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Research Paper

Transdermal Delivery of Human Growth Hormone Through RF-Microchannels

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Purpose. To evaluate the bioavailability and bioactivity of human growth hormone (hGH) delivered transdermally through microchannels (MCs) in the skin created by radio-frequency (RF) ablation.
Methods. The creation of MCs was observed in magnified rat and guinea pig skin after staining by methylene blue. Various doses of hGH in a dry form were applied on rat or guinea pig (GP) skin after the formation of MCs. The pharmacokinetic profile of systemic hGH in both animal models was monitored for 15 h post patch application. Bioactivity of the transdermally delivered hGH was verified by measuring IGF-I levels in hypophysectomized rats.
Results. The ordered array of MCs was clearly visible in the magnified rat and guinea pig skin. The MCs were very uniform in diameter and of equal separation. Creation of MCs in the outer layers of the skin enabled efficient delivery of hGH, with a bioavailability of 75% (rats) or 33% (GPs) relative to subcutaneous (s.c.) injection with plasma profiles resembling that of s.c. injection. Elevated levels of systemic insulin-like growth factor-1 (IGF-I) were observed after transdermal delivery of hGH to hypophysectomized rats indicative of the bioactivity of the transdermally delivered hGH *in vivo*.
Conclusions. Formation of RF-microchannels is a well-controlled process. These MCs permitted the transdermal delivery of bioactive hGH in rats and GPs with high bioavailability.

KEY WORDS: transdermal drug delivery; radio-frequency ablation; Via Derm; stratum corneum; human growth hormone.

INTRODUCTION

The number of peptide and protein drugs has increased dramatically in the past decades and is expected to grow further as a result of intense biotechnology research in academia and industry. Elucidation of appropriate delivery methods for this group of active molecules is extremely challenging, and currently most of these drugs are given by injection. However, various alternative strategies are being developed. These include oral methods that overcome the proteolysis in the GI tract (1), nasal delivery, buccal delivery (2), inhalation (3) or transdermal methods (4). Most of the methods developed so far have various limitations, such as drug molecular weight, low deliverable dose, or low bioavailability.

Recently, a new transdermal delivery technology was developed, being adapted from the well-known medical technology of radio-frequency (RF) ablation (5–8). It is based on an electronic device, termed ViaDerm, which generates an electrical current at high frequency in the range of radio frequencies (100–500 kHz). The passage of this current through cells in the upper skin layers, via an array of microelectrodes placed on the skin, brings about ionic vibrations within the skin cells leading to local heating, liquid evaporation, and cell ablation. Consequently, small microchannels (MCs), called RF-microchannels, are formed across the stratum corneum (SC) and epidermis, which are highly amenable to the transdermal delivery of water-soluble drugs into the systemic circulation (9).

Human growth hormone (hGH) is a 22-kDa protein with clinical use in children having short stature due to hGH-deficiency, renal insufficiency, Turner syndrome, and Prader-Willi syndrome. Recently, hGH was also approved by the FDA for children with severely short stature. Additionally, this drug is also indicated in adults who suffer from either acquired or childhood onset hGH-deficiency. hGH therapy, which demands years of good compliance to achieve its therapeutic effects, is currently administered by frequent subcutaneous (s.c.) injections. A depot injection was also developed that reduced the frequency of injection to once or twice a month. However, due to pain and irritation side effects (10), the success of this product is mediocre. Therefore, a user-friendly hGH delivery method is a keenly sought-after therapeutic.

The aim of the current study was to investigate if RF-generated MCs could support the transdermal delivery of hGH in rats and guinea pigs (GPs). Furthermore, *in vivo* bioactivity of hGH was assessed by monitoring the production of IGF-I, a key downstream mediator following hGH receptor activation.

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MATERIALS AND METHODS

Instruments

The device used to produce microchannels in the skin (ViaDerm, TransPharma Medical, Lod, Israel) was previously described in detail (9). The standard array of electrodes that was used produced MCs in the density of 100 MCs/cm^2 in a total area of 1.4 cm^2. In the hGH delivery studies, the device was applied twice on each skin area, so the MC density was 200 MCs/cm^2. Prior to ViaDerm application on the skin of animals, the hair was clipped using an Oster A5 clipper (cat. no. 78005-500, McMinnville, TN, USA), and shaved using a no. 40 blade and a Braun 3615 shaver. Immediately after ViaDerm application, TransEpidermal Water Loss (TEWL) was measured using a Dermalab instrument (Cortex Technology, Hadsund, Denmark).

Visualization of MCs

Fresh rat and guinea pig skin samples (Sprague-Dawley male rat, 350 g; Dunkin Hartley male guinea pig, 600 g; Harlan Laboratories Ltd., Rehovot, Israel) were excised from the animals, immediately pretreated with ViaDerm (100 MCs/cm^2), and then stained with 1% aqueous methylene blue (Carlo Erba Reagenti). The solution was applied for 15 s on the skin site, then wiped with soft tissue paper followed by isopropyl alcohol pads (Webcol, Kendall Company, Mansfield, MA, USA).

The control group consisted of application of the ViaDerm device on the skin in the absence of the power source but held with the same pressure followed by methylene blue staining. A Video Inspection System (S-T Industries Inc. model 20-8600, St. James, MN, USA) equipped with ×10 lens, was used in order to observe the created MCs.

Preparation of hGH Patches

Lyophilized hGH (Genotropin 16 or 36 IU/vial, Pharmacia & Upjohn, Puurs, Belgium) was used for the preparation of "printed" patches, in a proprietary owned process (11). This "print-like" method is based on accurately depositing small droplets of hGH solution on a transdermal backing liner at a total area of 1.4 cm^2 followed by a controlled drying process. This method permits accurate dosing and stable patches that contain a thin uniform layer of the protein in a dry form.

Animals

Study protocols were approved by the Institutional Animal Care and Use Committee of Assaf Harofeh Medical Center (Zriffin, Israel), and all procedures were conducted according to the Principles of Labotarory Animal Care (NIH Publication No. 85-23, revised 1985). Wild-type and hypophysectomized male Sprague-Dawley rats, 200–350 g, as well as wild-type male Dunkin Hartley guinea pigs 500–700 g (Harlan Laboratories Ltd.) were used. They were kept at constant temperature with a 12 h light:12 h dark cycle. Water and pelleted food (Koffolk, Tel Aviv, Israel) were freely available. The hypophysectomized rats were treated daily with s.c. injections of hydrocortisone sodium succinate (500 μg kg^{-1} day^{-1} Solu-Cortef®, (hydrocortisone sodium succinate for injection, USP, Pharmacia & Upjohn) and thyroxine sodium (15 μg kg^{-1} day^{-1}, Eltroxin, Bedford Labs, Bedford, OH, USA) from the day of arrival until beginning of the trial.

Procedures

The animals were anaesthetized by intraperitoneal (i.p.) injection of a combination of ketamine hydrochloride (85 mg/kg for rats and 70 mg/kg for GPs; Ketaset, Fort Dodge, IA, USA) and xylazine (3 mg/kg for rats and 6 mg/kg for GPs, Xyl-M2 veterinary, VMD, Arendonk, Belgium). Anesthesia was maintained using Isoflurane (0.5–1.5%, Isoflurane, Rhodia, Bristol, UK) or Halothane (0.5–2%, Rhodia, Bristol, UK) gas. Animals were placed in a dorsal recumbancy, and the abdominal hair was clipped and shaved. The application site was then wiped using an isopropyl alcohol pads (Webcol, Kendall). Thirty minutes later, TEWL measurements were used to check the skin integrity. Then, ViaDerm treatment was performed and followed by a second TEWL measurement 5 min post ViaDerm treatment. A hGH patch designated for each study protocol was then placed over the 1.4 cm^2 ViaDerm treated area. In each study, one group of animals received an hGH subcutaneous injection and was used as a reference group.

Blood samples were collected over 15–24 h, at time intervals specific for each study protocol, from the tail vein in rats and from a preinserted carotid cannula (PE-50, Portex Hythe, Kent, UK) in guinea pigs. Serum (rats) and plasma (GPs) were separated using a centrifuge (Hsiangtai Machinery Ind. Co. Ltd., Taipei Hsien, Taiwan) for 10 min at 6000 rpm and stored at –20°C until analysis. At the end of the study, the animals were euthanized after intracardial administration of pentobarbitone sodium (140 mg/kg, Pental, CTS Chemical Industries, Hod Hasharon, Israel).

Bioavailability of hGH in Rats and GPs Treated with ViaDerm

In order to study the bioavailability of hGH in rats, transdermal doses of 75, 150, 300, or 450 μg hGH were applied on normal rats' skin pretreated by ViaDerm application. Plasma hGH profiles were compared to those obtained following s.c. administration (150 μg hGH per rat). Each treatment group consisted of six animals. A test similar to the rat study described above was performed with GPs using transdermal doses of 50, 150, 300, and 400 μg per GP and an s.c. dose of 50 μg per animal. Each treatment group consisted of 5–6 animals.

Bioactivity of Transdermal Applied hGH

The bioactivity of the hGH was verified by measurement of IGF-I in hypophysectomized rats. A 200 μg hGH patch was directly applied to the 1.4 cm^2 ($n = 9$) skin area that was pretreated by ViaDerm application. The levels of hGH and IGF-I in the serum of these rats were compared to those found in the s.c. treated group (150 μg hGH, $n = 6$). A nontreated group and hGH on intact skin (800 μg) served as negative controls.

Analytical Methods

The dose of hGH placed on the printed patches was measured using high performance liquid chromatography (HPLC) analysis (EP 5.0, Somatropin assay). Briefly, the active material was extracted with 1 ml of 25 mM buffer phosphate, pH = 7, and was analyzed by size exclusion

(SE) HPLC using 30 cm column (internal diameter = 7.8 mm) TSK Gel G2000 SW 5 μm (TOSOH Bioscience, Stuttgart, Germany), precolumn TSK-Gel 6 cm × Ø 6 mm (TOSOH), phosphate/2-propanol mobile phase (97 volumes of 0.063 M buffer phosphate pH 7.0, with 3 volumes of 2-propanol), and detection at 214 nm.

hGH levels in rats and GP serum or plasma was measured using an enzyme-linked immunosorbent assay (ELISA) commercial kit (DSL-10-1900, Diagnostic Systems Laboratories, Inc., Webster, TX, USA). The kit is specific for human growth hormone and does not detect endogenous GP or rat GH. Areas under the concentration curves (AUCs) were calculated using a trapezoid method. Levels of IGF-1 were measured by the functional separation method, as previously described (12).

RESULTS

The photomicrograph of Fig. 1 shows a magnified image of rat (Fig. 1A) and guinea pig (Fig. 1B) skin samples after formation of MCs by ViaDerm and staining with methylene blue. The ordered pattern of microchannels can be observed. The diameter of all the MCs and the distances between MCs were uniform.

The TEWL values of the skin before and after ViaDerm application on rats and GPs were as follows: 2.9 ± 0.8 and 4.0 ± 0.8 *vs.* 39.2 ± 5.1 and 36.1 ± 5.6 g h^{-1} m^{-2} for rats and GPs before and after ViaDerm application, respectively. A significant increase in the TEWL was observed as a result of the formation of MCs in the skin.

Figure 2 depicts serum or plasma levels of hGH in rats (Fig. 2A) or GPs (Fig. 2B), respectively, after s.c. injection or transdermal delivery from patches containing increasing amounts of hGH. Table I summarizes the AUC and bioavailability level of the various transdermal doses compared to s.c. administration. A dose-dependent increase in the C_{max} and AUC was observed in both animal species up to a dose of 300 μg per 1.4 cm^2. A further increase in the amount of active material on the patch resulted in reduced bioavailability.

The serum hGH and IGF-1 levels are presented in Fig. 3. Delivery of hGH by s.c. injection or by application of hGH patch on ViaDerm treated skin resulted in a peak in the level of the hGH in the serum of the hypophysectomized rats. Both delivery methods resulted also in an increase in IGF-1 level. In the control group, there was no change in the levels of hGH and IGF-1.

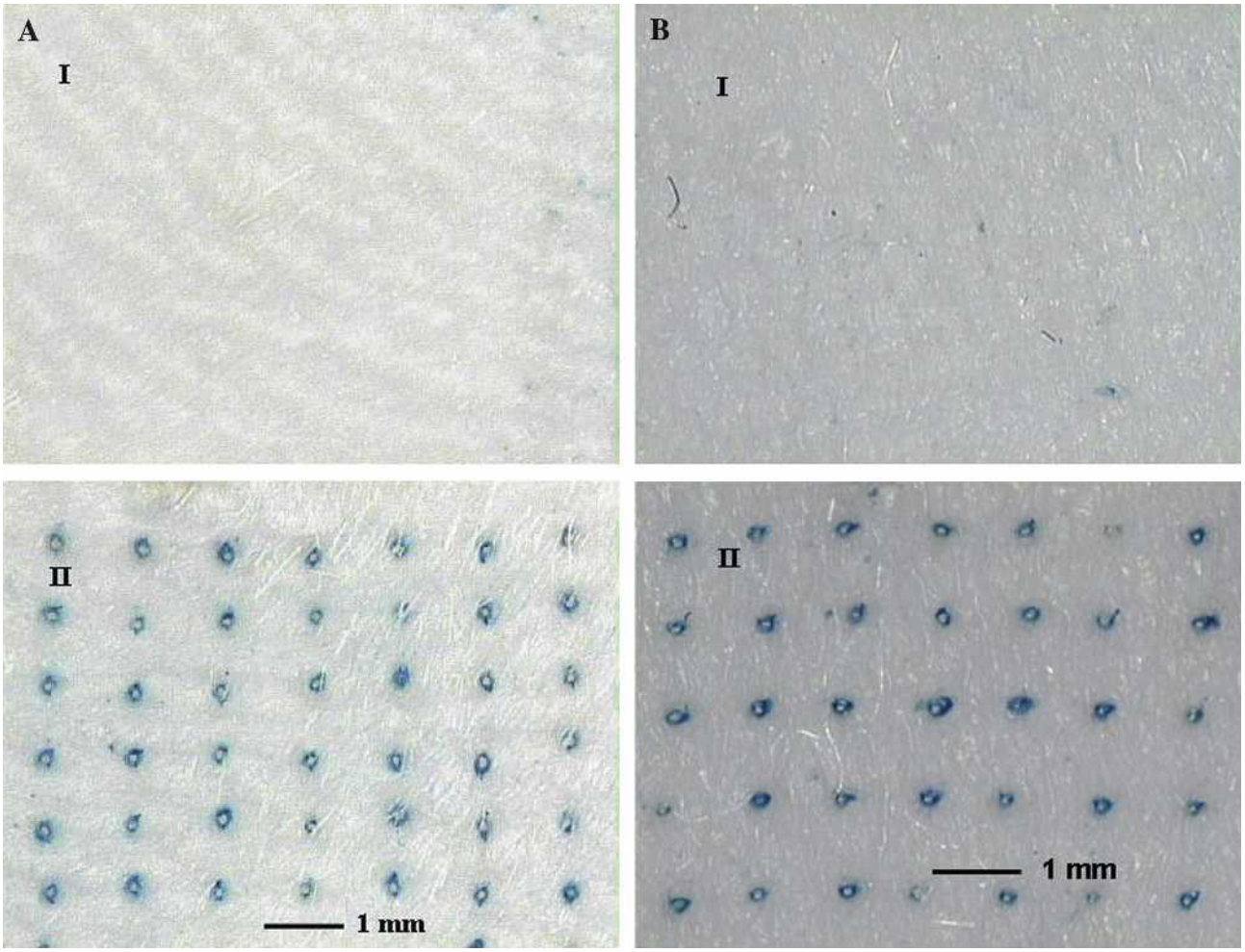

Fig. 1. Microchannels on the surface of ViaDerm treated (A) fresh rat and (B) guinea pig skin samples, after staining with methylene blue solution. The control group consisted of application of the ViaDerm device on the skin in the absence of the power source but held with the same pressure followed by methylene blue staining. (I) Control, (II) ViaDerm treated skin.

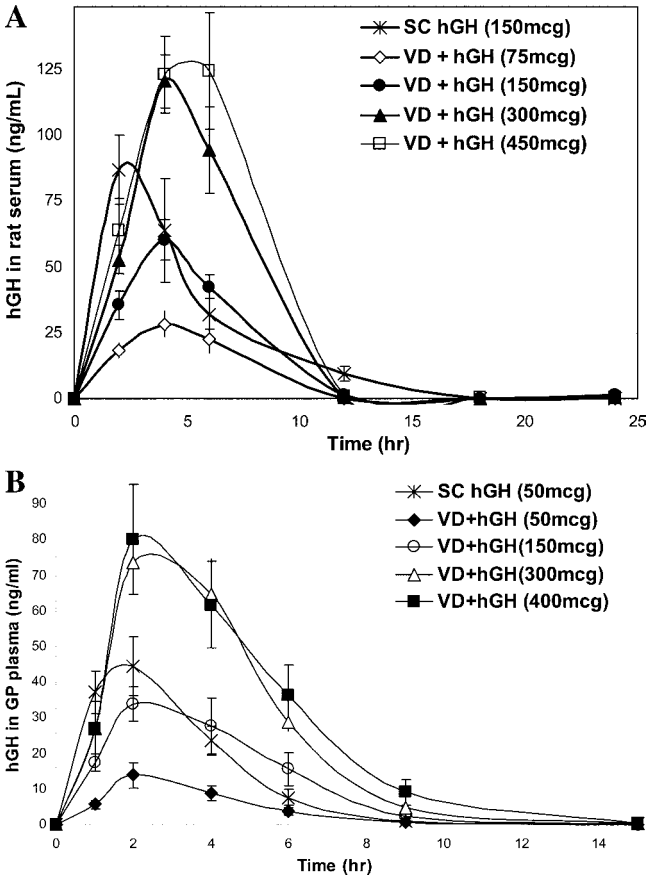

Fig. 2. hGH levels (ng/ml) in serum or plasma after application of increasing doses of transdermal hGH on 1.4 cm^2 ViaDerm treated area and s.c. injection of hGH: (A) Serum levels in rats. (B) Plasma levels in GP. Each data point represents the mean ± SEM of 5–6 animals.

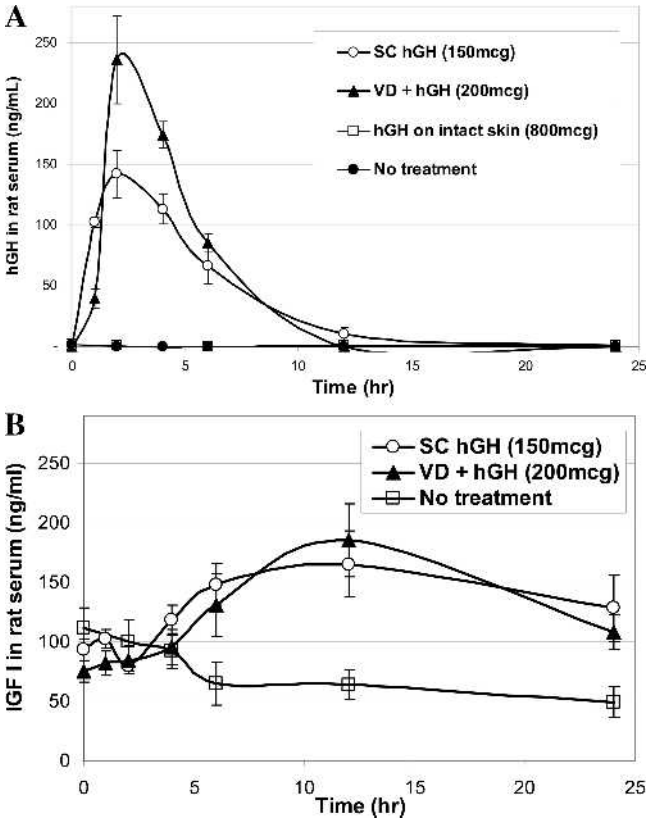

Fig. 3. Serum levels (ng/ml, mean ± SEM) of (A) hGH and (B) IGF-I after application of 200 μg hGH on 1.4 cm^2 ViaDerm treated area (n = 9), s.c. injection of 150 μg hGH (n = 7), or no treatment (n = 9) (control). Serum levels of hGH after application of 800 μg hGH on intact skin served as negative control (A).

DISCUSSION

The orderly pattern of RF-generated MCs in terms of their diameter and separated distances (Fig. 1) lays credence as to the reproducibility of the ViaDerm in creating MCs. Because methylene blue coloration of the MCs was a very rapid process, it would demonstrate the hydrophilic nature of the MCs. Indeed, previous studies showing the presence of extracellular fluid in MCs from porcine skin would support the theory of the hydrophilic nature of the MCs (9).

Table I. Mean AUC and Relative Bioavailability Values in Rats and Guinea Pigs

Mode of delivery	Dose micrograms (mcg)	AUC (ng.hr/ml.)	Bioavailability (% of s.c.)
Rats			
s.c.	150	489	100
Transdermal	75	184	75.3
Transdermal	150	376	76.9
Transdermal	300	727	74.3
Transdermal	450	884	60.3
Guinea pigs			
s.c.	50	176	100
Transdermal	50	57	32.4
Transdermal	150	175	33.1
Transdermal	300	362	34.3
Transdermal	400	404	28.7

The notion of using electricity for enhancement of transdermal drug delivery is not exclusive to the RF-microchannel technology. Iontophoresis uses an electrical field in order to drive ionized drug molecules across the SC barrier (13). In electroporation, short electrical pulses are used to create transient aqueous pores in the SC (14). Neither of these methods creates an orderly array of pores or MCs, by ablation of cells in specific locations, as presented in this study. Moreover, the ViaDerm device is user-friendly and minimally invasive. A human device safety study with 20 subjects was successfully completed with the electric parameters tested in this study. It was found that the ViaDerm device produced only slight irritation responses (minimal erythema and no edema) of a transient nature and the pain levels recorded were within the acceptable range for clinical use (in preparation).

It is important to note that the area covered with MCs is very small compared to the total skin area. MCs were created in a density of 200 MCs per cm^2 and less than 1% of the total treated area consists of MCs. Nevertheless, these MCs are highly amenable to the transdermal delivery of hGH, as non-ViaDerm treated skin is totally impermeable to hGH due to its large molecular size and hydrophilic nature (see also Fig. 3A, hGH on intact skin).

It is known that breaching the SC integrity is accompanied by an elevation in TEWL (15). Therefore, the formation

of MCs in the skin was verified by comparing TEWL values before and after treatment with the ViaDerm. There was a significant enhancement, of about 13- and 9-fold in rats and GPs, respectively, in TEWL values after ViaDerm application, despite the fact that the MCs occupy less than 1% of the skin area. It is also interesting to note that in rats and in guinea pigs the enhancement in TEWL was of a similar magnitude, despite of the differences in thickness of skin layers (16). The increase in TEWL serves as an indication for the creation of MCs, and as a predictor for the enhancement in transdermal drug delivery (17).

Low bioavailability is one of the major obstacles for the development of user-friendly delivery methods for peptides and proteins. The manufacturing processes of these active materials are usually complex with associated high costs. As compared to parenteral methods, this low bioavailability significantly reduces the feasibility of developing these alternative delivery methods as commercial products. If the bioavailability of the protein using the delivery method is low (less than 10–20%), there is a significant loss of protein resulting in higher manufacturing costs. This is despite the fact that more convenient methods will probably increase patient compliance and therefore drug efficacy (4). The bioavailability of hGH in this study relative to s.c. injection was found to be surprisingly high (75% in rats and 33% in GP; Table I). The RF-microchannel technology not only enabled the delivery of a high-molecular-weight protein (hGH) but also permitted a very efficient transdermal delivery of the drug.

This high bioavailability can be explained by the proposed mechanism of absorption of the hGH from a powder form. It is postulated that the highly water soluble hGH is dissolved by fluid that exudes from the created MCs. Consequently, a very high, local concentration of hGH solution is formed *in situ*. The delivery of the dissolved molecules is then mediated through the MCs into the viable tissues of the skin by diffusion across a steep concentration gradient. This leads to a high delivery rate and peak blood profile of the drug. The profile resembles that of s.c. injection, with a small delay in T_{max} that stems from the time required for dissolving the solid hGH and diffusion through MCs.

It is well-known that the SC functions as a rate controlling membrane in the case of transdermal delivery (18). In this study, we have demonstrated a clear increase in AUC in response to increasing amounts of drug on the patch. This dose response was linear up to a dose of 300 μg per 1.4 cm^2 and was observed in both rats and GPs. It is a reasonable hypothesis that following ViaDerm treatment, the SC no longer poses as a barrier to drug penetration through the aqueous microconduits. However, this linear increase in AUC did not persist at doses higher than 300 μg. It would appear that in both animal species, a dose of 300 μg/1.4 cm^2 can be defined as the "maximal efficient dose," at least when using the specific MCs density and electrodes that were used in this study. The factors that limit the delivered dose may be dissolution rate of hGH from the patches, the diffusion rate through the channels, the healing process of the channels and/or metabolism of the protein drug by skin derived proteases. These factors may also explain the differences in bioavailability observed in rats and GPs. It may be that these species differences stem from different healing rate and/or differences in proteases population and activity. This issue, as well as its relevance to human skin, should be further studied.

In addition to bioavailability, it is necessary to evaluate the effect of the processing method and delivery route on the integrity, conformation, and activity of the delivered protein drug (4). In this study, the bioactivity of the transdermally delivered hGH was clearly demonstrated using the hypophysectomized rat model. GH effects on cartilage growth are partly mediated by circulating IGF-1. A deficiency of GH is associated with low levels of IGF-1. In order to demonstrate the bioactivity of the hGH delivered through ViaDerm treated skin, hypophysectomized rats were used. The absence of hypophysa in these rats brings about minimal levels of endogenous GH, with concomitantly very low serum levels of IGF-1. Delivery of exogenous hGH in an active state elicits IGF-I release by the rat liver, which is expressed by a peak in serum IGF-I levels (19,20). Significant hGH doses in the plasma were measured in the ViaDerm treated rats reaching maximum levels within 4 h (Fig. 3A). The elevation in hGH, either in the s.c. or transdermal groups, was followed by an increase in IGF-1 (Fig. 3B), demonstrating that the hGH delivered transdermally was in an active form. The fact that the hGH retained its bioactivity throughout the patch manufacturing process and diffusion through skin layers underscores the notion that this delivery method might be used in a clinical setting.

In conclusion, this study demonstrates the functionality of the RF-microchannel technology as an alternative delivery method to s.c. injection of hGH. The similarities between the two methods in bioavailability, bioactivity, and serum drug profile offer much hope that the development of a commercial product based on this transdermal technology might be feasible.

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