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Transdermal Delivery of Etoposide Phosphate II: *In Vitro In Vivo* Correlations (IVIVC)

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ABSTRACT

A dependable *in vitro in vivo* correlation (IVIVC) is a vital tool to optimize drug formulation and expedite product development time. Although many IVIVC examples are available for oral delivery systems, IVIVC for transdermal delivery is far less common, especially for electrical-assisted delivery. The objective of this study was to develop an IVIVC for the iontophoretic delivery of the anticancer drug etoposide. Iontophoresis was performed at 4 current densities (100, 200, 300, and 400 $\mu A/cm^2$) both *in vitro* using a standard Franz-cell apparatus with excised porcine skin as membrane, and *in vivo* in a rabbit model. There was strong correlation between the *in vitro* % permeated across porcine skin and *in vivo* absorption (AUC, C_{max}) in the range 100-300 $\mu A/cm^2$. The correlation between *in vitro* flux and *in vivo* input rate (R_0) permitted to predict the R_0 from a different set of *in vitro* data (external validation). Convolution of such input rate accurately predicted *in vivo* plasma profiles (PE% <15) in the absorption phase, whereas the elimination phase was slightly under-predicted (PE% >20). *In vivo* absorption profiles obtained with deconvolution did not overlap directly with the *in vitro* profiles; however, correction for the lag time and the application of a scaling factor estimated from Levy' s plots resulted in excellent correlation.

Introduction

The U.S. Food and Drug Administration (FDA) formally recognized the importance of *in vitro in vivo* correlation (IVIVC) with the publication of the first Guideline in 1997 for extended release oral dosage forms.[1] The FDA defined an IVIVC as "a predictive mathematical model describing the relationship between an *in vitro* property of a dosage form (usually the rate or extent of drug dissolution or release) and a relevant *in vivo* response (i.e., plasma drug concentration or amount of drug absorbed)." A dependable IVIVC would indeed allow accurate and precise prediction of the *in vivo* kinetics of a drug substance from an *in vitro* property. The availability of such IVIVC would substantially reduce the time and costs associated with the selection of an optimal dosage form and speed up post approval changes.[2] Although the guideline addressed only oral dosage forms, in the same year, a workshop organized by the American Association of Pharmaceutical Scientists, U.S. FDA, and U.S. Pharmacopeial Convention[3] concluded that there are possible IVIVC for transdermal drug delivery systems (TDDS). However, the FDA did not release an official guideline for TDDS following the workshop, recognizing that only few studies were available at that time about IVIVC for transdermal systems. A 2012 white paper[4] acknowledges that not much progress was done in this field, and suggested that further research must be conducted to develop IVIVC for TDDS. Development of IVIVC for TDDS indeed would be useful to optimize TDDS with the fewest possible clinical trials in humans. In addition, it can possibly provide a surrogate for bioequivalence studies. Investigation of IVIVC for TDDS is an active area of research,[5-12] although it mostly focuses on passive delivery systems.

The objective of this study was to investigate different possible IVIVC approaches for an iontophoretic transdermal delivery system. Both the *in vitro* and the *in vivo* data were from a previous study exploring the possibility to deliver[13] the anticancer drug etoposide phosphate (ETP) via iontophoresis. ETP is the water-soluble form of etoposide, a drug active against various cancers.[14] ETP therapy requires multiple intravenous infusions that often force patients to refuse treatment because of the negative impact on their quality of life. Therefore, the availability of an alternative route of administration for ETP would increase patients' compliance and possibly therapeutic success. ETP is an ester pro-drug that converts only partially to the active moiety in the skin, therefore its toxicity is significantly less than the parent drug.

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Specifically, we developed and evaluated the following: (1) a Level A correlation of the *in vivo* absorption profiles—obtained by deconvolution of plasma data—with the *in vitro* permeation profiles; (2) a Level A correlation of the *in vivo* plasma profiles—predicted from the correlation between input rate (R_0) and *in vitro* flux (J_{ss})—with the observed one; and (3) a Level C correlation in which we determined a single point relationship between an *in vitro* parameter and an *in vivo* pharmacokinetic parameter either from skin or plasma.

Materials and Methods

Brief Description of Experiments

The *in vitro* and *in vivo* experiments used to build the IVIVC models were described in details in a previous communication.[13] Briefly, we delivered the drug ETP via iontophoresis at 4 current densities (100, 200, 300, and 400 μA/cm^2) both *in vitro* using a standard Franz-cell apparatus with excised porcine skin as membrane and *in vivo* in a rabbit model. Table 1 reports a summary of the most relevant experimental conditions. We also administered a 10-min IV infusion to each rabbit to determine the true clearance and volume of distribution, and the unit impulse response (UIR, disposition function). Data for external predictability analysis were obtained during the iontophoresis optimization process[15,16] under different iontophoretic conditions as reported in Table 1.

Estimation of In Vitro *Parameters*

The *in vitro* experiments provided permeability profiles and estimate of steady state flux and cumulative amount delivered. The *in vitro* flux of total etoposide, J_{ss}, was determined from the permeability profiles with the following equation:

$$J_{ss} = \frac{(Q_2 - Q_1)/A}{t_2 - t_1} = \frac{V(C_2 - C_1)/A}{t_2 - t_1} \quad (1)$$

where Q and C are the drug mass (nmol) and concentration gradient (nmol/L), respectively, in the receiver phase; A is the diffusion area (cm^2), V is the receiver solution volume (mL), and t is the time (h). The *in vitro* cumulative amount delivered was calculated from the plateau of the permeability profiles (Table 2).

Table 1
Summary of *In Vitro* and *In Vivo* Experimental Set Up and Conditions

Parameters	*In-vitro* Experiments	*In-vivo* Experiments
Instrument/assembly	Type 1 Franz diffusion cells	–
Temperature of receiving compartment	37 ± 0.5°C	–
Receiver volume	8 mL	–
Membrane/animal model	Excised porcine ear skin	New Zealand female albino rabbits A: Rabbit# 1, 2, and 3 B: Rabbit# 4, 5, 6, and 7
Average membrane thickness	1.03 ± 0.06 mm (n = 12)	–
Receiving compartment solution	Lactated Ringer's solution	–
Receiving compartment stirring speed	500 rpm	–
Iontophoretic device	IOMED® Phoresor II, model no. PM 700	IOMED® Phoresor II, model no. PM 700
Iontophoretic patch	TransQ^E (IOMED Inc., Salt Lake City, UT)	TransQ^E (IOMED Inc., Salt Lake City, UT)
Iontophoretic application area	1.0 cm^2	A: 10.1 cm^2 B: 13.4 cm^2
Iontophoretic conditions	Cathodal iontophoresis	Cathodal iontophoresis
Iontophoretic current densities	A: 100, 200, 300, and 400 μA/cm^2	A: 100, 200, 300, and 400 μA/cm^2 B: 300 μA/cm^2
Iontophoretic current application time	60 min	A: 60 min B: 30 min
Strength of donor compartment	30 mg/mL	30 mg/mL
Amount of ETP in donor	6 mg (0.2 mL)	A: 60 mg (2.0 mL) B: 75 mg (2.5 mL)

A, conditions used to generate the IVIVC models; B, conditions used for external predictability tests.

Estimation of Unit Impulse Response

The UIR is the plasma concentration resulting from the instantaneous administration of a unit amount of drug. The plasma concentrations obtained from the administration of the 10-min IV infusions of ETP (2.99 μmol/kg) were best fit with a 3-exponential disposition model as determined with analysis of residuals, parameters CV %, and Akaike Information Criterion. The estimated parameters were then used to build the 3-exponential UIR:

$$C(t) = A_1 e^{-\alpha_1 t} + A_2 e^{-\alpha_2 t} + A_3 e^{-\alpha_3 t} \quad (2)$$

Table 3 reports the values of the parameters for each individual rabbit.

Estimation of In Vivo *Parameters*

The *in vivo* pharmacokinetic parameters C_{max} and AUC were obtained by non-compartmental analysis with Phoenix 6.3 (Certara®, Princeton, NJ) for skin (using microdialysis technique) and plasma (using conventional blood sampling) data.

Because a zero-order process often describes iontophoretic delivery, we first calculated the *in vivo* amount of drug delivered as follow:

$$F \times dose\ applied = \mathrm{AUC}_{\mathrm{Iontophoresis}} \times \mathrm{Clearance}_{\mathrm{IV}} \quad (3)$$

where F is the fraction of drug permeated, $\mathrm{AUC}_{\mathrm{iontophoresis}}$ is the area under the plasma concentration curve following the iontophoretic administrations, and $\mathrm{Clearance}_{\mathrm{IV}}$ is the plasma clearance estimated in the same rabbit from the short IV infusion data. Then, assuming that delivery lasted for the duration of electrical current application (60 min), the zero-order delivery rate (R_0, amount/time) was calculated as follows:

$$R_0 = \frac{F \times dose\ applied}{Time\ of\ patch\ application} \quad (4)$$

The *in vivo* studies provided plasma concentration profiles and estimates of C_{max}, AUC, cumulative amount delivered, and input rate (Table 2).

Development of Transdermal IVIVC

Relationship Between In Vitro *and* In Vivo *Parameters (Level C)*

Both skin and plasma data obtained from all the 4 different current densities were considered for this approach. Individual *in vitro* parameter directly compared with individual *in vivo* parameter for either skin or plasma data as discussed in previous section considering all the current densities to evaluate relationship between *in vitro* and *in vivo* parameters.