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Research Paper

Transdermal Delivery of Heparin Using Pulsed Current Iontophoresis

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Purpose. In clinical practice heparin has to be administered by injection with obvious disadvantages; thus, transdermal delivery by electrically assisted methods have been studied. In this study we evaluated the efficacy of a Food and Drug Administration-approved pulsed current iontophoresis system in delivering heparin through living rat skin.
Methods. Fluorescent and radioactive heparin as well as a commercial heparin preparation were delivered through rat skin via a pulsed current iontophoresis system.
Results. Pulsed current iontophoresis allowed fluorescent heparin to cross the stratum corneum localizing in epidermis and dermis. Unfractionated, high-, and low molecular weight fraction pools, obtained by fractionating [^{35}S]-unfractionated heparin on a molecular weight sieve, were then separately tested. Pulsed current iontophoresis elicited the transdermal delivery of low molecular weight heparin, but not that of high molecular weight heparin. Finally, pulsed current iontophoresis of an unfractionated pharmaceutical heparin preparation significantly decreased plasmatic factor Xa activity.
Conclusions. We hypothesize that this technique could be used to administer low molecular weight heparin in a cost-efficient and safe manner without the need for syringes and needles.

KEY WORDS: cardiovascular disease; heparin; iontophoresis; rat skin; transdermal delivery.

INTRODUCTION

Heparin is a highly sulfated glycosaminoglycan with different biological activities that are exploited for treatment of various human diseases (1,2). Among these, most noteworthy are its anticoagulant and antithrombotic properties (3,4). There exist different molecular species of heparin, termed high and low molecular weight heparin, according to the molecular weight and the corresponding length of the polysaccharide chain. Pharmaceutical heparin preparations, i.e., those most commonly used in the prophylaxis of thromboembolic diseases, are a mixture of high and low molecular weight heparins. These preparations are referred to as unfractionated heparin (UFH). Several studies demonstrated that low molecular weight heparin has more antithrombotic activity with less anticoagulant activity than UFH or high molecular weight heparin (5). Hence, the opportunity to administer low molecular weight heparin in order to maximize therapeutic advantage (antithrombotic prophylaxis) and minimize side effects (bleeding due to inhibition of coagulation). Heparin (UFH as well as low molecular weight heparin) has to be administered by injection, either subcutaneous, intramuscular, or intravenous, with obvious practical disadvantages in terms of safety and patient compliance. Furthermore, injection of heparin has the potential risk of bleeding complications and the need for careful monitoring (6). Therefore, several studies and clinical trials are currently underway to determine the efficacy and safety of oral heparin formulations (7). However, because of the need for further studies (7) and the high cost of oral heparin preparations (in particular, low molecular weight heparin), the most common way of administering heparin is still through injection.

Transdermal delivery of heparin could be proposed as an alternative to injection. Electrically assisted methods have been studied for their efficacy in delivering heparin across the stratum corneum (SC). Skin electroporation showed interesting results (8,9), whereas conventional iontophoresis was significantly less efficient (8). If the efficacy of iontophoresis in delivering heparin could be improved, there would be significant advantages; in fact, iontophoresis is commonly used in hospital and outpatient settings to deliver a variety of therapeutics without major complications. In this study, we evaluated the efficacy of a powered iontophoresis drug delivery system approved for clinical use in humans by the U.S. Food and Drug Administration (FDA), in delivering unfractionated, high-, and low molecular weight heparin through living rat skin.

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ABBREVIATIONS: DIC, Differential Interference Contrast; FDA, U.S. Food and Drug Administration; FITC, Fluorescein Isothiocyanate Conjugated; PBS, Phosphate Buffered Saline; SC, *Stratum Corneum*; UFH, Unfractionated Heparin.

MATERIALS AND METHODS

Description of the Device for Pulsed Current Iontophoresis

The equipment used in this study (Transderm® Ionto System, Mattioli Engineering, Florence, Italy) is a powered iontophoresis drug delivery system indicated for the local administration of ionic drug solutions and can be used as an alternative to injections (FDA approval no. K042590, 10.14.2004). According to the information provided by the manufacturer, the electronic pulse generator produces bursts of pulses that are applied to the skin through electrodes applied to a plastic plate (the applicator). The design of the device provides one channel output. The intensity of the stimulation may be adjusted by a rotary switch that acts directly on the driving circuit. The pulse frequency and duration, as well as burst duration and period, are fixed and determined by electronic components; there are no programmable devices inside the unit. The output wave-form is biphasic: it has a positive and negative side with an average value very close to zero. The output circuit is coupled to the driving circuit by a transformer that completely insulates the patient from the electric supply; the driving waveform is of square type. Output waveform is close to an exponential decay figure. The output characteristics/specifications of the Transderm® Ionto System (on a 0–15,000 Ω load) are as follows:

Output current during burst	Adjustable at 1, 2, 3, 4, and 5 mA rms
Maximum phase charge (μC)	1
Average output current (average time >20 ms)	Adjustable at 0.5, 1, 1.5, 2, and 2.5 mA rms
Pulse frequency	2,200 Hz ± 10%
Pulse width	454 μs
Burst frequency	50 Hz ± 10%
Burst duty cycle	50%

Electric Parameters Used for Iontophoresis

The setting parameters chosen for this study were:

Average pulse current intensity	5 mA
Burst repetition frequency	50 Hz
Burst time	10 ms
Pulses per burst	22 positive and 22 negative
Pulse frequency	2,200 Hz
Surface of the electrode	0.5 cm^2
Treatment time	10 min

According to the manufacturer's specifications, with these parameters the equipment delivers 2,200 Hz pulses grouped in 50 Hz bursts of symmetrical alternate polarity. On a 20,000 Ω resistive load, each pulse is an alternate polarity exponential decay pulse with 8 mA maximum peak current intensity and 160 V peak pulse. Average pulse current intensity is 5 mA. On a 1,000 Ω resistive load, the pulse is still an exponential decay pulse, but, because of the longer fall time before the successive pulse of opposite polarity, its shape is closer to a square wave of ±5 mA. The resulting voltage is a square wave of ±5 V. On abraded skin (impedance, 800 Ohm) peak voltage is 6.4 V. The calculated average pulse current density on the electrode is 10 mA/cm^2.

Current intensity, voltage, and skin impedance under the conditions applied during the experiment (i.e., using an electrode surface of 0.5 cm^2) were monitored with an oscilloscope (Tektronik, Milan, Italy) and measured throughout the experiment. Measurement of the current intensity waveform showed bursts of alternate symmetric 5 mA square pulses matching those indicated by the manufacturer.

Separation of Heparin Molecular Species

[^{35}S]-labeled heparin was purchased from Amersham Biosciences (Little Chalfont, UK). Each vial of the product contains the followng: L-[^{35}S]cystine (1.5–9.2 GBq/mmol, 40–250 mCi/mmol); sodium [^{35}S] sulfate (up to 3.7 GBq/mmol, 100 mCi/mmol); [*N*-Sulfonate-^{35}S] heparin (up to 5.5 GBq/g, 150 mCi/g). The above-mentioned commercial preparation containing different molecular weight species was referred to as [^{35}S]UFH. [^{35}S]UFH was fractionated on a molecular weight sieve, using a column containing the resin Sephadex G-75 (Pharmacia Biotech AB, Uppsala, Sweden) following the procedure described by Cavari *et al.* (10). Briefly, [^{35}S]UFH was dissolved in 1 M NaCl and applied to the column. 1 M NaCl was used to elute the column using a peristaltic pump set at 4 ml/h. Fractions of 2 ml were collected and 1 μl of each fraction was counted for [^{35}S] radioactivity in a liquid scintillation counter. [^{35}S]-labeled heparin, at 8.75×10^7 cpm, was applied to the column, and 8.03×10^7 cpm was recovered in 52 fractions. We could separate two major peaks, identified on the basis of coelution with molecular weight markers. Pooling the fractions corresponding to each major peak, we obtained one pool containing high molecular weight heparin (average molecular weight, 12.9 kDa), and one pool containing low molecular weight heparin (average molecular weight, 4.5 kDa).

Drug Delivery Protocol

In the experiments (performed according to "Principles of Laboratory Animal Care"), we used male 6- to 8-month-old Wistar rats weighing 350 ± 50 g. Each experimental point was repeated three times (i.e., in three different animals). Before iontophoresis, rats were anesthetized via intraperitoneal injection of pentothal sodium 50 mg/kg (Abbott, Latina, Italy) (11) and depilated. The manufacturer recommended performing a mild abrasion on the area to be treated to ensure repeatability as a result of the standardization of the thickness of the SC. Abrasion was performed using a closed-loop abrasion system with corundum crystal, which is a component of the drug delivery system. In some experiments, however, the closed-loop abrasion system was disconnected, and iontophoresis was performed without prior abrasion.

Skin impedance was measured after shaving, and after abrasion (i.e., before beginning iontophoresis). The effect of heparin on skin impedance was also evaluated.

For the study of transdermal delivery of fluorescent heparin, 1 mg of fluorescein isothiocyanate (FITC)-conjugated heparin (Molecular Probes, Carlsbad, CA, USA), was

dissolved in 500 μl of phosphate-buffered saline (PBS). We applied 250 μl of the solution on each of two selected skin areas (one at the left and one at the right side of the spinal cord). One area was treated with pulsed current iontophoresis. In the other area, selected as a control area, no electric treatment was performed. Both areas (control and treatment) underwent shaving and abrasion as indicated.

For the study of transdermal delivery of [^{35}S]heparin, 1 ml of PBS containing [^{35}S]heparin was applied onto two areas of the skin as described above. As the amount of radioactivity differed according to sample preparation (i.e., [^{35}S]UFH contained the highest amount of radioactivity; high and low molecular weight fraction pools contained less radioactivity), we took care to apply onto the skin the same amount of radioactivity in each experimental point.

Evaluation of Transdermal Delivery of Fluorescent Heparin by Confocal Laser Scanning Microscopy and by Light Microscopy

Full-thickness biopsies were obtained from treated and control areas, and were immediately frozen; then, sections (15 μm thick) were obtained using a Frigocut 2700 cryostat (Reichert-Jung, Leica, Wetzlar, Germany) and placed on microscope slides (SuperFrost Plus, Kobe, Marburg, Germany). The sections were fixed by exposure to paraformaldehyde vapor for 30 min; then sections were rinsed once with PBS. For the study using confocal scanning laser microscopy, sections were covered in Acquovitrex solution (Carlo Erba, Florence, Italy) with glass coverslips and stored at −20°C until imaged. Sections were examined by a Bio-Rad MCR 1024 ES confocal laser scanning microscope (Bio-Rad, Hercules, CA, USA) equipped with a 15-mW Kr–Ar laser for fluorescence measurements and with differential interference contrast (DIC) optics for transmission images. Fluorescence was collected using a Nikon PlanApo ×10 and ×20 lens objective. Series of optical sections at 0.4 μm intervals were obtained and superimposed to create a single composite image. The laser potency, photomultiplier, and pinhole size were kept constant. For light microscopy observation, following their exposure to paraformaldehyde vapor for 30 min, sections were rinsed once with PBS and then they were stained by hematoxylin–eosin solution (Sigma-Aldrich, Milan, Italy). After staining, specimens were observed by a light microscope (Nikon Instruments, Milan, Italy) at different magnifications (×10 and ×20) and images documented by a digital camera connected to the microscope.

Measurement of Transdermal Delivery of [^{35}S]heparin

In the experiments with [^{35}S]heparin, after each treatment, both control and treated areas were cleaned with successive swabs, and each swab was counted for radioactivity. When no radioactivity was recovered from the swab, we proceeded to perform the biopsies. Radioactivity remaining on the electrodes after cleaning was also measured to ascertain the absence of contamination before proceeding to the next stage. Full-thickness biopsies were taken both from treated and from control areas. To further remove any [^{35}S]heparin that did not penetrate the tissues, biopsies were soaked in 7 ml of PBS, then the specimens were washed several times (5 ml/wash) with PBS. Each time, an aliquot of washing solution was counted for [^{35}S] radioactivity; when no radioactivity could be detected in the washing solution, we considered that all nontissue-associated heparin had been removed. Then, specimens were dried and weighed; to completely destroy the collagen bundles in the dermis and to dissolve the intercellular bridges and junctional complexes among the epidermal cells, each specimen was mechanically fragmented. Fragments were incubated for 48 h in a solution containing 10 mg/ml collagenase and 25 mM trypsin–EDTA, in continuous shaking inside an incubator for cell cultures at 37°C and with 5% CO_2. After 48 h incubation, specimens were transferred into vials for liquid scintillation, each containing 5 ml of scintillation liquid; radioactivity was measured by a liquid scintillation counter. Data were expressed as cpm/mg (of biopsy weight; average biopsy weight was 250 mg). Presented data are means ± SEM ($n = 3$).

Study of Plasmatic Factor Xa Activity

In the experiments designed to measure modification of plasmatic factor Xa activity, blood samples were drawn 1 h after pulsed current iontophoresis, i.e., at a time when heparins (both UFH and low molecular weight heparin), administered by subcutaneous injection, reach their peak levels and peak biological activities (12). In these experiments, we took as controls untreated rats, rats where UFH was applied onto the skin (after shaving and abrasion) without any electrical treatment, or rats where UFH was administered by subcutaneous injection. Factor Xa activity was determined by an activated partial thromboplastin time-based (aPTT) one-stage clotting time assay. Factor Xa-depleted plasma was used as the substrate and the clotting time with the rat plasma was compared to the clotting time of normal pooled plasma. Factor Xa activity, such as in humans, was expressed as percentage (13).

Statistical analysis was performed using Student's t test. Values were considered significantly different at $p < 0.05$.

RESULTS

In this study we investigated the transdermal delivery of heparin on living rat skin performed via an FDA-approved device. Observation by light microscopy after hematoxilyn–eosin staining showed no significant differences between treated and control skin areas (Fig. 1A and B). In panel B, control refers to an area that had been depilated and shaved, but where no abrasion or electrical treatment had been performed. On the surface of both specimens, the SC could be observed, and seemed well preserved. In fact, a recent review on this topic claims that SC must be intact for iontophoresis (14). As expected, the thickness of the SC was decreased in treated areas because of abrasion (Fig. 1A). However, we could not detect any lesion to this layer or to underlying layers of the epidermis. The partial detachment from the other skin layers that could be observed in control and in treated samples is a common histological artifact. Epidermis, both in control and in treated specimens seemed such as a strip composed by different layers of cells strictly adherent to each other. As expected, it presented a rippled course, a strong adhesion to the underlying dermis and well-preserved hair follicles and sebaceous glands emerging from

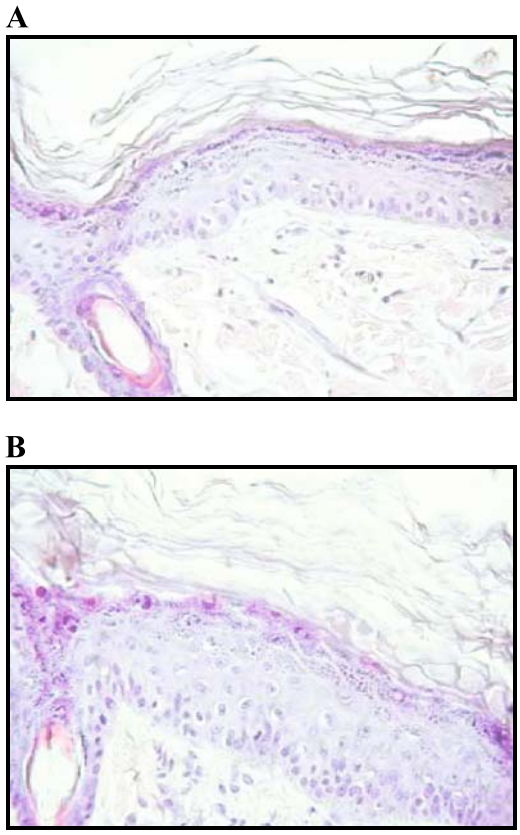

Fig. 1. Histology of rat skin treated or untreated with pulsed current iontophoresis. (A) Rat skin treated by pulsed current iontophoresis. SC, partially detached from the other layers of epidermis, was evident. Epidermis had an uniform thickness with no observable interruptions; numerous collagen bundles, spatially well organized, composed the dermis; various connective cells such as fibroblasts were interspersed among the collagen bundles; appendages seemed unaffected. Light microscopy; hematoxilyn–eosin staining. Total magnification ×200. (B) Control. This sample was taken from an area that had been depilated and shaved, but where no abrasion or electrical treatment had been performed. SC, partially raised and detached from the underlying layers, epidermis and dermis with appendages were evident. Light microscopy; hematoxilyn–eosin staining. Total magnification ×200.

the dermis. Dermis had a wide thickness and it showed, both in control and in treated specimens, numerous collagen bundles. The bundles were closely crowded and showed different spatial distribution; several connective cells such as fibroblasts were interspersed among collagen bundles. On the whole, epidermis, dermis, and appendages, such as hair follicles and sebaceous glands, did not show any significant modification in shape, consistency, or staining. No morphological sign of cell injury (i.e., abnormalities in shape or staining) could be detected in the treated samples. Taken together, these results demonstrate that treatment did not cause any tissue damage. This conclusion was consistent with observation of the area where the device was applied; only pale redness of the skin was noticed (not shown).

Skin impedance is critical in determining the efficacy of electrically assisted transdermal transport techniques. Because living skin impedance greatly varies according to different factors (15,16), we studied the effect of the entire treatment on skin impedance. The results reported in Fig. 2 demonstrate that rat skin impedance after shaving was about 1,200 Ω. This value is compatible with the age of the rats used in these experiments, given that skin resistance in rats increases with aging (17), and it is also compatible with values determined in Wistar male rats where abdominal hair was clipped (18). Abrasion significantly ($p < 0.05$ *vs.* "control") reduced rat skin impedance. UFH (commercial preparation for human use, 1,562 IU/kg), applied after abrasion, further reduced impedance ($p < 0.05$ both *vs.* "control" and *vs.* "abrasion"). These results are consistent with a previous study that used heparin in skin electroporation (9), where heparin enhanced the transdermal delivery of mannitol. In that study, it was proposed that charged macromolecules such as heparin enhanced electroporation-assisted delivery by stabilizing the increased permeability caused by high-voltage pulses.

Pulsed current iontophoresis allowed the transdermal transport of fluorescent heparin. The distribution of fluorescent heparin immediately after pulsed current iontophoresis is shown in Fig. 3A. Similar to that observed with high voltage electroporation (19,20), fluorescent heparin was detectable both in the SC and in the underlying epidermis layers after pulsed current iontophoresis. Fluorescence was more intense in the SC, where FITC-conjugated heparin was applied, and became gradually less intense, but still bright, in the epidermis. Fluorescence in the epidermis was distributed along intercellular pathways so as to clearly show both the outline of the corneocytes forming the SC and the outline of the keratinocytes in the underlying layers. Fluorescence was also observed in some areas of dermis, suggesting a diffusion of FITC-conjugated heparin through the dermoepidermal junction. No fluorescence was detected in association with appendages. Figure 3B shows that in the control area (i.e., where FITC-conjugated heparin was applied onto the skin after shaving and abrasion, but where no electrical treatment was performed), only a weak background green fluorescence level was detectable in the SC, whereas no fluorescence at all could be observed in the epidermis, dermis, and appendages.

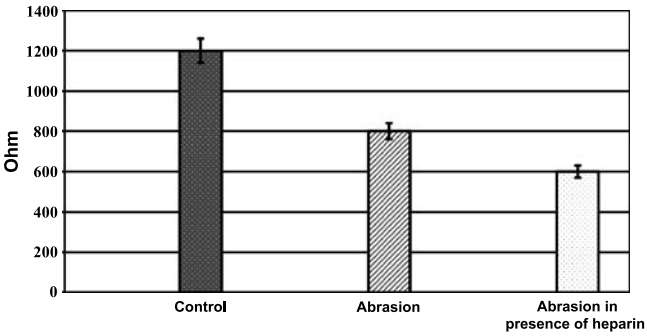

Fig. 2. Measure of rat skin impedance. Rat skin impedance was measured by an oscilloscope connected to the pulsed current iontophoresis system. Skin impedance was measured in control areas ("Control," i.e., shaved areas without any further treatment), in areas that had undergone abrasion ("abrasion"), and in areas where heparin (commercial UFH for human use, 1,562 IU/kg) was applied after abrasion ("abrasion in presence of heparin"). Electrical parameters and conditions were those described in the "Materials and Methods" section. Data are means ± SEM ($n = 3$).

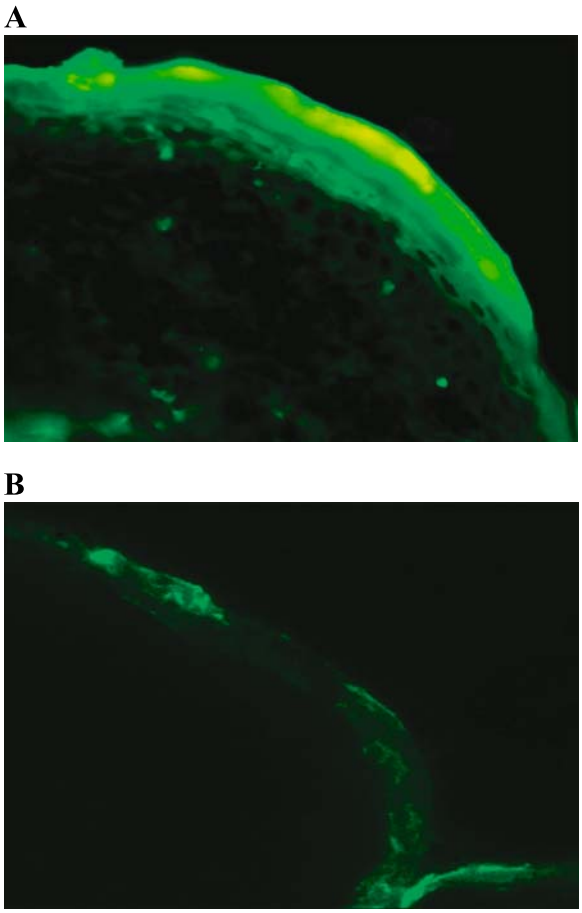

Fig. 3. FITC-conjugated heparin distribution in rat skin after pulsed current iontophoresis and in control area. (A) After pulsed current ionophoresis FITC-conjugated heparin was distributed on the SC and in all the underlying epidermis layers. The characteristic outline of corneocytes and of keratinocytes belonging to the different epidermis layers is evident; fluorescence is also observed in some areas of the dermis but not associated to appendages. Confocal laser scanning microscopy. Total magnification ×100. (B) Control. This sample was taken from an area that had been depilated, shaved, and abraded, but where no electrical treatment had been performed. A weak fluorescent background was present in the SC. No fluorescence at all was observed in the underlying epidermis layers nor in the dermis. Confocal laser scanning microscopy. Total magnification ×100.

Having demonstrated that pulsed current iontophoresis elicited the transdermal transport of fluorescent heparin, we tried to determine which molecular species of heparin was preferentially delivered through the skin. Thus, FITC-conjugated heparin as well as UFH are a mixture of high- and low molecular weight heparins. Previous studies performed using heparin in skin electroporation demonstrated that small heparin molecules were preferentially transported (8). First, we measured the transdermal transport of [^{35}S]UFH. This mixture of high- and low molecular weight species is representative of pharmaceutical preparations that are routinely used in clinical practice.

Figure 4 shows that there was minimal amount of [^{35}S] radioactivity still associated with biopsies after repeated washing (radioactivity in "control"; control areas refer to areas where shaving and abrasion were performed and [^{35}S]heparins applied, without any electrical treatment). After pulsed current iontophoresis of [^{35}S]UFH, we could observe an increase in the amount of [^{35}S] radioactivity recovered in the biopsies. However, this increase, reflecting transdermal passage of [^{35}S]UFH, albeit significant ($p < 0.05$ vs. "control") was not impressive. When the purified high- and low molecular weight fraction pools obtained by fractionating the same batch of [^{35}S]UFH on the Sephadex G-75 column were separately tested, the results were different from those obtained with [^{35}S]UFH. Pulsed current iontophoresis did not significantly elicit the passage of radioactive high molecular weight heparin. Less than 1% of radioactive high molecular weight heparin was recovered in the biopsies from treated areas (cpm applied, 1,465,704 ± 2,201; cpm recovered, 13,362 ± 776; biopsy weight, 262 mg). However, pulsed current iontophoresis significantly increased the transdermal transport of radioactive low molecular weight heparin. In fact, the amount of low molecular weight heparin-associated radioactivity recovered in the biopsies from treated areas (solid bars) was significantly greater than that recovered in the control biopsies. About 10% of applied radioactive low molecular weight heparin was recovered in the biopsies from treated areas (cpm applied, 1,495,511 ± 2,182; cpm recovered, 141,358 ± 2,439; biopsy weight, 251 mg).

Having demonstrated that low molecular weight heparin was the molecular species preferentially delivered into the skin following pulsed current iontophoresis, we investigated whether plasmatic factor Xa activity changed as a result of low molecular weight heparin passage into plasma. We decided to study factor Xa activity because we hypothesized that the skin acted as a molecular weight sieve allowing the preferential passage of low molecular weight heparin, and the more predictable pharmacokinetic profiles of low molecular weight heparins are largely based on the evaluation of the activity of this factor (12). In these experiments, we applied onto the skin 1,562 IU/kg of commercial UFH (for human use) commonly used, by subcutaneous administration, for the

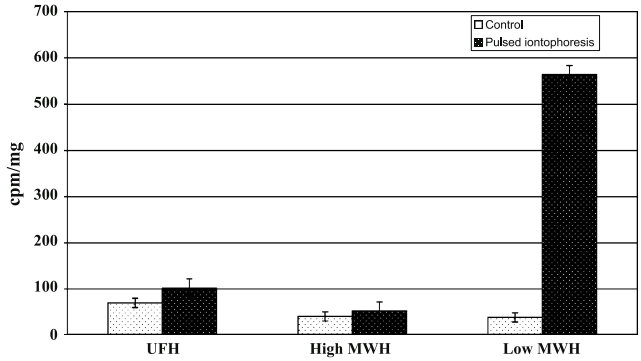

Fig. 4. Transdermal transport of [^{35}S]heparin species. "Control" and "pulsed iontophoresis", indicate the treatment for each sample. "Control" refers to areas where [^{35}S]heparin species were applied after shaving and abrasion, but where no electrical treatment had been performed. cpm: [^{35}S] radioactivity recovered in the biopsies; mg: weight of the biopsies. Data are means ± SEM (n = 3). UFH: unfractionated heparin; high and low molecular weight heparin (MWH): high and low molecular weight heparin fractions. Electrical parameters and conditions are those described in the "Materials and Methods" section.

long-term management of human patients (21). Plasmatic factor Xa activity in untreated rats, or in rats where UFH (1,562 IU/kg) was applied onto the skin, after shaving and abrasion, without any electrical treatment, was almost identical, i.e., 67%. Pulsed current iontophoresis in rats where UFH (1,562 IU/kg) was applied onto the skin, caused a significant decrease in plasmatic factor Xa activity that was reduced to 33.2%. In other experiments, pulsed current iontophoresis was applied onto the shaved rat skin without prior abrasion. Plasmatic factor Xa activity was reduced to 33.5%.

Considering that about 10% of applied radioactive low molecular weight heparin was transdermally delivered, we could estimate that the dose applied in this study (i.e., 1,562 IU/kg) corresponded to a delivered dose quite similar to that subcutaneously injected daily in human patients for the treatment of most common thromboembolic diseases (i.e., 12,000 IU for the average patient weighing 70 kg) (21). Consistent with this estimate, the subcutaneous injection of the same amount of UFH (corresponding to 100,000 IU in humans) completely inhibited factor Xa activity.

DISCUSSION

In this study, we demonstrate that pulsed current iontophoresis is a technique suitable for the transdermal delivery of heparin. Macroscopical and histological analysis of treated skin areas did not reveal signs of tissue or cell damage. Shaving and abrasion did not disrupt the SC and were not sufficient *per se* to allow the transdermal passage of unfractionated, high-, and low molecular weight heparins. Because we used an FDA-approved drug delivery system, we carefully followed the manufacturer's instructions to reproduce in rats the exact conditions applied in humans, including mild abrasion of skin areas to be treated. However, control experiments with fluorescent or radioactive heparin or with UFH (measuring factor Xa activity) demonstrated that abrasion did not allow the transdermal passage of heparins in rats.

Pulsed current iontophoresis showed limited efficacy with UFH, whereas it significantly increased by a factor of 10 the transdermal transport of low molecular weight heparin. These results are consistent with a previous report demonstrating that high-voltage skin electroporation caused the preferential transdermal transport of small heparin molecules (8). From our data, it seems that pulsed current iontophoresis of heparin produced results that are "in between" those produced by high-voltage electroporation, and those produced by conventional DC current iontophoresis (8). Although peak voltage on abraded skin was 6.4 V, i.e., a value much lower than that required for electroporation, we may hypothesize that heparin functioned as a chemical enhancer of electrically assisted transdermal transport as observed during high-voltage skin electroporation when heparin enhanced mannitol transport by interacting with transport pathways (9). Also, the results obtained with fluorescent heparin are consistent with this interpretation. Thus, unlike conventional DC current iontophoresis, high voltage electroporation favors the passage of molecules through newly formed transport pathways between cells (19), just like those observed with pulsed current iontophoresis. Thus, pulsed current iontophoresis of heparin could reproduce *in vivo* the conditions observed in human cadaver skin where electroporation followed by iontophoresis allowed the transdermal passage of a peptide hormone (22).

It is accepted that low molecular weight heparin has significant pharmacological advantages over UFH preparations commonly administered in daily clinical practice. Briefly, low molecular weight heparin shows more antithrombotic activity and less anticoagulant activity with lower risk of bleeding as a side effect (23). However, pure low molecular weight heparin preparations are difficult to produce and are significantly more expensive than UFH. In addition, although oral delivery of modified heparins is currently under investigation (7), heparin preparations are still administered by injection, using syringes and needles, with obvious complications in terms of sterilization and disposal of biohazardous material as well as patient compliance. All these practical hindrances (including high costs) limit the use of low molecular weight heparin in the chronic prophylaxis of most common thromboembolic diseases. This is most unfortunate, as several studies demonstrated that chronic prophylaxis with low molecular weight heparin significantly reduced morbidity and mortality in a large number of patients (24,25).

CONCLUSIONS

Having observed that pulsed current iontophoresis allowed the preferential transdermal transport of low molecular weight heparin, we hypothesize that this technique could be used to administer low molecular weight heparin in a cost-efficient and safe manner without the need for syringes and needles. Thus, pulsed current iontophoresis of common (low-cost) UFH preparations would result in the passage of low molecular weight species as if the skin functioned as a molecular sieve.

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