel surface area fraction, or more precisely for fraction of initial permeability of the microchannel pathway. Implicitly it is assumed that the *in vitro* situation f_{OMF} always holds the value of unity and Eq. 10 collapses to Eq. 1. Of note, in the strict sense the microchannel healing process causes a time-dependent drop in the microchannel skin surface area which is accompanied by the same area increase in the intact skin around the microchannels. However, since f_{MCP} is typically very small (e.g. 0.025) as compared to f_{ISP} (e.g. 0.975), the time-dependent increase in f_{ISP} is minute and ignored in Eq. 10, as shown by a lack of a time-dependent term in front of the $f_{ISP} \cdot J_{ISP}$ term. This simplification reduces the calculation work and does not negatively affect the results.

Once drug has crossed viable skin tissue and reaches local microvasculature it is swept away by blood flow to find itself in the central pharmacokinetic compartment.

Module 3 – Pharmacokinetic compartments

Drug is absorbed through viable tissue into the systemic circulation, or central pharmacokinetic compartment. The experimental data reported herein demonstrated an apparent 3-exponential decrease in NTX concentration as a function of time after a bolus IV administration. In consequence, a 3-compartment mammillary model was chosen to describe NTX distribution and elimination from the body. The differential equations that describe drug delivery to the central compartment, its distribution and elimination are as follows:

$$\frac{dC_1}{dt} = \frac{A \cdot J_{TOT,C}}{V_d} + \frac{k_{21} \cdot X_2}{V_d} + \frac{k_{31} \cdot X_3}{V_d} - k_{12} \cdot C_1 - k_{13} \cdot C_1 - k_{10} \cdot C_1 \quad (11)$$

$$\frac{dX_2}{dt} = k_{12} \cdot C_1 \cdot V_d - k_{21} \cdot X_2 \quad (12)$$

$$\frac{dX_3}{dt} = k_{13} \cdot C_1 \cdot V_d - k_{31} \cdot X_2 \quad (13)$$

where A is the total skin area of formulation (hydrogel) application, V_d is the initial volume of distribution (volume of distribution of the central compartment); k_{12} , k_{13} , k_{21} , k_{31} are micro rate constants describing drug transport between central (#1) and peripheral (#2 and #3) compartments; and k_{10} is the elimination constant. The drug plasma concentration (in the central compartment) is a single major variable of interest.

Method of solution

Scientist[®] software (Micromath, St Louis, MO) was used to numerically solve model equations (Eq. 8 – 13 and 22) transformed into LaPlace domain. The inverse transformation was performed by software using Piessens and Weeks algorithms. Scientist[®] initially uses a fast Weeks algorithm; however, in a situation when this method is not applicable it automatically detects it and employs to a slower but more reliable Piessens algorithm. The accuracy of numerical solutions obtained from equations written in a LaPlace domain was compared with those written in a time domain for a case where diffusion equations describing drug transport through MN-treated skin were replaced with true zero-order delivery. The agreement between the two methods was excellent.

Parameter estimation

Estimation of naltrexone diffusion coefficient in water—The estimate of the naltrexone diffusion coefficient in water was obtained by the use of the Stokes-Einstein equation:

$$D = \frac{k \cdot T}{6 \cdot \pi \cdot \eta \cdot r} \quad (14)$$

where k is the Boltzmann constant, T is the absolute temperature, η is the viscosity and r is the hydrodynamic radius of the solute. Using an experimentally determined viscosity value of 1.03 cP determined at 32 °C³⁰, and assuming the hydrodynamic molecular radius of 4.6 Å⁴⁷, a diffusion coefficient value of $4.7 \cdot 10^{-6} \text{ cm}^2 \cdot \text{s}^{-1}$ was calculated. This value is assumed to accurately approximate the diffusivity of naltrexone in microchannels filled with interstitial fluid. It has been formerly shown by McAllister et al. that MN-enhanced transdermal flux values for a variety of compounds of different sizes correlated well with prediction based on the Stokes-Einstein equation²⁶. It suggests that diffusivity of drug molecules in the microchannels resembles that seen in the bulk solution.

Estimation of naltrexone diffusion coefficient in the dermis—For the purpose of experimental assessment of drug diffusivity in the dermis, a study involving NTX permeation across skin devoid of epidermis was carried out. Figure 2 demonstrates a representative graph of cumulative amount of NTX plotted against time.

As evident from the plot, the flux is very high, which caused depletion of drug in the donor solution. At the end of the 48 h study, experimentally measured NTX concentration in the donor solution dropped, on average, by $50 \pm 18 \%$. Due to this fact, the plot lacks a late steady-state portion and flux was estimated not from the terminal, but rather, early-time

graph portion. The slope of the plot in the 6–12 h timeframe better approximates steady-state flux, in this case, as it allows for lag time but minimizes the effect of drug depletion in the donor solution. Flux values obtained by this method were plotted against the experimentally measured thickness of the dermis employed in the diffusion study. Figure 3 summarizes all data.

A clear trend is visible with thicker dermis corresponding to higher diffusional resistance of the barrier and decreased flux. The reciprocal relationship between flux and the thickness of the barrier (dermis) can be described by Eq. 15:

$$J = \frac{D_{VT}}{h_{VT}} \cdot C \quad (15)$$

where J is steady-state flux, h_{VT} is thickness of the dermis (viable tissue), D_{VT} is diffusion coefficient of naltrexone in the dermis, and C is donor solution concentration of NTX•HCl. Since the values of all the above terms are known with exception of the naltrexone diffusion coefficient in dermis, it can be estimated by non-linear regression of flux on dermis thickness by means of Eq. 15. The mean estimated value was found to be $3.46 \cdot 10^{-7} \text{ cm}^2 \cdot \text{s}^{-1}$, 95 % confidence interval of $(3.28 \cdot 10^{-7}, 3.65 \cdot 10^{-7})$. This value is approximately 14-fold lower than the diffusion coefficient of naltrexone in water estimated in the former paragraph.

Estimation of effective microchannel diameter—Effective microchannel diameter was estimated from *in vitro* diffusion data. Former studies³⁰ employed an *in vitro* diffusion set-up consisting of 0.95 cm^2 of 1.8 mm-thick Yucatan minipig skin possessing 100 microchannels and a donor solution of NTX•HCl in water at 110 mg/ml. The length of the microchannels in the skin was assessed to be approximately $650 \mu\text{m}$ (unpublished data). The steady-state flux through the microchannel pathway alone ($J_{\text{MCP-EXP}}$) was measured to be $29.0 \pm 4.4 \mu\text{g} \cdot \text{h}^{-1}$ ³⁰. By comparison of this experimental flux value ($J_{\text{MCP-EXP}}$) to the calculated flux value ($J_{\text{MCP-CAL}}$), which corresponds to the same experimental conditions, but with a fractional skin surface area of microchannel pathway of unity ($f_{\text{MCP-CAL}} = 1$), the estimate of the effective microchannel pathway skin fractional surface area ($f_{\text{MCP-EXP}}$) can be obtained. The value of $J_{\text{MCP-CAL}}$ was estimated by use of the following parameter values: $D_{\text{MC}} = 4.70 \cdot 10^{-6} \text{ cm}^2 \cdot \text{s}^{-1}$, $h_{\text{MC}} = 0.65 \text{ mm}$, $D_{\text{VT}} = 3.46 \cdot 10^{-7} \text{ cm}^2 \cdot \text{s}^{-1}$, $h_{\text{VT}} = 0.115 \text{ mm}$, $C = 110 \text{ mg/ml}$, and $f_{\text{MCP-CAL}} = 1$ according to Eq. 16:

$$J_{\text{MCP-CAL}} = f_{\text{MCP-CAL}} \cdot \frac{C}{\frac{h_{\text{MC}}}{D_{\text{MC}}} + \frac{h_{\text{VT}}}{D_{\text{VT}}}} \quad (16)$$

where $f_{\text{MCP-CAL}}$ is the microchannel pathway skin fractional surface area, C is NTX•HCl donor solution concentration, D_{MC} is naltrexone diffusivity in microchannels approximated by naltrexone diffusivity in water, h_{MC} is the length of the microchannel, D_{VT} is naltrexone diffusivity in the dermis, and h_{VT} is the thickness of the underlying dermis layer. Numerically, $J_{\text{MCP-CAL}}$ was found to equal $1139.8 \mu\text{g} \cdot \text{cm}^{-2} \cdot \text{h}^{-1}$.

On the other hand, the $J_{\text{MCP-EXP}}$ of $29.0 \mu\text{g} \cdot \text{cm}^{-2} \cdot \text{h}^{-1}$ corresponds to the following Eq. 17:

$$J_{\text{MCP-EXP}} = f_{\text{MCP-EXP}} \cdot \frac{C}{\frac{h_{\text{MC}}}{D_{\text{MC}}} + \frac{h_{\text{VT}}}{D_{\text{VT}}}} \quad (17)$$

where $f_{\text{MCP-EXP}}$ is the only unknown. Since the numerical value of the ratio of drug donor solution concentration to the total microchannel pathway resistance is the same in Eq. 16 and Eq. 17, the solution for $J_{\text{MCP-EXP}}$ can be simplified to Eq. 18:

$$f_{MCP-EXP} = \frac{J_{MCP-CAL} \cdot f_{MCP-EXP}}{J_{MCP-CAL}} \quad (18)$$

which yields the $f_{MCP-EXP}$ value of 0.025 or 2.5 %. This fractional skin surface area translates into an effective microchannel opening diameter of 176 μm . Noteworthy, this effective opening diameter is larger than the actual skin surface opening of the microchannel that is around 50–100 μm wide⁴⁸. The likely reason for this difference is the inclusion of lateral diffusion within the “effective” microchannel diameter. While all the equations presented herein are one-dimensional, in reality lateral diffusion will contribute to the overall flux, and it can be expected that “actual” microchannel diameter will not suffice to account for the flux obtained through the microchannel pathway. Therefore, it will be the effective microchannel diameter that will be used for further calculations.

Estimation of microchannel pathway fractional skin area *in vivo*—The *in vivo* Yucatan minipig transdermal experiment involved application of 4 patches. Each patch had a diameter of 4 cm and covered 400 microchannels. Therefore, the total skin surface area involved in diffusion was 50.24 cm^2 and skin surface area of all microchannel openings was 0.39 cm^2 , which gives the microchannel pathway fractional skin area $f_{MCP} = 0.0077$ or 0.77 % *in vivo*.

Estimation of the microchannel closure rate *in vivo*—Electrical impedance was formerly used to evaluate the permeability of the microchannel pathway *in vivo*^{38, 46, 47}. Skin electrical impedance in the context of these studies is understood as electrical resistance measured in the presence of an AC signal. It measures the difficulty of electrical current (ionic) transport across skin. In the experimental set-up used in the abovementioned studies, after MN treatment, three parallel and independent pathways for electric current transport can be distinguished: resistor, skin around the microchannels, and the microchannels themselves. Alternatively, in the absence of microchannels in the skin, only two parallel pathways are available: resistor and untreated skin. The latter case represents either a situation before MN treatment or a situation when microchannels are fully closed/healed after MN treatment. The resistor and skin, with or without microchannels, are truly unrelated pathways. However, in the strict sense, microchannels and skin are not since upon MN treatment microchannels take over a minute skin surface area. Practically, the drop in the intact skin surface area is negligible as a result of microchannel formation and the fractional area of skin before and after MN treatment remains close to unity $f_{MCP} \approx 1$. Based on earlier *in vitro* effective microchannel diameter estimates (167 μm), density of MN treatment (100/ cm^2), and electrode surface area (0.95 cm^2), the fraction of microchannel pathway skin surface area f_{MCP} in *in vivo* microchannel closure studies also equals 2.5 %. Of note, the intact skin electrical impedance value corrected for f_{MCP} establishes the upper limit for the microchannel pathway. In other words, when the microchannel closure process is complete the microchannel pathway electrical impedance values come back to the value corresponding to the intact skin. Overall, experimental electrical impedance measurements after skin MN-treatment provide a total electrical impedance value Z_{TOT} that is a function of Z_{RB} (resistor box), Z_{ISP} (intact skin pathway) and Z_{MCP} (microchannel pathway) according to Eq. 19:

$$Z_{tot} = \frac{1}{\frac{1}{Z_{RB}} + \frac{1}{Z_{ISP}} + \frac{0.025}{Z_{MCP}}} \quad (19)$$

where the implicit assumption about a negligible drop in f_{ISP} as a result of MN-treatment is built in. In the absence of MN-treatment the total electrical impedance equals:

$$Z_{tot} = \frac{1}{\frac{1}{Z_{RB}} + \frac{1}{Z_{ISP}}} \quad (20)$$

Since Z_{TOT} and Z_{RB} are known, and Z_{ISP} can be independently estimated from an experiment employing untreated skin under occlusion, the electrical impedance of the microchannel pathway can be calculated. This approach allows elimination of the confounding variables, such as the influence of the resistor settings and the hydration state of the skin. Moreover, to evaluate microchannel closure kinetics it is more intuitive to use the inverse of the electrical impedance (Z), the electrical admittance (Y), which directly correlates with permeability or fractional skin surface area of open microchannels. The interpretation of electrical admittance values resembles another popular method – transepidermal water loss (TEWL), in the sense that high values signify compromise in the barrier integrity, while low, baseline values are typical under normal physiological conditions. Since no reliable electrical impedance microchannel closure data is available for Yucatan minipig at present, an estimate was obtained from human^{38, 46} and rat⁴⁷ electrical impedance microchannel closure data as well as unpublished data. It is believed that an estimate based on human and rat electrical impedance microchannel closure data will provide an acceptable surrogate for its Yucatan minipig counterpart. The time-dependent changes of the microchannel pathway admittance values (after subtraction of baseline intact skin pathway value) followed an apparent exponential decrease. The subtraction of baseline intact skin pathway value from microchannel pathway admittance is necessary to assure that over a long duration, the signal drops to zero as required by Eq. 21. To estimate the apparent first-order microchannel closure rate constants (k_Y 's), the data was fitted to the following empirical model:

$$Y_{MCP} = Y_{MCP}^0 \cdot e^{-k_Y t} \quad (21)$$

where Y_{MCP}^0 stands for the initial microchannel pathway admittance after microneedle treatment. The k_Y value selected for present modeling was 0.041 h^{-1} , corresponding to $t_{1/2}$ of 17 h, which is in good agreement with published^{38, 46, 47} and unpublished results implying microchannel closure half-lives on the order of 12–20 h.

Finally, it was assumed that the time-dependent microchannel closure in the skin (drop in the microchannel pathway permeability) directly correlates with electrical admittance of the microchannel pathway such that $k_Y = k_{OMF}$. Hence:

$$f_{OMF} = f_{OMF}^0 \cdot e^{-k_{OMF} t} \quad (22)$$

where f_{OMF}^0 numerically equals the value of unity and corresponds to the initial open microchannel surface area fraction, or more precisely the initial permeability of the microchannel pathway obtained immediately after skin MN treatment.

Formerly published reports support this assumption and indicate that a good correlation between electrical admittance and skin permeability of hydrophilic/charged solutes exist. Specifically, G. Yan et al. found a good linear correlation ($R^2 = 0.94$) between acyclovir flux and skin conductance²⁴. This is not surprising since the presence of microchannels in the skin establishes the pathway of least electrical and diffusional resistance. Hence, the electrical impedance measurements can be expected to reliably probe the barrier domain for transport of NTX•HCl across MN-enhanced skin. Also, a report by Karande et al. demonstrated that even for non-MN-treated skin, in the presence of chemical enhancers, the inverse correlation between electrical impedance and permeability of hydrophilic solutes was good ($R^2 = 0.8$)⁴⁹. These authors stated that permeability can be related to skin

electrical impedance through the porous pathway theory with the fundamental underlying hypothesis that polar uncharged solutes and ions (carrying current) migrate through the stratum corneum via identical pathways.

Adjustment of naltrexone diffusivity per formulation microviscosity—

Previously published work³⁰ discussed the influence of propylene glycol on the MN-assisted NTX•HCl flux. It was demonstrated that the inclusion of PG in the aqueous donor solution caused a marked decrease in the transport rates *in vitro*. Hence, naltrexone diffusivity values corrected for the presence of 10 % PG in the formulation used in the current *in vivo* study are the following: $D_{MC} = 3.34 \cdot 10^{-6} \text{ cm} \cdot \text{s}^{-1}$ and $D_{VT} = 2.46 \cdot 10^{-7} \text{ cm} \cdot \text{s}^{-1}$.

Estimation of naltrexone pharmacokinetic parameters—In order to assess parameter values related to NTX distribution and elimination from the body, IV bolus drug administration was carried out. The plasma levels of NTX in Yucatan minipig were observed to follow an apparent three-exponential decrease and can be adequately described by a three-compartmental model as shown in Figure 4. To obtain the pharmacokinetic parameter values the experimental data from three independent experiments with the following doses, 1 mg/kg, 9 mg/kg, and 10 mg/kg were fitted simultaneously to the following differential equations:

$$\frac{dC_1}{dt} = \frac{k_{21} \cdot X_2}{V_d} + \frac{k_{31} \cdot X_3}{V_d} - k_{12} \cdot C_1 - k_{13} \cdot C_1 - k_{10} \cdot C_1 \quad (23)$$

$$\frac{dX_2}{dt} = k_{12} \cdot C_1 \cdot V_d - k_{21} \cdot X_2 \quad (24)$$

$$\frac{dX_3}{dt} = k_{13} \cdot C_1 \cdot V_d - k_{31} \cdot X_2 \quad (25)$$

where at time zero the initial drug concentration in the central compartment (C_1) was calculated as dose/ V_d . Such analysis resulted in parameter values summarized in Table 1. The three progressive doses reported herein were selected to provide quantifiable plasma levels over a reasonable time span for modeling purposes. Given the preliminary nature of the IV bolus studies and limited number of repetitions, the estimate values are to be taken with caution: $V_d = 43.86$ (31% CV); $k_{12} = 3.192$ (53% CV); $k_{21} = 2.002$ (30% CV); $k_{13} = 0.098$ (31% CV); $k_{31} = 0.050$ (21% CV); $k_{10} = 2.596$ (27% CV). Further studies will provide more certainty in the estimated parameter values. Systemic clearance of NTX, defined as $k_{10} \cdot V_d$ equals $1.9 \text{ l} \cdot \text{min}^{-1}$ or $47 \text{ ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$. Hence, weight-normalized systemic NTX clearance value is practically equivalent to its human counterpart of around $50 \text{ ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$.

Results

Experimental data demonstrating NTX plasma levels in Yucatan minipig following a “poke and patch” method and the model fit (Eq. 8–13 and 22) to the PK data are shown in Figure 5. No steady-state NTX plasma level was observed. Initially, the drug concentration increased quickly to reach a C_{max} of approximately 2.5 ng/ml at 4 h. A sharp drop in the drug plasma level followed and within approximately 24 h, the NTX concentration became only half of the C_{max} . Finally, in the 48–72 h timeframe the level seemed to plateau at 0.6 ng/ml. After removal of the transdermal patches at 72 h, the NTX level dropped rapidly below the lower limit of quantification. No NTXol was detected in the transdermal drug delivery study. The present model captured the PK profile shape of NTX well and permitted estimation of the hypothetical “effective” *in vivo* dermis thickness (h_{VT}) separating the

microchannel from the skin depth at which sink conditions exist. The regression analysis yielded a 0.74 mm point estimate for $h_{VT'}$, which corresponds to a 1.39 mm total skin thickness (0.65 mm microchannel length, h_{MC} , plus 0.74 mm dermis underneath the microchannels, $h_{VT'}$). Additionally, a $4.02 \cdot 10^{-12} \text{ cm}^2 \cdot \text{s}^{-1}$ estimate for *in vivo* D_{SC} was obtained. All parameters are summarized in Table 1. Next, simulations were carried out using the current model to elucidate the difference in NTX•HCl permeation under *in vitro* and *in vivo* conditions (Figure 6) as well as the contributions of the microchannel pathway flux and the intact skin flux to the total flux *in vivo* (Figure 7). The impact of the hypothetical decrease in the rate of microchannel closure on the pharmacokinetic profile was also demonstrated in Figure 8.

Discussion

The overall goal of employing the mathematical model reported herein to describe the plasma levels of NTX following the “poke and patch” treatment was to evaluate if recognition of two parallel flux and microchannel closure processes would suffice to adequately describe the PK profile, and to obtain a reliable value of the $h_{VT'}$ parameter. The knowledge of the $h_{VT'}$ parameter can help in better design of *in vitro* experiments to reflect *in vivo* conditions as closely as possible. All the other parameter values are known from independent studies and summarized in Table 1. Of those, two parameter estimates are to be taken with caution: k_{OMF} and D_{SC} . The first was obtained through the investigation of the microchannel closure process in humans^{38, 46} and rats⁴⁷, and is employed as a surrogate for its Yucatan minipig counterpart, which is lacking at present. The second, D_{SC} , obtained through an *in vitro* permeation study³⁰ turned out to be an overestimate. If its value held true under *in vivo* conditions, a plateau observed at late time points (48–72 h) in Figure 5 would need to equal approximately 1.1 – 1.5 ng/ml. A possible reason for this mismatch is the negative effect of storage and the freeze-thaw process on Yucatan minipig skin used in *in vitro* experiments. Transport of ionized species is expected to be very sensitive to even the slightest defects in the skin, which can cause a significant overestimate of permeation⁵⁰. Such defects are not anticipated to play a major role in the transport of unionized and moderately lipophilic species, for which the SC lipid domain is the preferred route of permeation. Hence, the *in vivo* value of D_{SC} was re-estimated through regression analysis. Two parameter values, $h_{VT'}$ and D_{SC} were fitted simultaneously through regression of the NTX plasma concentration vs. time curve, while keeping all other parameter values, obtained independently, constant. Hence, little “flexibility” in the fit was allowed. The least-squares fit of the model equations to experimental data is denoted as the solid line in Figure 5. In general, good correlation was achieved which indicates that the model presented herein can dependably represent *in vivo* drug delivery conditions from a “poke and patch” method. The regression estimate of the *in vivo* D_{SC} was found to be $4.02 \cdot 10^{-12} \text{ cm}^2 \cdot \text{s}^{-1}$, or 95% CI ($4.99 \cdot 10^{-13}$, $7.54 \cdot 10^{-12}$), suggesting a point estimate value about 3-fold lower as compared to the estimate obtained from the *in vitro* studies³⁰, but within a large confidence interval range. More importantly, though, the regression analysis revealed the 0.74 mm, 95% CI (0.64, 0.84), estimate for $h_{VT'}$. The accuracy of the $h_{VT'}$ estimate depends on the method of its estimation. Herein, as mentioned before, point estimates of all other parameters were fixed while performing the least-square fit. This approach assumes that these estimates were accurate and no uncertainty from their estimation was included in the fitting process. An alternative, more conservative, approach would include simultaneous fitting of individual parameters from independent experiments together with the herein described model, which would lead to a larger confidence interval for $h_{VT'}$. This approach was not used in the present analysis given the preliminary nature of the findings, and the emphasis put on the recognition of the critical factors involved in the drug transport through closing microchannels *in vivo*. Yet another approach could involve simultaneous fitting of several parameters besides $h_{VT'}$, based on the transdermal data alone. However, such method was

abandoned due to high correlation between parameters of interest and, hence, low credibility of the output. The value of 0.74 mm may seem large, especially in light of the fact that skin microvasculature is located in the direct vicinity of the microchannel. For untreated skin, the distance that a permeating drug molecule needs to cross to reach cutaneous microcirculation is often assumed to be approximately 200 μm , which anatomically corresponds to the thickness of avascular epidermis. It is important to recognize that while the assumption of absolute clearance at a certain skin depth works well for many scenarios involving passive permeation through untreated skin, it does not realistically represent the *in vivo* drug transport process where barrier properties of the skin are substantially compromised^{51–54}. One such case is skin pretreatment with MN. The existence of *in vivo* capillary clearance makes the *in vivo* permeation process different from its *in vitro* counterpart. Additionally, the rate of systemic drug uptake *in vivo* may no longer be controlled by diffusion through dermis alone, but also be dependent on blood capillary permeability and the perfusion of dermal capillaries (blood flow-limited case). Therefore, the relatively large estimate of the h_{VT} parameter obtained herein may contain the contributions from blood capillary permeability and blood perfusion in addition to the diffusion through the dermis. As such, the h_{VT} parameter may be seen as a “lump” parameter which does not explicitly distinguish between contributions of the abovementioned processes. It can be expected that a more complicated clearance distribution within the dermis would more realistically describe diffusion-clearance processes^{55–58}. However, the simple modeling approach used in the present work has the advantage of providing a direct guidance with respect to the selection of skin thickness for *in vitro* diffusion studies that, after correction for the microchannel closure process, will demonstrate very good agreement with *in vivo* permeation. Additionally, this is the first case where this type of approach was used and the value of “effective” dermis thickness *in vivo*, h_{VT} , is reported. *In vitro*, the h_{VT} value can be interpreted as an effective dermis thickness between a microchannel and a receiver solution which provides true sink conditions. *In vivo*, this value can be understood as effective dermis thickness between a microchannel and a *hypothetical* dermis level at which an absolute clearance exists. Hence, the total *in vitro* skin thickness that provides good agreement with *in vivo* PK data, after accounting for microchannel closure kinetics, is 1.39 mm (0.65 mm microchannel length plus 0.74 mm dermis underneath the microchannels). It should be noted that a simple modeling approach used herein does not accommodate for several other factors that could affect drug transport *in vivo*, such as microchannel shape, length, or non-first order kinetics of microchannel closure. A more elaborate model would be required to address the above factors. For example, microneedle geometry may not only affect the fractional skin surface area of the microchannel pathway, but also the apparent value of h_{VT} . This is due to the fact that the use of one-dimensional diffusion modeling in the *in vivo* situation may be an oversimplification. At this time, it was judged that the present approach was sufficient to elucidate key factors related to NTX•HCl delivery *in vivo*. The data analysis suggests that a single major obstacle for obtaining long-term, near steady-state drug profiles *in vivo* after the use of a “poke and patch” method is the relatively rapid closure of microchannels. Delaying the onset of microchannel closure or decreasing the rate of microchannel closure could potentially greatly extend the timeframe of drug delivery via this route. This applies to a “poke and patch” method where the formulation is non-rate-limiting. Other uses of MN, e.g. inclusion of drug in polymeric dissolvable MN, would require a different treatment and optimization.

Another interesting aspect of the results discussed herein is their clinical relevance. Some differences in PK parameters are expected to exist between pigs and humans, but the general findings with respect to elucidation of key parameters governing skin drug transport following a “poke and patch” method in Yucatan minipig are clinically relevant. There are several reasons for this inference. First, the Yucatan minipig skin is characterized by anatomy and physiology similar to human skin. The porcine and human skin are both

sparsely hairy, have a thick epidermis, a dermis with a well-differentiated papillary layer and a large content of elastic tissue. Porcine skin layer thicknesses: approximately 25 μm for SC^{59, 60}, 70 μm for epidermis^{59, 60} correlate well with their human counterparts. The proteinaceous composition of the SC is grossly similar and the composition of glycosphingolipids and ceramides in pig resembles human epidermis. Dermal collagen fiber arrangement, vascularization and anatomy of the blood vessels were also found to be remarkably similar⁶¹. Moreover, the enzyme classes present in the skin of the domestic pig mirror that in human. A main morphological difference between both skin types includes a scaly, thickened porcine SC in the dorsal region. In terms of the physiology differences - dissimilar occurrence and distribution of apocrine and eccrine glands were reported. These differences manifest themselves in skin surface pH, which is close to 5 in humans and 6–7 in pigs⁶². Overall, the similarities observed between human and porcine skin are much more pronounced than between any other common laboratory animals, such as mice, rats, guinea pigs, dogs and monkeys⁶³. Second, the estimate of the Yucatan minipig microchannel closure rate was obtained from available human and rat data and its use in the present modeling showed good agreement with minipig “poke and patch” PK. This suggests that the kinetics of the underlying skin healing process leading to the closure of the microchannels over time were comparable. Third, drug diffusion coefficients in mammalian skin were found to be similar⁵⁸. Lastly, although one can argue that the experimental details used in the present animal study, such as the number of microneedle treatments or skin surface area covered with transdermal formulation may not be very practical for human use, these differences in the experimental set-up can be easily accounted for by the mathematical model. Hence, the model could be used for performing simulations/analysis of PK data from a clinical “poke and patch” study of different design. A few model simulations highlighting the insights obtained from the current Yucatan minipig “poke and patch” study are presented below. First, it is instructive to compare the microchannel pathway flux J_{MCP} uncorrected (*in vitro* conditions) and corrected for the microchannel closure process (*in vivo* conditions) through experimental skin surface area. Figure 6 demonstrates the drastic difference between *in vitro* and *in vivo* conditions. Not only does *in vivo* flux decrease sharply over time but also never quite reaches the steady-state flux level of almost 0.5 mg of NTX•HCl per hour in absence of microchannel healing. The more advanced healing process is the greater limitation if drug transport via the microchannel pathway exists. Sometimes a “lifetime” value has been assigned to microchannels, which could then be employed to determine the time window for MN-assisted drug delivery. While this approach provides some basic information with respect to timescale of the events, it fails to recognize the time-dependent changes in permeability of the microchannels. The recognition of “open/close” microchannel state depends either on arbitrary assumptions (e.g. 5–10% “open” microchannel is considered “closed”) or statistical difference in signal between MN-treated and untreated skin which, in turn, depends on the sensitivity and precision of the measurement technique (TEWL, electrical impedance, dye staining). Even more importantly, though, the consequence of microchannel transition between arbitrary “open” and “close” states does not have any bearing on the drug transport. Adopting a 10% “open” mark as borderline between the “open/close” state in the present study gives microchannels a “lifetime” of around 56 h. However, it is evident from the graph that while the J_{MCP} keeps decreasing at 56 h, there is no discontinuity in the flux at that time. The drop in J_{MCP} between 55 and 57 h is of lesser practical significance than at any time before 56 h, say, 12 and 14 h. What is of major importance is the apparent exponential decrease in permeability of the microchannels, implying that, after achieving a maximum, flux will continue to decrease by one half every 17 h. In the current study, within an “open” microchannel timeframe of 56 h, J_{MCP} will diminish as much as 8.6 times. Hence, the microchannel closure process cannot be successfully described by an on-off approach assigning either an “open” or “closed” state, but rather gradual changes in the permeability must be recognized.

Secondly, it can be visualized what the contributions of intact skin pathway and microchannel pathway are to the total flux *in vivo* across 50.24 cm² of skin area employed in the study. In the experiment reported herein, J_{ISP} was so low that it could not be detected directly with analytical methods available (below LLOQ), and its value was inferred indirectly through a least-squares fit to the model. Figure 7 demonstrates that the total flux is almost synonymous with J_{MCP} , except for late times when microchannels are almost completely closed and a low J_{ISP} value comes into play. It is also evident from the comparison of Figure 6 and Figure 7 that the time needed to reach J_{ISP} steady-state is much longer as compared to the time needed to reach J_{MCP} steady-state. Next, an interesting comparison can be made by allowing for hypothetical changes in the microchannel closure half-life ($t_{1/2}$). Experimental $t_{1/2}$ was evaluated to be around 17 h, and is too short to provide a sustained delivery of drug over the period of several days. However, if the rate of microchannel healing can be slowed four times ($t_{1/2}$ of 69 h), a substantial improvement in the flux and drug plasma concentration can be anticipated as demonstrated in Figure 8. In such case, a drop of NTX plasma concentration from around 3 to 2 ng/ml can be expected, instead of the experimentally observed drop from 2.7 to 0.4 ng/ml during 72 h. An alternative solution could involve the delay in the onset of the healing process rather than the decrease in the rate of healing. The approaches that can be utilized to achieve these goals are beyond the scope of the present work. It should be noted, however; that attempts have been made to elongate lifetime of the microchannels. Recent publications by Banks et al.³¹ and Brogden et al.⁶⁴ suggest that topical use of diclofenac, a COX enzyme inhibitor, can slow down the microchannel healing process, possibly through reduction in the inflammatory response to the micro-injury. Generally, the use of methods that can delay the onset and/or slow down the rate of microchannel closure can have a vital effect on the delivery of therapeutic agents. Finally, Figure 8 also presents the delivery profile of NTX anticipated in the absence of the microchannel closure process. This profile establishes the upper limit of *in vivo* drug delivery. As a result of the hypothetical microchannel closure half-life's extension from 17 h to 69 h to infinity (no healing), the shape of the drug delivery profile changes. While in the absence of the healing process the profile resembles a familiar shape of near zero-order delivery typically observed in classical transdermal drug delivery, with the microchannel closure process on, the shape gradually changes to resemble an oral delivery profile. Although the underlying processes behind the oral absorption processes and MN-enhanced percutaneous drug transport are different, they manifest themselves in drug plasma concentrations in a similar way. When sustained drug delivery over several days is desired, the decrease in the rate of microchannel healing could provide an extended window of high-flux NTX delivery.

In the present work, NTX•HCl delivery in Yucatan minipig with a “poke and patch” method was investigated. After an initial spike in plasma drug concentration, a rapid drop and plateau followed. A mathematical model, which incorporated the concepts of two-pathway parallel drug transport, microchannel closure process, and drug disposition and elimination *in vivo*, was developed. With the majority of the model parameters estimated independently, the overall fit of the model to experimental data was good implying that it account for key processes related to MN-enhanced drug delivery *in vivo*. Additionally, an estimate for *hypothetical* dermis thickness separating a microchannel from dermis level at which an absolute clearance exists, was obtained. Its relatively large value suggests that the effect of diffusional resistance of the dermis *in vivo* (and possibly permeability of capillaries and blood perfusion) should not be ignored and must be accounted for in order to obtain a good *in vitro-in vivo* correlation. Based on the current study, it seems that the relatively fast rate of microchannel closure is the single major hurdle for obtaining a sustained-delivery drug profile *in vivo* after a “poke and patch” method. The application of the present model to other drugs delivered by MN-enhanced skin could shed some light on the generality of the present findings. While the overall structure of the model should easily accommodate

different diffusion coefficients of drugs, density of MN treatment, microchannel closure times and pharmacokinetic parameters, it would be very interesting to see if the value of the h_{VT} parameter remains approximately constant for small permeants. The limitations of the present model include lack of explicit recognition of the microchannel length effect on the transport, ignorance of the possible dermal metabolism, protein binding, local blood flow, permeability of the capillaries, and microvasculature spatial distribution. Future developments could address the above factors by expansion of the current model.

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Abbreviations

NTX•HCl	naltrexone hydrochloride
NTXol•HCl	naltrexol hydrochloride
MN	microneedles
PK	pharmacokinetic
IVIVC	<i>in vitro-in vivo</i> correlation
PG	propylene glycol
J_{TOT}	total flux (<i>in vitro</i>)
f_{ISP}	fractional skin surface area of the intact skin pathway
J_{ISP}	flux through the intact skin pathway
f_{MCP}	fractional skin surface area of the microchannel pathway
J_{MCP}	flux through the microchannel pathway
C_m	drug concentration in the membrane
D_m	drug diffusion coefficient in the membrane
t_d	diffusion time
J_{TOT,C}	total flux corrected for the microchannel closure process (<i>in vivo</i>)
f_{OMF}	open microchannel surface area fraction
A	total skin area to which a transdermal formulation is applied
V_d	initial volume of distribution (volume of distribution of the central compartment)
k₁₂, k₁₃, k₂₁, k₃₁	micro rate constants describing drug transport between central (#1) and peripheral (#2 and #3) PK compartments
k₁₀	drug elimination constant from the central (#1) compartment
k	Boltzmann constant
T	absolute temperature
η	viscosity
r	hydrodynamic radius of the permeant
J	steady-state flux

h_{VT}	thickness of the dermis (viable tissue)
D_{VT}	diffusion coefficient of naltrexone in the dermis
C	donor solution concentration
$J_{MCP-EXP}$	experimental flux value of the microchannel pathway
$J_{MCP-CAL}$	calculated flux value of the microchannel pathway
$f_{MCP-EXP}$	experimental fractional skin surface area of the microchannel pathway
$f_{MCP-CAL}$	calculated fractional skin surface area of the microchannel pathway
D_{MC}	diffusion coefficient of naltrexone in the microchannels
h_{MC}	length of the microchannel (=thickness of the microchannel layer)
$h_{VT'}$	thickness of the dermis layer underlying the microchannel
Z_{TOT}	total electrical impedance
Z_{RB}	electrical impedance of the resistor box
Z_{ISP}	electrical impedance of the intact skin pathway
Z_{MCP}	electrical impedance of the microchannel pathway
Y	electrical admittance
Y_{MCP}	electrical admittance of the microchannel pathway
Y^0_{MCP}	initial electrical admittance of the microchannel pathway after MN treatment
k_Y	first-order rate constant of microchannel pathway electrical admittance decline
f^0_{OMF}	initial open microchannel surface area fraction
k_{OMF}	first-order rate constant of microchannel closure
CV	coefficient of variation

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Figure 1.

A schematic diagram of the model used to analyze the MN-assisted transdermal delivery of NTX•HCl in Yucatan minipig. Hydrogel drug formulation is placed on the MN-treated skin area. Skin allows drug transport through two parallel pathways: microchannel pathway (MCP) and intact skin pathway (ISP). Drug is distributed amongst central (1) and two peripheral compartments (2 and 3), and eliminated from the central compartment.

Figure 2.

An individual ($n = 1$), representative permeation profile of NTX•HCl across dermis. Filled squares (■) denote experimental data points while the solid line shows linear approximation of flux at the 6–12 h timeframe, extrapolated over the whole experiment duration. High flux resulted in approximately 50% drug depletion in the donor solution by the end of the 48 h study which manifests itself as a decline in flux at later time points.

Figure 3. NTX flux across dermis. Fourteen ($n = 14$) individual flux values denoted as filled diamonds (◆) are plotted against increasing thickness of the dermis. The solid line represents the least-squares fit to Eq. 15.

Figure 4. Yucatan minipig plasma NTX levels after IV bolus administration of 1 mg/kg dose (◆), 9 mg/kg dose (▲), and 10 mg/kg dose (●). Solid lines represent a simultaneous least-squares fit according to Eq. 23–25 for each dose level.

Figure 5.

Yucatan minipig plasma NTX levels after a “poke and patch” method. At 72 h transdermal patches were removed. Points (◆) represent mean of three ($n = 3$) determinations, while the solid line represents a least-squares fit according to Eq. 8–13 and 22 in the 0–72 h timeframe.

Figure 6. Simulation demonstrating time-dependent changes in microchannel pathway flux under *in vitro* (dotted line) and *in vivo* (solid line) conditions. Microchannel closure causes the drop in flux *in vivo*.

Figure 7. Simulation demonstrating contributions of intact skin pathway flux (gray line) and microchannel pathway flux (dotted line) to the total flux (solid black line) *in vivo*.

Figure 8. Simulation demonstrating an effect of a hypothetical change in the microchannel closure time on the plasma concentration of NTX. Solid line corresponds to microchannel closure $t_{1/2}$ of 17 h (experimental), dotted line - $t_{1/2}$ of 69 h (hypothetical), and gray line - $t_{1/2}$ of infinity (hypothetical, no microchannel closure).

Table 1

Model parameters. All the parameter values (except for two which are denoted by asterisks) were obtained through independent experiments and were kept constant during performing a least-squares fit of the model to the *in vivo* transdermal data. The two fitted parameters (denoted by asterisks) are D_{SC} (*in vivo*) and h_{VT} .

Parameter	Point estimate	95% CI	Unit
D_{SC} (<i>in vitro</i>)	$4.02 \cdot 10^{-12}$	n/a	$[cm^2 \cdot s^{-1}]$
D_{SC} (<i>in vivo</i>)*	$1.45 \cdot 10^{-12}$	$(4.99 \cdot 10^{-13}, 7.54 \cdot 10^{-12})$	$[cm^2 \cdot s^{-1}]$
h_{SC}	20	n/a	$[\mu m]$
D_{VT}	$2.46 \cdot 10^{-7}$	n/a	$[cm^2 \cdot s^{-1}]$
h_{VT} *	740	(640, 840)	$[\mu m]$
A	50.24	n/a	$[cm^2]$
f_{ISP}	1	n/a	[-]
f_{MCP}	0.0077	n/a	[-]
$k_{OMF} = k_Y$	0.041	n/a	$[h^{-1}]$
V_d	43.86	n/a	[l]
k_{12}	3.192	n/a	$[h^{-1}]$
k_{21}	2.002	n/a	$[h^{-1}]$
k_{13}	0.098	n/a	$[h^{-1}]$
k_{31}	0.050	n/a	$[h^{-1}]$
k_{10}	2.596	n/a	$[h^{-1}]$