In Vitro Percutaneous Absorption of Ketoprofen and Testosterone: Comparison of Pluronic Lecithin Organogel vs. Pentravan Cream

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ABSTRACT

An *in vitro* human percutaneous absorption study was conducted to assess the delivery of ketoprofen and testosterone from two base formulations, a Pluronic lecithin organogel and Pentravan Cream. Each formulation was applied to *ex vivo* human trunk skin (from three skin donors) on triplicate sections mounted onto Franz Diffusion Cells. Following a 5-mcL/cm^2 applied dose, serial dermal receptor solutions were collected over 48 hours. For both compounds, a greater rate and extent of absorption was found from the Pentravan formulation than from the Pluronic lecithin organogel formulation: 3.8-fold greater for ketoprofen, 1.7-fold greater for testosterone, for amount absorbed.

INTRODUCTION

The first article on the innovative transdermal delivery system "Pluronic lecithin organogel," or PLO gel, appeared in this journal in the late 1990s.[1-3] PLO gel has been a popular base formulation used by compounding pharmacists for topical and systemic delivery of medications through the skin ever since. Over the ensuing decade, PLO gel has been utilized to deliver a variety of medications, from nonsteroidal anti-inflammatory drugs (NSAIDs) to hormones. The library of formulations using PLO gel is extensive, and the clinical evidence of the efficacy of this liposomal delivery system is evident in the literature.[4-6]

In the decade that followed its introduction, alternatives to PLO gel have been provided by several suppliers of pharmaceutical compounding ingredients. These cream versions of PLO gel were first created in response to cosmetic concerns expressed by patients. With the original PLO gel design, in order to ensure safe and effective use, the site of application was frequently covered to prevent the medication from transferring to clothing and other individuals during the time of absorption. A vanishing cream version of PLO removed this concern by using the oil phase of PLO gel as the oil phase of a standard oil-in-water vanishing cream. Pentravan was designed to maintain the liposomal technology of PLO while removing the cosmetic and preparation drawbacks. Included in the design and development of the Pentravan base vehicle were consider-

ations for ease in preparation, stability in its physical properties, and increased drug loading capacity, to accommodate higher concentrations when needed.

This study compares the original PLO gel, to a "next generation" of compounding vehicles. The study examined two drugs, ketoprofen, one of the original active ingredients used to develop PLO gel, along with testosterone. Each drug was evaluated in both the PLO gel and Pentravan formulations on *ex vivo* human skin using Franz Diffusion Cells[7] and the finite dose model.[8]

The *in vitro* Franz human skin finite dose model has proven to be a valuable tool for the study of percutaneous absorption and the determination of the pharmacokinetics of topically applied drugs. The model uses *ex vivo* human skin, most often trunk skin, mounted in specially designed static diffusion chambers allowing the skin to be maintained at a temperature and humidity that match typical *in vivo* conditions. A finite dose (for example, 2 mg/cm^2 – 10 mg/cm^2) of formulation is applied to the outer surface of the skin, and drug absorption is measured by monitoring its rate of appearance in the dermal receptor

solution bathing the inner surface of the skin. Data defining total absorption, rate of absorption, as well as skin content can be accurately determined in this model. The method has historic precedent for predicting *in vivo* percutaneous absorption kinetics accurately.[9-10]

MATERIALS AND METHODS

Test Articles and Reagents

The test formulations consisted of 10% ketoprofen in PLO gel (Lot Keto10PLO), 10% ketoprofen in Pentravan cream (Lot Keto10Pentra), 10% testosterone in PLO gel (Lot Testos10PLO), and 10% testosterone in Pentravan cream (Lot Testos10Pentra). These formulations were prepared by Fagron US (formerly Gallipot, Inc., Saint Paul, Minnesota). Neat Ketoprofen USP (Gallipot, Inc, Lot 1105114H13, Correction factor 0.997) and Neat Testosterone USP (Micronized C-III: Gallipot, Inc., Lot 1107314B15, Correction factor 0.994), for use as analytical standards, were also provided by Fagron US. Distilled deionized (DDI) water was prepared by the research laboratory, distilled using a Thermo Scientific Barnstead Megapure 6A water still (Series 1924, Model A440697 208V;

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Dubuque, Iowa) and DDI using a Barnstead deionizer (Model D11911). Phosphate buffered saline (PBS) was obtained as a concentrate from Fisher Scientific (Pittsburgh, Pennsylvania) as EMD brand 10X PBS Lot 3261C467, and diluted with DDI water by the research laboratory. Oleth-20 (Lot 0000454925, Product 041581) was obtained from Croda Inc. (Edison, New Jersey), Gentamicin sulfate was obtained either as Lot 01-681-DK from Hospira, Inc. (Lake Forest, Illinois), or as Lot 110M0800 from Sigma-Aldrich (Saint Louis, Missouri). Tritiated water (^3H_2O) (Lot 3615509, production date 12/19/08) was purchased from PerkinElmer (Boston, Massachusetts) at a 1 microCurie/mL specific activity, which was diluted with DDI prior to use. Scintillation spectroscopy was performed using a PerkinElmer (Boston, Massachusetts) Tri-Carb 3100TR Scintillation Counter. Receptor solution samples were concentrated by vacuum centrifugation using Thermo Scientific (Asheville, North Carolina) Savant Speed-Vac Models ISS100, ISS110, and SPD2010. Sample processing reagents and high-performance liquid chromatography (HPLC) reagents used for sample analysis were obtained from two suppliers, either from Fisher Scientific (Pittsburgh, Pennsylvania) or from VWR International (Radnor, Pennsylvania), and included acetonitrile (Fisher Optima grade Lots 110207 and 110480; EMD brand HPLC grade Lot 49162); ethanol (Fisher HPLC grade Lots 112500 and 106073); methanol (Honeywell HPLC grade Lots DC873 and DD373); isopropyl alcohol (JT Baker USP grade Lot J44B08); dipotassium hydrogen phosphate (K_2HPO_4) (JT Baker USP grade Lots C04H03 and E41476); hydrochloric acid (Fisher ACS grade Lot 091250); sodium hydroxide (BDH brand ACS grade Lot 78450); and Scintiverse (Fisher Scintanalyzed Lots 100719 and 111750). Human *ex vivo* skin (pivotal study donors WF030307, JG121609, and NL060710) was obtained from the New York Firefighters Skin Bank (New York, New York).

Diffusion Cell Preparation

Human, *ex vivo*, trunk skin without obvious signs of skin disease was used in this study. The skin was dermatomed at collection, cryopreserved, sealed in a water-impermeable bag, and stored at -70°C until the day of the experiment. Prior to use, it was thawed in ~37°C water, then rinsed in water to remove any adherent blood and other materials from the surface.

The skin from each donor was then cut into multiple smaller sections large enough to fit on 1-cm^2 Franz Diffusion Cells. The dermal receptor compartment was filled to capacity with the receptor solution of phosphate buffered saline with 0.008% gentamicin (to minimize microbial growth), and the epidermal chamber (chimney) was left open to the ambient laboratory environment. The cells were then placed in a diffusion apparatus in which the dermal receptor solution was stirred magnetically at 600 RPM and its temperature maintained to achieve a skin surface temperature of 32.0°C ± 1.0°C (surface temperature determined using a non-contact, calibrated surface, infrared temperature indicator). The ambient laboratory conditions were controlled within a target range for relative humidity of 45% (± 10%), and within a target range for temperature of 21°C ± 4°C, both monitored with digital recording systems.

Randomly selected skin donors consisted of two males (Caucasian; 46 years of age and 61 years of age) and one female (Hispanic; 50 years of age) from which three skin sections were prepared from each donor and for each test formulation. To assure the barrier integrity of each skin section, its permeability to tritiated water was determined before application of the test products. Following a brief (0.5 to 1 hour) equilibrium period, ^3H_2O (~ 0.5 mcCi/mL) was layered across the top of the skin so that the entire exposed surface was covered (approximately 200 to 500 mcL). At five minutes after application, the ^3H_2O aqueous layer was removed by blotting with laboratory tissue. At 30 minutes after application, the receptor solution was collected and analyzed for radioactive content by liquid scintillation counting. Specimens of torso skin in which absorption of ^3H_2O are less than 1.56 mcL/cm^2 are considered acceptable based on historical data.[11-12] All skin sections dosed were within the acceptance criterion, and the donors were within the normal water absorption range observed from the general population.

Dosing and Sample Collection

Prior to administration of the topical test formulations to the skin sections, a pre-dose receptor solution sample was collected, and the entire receptor compartment was refilled with PBS containing 0.008% gentamicin for those skin sections to be dosed with the ketoprofen formulations, and 0.1x-PBS containing 0.1% oleth-20 and 0.008% gentamicin for those skin sections to be dosed with the testosterone formulations. The oleth-20 was added to ensure sufficient sink solubility for testosterone in the aqueous media. The chimney was then temporarily removed from the Franz Diffusion Cell to allow full access to the epidermal surface of the skin.

Subsequently, a pre-determined volume amount representing a 5-mg amount of formulation/cm^2/skin section was drawn up and dispensed by using a calibrated positive displacement pipette and evenly dispersed and rubbed into the skin surface using a glass rod. The glass rod was retained for analysis of formulation residue, to correct for the actual applied dose. Non-dosed diffusion (control) cells, with skin from each donor were also included in the study.

Approximately five to ten minutes after dose application, the donor compartment (chimney) of the Franz Diffusion Cell was replaced. At 2, 4, 8, 12, 24, 32, and 48 hours after dose application, the receptor solution was removed in its entirety, and a 1-mL volume aliquot (ketoprofen-dosed skin sections) and a 4-mL volume aliquot (testosterone-dosed skin sections) were saved for subsequent analysis. All samples were stored at -20°C pending processing and analysis.

The glass rods were extracted overnight in 1 mL isopropanol. The receptor solutions from the testosterone-dosed skin sections were concentrated by vacuum centrifugation, and reconstituted in 400 mcL 50:50 ethanol:water (v/v). All other samples were assayed directly.

Analytical

Quantification of ketoprofen was by HPLC/ultraviolet (UV). A solvent system consisting of A) 0.2% K_2HPO_4 in DDI, pH 7.5, and B) acetonitrile, using a gradient starting as 77% A to 60% A over 2 minutes, was run through a Phenomenex

(Torrance, California) Gemini C18 column (50 × 3.0 mm, 3 microns) at a flow rate of 0.4 mL/min. Samples, including standard curves and quality control samples, were injected at 2 mcL. Eluting peaks were measured at 256 nm (4 nm bandwidth) referenced to 500 nm (50 nm bandwidth). Limit of quantification was set at 0.1 mcg/mL.

Quantification of testosterone was by HPLC/UV. A solvent system consisting of an isocratic mixture of methanol (75%) and DDI (25%) was run through a Phenomenex Luna C18(2) column (100 × 4.6 mm, 3 microns) at a flow rate of 0.750 mL/min. Samples, including standard curves and quality-control samples, were injected at 10 mcL. Eluting peaks were measured at 245 nm (4 nm bandwidth) referenced to 450 nm (50 nm bandwidth). Limit of quantification was set at 0.05 mcg/mL.

Regulatory Applicability

The study was conducted in a manner that is compatible with Organisation for Economic Co-operation and Development Principles of Good Laboratory Practice, 13 and the receptor sample analytical methodology validation was performed in a manner compatible with the International Conference on Harmonisation Harmonised Tripartite Guideline.[14] All data were reviewed and verified by quality-control staff. Adherence to the protocol and laboratory standard operating procedures were reviewed and verified by quality-assurance staff.

RESULTS

Rate of percutaneous absorption is presented as the flux of ketoprofen and testosterone that appears in the receptor solution under the skin over time. Analytical source data were corrected for actual applied dose and receptor chamber volume to obtain the actual amount absorbed between each sampling time point. Individual diffusion cell penetration values were calculated then averaged across replicates for a donor mean ± standard deviation (SD). The donor means were then averaged to obtain a population mean ± standard error of the mean (SEM). Statistical comparisons were made using the student's T-test for 2-tailed equal variance. In addition, the Pentravan/PLO ratio was

evaluated using the z-test for assessing significance for greater total absorption.

Table 1 lists the measured flux of ketoprofen across the three donors evaluated. Table 2 lists the measured flux of testosterone across the three donors evaluated. This data is also presented in Figures 1 and 2. As flux (rate of penetration) is not a discrete, directly measureable value, but rather is a time-averaged value determined across a sampling period, by convention, flux is reported at the mid-point of sample collection for that sample period.

CONCLUSIONS AND DISCUSSION

There are numerous critical considerations regarding the quality and utility of a formulation base for topical delivery of medications.[15-17] These include its versatility, the ability to dissolve a wide range of medications with different chemical properties, the ability to accommodate drug-loading levels for use at higher concentrations, adequate shelf-life stability, drug release from the formulation, rate and extent of drug delivery, patient acceptability, ease in preparation, and affordability. The next generation of compounding vehicles must be expected to adequately balance these considerations, and acceptably satisfy the intended therapeutic needs. Of these considerations, this study only addresses the rate and extent of drug delivery for two compounds: ketoprofen and testosterone.

TABLE 1. Mean Flux (mcg/cm^2/hr) Results: Ketoprofen. (Mean ± SEM, n=3 Donors)

| TIME (HR)[a]	KETOPROFEN 10% IN PENTRAVAN LOT KETO10PENTRA	KETOPROFEN 10% IN PLO LOT KETO10PLO
1.0	0.165 ± 0.165	0.097 ± 0.043
3.0	0.755 ± 0.538	0.260 ± 0.152
6.0	1.384 ± 0.774	0.389 ± 0.187
10.0	1.722 ± 0.775	0.450 ± 0.195
18.0	1.677 ± 0.522	0.420 ± 0.149
28.0	1.627 ± 0.422	0.421 ± 0.141
40.0	1.249 ± 0.263	0.337 ± 0.091
Receptor (mcg/cm^2)	67.29 ± 21.37	17.92 ± 6.16
Receptor (%)[b]	13.12 ± 1.05	3.63 ± 1.23

[a]Time as midpoint between samples; [b]Percent of applied dose; PLO = Pluronic lecithin organogel

**TABLE 2. Mean Flux (mcg/cm^2/hr) Results: Testosterone. (Mean ± SEM, n=3 Donors)**

TIME (HR)[a]	TESTOSTERONE 10% IN PENTRAVAN LOT TESTOS10PENTRA	TESTOSTERONE 10% IN PLO LOT TESTOS10PLO
1.0	0.025 ± 0.016	0.005 ± 0.003
3.0	0.075 ± 0.038	0.027 ± 0.008
6.0	0.097 ± 0.047	0.039 ± 0.009
10.0	0.106 ± 0.305	0.047 ± 0.009
18.0	0.095 ± 0.032	0.053 ± 0.009
28.0	0.095 ± 0.026	0.058 ± 0.009
40.0	0.082 ± 0.019	0.051 ± 0.007
Receptor (mcg/cm^2)	4.22 ± 1.36	2.31 ± 0.35
Receptor (%)[b]	0.86 ± 0.28	0.48 ± 0.07

[a]Time as midpoint between samples; [b]Percent of applied dose; PLO = Pluronic lecithin organogel

FIGURE 1. Mean flux (mcg/cm^2/hr) results: Ketoprofen. (Mean ± SEM)
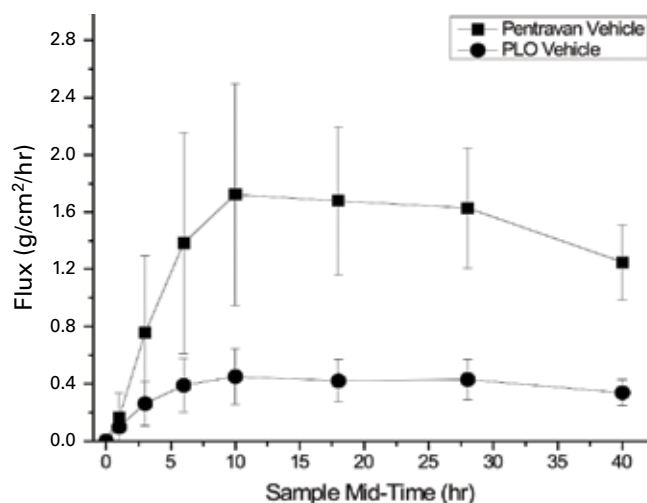
FIGURE 2. Mean flux (mcg/cm^2/hr) results: Testosterone. (Mean ± SEM)
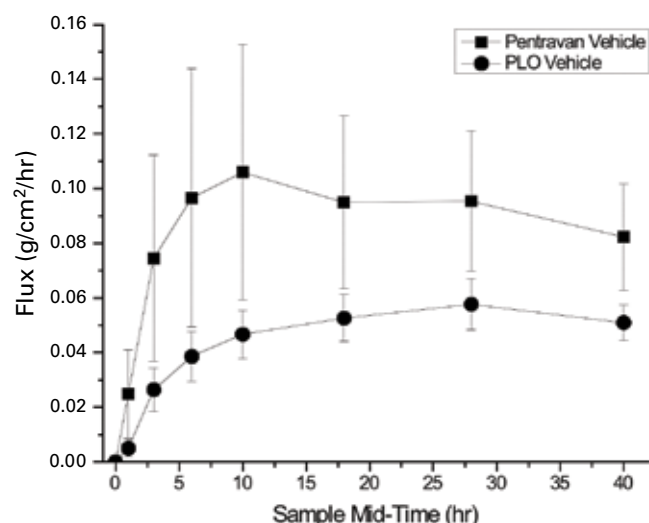
This study was designed to evaluate the potential therapeutic utility, based on delivery of ketoprofen and testosterone, from a novel Pentravan base compounding vehicle and comparing it to the traditional PLO gel vehicle. This *in vitro* study was conducted to evaluate the performance of these compounding vehicles when applied to *ex vivo* human skin, using the Franz finite dose model, which has been demonstrated to correlate with clinical study results.

The data demonstrates that both ketoprofen and testosterone do penetrate into and through human skin from both of the compounding vehicles evaluated. The penetration profile for ketoprofen was similar in both formulations and is characterized by a gradual rise to a peak rate of penetration approximately 10 hours after dose application, followed by a relatively sustained or slow decline in flux thereafter. The penetration profile for testosterone varied somewhat between the two formulations with a peak rate of penetration being seen at 10 hours after application from the Pentravan formulation, and at approximately 28 hours after dose application from the PLO formulation.

For both test compounds, the Pentravan formulation delivered more drug, both in rate and amount absorbed, through the skin than the PLO formulation. This greater delivery was found to be statistically consistent (P <0.001 using the z-test) for both compounds based on the ratio of Pentravan/PLO for total absorption. Based on the magnitude of difference, in the case of ketoprofen, 3.8-fold more drug was delivered (P=0.091) from the Pentravan formulation than from the PLO formulation. For testosterone, the difference was not significant (P=0.2446), but the results did demonstrate a 1.7-fold greater delivery from the Pentravan formulation than from the PLO formulation.

The results observed with the Pentravan formulation suggest that it may provide improved performance characteristics relative to the PLO gel, which may be particularly important in situations where the PLO gel may not be facilitating the desired clinical response. Although this study only evaluated two popular formulations for topically applied compounding preparations, it is reasonable to anticipate that, relative to the PLO gel, Pentravan might likely provide similarly enhanced delivery and performance with most other topically applied NSAIDS and hormones in a related chemical class to each of the compounds evaluated in this study.

FINANCIAL DISCLOSURE

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