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Research Paper

Transdermal Delivery of Interferon Alpha-2B using Microporation and Iontophoresis in Hairless Rats

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Purpose. To demonstrate transdermal delivery of interferon alpha-2b (IFNα2b) in hairless rats through aqueous microchannels (micropores) created in the skin and enhanced by iontophoresis.
Materials and Methods. The Altea Therapeutics PassPort™ System was configured to form an array of micropores (2.0 cm^2; 72 micropores/cm^2) on the rat abdomen. The transdermal patch (Iomed TransQ1-GS-hydrogel) was saturated with an IFNα2b solution (600 μg/ml) and applied for 4 h. Delivery was evaluated with and without cathodic iontophoresis (0.1 mA/cm^2). Intravenous delivery (0.4 μg/100 g body weight) was performed to support pharmacokinetic calculations.
Results. IFNα2b was not delivered through intact skin by itself (passive delivery) or during iontophoresis. However, passive delivery through micropores was achieved *in vivo* in rats. A dose of 397±67 ng was delivered over 6 h, with steady state serum concentrations reaching a plateau at 1 h post-patch application. These levels dropped rapidly after patch removal, and returned to baseline within 2 h of patch removal. Iontophoresis-enhanced delivery through micropores resulted in a two-fold increase in the dose delivered (722±169 ng) in the hairless rat.
Conclusions. *In vivo* delivery of IFNα2b was demonstrated through micropores created in the outer layer of the skin. Iontophoresis enhanced delivery through microporated skin in hairless rats.

KEY WORDS: interferon alpha; iontophoresis; microporation; protein delivery; transdermal delivery.

INTRODUCTION

The advent of new therapeutic molecules, including bioactive proteins and peptides, has given rise to new formulation and drug delivery challenges. Such molecules are administered parenterally because the oral bioavailability is typically very low. Their short circulating half-life often leads to the need for frequent injections resulting in a "peak-valley" profile in the blood stream. Techniques such as PEGylation result in a longer-circulating half-life for these peptides and/or proteins, but are often associated with a decrease in potency and in some cases, increased risk of side effects. Interferon alpha 2b (IFNα2b) is an antiviral protein that is produced by the body in response to a viral attack (specific activity: 260 MIU per mg) (1).

Recombinant human IFNα2b is produced with recombinant DNA techniques using an E-coli expression system, and is approved for treatment of viral hepatitis B and C and other indications. It is administered subcutaneously or intramuscularly at a starting dose of 3 MIU given three times a week for 6 months. Some studies have suggested that extension of the treatment from 6 months to 1 year may result in an improved patient response rate (2). This thrice-weekly regimen coupled with its short half-life (5–6 h in humans) results in a pharmacokinetic profile that exhibits a "peak and valley" pattern of blood levels (3). Increasing the dose to achieve longer lasting levels leads to undesirable side effects (2–6). Alternate routes of drug delivery that have been investigated in animal models include oral, nasal, and pulmonary (7–9), but these suffer from poor bioavailability and were not able to achieve and maintain steady-state daily blood levels. IFNα2b has also been administered by continuous subcutaneous infusion over a period of 3 months (3), but wearing an infusion pump is inconvenient and limits daily activities. Transdermal delivery systems present an attractive, non-invasive delivery method to achieve steady blood levels and have the potential to limit the side effects of interferon therapy. However, conventional transdermal delivery systems are limited to use with potent small molecule lipophilic drugs. This is mainly due to the barrier function of the stratum corneum, the outer layer of dead skin cells, which is rate-limiting for transdermal transport. Various technologies have been used to overcome the barrier of the stratum corneum layer including the use of microfabricated needles/projections to create microchannels in the skin (10–12). In one study, the authors reported that the microprojection patch technology was capable of delivering therapeutically

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relevant amounts of an antisense oligodeoxynucleotide (7 kDa), through the skin in hairless guinea pigs (11).

In the present study, we have evaluated a novel transdermal delivery technology, thermal microporation, which involves the thermal ablation of microscopic areas of the stratum corneum to form an aqueous pathway into the viable tissues of the skin. This technique has previously been investigated in clinical studies for the rapid extraction of interstitial fluid from the skin for glucose monitoring, and in animals and human subjects for transdermal delivery of insulin (13–16). However, this is the first publication establishing the applicability of this method for delivery of a large protein drug molecule. Microporation using thermal ablation involves the rapid localized application of thermal energy to the skin surface that results in the ablation of the stratum corneum cells in a microscopic area (17). Thermal ablation is accomplished by contacting an array of electrically resistive filaments to the skin surface and briefly heating the filaments by applying a short controlled pulse of electric current. The thermal energy delivered to the skin from each filament causes the ablation of the stratum corneum and the subsequent creation of a micropore into the viable epidermis. Conditions can be adjusted to create micropores typically in the range of 50–200 μm wide and 30–50 μm deep. The dimensions of the micropores are controlled by several factors, including the temperature-time profile and the geometry of the filaments that contact the skin. Typically, preclinical animal models are used that either have no fur (hairless rats, hairless mice, or hairless guinea pigs), or have regions with sparse hair (abdomen of mini-pigs). However, in certain cases that require specific animal models be used (e.g., balb/C mice for vaccine studies) fur may be closely trimmed to allow proper filament to skin contact. For discussion about the effect of animal model hair on microporation for vaccine delivery, see Bramson *et al*. (18). For clinical and eventual commercial use, the product is intended to be used in a similar manner to conventional transdermal patches in which hair is trimmed to allow good skin adhesive contact. Skin sites with no hair or sparsely populated hair are selected for microporation and patch application, or alternatively, hair can be closely trimmed.

Literature reports have shown that transdermal delivery through permeabilized skin can be modulated or controlled using electrical methods such as iontophoresis (11,19–22). Iontophoresis involves the application of a small amount of physiologically acceptable electrical current to drive ionic drugs into the body (23). Iontophoresis has been typically used to deliver relatively small macromolecules (e.g. peptides, oligonucleotides etc. typically <10 kDa) through intact skin (24,25). Attempts to deliver large molecules across intact skin for systemic delivery with iontophoresis have not shown promise (26). In this study, we have assessed the *in vivo* transdermal delivery of IFNα2b through micropores, and have evaluated its enhancement by iontophoresis.

MATERIALS AND METHODS

Materials

Hairless rats (300–400 g) were obtained from Charles River (Wilmington, MA). Hairless rats were housed and experiments were conducted under IACUC-approved protocols, at the animal facility at Mercer University. Trans-Q 1GS, transdermal patches with electrodes for iontophoretic delivery, (Iomed Inc., Salt Lake City, Utah) were used for all transdermal studies. Human IFNα2b ELISA kits were obtained from PBL Biomedical Labs (Piscataway, NJ). Phosphate buffer, sodium chloride, EDTA, and polysorbate 80) were purchased from Fisher Scientific (Pittsburgh, PA).

Microporation

An experimental microporation system (Altea Therapeutics Corporation) was used to create an array of micropores on the skin surface of the rodent abdomen. This system included a laptop computer, microprocessor control circuitry, and a 3-axis stepper motor assembly with microscopic tip holder and skin interface plate. The circuitry controlled the electrical current pulse width, number of pulses and temperature of the filament that is placed in brief contact with the skin surface. The software user interface allowed the programming of various microporation parameters including tip temperature, pulse width, number of pulses, pulse spacing and tip contact pressure. The tip temperature was calibrated with the use of a temperature sensing, non-contact optical detector to a nominal peak temperature of approximately 750°C using an optical pyrometer (Leeds and Northrup, Model 8622). The microporation system created an array of micropores (72 micropores/cm^2 in a 2 cm^2 area), where each micropore was formed as an elliptically-shaped area of ablated stratum corneum approximately 80 μm wide, 300 μm long and 40 μm in depth, as determined by light microscopy. Due to the precision of the filament geometry and tip contact pressure, the micropore dimensions were fairly consistent across an array, with a variability based on excised skin measurements of approximately 20–30% (%CV). Histological evaluation of microporated rat skin was not performed, but in some cases, a slight erythema was evident in the skin surrounding each micropore, indicating local histamine release.

In previous studies, the microporation parameters utilized for this study were used in clinical investigations to painlessly microporate human skin (13–16). Rats were handled in accordance with the IUCAC protocol approved by the Mercer University Animal Committee. Each rat was anesthetized using ketamine (75 mg/kg) and xylazine (10 mg/kg), administered intraperitoneally. Before microporation, each rat was closely inspected to ensure that the skin in the abdominal region was intact. The skin was then wiped clean with an alcohol swab and lightly dried with lint free tissue.

Transdermal Patch and IFNα2b Formulation

Aqueous solutions of IFNα2b (MW=19.271 kDa) with interferon alpha concentrations ranging from 11.5 μg/ml to 1,000 μg/ml were prepared in 50 mM, 7.5 pH phosphate buffer containing 75 mM sodium chloride, 0.3 mM EDTA and 0.01% polysorbate 80. The gel sponge portion (7.5 cm^2) of the Trans-Q® 1 GS patch was saturated with 1 ml of drug solution, with drug loading ranging from 11.5 μg to 1.0 mg depending on the particular experiment. After the

microporation process was complete, the rats (n=3) were placed on the table with the abdomen facing up. Patches were applied on the microporated area and covered with an adhesive covering to ensure skin contact throughout the study period. Patches were removed after 4 h and the microporated area was washed under running water and blotted with lint free tissue. Animals were placed in a restrainer throughout the duration of the experiment. Blood samples were taken from the tail-vein of the rat and sampling continued for 2 h after patch removal (0, 1, 2, 3, 4, 4.5, 5 and 6 h samples).

Transdermal Iontophoresis

For the iontophoresis experiments, the electrode in the Trans-Q patch was used as the cathode and the reference electrode supplied with it (counter electrode) was used as the anode. The Trans-Q (cathode) and counter electrode (anode) patches were placed on the rat abdomen apart from each other. A constant current of 0.1 mA/cm^2 was applied using a Dupel® dual channel iontophoretic system (Empi, MN) for 4 h. Blood samples were taken from the tail-vein of the rat and sampling was continued for 2 h after patch removal (0, 1, 2, 3, 4, 4.5, 5 and 6 h samples).

Intravenous Injection

Catheters filled with 100 IU of heparin sodium, in isotonic saline were inserted surgically into the right jugular vein of the rats (n=3). IFNα2b solution was made in pH 7.4. 50 mM phosphate buffer so that 50 μl contained 0.4 μg IFNα2b. This solution was prepared under aseptic conditions in a laminar flow hood and sterile-filtered through a 0.2 μm filter. A dose of 50 μl/100 g weight of rat (4 μg/kg) was administered through the catheter and flushed with heparin sodium in isotonic saline. Blood samples (350–400 μl) were taken from the tail vein at 5, 10, 15, 30, 45, 60, 90, 120, 150, 180 and 240 min.

Analytical Procedures

Whole blood was allowed to clot and then was centrifuged at 10,000 rpm for 5 min to obtain the serum as the supernatant. Serum samples were analyzed for IFNα2b using a commercially available Enzyme Linked Immuno-Sorbent Assay (ELISA) kit for human interferon alpha (PBL Biomedical Labs, Kit # 41100). The ELISA methodology involved a heterogeneous antibody sandwich in which interferon from the sample was captured by the antibody immobilized on the microtiter plate wells, and a detection antibody was used to complete the sandwich. A signal generator of conjugated horseradish peroxidase (HRP), together with tetramethyl benzidine (TMB) was used to develop the assay. The ELISA assay had no cross reactivity to rat IFN-α. A viral inhibition bioassay (PBL Biomedical Labs, NJ), based on the cytopathic effect of IFNα2b 2b on the vesicular stomatitis virus in the MDBK (bovine kidney) cell line, was performed to identify the fraction of bioactive interferon. The standards used in the bioassay were calibrated to the international reference standards provided by the National Institutes of Health. All data were statistically treated for significance using ANOVA at the p<0.05 level.

Pharmacokinetic Analysis

Pharmacokinetic parameters were determined from the serum drug concentration vs. time data. Intravenous data

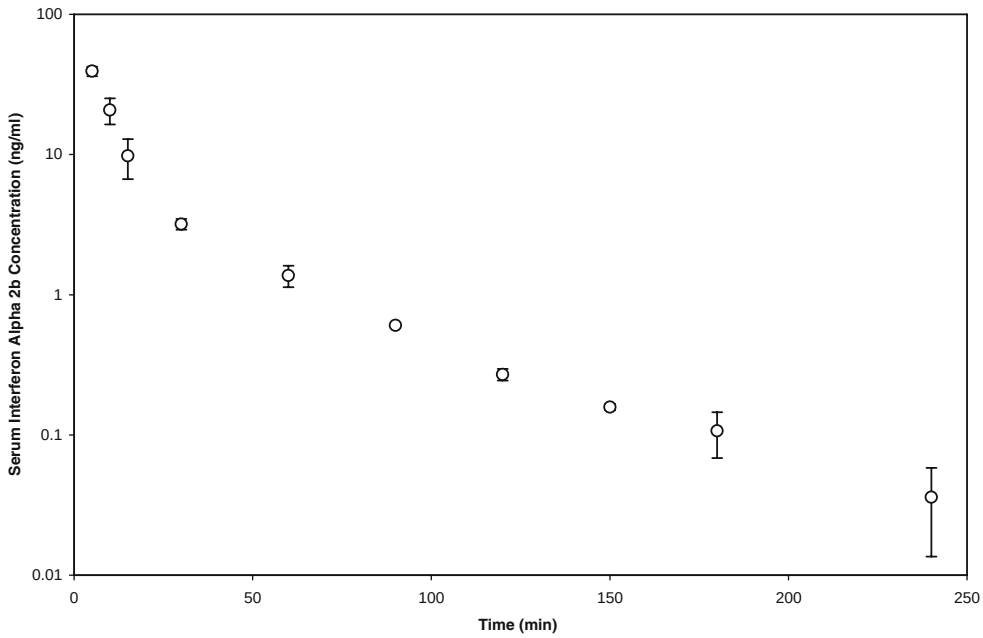

Fig. 1. Bolus intravenous injection of IFNα2b (4 μg/kg) (Mean±S.E., n=3).

Table I. Pharmacokinetic Parameters Following IV Dose, as Calculated using WinNonlin® Software by Non-compartmental Analysis (n=3)

Parameter	Units	Value	±SE
[a]k	Min^{-1}	0.0236	0.0013
[b]$t_{1/2}$	Min	29.6	1.76
[c]$AUC_{(0–infinity)}$	Min*ng/ml	745	66
[d]Cl_{iv}	Ml/min/kg	4.9	0.51
[e]$AUMC_{(0–infinity)}$	Min*min*ng/ml	13,068	1,784
[f]$MRT_{(infinity)}$	Min	17.4	1.2

[a] Elimination rate constant obtained from the terminal portion of serum concentration-time plot (points used: 60, 90, 120, 150, 180 min)
[b] Elimination half-life of IFNα2b calculated from k
[c] Area under curve from 0 to infinity for serum concentration-time plot calculated using linear trapezoidal rule
[d] Observed clearance from IV data
[e] Area under the first moment curve for concentration-time plot calculated using linear trapezoidal rule
[f] Mean residence time calculated as AUMC/AUC.

were fit into a non-compartmental pharmacokinetic model using WinNonlin® software version 3.1 (Pharsight, Mountain View, CA) and pharmacokinetic parameters (k, $t_{1/2}$, Cl, AUC, AUMC, MRT) were obtained. The area under the serum drug concentration vs. time curve ($AUC_{(transdermal)}$) and the area under the first-moment curve ($AUMC_{(transdermal)}$) after transdermal delivery were calculated using the linear trapezoidal rule. The mean residence time ($MRT_{(transdermal)}$) for transdermal delivery was calculated by $AUMC_{(transdermal)}/AUC_{(transdermal)}$.

RESULTS AND DISCUSSION

Figure 1 shows the serum concentration vs. time profile for intravenous administration (400 ng/100 g of body weight) of IFNα2b. The pharmacokinetic parameters (Table I) were calculated by fitting the serum concentration vs. time data to a non-compartmental model using the WinNonlin® software. The Cl_{iv} (Table I) was used to calculate the transdermal dose delivered ($AUC_{transdermal}$* Cl_{iv}). After an initial distributive phase, IFNα2b disappeared from the serum with an observed half-life of 29.6±1.8 min. The $AUC_{(0–infinity)}$ was calculated to be 745 ±66 min*ng/ml. According to published literature, plasma interferon concentrations decline rapidly after IV injection irrespective of the animal model used. The half-life obtained in this study is in close agreement with other studies performed in mice and rats that have been reported in the literature (27–29).

Figure 2 shows the transdermal delivery of IFNα2b in hairless rats. There was no delivery detected through intact skin. Delivery through microporated skin from a patch containing 600 μg IFNα2b resulted in a total amount delivered of 397±62 ng ($AUC_{(transdermal)}$*Cl_{iv}) at 6 h (0.067% of patch loading delivered into the systemic circulation). When iontophoresis was applied to intact skin, there was no delivery of interferon detected (Fig. 3). However, when iontophoresis was applied to microporated skin, the amount delivered was 722±169 ng ($AUC_{(transdermal)}$*Cl_{iv}) in 6 h (0.12% of patch loading delivered into the systemic circulation). Iontophoresis increased the transport of IFNα2b through microporated skin two-fold with significantly more amounts being delivered into the systemic circulation (p<0.05) (Table II). Iontophoresis did not have any effect on the bioactivity of the IFNα2b as determined in an *in vitro* Franz cell set up. The donor solutions that were exposed to the iontophoretic current as well as the receptor solutions containing the transported IFNα2b were tested for bioactivity (performed at PBL Biomedical Labs) and were found to be bioactive (260–277 MIU/mg).

The results presented here indicate that microporation enabled the iontophoretic delivery of a large protein (MW for IFNα2b is 19.27 kDa) through the skin into the systemic circulation, whereas there was no iontophoretic delivery through intact skin. This is most likely because the pathways

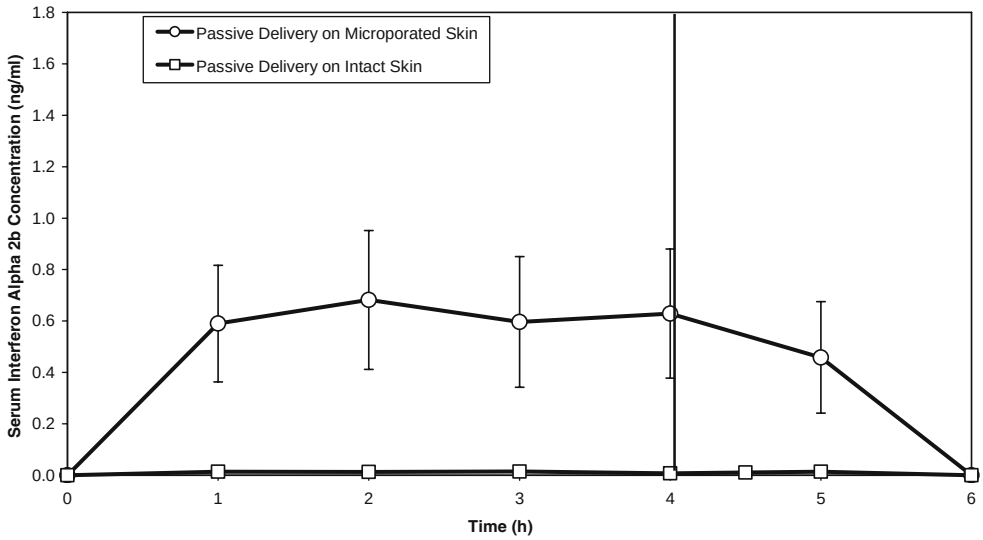

Fig. 2. Transdermal delivery of IFNα2b in hairless rats by microporation compared to delivery through intact skin. Patch contained 600 μg IFNα2b. Patch removed at 4 h (Mean±S.E., n=3).

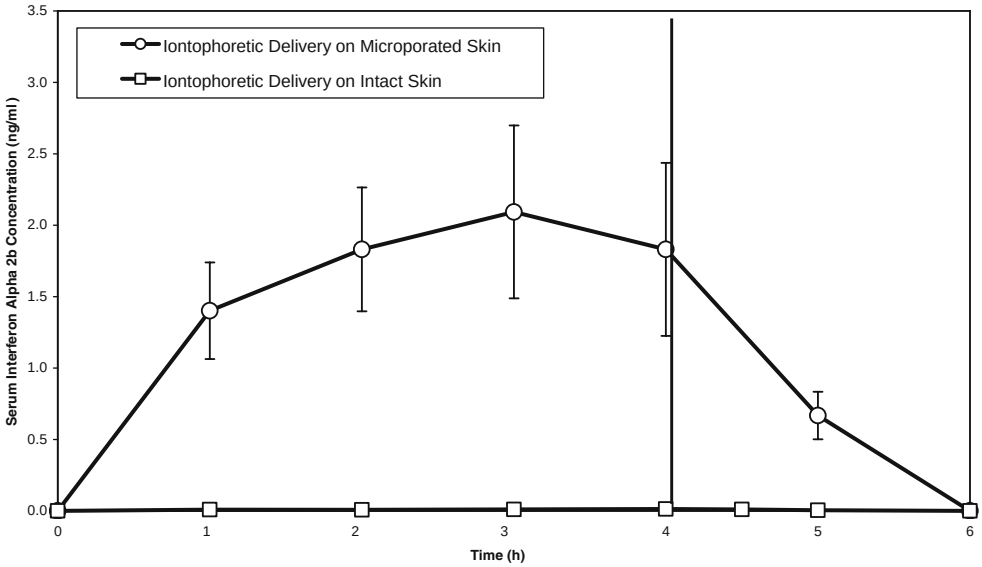

Fig. 3. Transdermal delivery of IFNα2b under iontophoresis: microporated versus intact skin (Mean±S.E., *n*=3). Patch removed at 4 h.

created by microporation (micropores) are larger than iontophoretic pathways (skin appendages). It is generally accepted in the transdermal literature that the molecular weight limitation for transdermal transport of molecules through intact skin is approximately 8–10 kDa (23). However, there have been some reports of the delivery of very large molecules via iontophoresis through intact skin These studies have been limited to compounds with a local site of action such as botulinum toxin A (MW 150 kDa present as a 900 kDa complex) which was tested for efficacy to control hyperhidrosis (30). In some cases, abrasion of the skin was required to achieve the sufficient local delivery of the large compound. For example, heparin and hyaluronic acid (MW 800 kDa), were delivered by iontophoresis through abraded skin (31, 32). However, there is no evidence of therapeutic amounts of such large molecular weight compounds being delivered to the systemic circulation without disruption or removal of the stratum corneum. Several other techniques have been utilized to disrupt or overcome the stratum corneum to deliver large molecules using microneedles, microprojections, or microblades (11,12,33). Delivery of large molecule drugs through microporated skin has been shown as a viable approach for systemic delivery and in addition, the microporation approach has been shown to deliver very large molecules (adenoviral vaccines) intended for a local site of action (18).

Passive delivery of IFNα2b through microporated skin was found to be dependent on the patch loading. Three different concentrations of IFNα2b were studied: 11.5 μg/ml, 600 μg/ml, and 1,000 μg/ml of IFNα2b in the 1.0 ml fill volume patch (Fig. 4). For the 1000 μg dose patch, 762±25 ng of IFNα2b was delivered which is two times the amount delivered for the 600 μg dose patch (397±67 ng) and ten times the amount delivered for the 11.5 μg dose patch

Table II. Pharmacokinetic Parameters Following Transdermal Delivery Calculated using Non-compartmental Analysis (*n*=3)

Parameters	Units	Passive Delivery through Microporated Skin (600 μg dose)	±SE	Iontophoretic Delivery through Microporated Skin (600 μg dose+0.1 mA/cm^2)	±SE
[a]Cmax	ng/ml	0.68	0.27	2.09	0.6
[b]Tmax	Min	120	–	180	–
[c]$AUC_{(transdermal)}$	min*ng/ml	254	47	501	154
[d]$AUMC_{(transdermal)}$	min*min*ng/ml	1,354	217	2,296	574
[e]$MRT_{(transdermal)}$	Min	5.4	.06	4.7	0.4
[f]Dose Delivered	Ng	397	62	722	170

[a] Maximum serum concentration of IFNá2b observed
[b] Time at which C_{max} occurs
[c] Area under the serum concentration-time plot calculated by linear trapezoidal rule
[d] Area under the first moment curve for concentration-time plot calculated using linear trapezoidal rule
[e] Mean residence time calculated as $AUMC_{(transdermal)}/AUC_{(transdermal)}$
[f] $AUC_{(transdermal)}*Cl_{iv}$

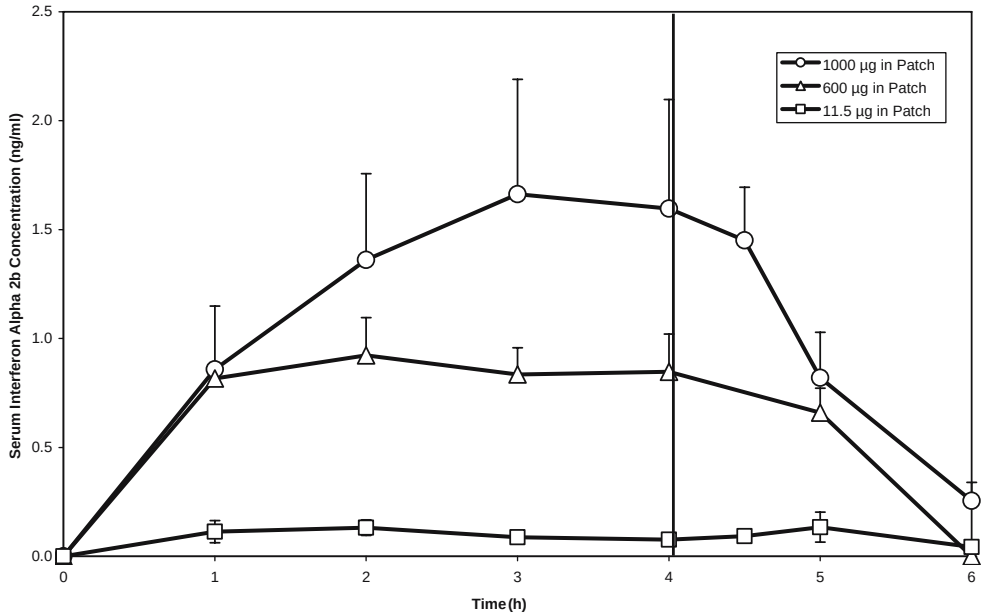

Fig. 4. *In vivo* delivery of IFNα2b through microporated skin: effect of interferon concentration in the patch. Serum interferon concentration (Mean±S.E., n=3). Patch removed at 4 h.

(70±11.2 ng). The relationship between the amount of IFNα2b delivered and the patch loading for a fixed volume (or equivalently the IFNα2b concentration in the patch) was linear (R^2=0.97) Transdermal delivery of IFNα2b through micropores from an aqueous solution is concentration dependent which infers a concentration gradient mechanism of delivery. In addition, previous work showed that the amount of IFNα2b delivered was linearly related to the micropore density (number of micropores per cm^2) using an *in vitro* Franz cell model (data not shown).

The current study was not designed to demonstrate high bioavailability for several reasons. First, it was intended to have excess drug loading (fluid volume) in the patch to avoid the potential effect of drug depletion on the pharmacokinetic profile. Second, the potential effect of patch misalignment over the microporation site was avoided by using a drug reservoir and electrode that had an area that was larger than the microporation site (2 cm^2), an approach that was consistent with the smallest commercially available iontophoretic patch size (7.5 cm^2 gel sponge for TransQ® 1-GS). Various steps can be taken to increase bioavailability, mainly by minimizing the area and thickness of the iontophoretic patch, or by optimizing the drug formulation for bioavailability.

For the marketed IFNα2b product, Intron® A, the approved route of delivery is by subcutaneous injection and the typical dose is 3×10^6 IU (corresponding to 11.5 µg) given three times a week. In the present study, the amount of IFNα2b delivered from the highest concentration patch (1,000 µg/ml) was 0.762 µg, a dose 15-fold lower than the 11.5 µg dose intended for human use, and the rat weights were approximately 150-fold lower than a human weighing 60 kg. In addition, if the patch was applied for 24 h as would be the case for a daily interferon patch product, then the dose delivered would be four times higher, or 3.0 µg. The C_{max} of the mean serum interferon alpha curve for the 1,000 ug/ml patch was 1.66 ng/ml, or 431 IU/ml and 0.113 ng/ml, or 29 IU/ml, for the 11.5 µg/ml patch. This compares to a C_{max} of 990 IU/ml for a subcutaneous injection of 1.67 MIU, and 1,150 IU/ml for a subcutaneous injection of 3.2 MIU (8,27). Transdermal administration of interferon alpha has been shown to result in steady serum interferon levels during patch application as opposed to the peak-trough pharmacokinetic profile observed with subcutaneous injection.

CONCLUSIONS

Transdermal delivery through microporated skin offers a needle-free, painless route of administration that has demonstrated steady blood levels during patch application. In this study, microporation enabled the delivery of IFNα2b in hairless rats *in vivo* at serum levels comparable to subcutaneous injection based on published data. The transdermal delivery of interferon alpha was shown to be concentration dependent as demonstrated by an increase in serum levels achieved as the interferon concentration in the patch was increased. Enhancement and modulation of the delivery through microporated skin was achieved by iontophoresis *in vivo*.

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REFERENCES

1. S. Pestkaand and A. Meager. Interferon standardization and designations. *J. Interferon. Cytokine Res.* **17**(Suppl 1):S9–S14 (1997).
2. P. Jouet, F. Roudot-Thoraval, D. Dhumeaux, and J. M. Metreau. Comparative efficacy of interferon alfa in cirrhotic and noncirrhotic patients with non-A, non-B, C hepatitis. Le groupe Francais pour l'etude du traitement des hepatites chroniques NANB/C. *Gastroenterology* **106**:686–690 (1994).
3. S. Schenker, D. Cutler, J. Finch, C. H. Tamburro, M. Affrime, R. Sabo, and M. Bay. Activity and tolerance of a continuous subcutaneous infusion of interferon-alpha2b in patients with chronic hepatitis. *C. J. Interferon. Cytokine Res.* **17**:665–670 (1997).
4. V. Bocci, F. Carraro, A. Naldini, P. P. Cagol, E. M. Pasqual, C. Prevaldi, and D. Casara. Distribution of human recombinant interferon-alpha 2 in rat plasma, liver, and experimental liver metastases. *Mol. Biother.* **2**:233–234 (1990).
5. A. Bonettiand and S. Kim. Pharmacokinetics of an extended-release human interferon alpha-2b formulation. *Cancer Chemother. Pharmacol.* **33**:258–261 (1993).
6. Y. Yoshikawa, Y. Komuta, T. Nishihara, Y. Itoh, H. Yoshikawa, and K. Takada. Preparation and evaluation of once-a-day injectable microspheres of interferon alpha in rats. *J. Drug Target.* **6**:449–461 (1999).
7. J. S. Patton, P. Trinchero, and R. M. Platz. Bioavailability of pulmonary delivered peptides and proteins: a-interferon, calcitonins and parathyroid hormones. *J. Control. Release* **28**:79–85 (1994).
8. L. Paulesu, F. Corradeschi, C. Nicoletti, and V. Bocci. Oral administration of human recombinant interferon-a2 in rats. *Int. J. Pharm.* **46**:199–202 (1988).
9. C. K. Shimand and S. R. Kim. Administration route dependent bioavailability of interferon-a and effect of bile salts on the nasal absorption. *Drug Dev. Ind. Pharm.* **19**:1183–1199 (1993).
10. S. Henry, D. V. McAllister, M. G. Allen, and M. R. Prausnitz. Microfabricated microneedles: a novel approach to transdermal drug delivery. *J. Pharm. Sci.* **87**:922–925 (1998).
11. W. Lin, M. Cormier, A. Samiee, A. Griffin, B. Johnson, C. L. Teng, G. E. Hardee, and P. E. Daddona. Transdermal delivery of antisense oligonucleotides with microprojection patch (Macroflux) technology. *Pharm. Res.* **18**:1789–1793 (2001).
12. D. V. McAllister, M. G. Allen, and M. R. Prausnitz. Microfabricated microneedles for gene and drug delivery. *Annu. Rev. Biomed. Eng.* **2**:289–313 (2000).
13. D. P. Joshi, C. A., S. L. Chang, S. E. Mills, A. M. Smith, and A. K. Banga. Steady insulin infusion visa micropores through the stratum corneum in hairless arts. *AAPS PharmSci.* **5**:T3106 (2003).
14. A. Smith, D. Yang, H. Delcher, J. Eppstein, D. Williams, and S. Wilkes. Fluorescein kinetics in interstitial fluid harvested from diabetic skin during fluorescein angiography: implications for glucose monitoring. *Diabetes Technol. Ther.* **1**:21–27 (1999).
15. A. M. Smith, J. A. Eppstein, H. K. Delcher, M. S. McRae, T. J. Woods, D. J. Williams, and M. R. Hatch. Transdermal insulin infusion through thermally created micropores in humans. *Diabetes* **50**: A132 (2001).
16. A. M. Smith, T. J. Woods, D. J. Williams, H. K. Delcher, J. A. Eppstein, and M. S. McRae. Transdermal basal insulin delivery through micropores. *Diabetes* **51**:A47 (2002).
17. J. Eppstein, M. R. Hatch, and D. Yang. Microporation of human skin for drug delivery and monitoring applications. US Patent 5885211 (1999).
18. J. Bramson, K. Dayball, C. Evelegh, Y. H. Wan, D. Page, and A. Smith. Enabling topical immunization via microporation: a novel method for pain-free and needle-free delivery of adenovirus-based vaccines. *Gene Ther.* **10**:251–260 (2003).
19. M. Al-Khalili, V. M. Meidan, and B. B. Michniak. Iontophoretic transdermal delivery of buspirone hydrochloride in hairless mouse skin. *AAPS PharmSci.* **5**:E14 (2003).
20. N. Sekkat, Y. N. Kalia, and R. H. Guy. Control of drug delivery across an impaired skin barrier by iontophoresis. In *Proceedings of the 28th International Symposium on Controlled Release of Bioactive Materials and 4th Consumer & Diversified Products Conference*, San Diego, CA, United States, June 23–27, 2001, pp. 1:185–186.
21. N. Sekkat, Y. N. Kalia, and R. H. Guy. Porcine Ear Skin as a Model for the Assessment of Transdermal Drug Delivery to Premature Neonates. *Pharm. Res.* **21**:1390–1397 (2004).
22. P. G. Green. Iontophoretic delivery of peptide drugs. *J. Control. Release* **41**:33–48 (1996).
23. A. K. Banga. *Electrically Assisted Transdermal and Topical Drug Delivery*, Taylor & Francis, London, 1998.
24. A. V. Badkar and A. K. Banga. Electrically enhanced transdermal delivery of a macromolecule. *J. Pharm. Pharmacol.* **54**:907–912 (2002).
25. S. L. Chang, A. M. Smith, S. E. Mills, D. P. Joshi, A. V. Badkar, and A. K. Banga. Transdermal delivery of human parathyroid hormone 1–34 by thermally created micropores and electrotransport. *AAPS PharmSci.* **3**:(2001).
26. N. G. Turner, L. Ferry, M. Price, C. Cullander, and R. H. Guy. Iontophoresis of poly-L-lysines: the role of molecular weight? *Pharm. Res.* **14**:1322–1331 (1997).
27. V. Bocci, F. Corradeschi, A. Naldini, and E. Lencioni. Enteric absorption of human interferons a and b in the rat. *International Journal of Pharmaceutics* **34**:111–114 (1986).
28. O. Bohoslawec, P. W. Trown, and R. J. Wills. Pharmacokinetics and tissue distribution of recombinant human alpha A, D, A/D(Bgl), and I interferons and mouse alpha-interferon in mice. *J. Interferon Res.* **6**:207–213 (1986).
29. N. H. Greig, T. T. Soncrant, K. M. Wozniak, and S. I. Rapoport. Plasma and tissue pharmacokinetics of human interferon-alpha in the rat after its intravenous administration. *J. Pharmacol. Exp. Ther.* **245**:574–580 (1988).
30. G. M. Kavanagh, C. Oh, and K. Shams. BOTOX delivery by iontophoresis. *Br. J. Dermatol.* **151**:1093–1095 (2004).
31. S. Pacini, T. Punzi, M. Gulisano, F. Cecchi, S. Vannucchi, and M. Ruggiero. Transdermal delivery of heparin using pulsed current iontophoresis. *Pharm. Res.* **23**:114–120 (2006).
32. S. Pacini, T. Punzi, M. Gulisano, and M. Ruggiero. Pulsed current iontophoresis of hyaluronic acid in living rat skin. *J. Dermatol. Sci.* **44**:169–171 (2006).
33. A. K. Banga. New technologies to allow transdermal delivery of therapeutic proteins and small water soluble drugs. *Am. J. Drug Deliv.* **4**:221–230 (2006).