Enhanced Percutaneous Permeation After Fractional Thermo-Ablation: an Ex Vivo Study

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ABSTRACT

Background: *Thermo mechanical ablation (TMA) is a novel platform for fractional ablation and non-ablation for skin treatment with no pain.*

Objective: *This study aimed to evaluate the effects of TMA pre-treatment to facilitate transdermal drug delivery.*

Methods: *Novoxel's proprietary Tixel® TMA system was evaluated in this study by a diffusion cell system using excised porcine ear skin. A 1 cm^2 tip, composed of a matrix of metallic pyramids, applied on freshly excised pig ear skin samples was operated at an ablative temperature of 400^oC for 6ms and 9ms. Verapamil hydrochloride 1% was used as a hydrophilic model drug known to have a poor absorption across mammal skin.*

Results: *The present study has demonstrated that Tixel treatment for 9 or 6 ms increased skin permeability for verapamil along a 24-hour application period, with a highly significant difference relative to non-treatment control ($p<0.05$). After 24 hours, the cumulative amount of the drug that had penetrated into 9ms Tixel-treated skin was 728.6 (±358.0 SD) µg/cm^2 (n=11) compared to 33 (±25 SD) µg/cm^2 that penetrated across untreated skin (n=8).*

Conclusions: *Fractional TMA effectively enhanced verapamil skin penetration.*

Keywords: verapamil, ablation, transdermal delivery, permeation, Franz cell

INTRODUCTION

Verapamil hydrochloride is a calcium-channel blocker which is classified as a class IV anti-arrhythmic agent. It is generally used in the control of supraventricular tachyarrhythmias, in the management of classical and variant angina pectoris, and in the treatment of hypertension. Although treatment with verapamil is well tolerated, adverse effects connected with its pharmacological effects on cardiac conduction can arise and may be sometimes severe. Adverse reactions include bradycardia, heart block, heart failure, transient asystole, and constipation.

Topical verapamil hydrochloride has been a widely investigated drug for Peyronie's disease by injection or by transdermal gels. Peyronie's disease is a fibrosis of the penile tunica albuginea estimated to effect from 3%, as found in a German study, to approximately 9% of men, as determined in a population of men screened for prostate cancer [1]. It has been shown that its intralesional injection in Peyronie's plaque had a beneficial effect on alleviating the pain and improvement of the curvature and the sexual performance [2]. The research has demonstrated that the drug has the ability to increase collagenase activity, resulting in breaking down and remodeling the excess collagen. On the basis of the injectable verapamil study, a transdermal verapamil 15% gel has been prescribed and marketed under FDA approval [3]. Apart of the advantageous use of verapamil in Peyronie's disease, microneedle-mediated transdermal verapamil delivery was also proposed as a beneficial technology for patients with hypertension. Application of stainless steel microneedles increased transdermal delivery of verapamil hydrochloride across porcine ear skin [4].

Verapamil hydrochloride is 491.06Da molecule with hydrophilic properties, making it an ideal molecular model for evaluating the TMA system as a transdermal drug enhancer delivery system. The study was performed in a Franz diffusion cell system using pig ear skin as a skin model and a sensitive HPLC assay to evaluate drug transport across the skin.

MATERIAL AND METHODS

Materials

Verapamil hydrochloride (Euroasian Chemicals Ltd., Mumbai, India; MfD: 02/2014) was freshly prepared before each experiment at 1% solution with pure water. High-performance liquid chromatography (HPLC) grade solvents were obtained from J.T. Baker (Mallinckrodt Baker, INc., Phillipsburg, NJ, USA).

Skin preparation

The animal protocol was reviewed and approved by the Institutional Animal Care and Use Committee, which complies with the Israeli Law of Human Care and Use of Laboratory Animals, 1994.

Full-thickness porcine skins were excised from fresh ears of slaughtered white pigs (100 kg, aged 6 months, breeding of Landres and Large White, Kibbutz Lahav, Israel). Skin sections were cut from the outer side only and subcutaneous fat was removed with a scalpel. The skin sections were measured for transepidermal water loss (TEWL - Dermalab® Cortex Technology instrument, Hadsund, Denmark). Only pieces that the TEWL levels were less than 10 g/m^2/h were used.

Skin pretreatment with Tixel

Excised skins were treated with Tixel Thermo-Mechanical Ablation (TMA) System (Tixel, NOVOXEL ltd, Israel). A S-Tip, made of a matrix of gold-plated sharp stainless steel pyramids, was placed in contact with the ex vivo skin samples and was operated at 400^oC for 6ms or 9ms [5]. Control skins with no pretreatment were also collected.

In vitro skin permeation study

Immediately after skin treatment, the permeability of verapamil through porcine skin was determined in vitro with a Franz diffusion cell system (Crown Bioscientific, Inc., Clinton, NJ) [6]. Treated and control skins were placed on the receiver chambers with the stratum corneum facing upwards, and the donor chambers were then clamped in place. The excess skin was trimmed off, and the receiver chamber, defined as the side facing the dermis, was filled with phosphate buffered saline (PBS containing 10 mM PO_4^{-3}, 137 mM NaCl, and 2.7 mM KCl, pH 7.4). The diffusion area was 1.767 cm^2 (15 mm diameter orifice), and the receptor compartment volume was 12 ml. The solutions in the water-jacketed cells were thermostated at 37°C and stirred. After 15 min of skin washing at 37°C, the buffer was removed from the cells. Aliquots (0.5 g each) of verapamil hydrochloride 1% solution in purified water were placed in the donor chamber, and the receiver chambers were refilled with a fresh phosphate buffered saline (PBS, pH=7.4). Samples (2 ml) were withdrawn from the receiver solution at predetermined time intervals (3, 5, 7, 9, 12, 20 and 24 hours) and kept at -20°C until analyzed by HPLC. The cells were replenished up to their marked volumes with fresh buffer solution each time.

HPLC analysis of samples from receiver solutions

Aliquots of 20 µl from each vial were injected into HPLC system (Shimadzu VP series including diode-array detector for peak spectrum identification), equipped with a pre-packed C_{18} column (Betasil C18, 5µm, 250X4.6mm, ThermoHypersil, UK) heated to a temperature of 30°C. The quantitation of verapamil was performed by integration of peaks detected at 201 nm. The samples were chromatographed using an isocratic mobile phase consisting of phosphate buffer (0.02M, pH 3.0) - acetonitrile (50:50) at a flow rate of 1.0 ml/min. A calibration curve (peak area versus drug concentration) was constructed by running working standard of verapamil HCl solutions for every series of chromatographed samples. Calibration curves were linear over the range 0.1-100 µg/ml. Data were expressed as the permeating drug quantity per unit of the skin surface area, Q_t/S (S = 1.767 cm^2). Cumulative drug permeations (Q_t) were calculated as in [7].

Statistical analysis

The statistical differences between the skin permeation profiles of the formulations were analyzed employing the two-way unweighted means analysis of variance (ANOVA) test. The differences among group means were considered significant for *p* values < 0.05. Results are given as average ± standard deviation.

Histology

Treated skin samples were fixed in formalin, embedded in paraffin and stained for H&E for histopathological examination.

RESULTS and DISCUSSION

The penetration of verapamil hydrochloride increased about 5 and 20 times after skin had been pretreated with Tixel TMA system for 6 *ms* and 9*ms*, respectively (Q_{24} = 168.16± 93 µg/cm^2 (n=8) and 728 ±358 µg/cm^2 (n=11) compared to Q_{24}=33.75 ± 25.3 µg/cm^2 (n=5) in untreated skin. The statistical tests revealed highly significant differences between the experimental groups ($p<0.05$) (Fig 1)

In addition, lag time to get quantitative permeation across the skin decreased significantly from 9-10 hours in untreated skin to 3-5 hours in Tixel-treated skin (fig 2).

Examination of the histological sections for 9ms pretreatment showed a focal dermal coagulation and partial epidermis evaporation (fig 3).

CONCLUSION

This study demonstrates that TMA pretreatment of skin enhances the in vitro absorption of verapamil hydrochloride, a hydrophilic drug.

ACKNOWLEDGEMENT

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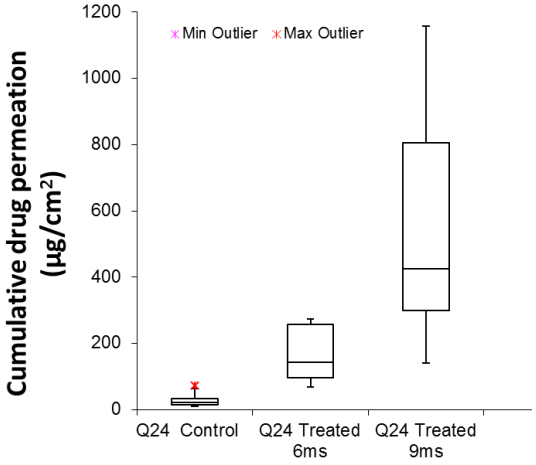

Figure 1: Cumulative amount of Verapamil along a 24h application period. The boxplots show significant increased Verapamil transdermal delivery in pre-treated ex vivo porcine skin compared to untreated skin ($p<0.05$ for both).

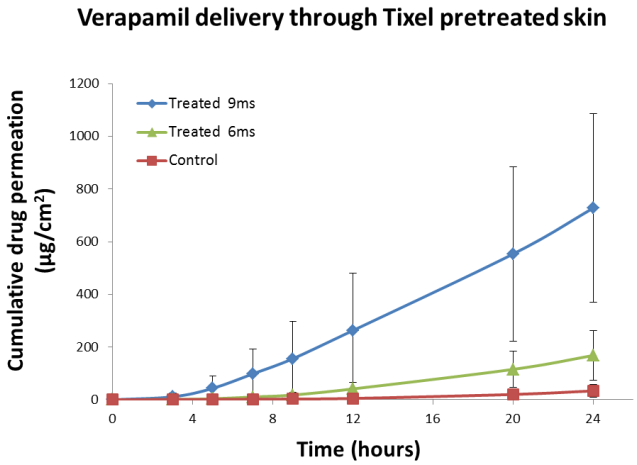

Figure 2: Verapamil delivery through Tixel pretreated skin at 6ms and 9ms pulse and untreated skin. The penetration of verapamil hydrochloride increased about 5 and 20 times after skin had been pretreated with Tixel TMA system for 6 *ms* and 9*ms*, respectively. Lag time to get quantitative permeation across the skin decreased significantly from 9-10 hours in untreated skin to 3-5 hours in Tixel-treated skin.

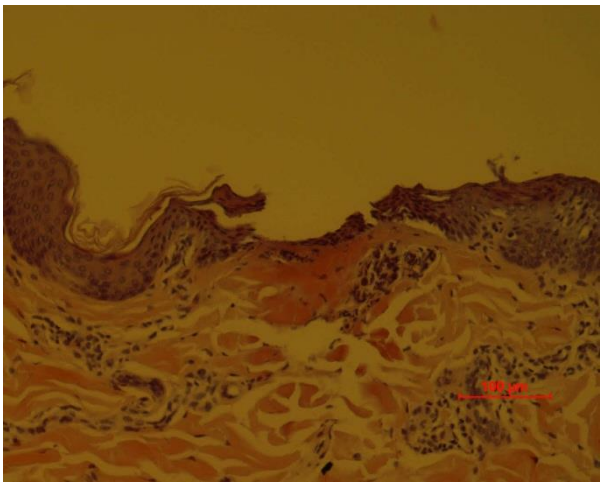

Figure 3: Histology of the ex vivo skin immediately after TMA treatment with 9ms pulse.