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Synergistic effect of iontophoresis and soluble microneedles for transdermal delivery of methotrexate

Viswatej Vemulapalli, Ye Yang, Phillip M. Friden and Ajay K. Banga

Abstract

The aim of this study was to investigate the transdermal iontophoretic delivery of methotrexate, alone or in combination with microneedles, in-vitro and in-vivo using intracutaneous microdialysis in the hairless rat. The average depth of the microdialysis probe in the skin was found to be 0.54 mm. Methotrexate was stable in the presence of an applied electric field as determined by cyclic voltammetry. A current density of 0.4 mA cm^{-2} applied for 60 min was used in combination with maltose microneedles to enhance delivery of methotrexate across the skin. Delivery was enhanced by iontophoresis and microneedles, both in-vitro and in-vivo. A synergistic 25-fold enhancement of delivery was observed in-vivo when a combination of microneedles and iontophoresis was used compared with either modality alone.

Introduction

Methotrexate (4-amino-N^{10}-methyl pteroyl-L-glutamic acid; MTX) is a folic acid antagonist with anti-neoplastic activity that is used in the treatment of psoriasis and rheumatoid arthritis. MTX acts by competitively inhibiting the enzyme dihydrofolate reductase, resulting in the inhibition of DNA synthesis. Psoriasis is a common chronic disorder characterized by hyperproliferation of epidermal cells, leading to erythematous papules and plaques. Since MTX inhibits mitotic activity, it is used in the treatment of psoriasis. MTX is generally administered either parenterally or orally for the treatment of psoriasis. The general dose range of MTX for psoriasis and rheumatoid arthritis is 7.5–25 mg per week. The systemic use of MTX is known to induce hepatotoxicity, suppresses bone marrow function and causes other adverse effects, such as nausea, vomiting, anaemia, fatigue, headache and thrombocytopenia, when used over a prolonged period of time.

Topical delivery of MTX at the psoriatic site has the potential to reduce the systemic side effects associated with this drug, and avoids first-pass elimination. Efforts have been made to enhance the delivery of MTX across the skin by formulating the drug in gels and creams and using enhancement methods (Vaidyanathan et al 1985; Alvarez-Figueroa & Blanco-Mendez 2001; Alvarez-Figueroa et al 2001; Sutton et al 2001; Syed et al 2001; Wong et al 2005). However, MTX (mol wt 454.44 g mol^{-1}), being hydrophilic (log P −1.85), is ionized at physiological pH, which limits passive permeation across the skin. Iontophoresis (ITP) is an active energy process which uses small amounts of physiologically acceptable electric current to drive ionic drugs into the body (Banga 1998). As MTX is negatively charged at physiological pH it could be delivered by cathodal ITP by means of electrorepulsion. Recently, a clinical study on MTX delivery by ITP suggested that a short application of current is sufficient for clinical efficacy and treatment of recalcitrant psoriasis (Tiwari et al 2003). Thus, detailed evaluation of enhancement technologies and relevant parameters has become important.

Microneedles have recently been investigated to create micron-sized holes in the skin by disrupting the stratum corneum. This minimally invasive technique is painless, as the microneedles pass only through the stratum corneum and viable epidermis, whereas the nerves that produce stimuli to the pain are in the deeper dermis. Microneedle-mediated drug delivery can be achieved by applying the drug formulation on microporated skin or by directly coating the microneedles with the drug (Cormier et al 2004; Davis et al 2004; Prausnitz 2004; Park et al 2005; Coulma et al 2006; Martanto et al 2006). Microneedles,

Department of Pharmaceutical Sciences, College of Pharmacy and Health Sciences, Mercer University, Atlanta, GA 30341, USA

Viswatej Vemulapalli, Ye Yang, Ajay K. Banga

Transport Pharmaceuticals Inc., Framingham, MA 01701, USA

Phillip M. Friden

Correspondence: Professor Ajay K. Banga, Department of Pharmaceutical Sciences, College of Pharmacy and Health Sciences, Mercer University, Atlanta, GA 30341, USA. E-mail: banga_ak@mercer.edu

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alone or in combination with ITP, have been used to deliver MTX into or across the skin. Microneedles can be made of a variety of materials such as silicon or metal, and can be solid or hollow. We used soluble microneedles made of maltose in this study.

Microdialysis allows continuous sampling of low-molecular-weight compounds in the extracellular fluid of the specific tissue into which a probe is inserted (Stagni & Shukla 2003). It involves minimal tissue damage during probe insertion, and barrier integrity recovers quickly (Chaurasia 1999; Cano-Cebrian et al 2005). In this study, microdialysis was used to monitor the delivery of MTX in the skin when ITP was used alone or with microneedles.

The aim of this work was to determine the effect of ITP, alone and in combination with soluble maltose microneedles, in-vitro and in-vivo, by means of intracutaneous microdialysis.

Materials and Methods

Methotrexate was obtained from Sigma (St Louis, MO, USA). All other chemicals and HPLC-grade solvents were obtained from Fisher Scientific (Pittsburgh, PA, USA).

Microneedles

Sharp-tipped, three-dimensional solid tetrahedron-shaped microneedles were developed and supplied by Texmac Inc. (Charlotte, NC, USA) using micromoulding technology, as described by Miyano et al (2005). Depending on the desired length of the microneedles, moulds from 200 μm to 2 mm are available. Two-line microneedles (28 microneedles 500 μm long per line, stacked in two parallel lines) made of maltose were used in these experiments. The microneedles were 508.46 ± 9.32 μm long with a radius of curvature of 3 μm at the tip. The microchannels created in the skin by the microneedles measured 55.42 ± 8.66 μm in diameter, as characterized by Kolli & Banga (2007). Microneedles did not contain any drug; the drug formulation was applied later to the microporated skin in all cases.

Scanning electron microscopy

Microneedles were mounted on aluminum stub using a double-sided carbon sticky tape and sputter coated using Emscope (SC 500 M) with gold target. The microneedles were examined using a field emission scanning electron microscope (SEM, Topcon, DS-130F, NJ, USA). The primary beam accelerating voltage was 10 KV and secondary electron images were collected digitally.

In-vitro permeation studies

The Institutional Animal Care and Use Committee of Mercer University approved all the animal procedures. Male hairless rats (8–10 weeks old) were purchased from Charles River, Wilmington, MA, USA.

The rats were asphyxiated using carbon dioxide, and full-thickness abdominal skin was excised immediately, with an average thickness of 1.15 mm. Skin was equilibrated in the receptor buffer (see below) and mounted on Franz transdermal diffusion cells (0.64 cm^2). The donor compartment (0.5 mL) contained MTX in phosphate buffer (pH 7.4, 250 mM). The receptor compartment contained phosphate buffer strength supplemented with 75 mM NaCl to drive the electrochemistry. MTX (pKa values 5.6, 4.8 and 3.8) is negatively charged at physiological pH and was therefore delivered by cathodal ITP. The cathode (silver–silver chloride; In vivo Metric, Healdsburg, CA, USA) was placed in the donor chamber together with drug solution, and the anode (silver wire, 0.5 mm diameter, 99.9%, from Aldrich, St Louis, MO, USA) was placed in the receptor chamber. Microneedles were used alone or in combination with current density of 0.4 mA cm^{-2} for 60 min. Samples (0.3 mL) were taken from the receptor chamber at predetermined time points following ITP and were replaced with the equivalent volume of receptor buffer. All experiments were done at least in triplicate. The samples were then analysed for MTX content by HPLC.

In-vivo studies

Male hairless rats (8–10 weeks old, weighing 270–320 g) were anaesthetized using ketamine and xylazine. Approximately 200 mg MTX gel (1% hydroxy ethyl cellulose (Hercules, Wilmington, DE, USA)) was placed in a drug cartridge specifically designed for iontophoretic delivery (Figure 1) and was kept in contact with the skin for 1 h. The cathode from a constant-current power supply (Keithley 2400, Cleveland, OH, USA) was connected to the drug cartridge and the anode was connected to a Trans Q (IOMED, Salt Lake City, UT, USA) inactive electrode which was used as the counter electrode. Cathodal ITP was performed using a current density of 0.4 mA cm^{-2} for 1 h. For microneedle studies, maltose microneedles (500 μm) were used and inserted into skin for about 90 s until the microneedles dissolved. For combination studies, ITP was applied after skin was microporated, and continuous sampling was performed using intracutaenous microdialysis. The linear probe was inserted into the skin, left

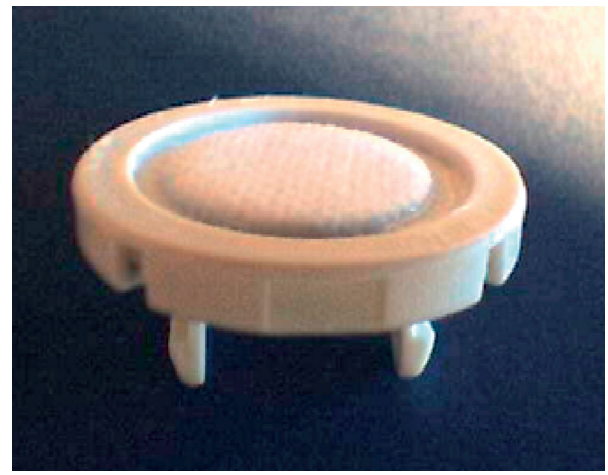

Figure 1 The iontophoretic drug cartridge (Transport Pharmaceuticals, Framingham, MA, USA). The surface area of the raised pad is 1.77 cm^2.

for 1 h to recover and retrodialysis was then performed. The linear probe was washed with phosphate-buffered saline (PBS, 1X, Fisher Scientific, Pittsburg, PA, USA) until the drug was eliminated from the probe and skin. Washed samples were collected and analysed to make sure that no drug was present before starting the experiment. The actual depth of the probe was measured by ultrasound imaging using the Dermascan instrument (Dermascan C, Cortex Technology, Hadsund, Denmark). Dialysate samples were collected every 15 min for the first 2 h and every 30 min for the next 2.5 h, and were analysed on the same day using HPLC without any further extraction, as serum proteins do not enter the microdialysis probe (20 kDa cut-off).

Microdialysis system

The microdialysis studies described above were carried out using a CMA 102 microdialysis pump AB and a CMA 142 microfraction collector (CMA/Microdialysis, Stockholm, Sweden). Linear microdialysis probes (BASi, West Lafayette, IN, USA) with 10 mm membrane window was used for the insertion into the skin.

A linear probe was inserted in the abdominal area of anaesthetized rats using a 22G needle placed intradermally, and the probe passed through the needle. The needle was then pulled so that the probe lay in the skin. The openings in the skin created by needle insertion were sealed using Wetbond (3M, St Paul, MN, USA). After probe insertion, the skin was allowed to recover for 1 h and then retrodialysis was conducted to determine the probe recovery factor (RF) before initiating the experiment. The linear probe was perfused with PBS (1X) and samples were collected every 15 min at a flow rate of 2 μL min^{-1} for 4.5 h. The recovery was performed until three similar recovery values were obtained.

RF was calculated using the formula: $RF = [C_p - C_d] / C_p$, where C_p and C_d are the concentrations in the perfusate and microdialysate, respectively.

Irritation monitoring

Any potential irritation was monitored by measuring transepidermal water loss (TEWL; Dermalab, Cortex Technology) and laser Doppler velocitimetry (LDV, Perimed, Japan) at the insertion site of the intradermal probe, before and after application of the cartridge used for drug loading. TEWL measures any changes in barrier integrity properties (Ahaghotu et al 2005) and stratum corneum disruption by microneedles. The TEWL instrument (open chamber) was sensitive and robust enough to detect changes in the barrier properties by skin irritants, as described by Tupker et al (1990). TEWL was used both in-vitro and in-vivo. Any erythema was monitored using a chromameter (Minolta, Japan).

Probe depth

The depth of the intracutaneous microdialysis probe in the skin was measured after insertion using a 20 MHz three-dimensional probe (Dermascan C Ver. 3). The probe has a resolution of 60 × 200 micron and capability of 10–15 mm penetration with an ultrasound velocity of 1580 m s^{-1} and was placed perpendicular to the skin with a thin layer of ultrasound gel. The automatic mode was used to scan the microdialysis probe; the average insertion depth was calculated using the Dermascan C software.

Cyclic voltammetry

Cyclic voltammetry (CV; Model CS 1200, Cypress systems, Lawrence, Kansas) is a commonly used technique to measure the redox of an active system. In addition to the redox potential, CV provides information about electron transfer between the electrode and the analyte, and also the stability of the drug in the presence of the analyte. CV was used here to show that the drug is stable under the electric field applied.

Silver–silver chloride was used as the reference electrode; silver wire was used as the auxiliary or counter electrode, and a carbon electrode as the working or indicator electrode. In this method, electrodes are immersed in the test solution and voltage is increased linearly and then decreased to the starting point. As a result, oxidation and reduction of the analytes is obtained. Similarities in the scan showed that MTX is not oxidized or reduced under the electric field, and is thus stable.

HPLC analysis

A reverse-phase HPLC method was used (Chatterjee et al 1997) for both in-vitro receptor samples and microdialysis samples. Samples of receptor fluid were analysed using a Waters Alliance HPLC system (Milford, MA, USA) with a reverse-phase column (C_{18}, 150 × 4.6 mm ID; 4 μm) and UV detection at 303 nm. The mobile phase consisted of 0.1 M monobasic sodium phosphate and 0.1 M Tris HCl in 23% methanol (v/v) in the ratio of 10:90, delivered at a flow rate of 1.5 mL min^{-1}. The retention time of MTX was approximately 5.5 min. The standard curve was linear over the range 0.125–10 μg mL^{-1} ($r^2 > 0.999$). The inter-day and intra-day s.d. of the HPLC assay were below 5%.

Statistical analysis

Data are presented as mean (± s.e.). Statistical analysis was performed using single-factor analysis of variance. A P value below 0.05 was considered to be significant.

Results and Discussion

The ultrasound image (Figure 2) shows the probe in the skin. The average depth of the linear microdialysis probe in the skin was found to be 0.54 mm. MTX was shown to be stable in the presence of an applied electric field, based on CV results.

The similarity of the two scans in Figure 3A and B (phosphate buffer with and without MTX during current application) indicates that MTX is electroactively stable in the buffer.

A current density of 0.4 mA cm^{-2} applied for 60 min was used for the ITP studies to maximize MTX delivery.

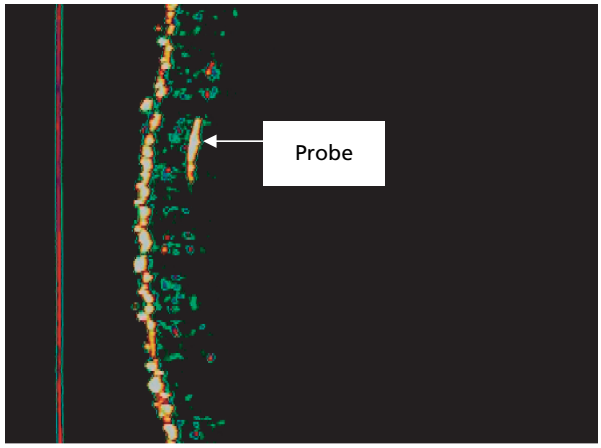

Figure 2 Ultrasound images showing the intradermal microdialysis probe in hairless rat skin

The two-line soluble maltose microneedles (500 μm each) were applied with 150–200 g pressure and were dissolved in skin within about 90 s of insertion. Figure 4 shows SEM pictures of soluble microneedles before and after insertion. TEWL was measured on in-vitro Franz cells before and after insertion of microneedles and also on intact skin as a passive control. TEWL values increased from 14.1 to 21.2 g cm^{-2} h^{-1} after microneedle insertion, suggesting that microneedles dissolved and disrupted the stratum corneum. Values for control skin were constant at about 14.5 g cm^{-2} h^{-1}. For these in-vitro studies, delivery by microneedles alone was significantly higher than by ITP alone ($P < 0.01$) (Figure 5). However, the delivery was not significantly different between microneedles alone or in combination with ITP.

For in-vivo studies, the average concentration of MTX in the dialysate (adjusted for recovery) was 42.5 μg mL^{-1} with ITP alone, 3.2 μg mL^{-1} with microneedles alone and 81.4 μg ml^{-1} with ITP in combination with microneedles (Figure 6). The increase in the concentration of MTX in the dialysate was 14 fold with ITP alone ($P < 0.05$) and 25 fold with ITP and microneedles ($P < 0.05$) when compared with the concentration with microneedles alone. The MTX concentration in the dialysate decreased after ITP was stopped and the drug cartridge removed after 1 h. In these studies, chromameter and LDV did not demonstrate any significant change in erythema or blood flow. However, TEWL values increased from an average baseline reading of 6.2 g cm^{-2} h^{-1} to 11.68 g cm^{-2} h^{-1} with ITP alone, from 7.6 to 10.4 g cm^{-2} h^{-1} with microneedles alone, and from 7.2 to 10.64 g cm^{-2} h^{-1} with the combination of ITP and microneedles (not significant).

Reasons for the discrepancy in permeation data between in-vitro and in-vivo studies for the effect of microneedles in combination with ITP are not clear but may be related to several factors. Skin hydration is known to increase permeation (Menon et al 1994; Barry 2001) so delivery through microporated skin is higher in-vitro (because of hydration of the skin by receptor media) and so combination with ITP does not enhance delivery further. It has also been suggested that microneedles may penetrate deeper in-vitro, as they are pressed on the skin over a hard surface (unlike the cushioning effect during in-vivo studies) (Teo et al 2005). This increased permeation may explain why there was no further increase in delivery by using the combination approach. The diffusion length of the viable layer (viable epidermis and dermis) was shorter in-vivo than in-vitro, as the effective area of the polar route in the stratum corneum is larger in-vitro (Yamashita et al 1994). Perhaps more importantly, the drug only has to cross the epidermis for in-vivo delivery (as blood circulation is under the epidermis) whereas it has to cross the entire length of skin to be detected in the receptor chamber during in-vitro studies. In-vivo studies measured the drug in the skin (intracutaneous probe) whereas in in-vitro studies drug was measured across the skin (receptor).

Blood samples were collected during the in-vivo microdialysis experiments and the plasma extracted and analysed using a method modified from the literature (Cociglio et al 1995). MTX was detected in plasma only in blood samples from the combination experiments (ITP plus microneedles, Figure 7).

The flux achieved with MTX using the two-layer microneedles (54 microneedles) in combination with ITP was 18.2 μg cm^{-2} h^{-1}. Extrapolated to a full array of microneedles with 250 pores per cm^2, this equates to a flux of 85.6 μg cm^{-2} h^{-1}. Steady-state plasma concentration (C_{ss}) was predicted from our in-vitro study using the equation: $C_{ss} = [J_{ss} \times A]/Cl$,

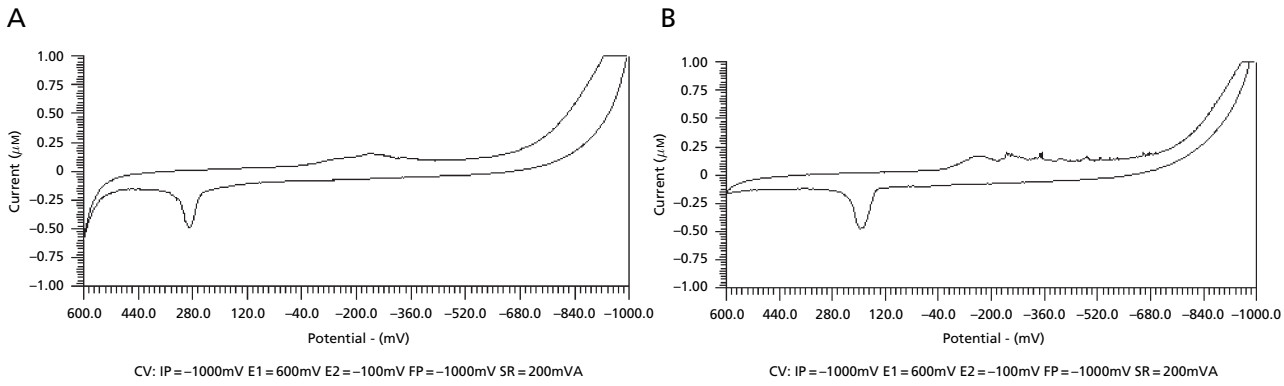

Figure 3 Cyclic voltammograms of 250 mM phosphate buffer, pH 7.4, with MTX 5 mg mL^{-1} (A) or buffer only (B).

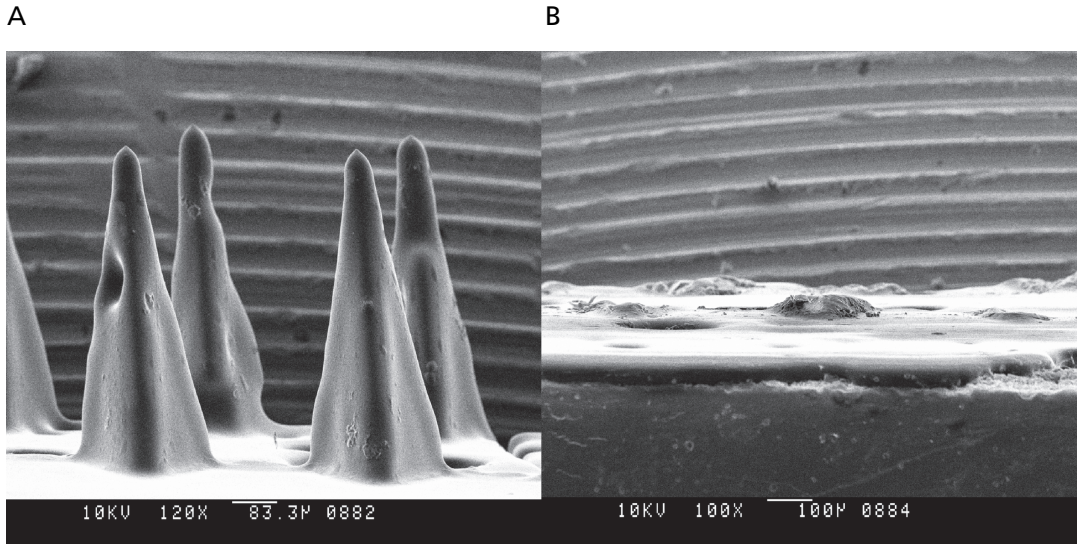

Figure 4 Scanning electron micrographs of soluble maltose microneedles before (A) and after insertion (B) into hairless rat skin.

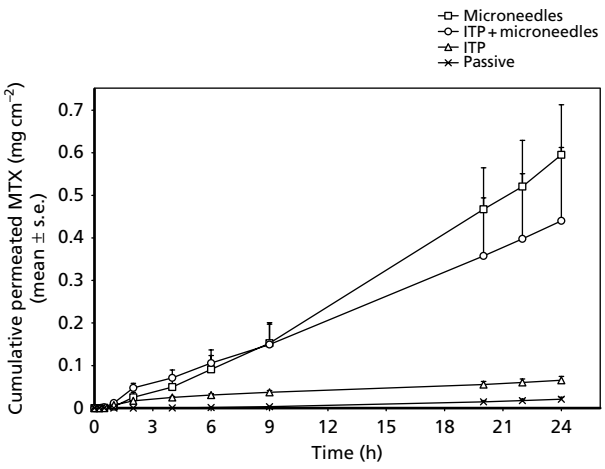

Figure 5 Accumulation of MTX permeated in-vitro by passive, iontophoretic (ITP) or microneedle-induced delivery or a combination of ITP + microneedles.

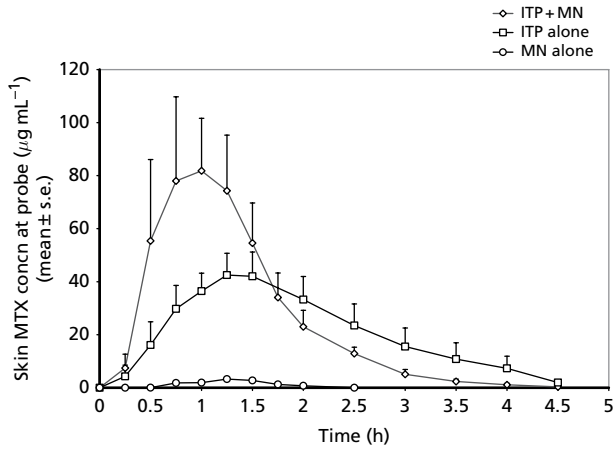

Figure 6 Concentration vs time profile of MTX delivered into skin in-vivo by iontophoresis (ITP), microneedles (MN), or ITP and MN combined, measured by an intradermal microdialysis probe.

where J_{ss} is the steady-state flux (μg cm^{-2} h^{-1}), A is surface area for drug absorption and Cl is the clearance of drug from the body (Guy & Hadgraft 1992). It was assumed that data from rat skin will be applicable to human skin. The Cl of MTX was reported to be 118 mL h^{-1} (Perwaiz et al 1998) and we used C_{max} values (maximum plasma concentration) from the literature for this calculation, since MTX is administered as a single dose rather than an infusion, and the dose administered orally or intramuscularly is the same as the mean absolute bioavailability (Grim et al 2003). We calculated that a patch size of 32.5 cm^2 would be required to achieve the desired level of 337 ng mL^{-1} in a 70 kg adult. The desired C_{max} of 337 ng mL^{-1} is based on oral dosing. The topical dose will actually be much lower, so in reality a much smaller patch can deliver the dosage. Topical dosage for MTX is not known but a clinical study by Tiwari et al (2003) suggests that a current density of 0.6 mA cm^{-2} for 15 min once a week for a total of 4 weeks would be clinically effective for the treatment of psoriasis.

Microneedle technology is relatively new compared with other enhancement techniques such as ITP. This technology has not been marketed yet but costs are expected to be low. Furthermore, the mould-based fabrication method used for the preparation of maltose needles is relatively cheap when compared with the cost of making silicon microneedles by microlithographic techniques. As the microneedle technology matures, costs will continue to go down, especially as manufacturing processes are scaled up. Several companies are currently developing these microneedles (Teo et al 2006).

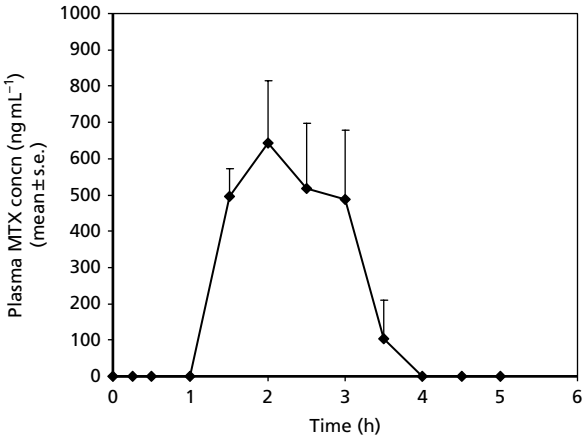

Figure 7 Plasma concentration of MTX following delivery by the combination of iontophoresis plus microneedles.

Conclusions

ITP alone or in combination with microneedles can significantly increase transdermal delivery of MTX in-vivo. These findings suggest that these techniques may enhance the clinical efficacy of MTX in the treatment of psoriasis and other skin disorders. Transdermal ITP in combination with microneedles has the advantages of delivering MTX locally without systemic side effects, is non-invasive, and allows better control over the amount of drug delivered. The application of microneedles enhanced MTX delivery over passive delivery, and the combination of microneedles and ITP was the most effective in our in-vivo studies.

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