Transdermal Delivery of Heparin and Low-Molecular Weight Heparin Using Low-Frequency Ultrasound

Samir Mitragotri[1,4] and Joseph Kost[2,3]

Received January 10, 2001; accepted May 3, 2001

Purpose. Heparin and low-molecular weight heparin (LMWH) are the most commonly used anticoagulants and are administered by intravenous or subcutaneous injections. However, injections of heparin have the potential risk of bleeding complications and the requirement of close monitoring in some cases. We hypothesized that transdermal delivery of heparin may provide an attractive alternative to injections. However, the dose of transdermally delivered heparin is limited by low skin permeability.

Methods. We increased skin permeability to heparin and LMWH using low-frequency (20 kHz) ultrasound. Biologic activity of transdermally delivered heparin was measured by using activated clotting time assays and by using anti-Xa (aXa) activity. Structural integrity of heparin was also assessed by using gel electrophoresis.

Results. Low-frequency ultrasound increased permeability of pigskin *in vitro* and rat skin *in vivo* and allowed delivery of biologically active doses of heparin and low-molecular weight heparin transdermally. A prolonged contact of transdermally delivered heparin with pigskin was found to reduce the biologic activity of heparin, although no such deactivation was observed during short exposures. Transdermally delivered LMWH resulted in sustained aXa levels in the blood. This result was in strong contrast to subcutaneous or intravenous injections of LMWH, which resulted in only temporary elevations of aXa level.

Conclusions. Transdermal delivery of low-molecular weight heparin is a potential alternative to injections.

KEY WORDS: heparin; ultrasound; low-molecular weight heparin; sonophoresis; thrombosis.

INTRODUCTION

Heparin and low-molecular weight heparin (LMWH) are the most commonly used anticoagulants and are administered by injections for the treatment of venous thromboembolism (1). Applicability of injections is limited by pain and complications associated with the fluctuations of heparin concentration in the blood. Transdermal drug delivery offers an attractive alternative to injections due to minimization of pain and possible sustained release of drugs (2). We hypothesized that the transdermal route of administration can be especially beneficial for the delivery of heparin and LMWH because of (i) sustained drug delivery, (ii) possibility of controlling the rate of administration, (iii) feasibility of on-demand termination, (iv) convenience, and (v) minimization of pain. However, transdermal transport of heparin is slow because of the low permeability of stratum corneum, the outermost layer of the skin. Attempts have been made to enhance transdermal heparin transport by using enhancers such as electroporation (3); however, applications of this method *in vivo* have yet to be shown.

Ultrasound under a variety of conditions has been used for enhancing transdermal drug transport (4–8). This phenomenon is referred to as sonophoresis. In one particular mode of sonophoresis, a short application of ultrasound is used to permeabilize skin for several hours (9). We hypothesized that increased skin permeability may be used to deliver heparin or LMWH across the skin. In this article, we present our *in vitro* and *in vivo* data to support this hypothesis.

MATERIALS AND METHODS

In Vitro Experiments

In vitro experiments were performed by using pigskin. All animal procedures were performed by using institutionally approved protocols. Pigskin was harvested from Yorkshire pigs immediately after sacrificing the animal. Skin samples without any detectable scratches or abrasions were cut into small pieces and stored in a −80°C freezer for use within 12 weeks. Sonophoresis experiments were performed by using a vertical Franz diffusion cell (receiver volume = 12 mL, area = 1.77 cm^2), which consists of a donor and a receiver compartment. A small stir bar and an Ag/AgCl disk electrode (E242 Invivo Metrics, Healdsburg, CA) were added to the receiver chamber. In addition, the receiver chamber was filled with PBS (phosphate-buffered saline, 0.01 M phosphate, 0.0027 M potassium, 0.137 M sodium chloride; Sigma Chemicals Co., St. Louis, MO). Pigskin was thawed and was mounted in the diffusion cell with the epidermis side facing up. The donor and the receiver compartments were clamped, making sure there were no bubbles in the receiver chamber.

Before applying ultrasound, structural integrity of the skin was checked by measuring its conductivity using methods described later. Skin samples with a resistivity < 50 kohm-cm^2 were assumed to be defective and not used. The donor compartment was filled with a 1% w/v solution of sodium lauryl sulfate (SLS; Sigma Chemicals) in PBS. Ultrasound was applied according to the procedure described later. At the end of sonication, the donor chamber was rinsed thoroughly with PBS to remove residual surfactant. A solution of heparin (H3393, 300 mg/mL; Sigma Chemicals) along with radiolabeled heparin (^3H labeled, obtained from New England Nuclear, Boston, MA (>98% pure), dissolved in PBS at a concentration of 10 μCi/mL) was added to the donor compartment. Samples were taken from the receiver compartment periodically to measure the amount of heparin transported transdermally. Concentration of radiolabeled heparin was measured by using a scintillation counter (Tricarb 2000, Packard Instruments, Meriden, CT). Skin permeability to heparin was calculated based on the equation, $P = V\Delta C/\Delta t / (AC_d)$, where V is the receiver volume, A is the skin area (1.77 cm^2), $\Delta C/\Delta t$ is the measured increase in the heparin concentration in the receiver compartment over a period of time Δt, and C_d is the heparin concentration in the donor compartment. To assess the contribution of the free label (tritiated water) to the experimentally measured heparin concentra-

[1] Department of Chemical Engineering, University of California, Santa Barbara, California 93106.
[2] Sontra Medical, Inc., Cambridge, Massachusetts 02139.
[3] Department of Chemical Engineering, Ben Gurion University, Beer Sheeva, Israel.
[4] To whom correspondence should be addressed. (e-mail: samir@engineering.ucsb.edu)

tion, some samples were lyophilized and were reconsituted in water. The measured radioactivity in the reconstituted samples was comparable with the original samples, Hence, the contribution of the free label was negligible.

In Vivo Experiments

All animal procedures were performed by using institutionally approved protocols. Detailed protocols for animal experiments have been described previously (10). Briefly, rats (Sprague-Dawley, either sex) were anesthetized with a mixture of ketamine (60 mg/kg) and xylazine (10 mg/kg) injected intraperitoneally or intramuscularly. After anesthesia was confirmed, a flanged glass cylinder (Permegear, Hellertown, PA, diameter 15 mm, height 2 cm) was glued on the rat's shaved lateral flank by using a minimal amount of cyanoacrylate adhesive (Permabond International or Vet Bond) on the outer edge of the flange. The chamber was filled with 1% SLS. Ultrasound was applied according to methods described in Ultrasound Application. Throughout the sonication, skin conductivity was measured by using methods described later. At the end of sonication, the donor chamber was rinsed thoroughly with PBS to remove residual surfactant. LMWH solution (1 mL) (Dalteparin, 150 mg/mL, provided by Prof. Robert Linhardt) was then placed on the skin. A catheter was placed in the jugular vein of the rat. Blood samples were taken from the catheter periodically to measure the concentration of LMWH in the blood. Concentration of LMWH was measured by using anti Xa activity (Sigma Chemicals). In some experiments, effect of ultrasound on transdermal inulin transport was measured. In these experiments, rat skin was exposed to ultrasound by using methods described later. At the end of sonication, the coupling medium was removed and was replaced with a solution of radiolabeled inulin (^{3}H labeled, obtained from New England Nuclear, dissolved in PBS at a concentration of 10 μCi/mL). A catheter was placed in the bladder and urine samples were taken periodically. The amount of inulin in the urine was measured by using a scintillation counter (Tricarb 2000, Packard Instruments, Meriden, CT).

Ultrasound Application

Ultrasound was applied only once to each skin site by using a sonicator (VCX 400, Sonics and Materials, Newtown, CT) operating at a frequency of 20 kHz. Before each new experiment, the sonicators were "tuned" according to a procedure specified by the manufacturer to ensure that the applied signal optimally matched the resonant frequency of the piezoelectric crystal. The horn was positioned 1 cm above the skin inside the donor chamber. The sonicators were operated in a pulsed mode (5 s on, 5 s off). Ultrasound intensity was 7 W/cm^2. This intensity corresponds to spatially averaged pulse average values (I_{SAPA}), that is, the intensity corresponds to that during the on period of ultrasound. Measurement of ultrasound intensity was performed by using a calorimetric method and is described elsewhere (11). Ultrasound was applied until the skin conductivity increased to 0.6 (kohm-cm^2)$^{-1}$.

Electrical Resistance Measurements

To measure the electrical resistivity of the skin, a 100-mV AC electric field (10 Hz), was applied across the skin for a short time by using a signal generator (model 33120A; Agilent, Palo Alto, CA). Current measurements were made with an ammeter (Micronta, Tandy Corporation, Fort Worth, TX). Skin electrical resistance was then calculated from Ohm's law. Because the measured skin resistance is the sum of the actual skin resistance and the saline resistance, the latter was subtracted from the measured skin resistance to obtain the actual skin resistance. Skin resistivity was obtained by multiplying the skin electrical resistance (measured experimentally) by the skin area (1.77 cm^2). Skin conductivity was calculated by taking the reciprocal of resistivity.

Heparin Activity/Clotting Assay

Biologic activity of transdermally delivered heparin was measured by using activated clotting time (ACT) assay (Hemacron, Edison, NJ) and anti-Xa assay (Sigma Chemicals). ACT tests were performed for *in vitro* experiments. For this purpose, freshly harvested pig blood was first preserved with sodium citrate (Sigma Chemicals) to prevent clotting. The blood was constantly agitated on an agitator and was used within 2 h of harvesting. In a 37°C prewarmed assay tube (Hemochron, Edison, NJ), 200 μL blood was added along with 200 μL of $CaCl_2$, which neutralizes the anticoagulant effect of sodium citrate. Immediately, 0.5 mL of the sample to be tested (obtained from the receiver compartment) was added to the tube, and the timer on the Hemachron was initiated. The tube was shaken briefly to mix the contents and then inserted into the Hemachron to measure the clotting time. Before testing samples collected from the receiver compartment, a standard curve of clotting time vs. known heparin concentrations (0, 0.5, 1, 2, 3, and 4 U/mL) was generated. The clotting times of the samples were interpolated on this curve to calculate their activity. If the sample concentration was expected to be high (outside the linear range of 0–4 U/mL), it was diluted before being tested.

Gel Electrophoresis

To test whether heparin was degraded in the skin, gels were run on the samples obtained from the receiver compartment at the end of the *in vitro* experiment. The methods of electrophoresis are similar to those reported in Ref. 12. In these experiments, aliquots (500 μL) were taken from the receiver compartment and were added to trichloroacetic acid (Sigma Chemicals) to precipitate proteins. The solution was then centrifuged at 14,000 *g* to form a pellet of the precipitated proteins. Samples of the supernatant were used for gel electrophoresis. A control lane with the donor heparin solution was placed next to the sample for comparison. The heparin samples (40 μL) were mixed with the buffer (10 μL; 0.31 M Tris-HCl, pH 6.8, 0.05% w/v bromophenol blue, 50% v/v glycerol) and 30 μL of each sample was loaded onto a 12% acrylamide gel (Biorad, Hercules, CA). Gel electrophoresis was performed at 100 V in an electrophoresis cell (Biorad, Hercules, CA) filled with running buffer (0.3% w/v Tris base, 1.4% w/v glycine, pH 8.3). The run was stopped when the marker was about 2 or 3 cm from the edge of the gel (about 2 h). Azure blue dye (0.08% w/v in PBS; Sigma Chemicals) was used to stain heparin for 1 h under constant agitation. Destaining was performed for about 1 h by using 10% v/v

methanol and 10% v/v glacial acetic acid in water. The gels were imaged with a computer scanner and the intensity was measured by using Adobe Photoshop. The intensity of each lane was analyzed from the top of the gel to the bottom in 1-mm contiguous sections. Intensity vs. distance plots were generated and were used to test the integrity of heparin.

RESULTS AND DISCUSSION

Ultrasound-Induced Skin Permeabilization

Skin permeabilization due to ultrasound application was tested *in vitro* by using pigskin and *in vivo* by using rat skin. Ultrasound was applied only once to each piece of skin *in vitro* or an animal *in vivo* to increase skin permeability. Application of ultrasound was performed in the presence of SLS because this combination is much more effective in enhancing transdermal transport compared with ultrasound alone (9). Enhanced skin permeability was monitored by using skin conductivity. Conductivity of pigskin *in vitro* before ultrasound application was about 0.01 (kohm-cm^2)$^{-1}$. Typical skin conductivity after a 10-min application of ultrasound (7 W/cm^2, 5 s on, 5 s off) was about 0.6 (kohm-cm^2)$^{-1}$. Skin conductivity remained steady for at least 24 h after ultrasound application. Thus, a short application of ultrasound increased skin permeability for a prolonged period of time.

Similar observations were made *in vivo*. Specifically, the skin conductivity before ultrasound application was about 0.01 (kohm-cm^2)$^{-1}$. This conductivity was enhanced by about 50-fold because of ultrasound application. The ultrasound application time required to permeabilize skin was much shorter *in vivo* (2 min) than that for pigskin *in vitro* (10 min). The difference between the sonication time *in vivo* and *in vitro* may be attributed to the difference in the animal model or to the presence of subcutaneous tissues *in vivo*. After application of ultrasound, the skin permeability remained high for at least 8 h (the duration of the experiment). The permeability would eventually recover to its baseline value due to the recovery processes in the skin. For example, we used the same protocol to increase skin permeability of human volunteers (13). In these experiments, the skin remained permeable for about 15 h, after which, it recovered to its normal permeability within 20 h (13).

In Vitro Heparin Delivery

Initial experiments were performed *in vitro* to assess the feasibility of transdermal heparin delivery. In the *in vitro* experiments, skin was permeabilized as described above, and heparin was then placed on the skin (300 mg/mL, with trace radioactivity). Figure 1 shows the amount of heparin delivered transdermally over time (circles). Figure 1 also shows the amount of heparin delivered in controls (squares). The transdermal flux of heparin (indicated by the slope of the curve) is about 21-fold higher through the sonicated skin compared with that through nonsonicated skin. Although it is difficult to ascertain the contribution of heparin fragments to total heparin permeability, the measured heparin permeability through sonicated skin after ultrasound application (2.1×10^{-3} cm/h) was actually higher than that of other hydrophilic

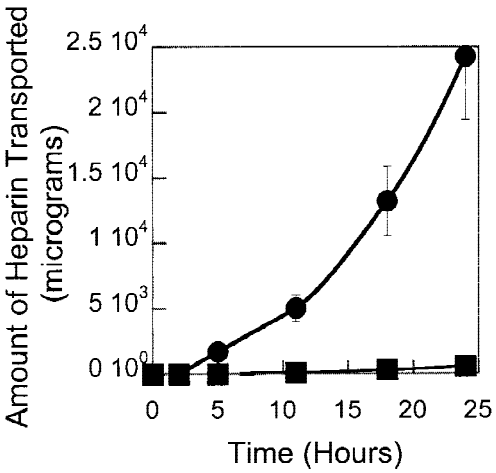

Fig. 1. Transdermal transport of heparin through ultrasound-treated pigskin (n = 5) (circles). Ultrasound (20 kHz, 7 W/cm^2, 5 s on, 5 s off) was applied until skin reached a conductivity of 0.6 (kohm-cm^2)$^{-1}$. Transport through control skin samples is also shown (squares). Error bars indicate standard deviations.

molecules examined including mannitol (MW = 180 Da) and inulin (MW ~ 5000 Da) under the same conditions. For example, skin permeability after ultrasound application under the same conditions was about 6×10^{-4} cm/h for mannitol and 3×10^{-4} cm/h for inulin. Thus, no significant size dependence of skin permeability was observed in the presence of ultrasound under conditions used in this article. Accordingly, contribution of small heparin fragments to this permeability is unlikely to be dominant, although detailed chromatographic studies are necessary to arrive at a firm conclusion on this issue. About 24 mg of heparin was delivered transdermally (across 1.7 cm^2) in 24 h, thus corresponding to a bioavailability of about 16%. This bioavailability can be increased by further optimization. With the permeability reported in this article, about 72 mg of heparin can be delivered in a day (24 h) from a patch having an area of 10 cm^2 and containing heparin solution at a concentration of 150 mg/mL. This dose is comparable with a typical therapeutic dose given for deep vein thrombosis in humans (a dose of about 1 mg/kg/d for a 70-kg person, or about 25,000 U/d bolus [approximately 150 U/mg of heparin]) (1,14).

We performed additional tests to assess whether transdermally delivered heparin is biologically active. For this purpose, we collected aliquots from the receiver solution and measured ACT and aXa activity by using methods described earlier. On the basis of the measurements of radioactive heparin, we predicted the magnitude of aXa activity and ACT in the receiver solution (assuming 100% biologic activity). We then compared the predicted values of ACT and aXa activity with those measured experimentally. Biologic activity of heparin in the receiver compartment was detectable in all samples. However, we found that the aXa activity and ACT of receiver samples were significantly lower than their predicted values. Specifically, experimentally measured aXa and ACT accounted for about 25–40% of the expected activity. Loss of heparin activity after transdermal transport was also obtained in the case of transdermal heparin delivery using electroporation (3). This issue was assessed in depth and is discussed next.

Biologic Activity of Heparin

We hypothesized that the lower-than-expected activity may be attributed to selective transport of low-molecular weight fractions of heparin. Because larger weight fractions of heparin possess a higher aXa and ACT activity, a reduction in the average molecular weight of heparin should result in a loss of aXa and anticlotting activity (15). To assess this hypothesis, we measured transdermal transport of three polysaccharides covering a wide range of molecular weights. Specifically, we measured skin permeability to inulin (MW ~5000), dextran (MW = 5000), and dextran (MW = 70,000 Da) after ultrasound pretreatment. We found that skin permeability to these molecules is about 3 (±2) × 10^{-4} cm/h, 4 (±2) × 10^{-4} cm/h and 6 (±4) × 10^{-4} cm/h, respectively. No systematic decrease in skin permeability with molecular weight was observed over a molecular weight range of 5000–70,000 Da. Hence, selective transport of low-molecular weight fractions is unlikely to explain disappearance of aXa activity in the receiver compartment.

Another possible explanation for the disappearance of aXa activity is the possibility of metabolism (depolymerization) of heparin molecules during their transdermal transport. Because the existence of long chains is critical in maintaining aXa and anticlotting activity (14), depolymerization can result in a loss of aXa activity. To test this hypothesis, we performed additional experiments in which heparin was incubated with skin and skin lysate (obtained by homogenization of 250 μg of skin in 1 mL of PBS) for 24 h. Heparin was added to this lysate such that the initial concentration of heparin ranged from 0.5 to 300 U/mL in various experiments. Control samples were performed in which heparin at the same concentrations was incubated with PBS. The resulting solutions were tested for aXa and ACT. Results of these tests are shown in Fig. 2 (circles: aXa activity; squares: ACT). Heparin samples incubated with skin or skin lysate resulted in a similar loss of biologic activity after 24 h. This deactivation effect was concentration dependent. No significant loss of activity was observed when the initial concentration of heparin exceeded 10 U/mL. However, about 75% aXa activity disappeared on a 24-h contact with skin when the initial heparin concentration was 1 U/mL. Figure 2 also shows that ACT was more prominently affected by skin lysate compared with aXa activity.

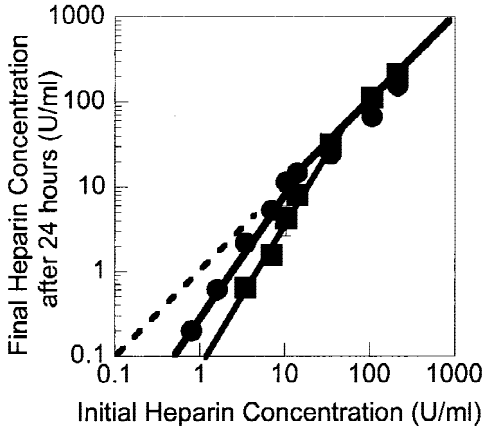

Fig. 2. Loss of aXa activity (circles) and ACT (squares) of heparin due to incubation with skin lysate with 24 h. X-axis corresponds to the concentration of heparin before addition of skin lysate. Y-axis corresponds to the concentration of heparin as measured by aXa activity or ACT after incubation with skin lysate. Each experiment was repeated at least five times. Error bars indicate standard deviations.

To assess whether the loss of activity may be attributed to depolymerization, these samples were also run on a PAGE gel. Because the change in the size distribution of heparin molecules should affect their migration through the gel, this method can be used as a first screen to quickly determine structural alterations in skin-exposed and control heparin samples. The samples exposed to skin lysate appeared different than unexposed ones. Specifically, the gels showed a reduced concentration of high-molecular weight chains (data not shown). The intensity of the gels of the treated samples was also less than that of the untreated samples. This difference may have originated from potential desulfation of heparin molecules in the skin. The difference between the treated and the untreated sample was much smaller when the initial concentration of heparin exceeded 10 U/mL (for the same amount of skin lysate, data not shown). This observation is consistent with the data shown in Fig. 2.

In Vivo Heparin Delivery

Before launching a detailed investigation of the mechanisms of heparin metabolism, we assessed its relevance to transdermal drug delivery *in vivo*. Note that the above mentioned tests were performed after heparin was in contact with skin (or skin lysate) for 24 h. In an *in vivo* situation, transdermally delivered heparin would be cleared by the blood vessels relatively quickly. Based on *in vitro* experiments, skin permeability to heparin after ultrasound treatment is about 2 × 10^{-3} cm/h. Because the primary transport barrier is stratum corneum (SC), most of the residence time should originate from heparin transport across the SC. The thickness of the SC is about 15 μm. Hence, the estimated residence time of heparin in the SC should be about 0.75 h ($\tau = \ell/P$). This calculation is performed assuming that the epidermis does not offer a significant barrier to heparin transport. This assumption requires further validation. To assess whether a 0.75-h-long skin contact is sufficient to deactivate heparin, we repeated the activity measurements of heparin in contact with skin for various times in the range of 0–24 h (original heparin concentration of 1 U/mL). The results of these studies are shown in Figure 3, which indicates that >90% of heparin is biologically active after 0.75-h-long contact with skin. These findings suggest that the deactivation observed during the *in vitro* experiments does not apply to the *in vivo* situation; however further investigations of this phenomenon should be performed. In view of these results, we next performed *in vivo* studies to confirm the efficacy of sonophoresis.

In these experiments, rat skin was permeabilized by using ultrasound, and a solution of LMWH was then placed on the skin. LMWH was used instead of heparin because we thought that a lower molecular weight of LMWH should facilitate its transdermal delivery. Figure 4 shows aXa activity in the rat blood as a function of time. No significant aXa activity was observed when LMWH was placed on nontreated skin. As can be seen from Fig. 4, significant amount of LMWH was transported transdermally after ultrasound pretreatment. aXa activity in the blood increased slowly for about 2 h, after

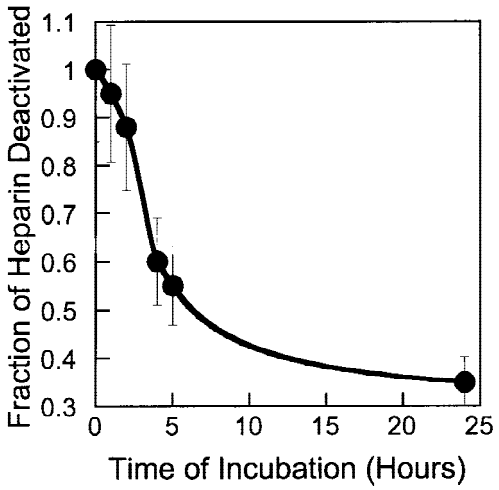

Fig. 3. Time dependence of heparin deactivation (n = 5). Deactivation is measured by the loss of aXa activity. Heparin was incubated with skin lysate for various times. Error bars correspond to standard deviations.

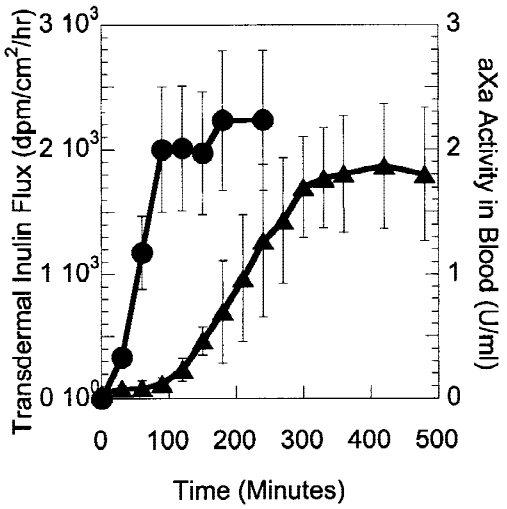

Fig. 5. A comparison of transdermal inulin flux (circles) after sonophoresis with aXa concentration in the blood after transdermal LMWH delivery (n = 4 each) (triangles). Error bars indicate standard deviations.

which, it increased rapidly before achieving a steady state after 5 h. The occurrence of a lag time of 2 h is rather surprising given the high skin permeability after ultrasound pretreatment. To assess the origin of this lag time, we performed additional experiments using inulin. In these experiments, skin was permeabilized by using a similar protocol and inulin was then placed on the skin. Figure 5 shows transdermal inulin flux (closed circles) and transdermal aXa activity in the blood after transdermal LMWH delivery (open circles, reproduced from Fig. 4). Figure 5 clearly shows that inulin quickly appears in the urine and achieves a steady state relatively quickly. Because inulin is not metabolized in the body, it allows determination of true transport lag time. Figure 5 shows that the lag time associated with transdermal transport is about 30 min and does not account for the lag time observed in case of LMWH. Additional studies are required to assess the origin of this excessive lag time of transdermal LMWH transport. A lag time of 2 h may not be an issue in clinical applications because transdermal LMWH treatment is effective over a period significantly longer than 2 h.

Figure 6 compares the time variation of aXa activity in the blood achieved by transdermal delivery (squares) with that obtained by an intravenous injection (triangles) and a subcutaneous injection (circles) of 150 U/kg of the same LMWH (taken from Ref. 15). The comparison reveals that the aXa activity in the blood after a subcutaneous injection increases rapidly and thereafter decreases to low levels within 5 h. Similar behavior is observed in the case of an intravenous injection except that the aXa activity is observed in the blood immediately after the administration. No significant activity was observed beyond 5 h. On the other hand, aXa activity during transdermal drug delivery was observed well beyond 5 h. Bioavailability for transdermal drug delivery (fraction of

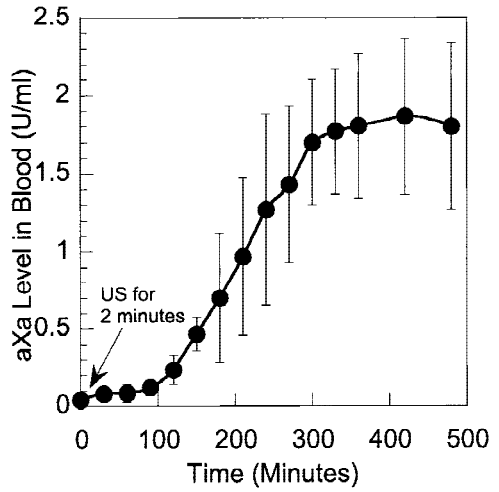

Fig. 4. aXa concentration in the blood after transdermal LMWH delivery (n = 4). Error bars indicate error bars. No significant activity in the blood was observed when LMWH was placed on untreated skin.

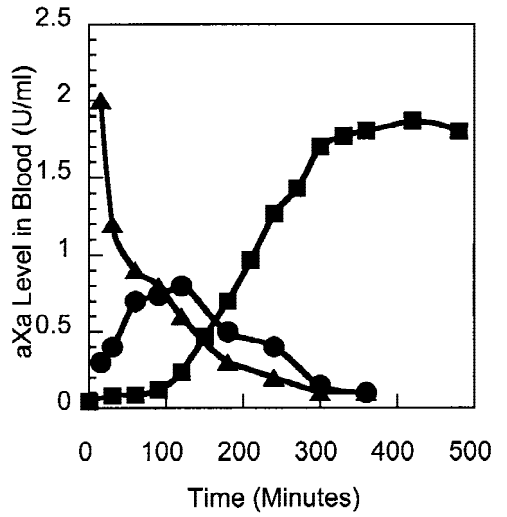

Fig. 6. Comparison of aXa profiles in the blood after transdermal delivery (squares) with a single subcutaneous injection (circles) and intravenous injection (triangles). The data for transdermal drug delivery is reproduced from Fig. 4. The data for subcutaneous and intravenous injections were taken from Ref. 15.

drug applied on the skin that appeared in the blood) was calculated by using the area under the curves for subcutaneous and transdermal delivery methods. Bioavailability of transdermal delivery is about 2% in 8 h (i.e., an estimated bioavailability of about 6% in 24 h). Although these data show sustained delivery of LMWH across the skin, further improvements in the bioavailability are necessary to increase the practicality of this method. This sustained aXa activity in the blood should improve the effectiveness of antithrombotic therapy.

The data presented here show the feasibility of a noninvasive method of heparin or LMWH delivery. Preliminary experiments with human volunteers have shown that application of ultrasound and SLS under the same conditions reported in this article does not damage the skin (13), although additional experiments should be performed to arrive at a firm conclusion about the safety of this method. We envision that the patient may use a product based on this technology in the following way. The patient would apply ultrasound to the skin once in the morning and place the patch on the sonicated area. The patch would deliver heparin or LMWH throughout the day to provide sustained levels of heparin concentrations in the blood. The dose of heparin may be controlled through skin permeability, area, or LMWH concentration in the patch (14). Additional studies focused on detailed pharmacokinetics should be performed before testing this method in a clinical setting.

ACKNOWLEDGMENTS

We thank Long Le for technical assistance and Prof. Robert Linhardt for providing low-molecular weight heparin.

REFERENCES

1. A. Frydman. Low-molecular weight heparins: an overview of their pharmacodynamics, pharmacokinetics and metabolism in humans. *Hemastatis*. **26**:24–38 (1996).
2. R. L. Bronaugh and H. I. E. Maibach. *Percutaneous Absorption: Mechanisms-Methodology-Drug Delivery*, Marcel Dekker, New York, 1989.
3. M. R. Prausnitz, E. R. Edleman, J. A. Gimm, R. Langer, and J. C. Weaver. Transdermal delivery of heparin by skin electroporation. *Biotechnology* **13**:1205–1209 (1995).
4. S. Mitragotri, D. Blankschtein, and R. Langer. Ultrasound-mediated transdermal protein delivery. *Science* **269**:850–853 (1995).
5. S. Mitragotri, D. Edwards, D. Blankschtein, and R. Langer. A mechanistic study of ultrasonically enhanced transdermal drug delivery. *J. Pharm. Sci.* **84**:697–706 (1995).
6. D. Bommannan, H. Okuyama, P. Stauffer, and R. H. Guy. Sonophoresis. I. The use of high-frequency ultrasound to enhance transdermal drug delivery. *Pharm. Res.* **9**:559–564 (1992).
7. D. Bommannan, G. K. Menon, H. Okuyama, P. M. Elias, and R. H. Guy. Sonophoresis. II. Examination of the mechanism(s) of ultrasound-enhanced transdermal drug delivery. *Pharm. Res.* **9**:1043–1047 (1992).
8. K. Tachibana. Transdermal delivery of insulin to alloxan-diabetc rabbits by ultrasound exposure. *Pharm. Res.* **9**:952–954 (1992).
9. S. Mitragotri, D. Ray, J. Farrell, H. Tang, B. Yu, J. Kost, D. Blankschtein, and R. Langer. Synergistic effect of ultrasound and sodium lauryl sulfate on transdermal drug delivery. *J. Pharm. Sci.* **89**:892–900 (2000).
10. S. Mitragotri and J. Kost. Low-frequency sonophoresis: a noninvasive method for drug delivery and diagnostics. *Biotech. Prog.* **16**:488–492 (2000).
11. S. Mitragotri, J. Farrell, H. Tang, Terahara, J. Kost, and R. Langer. Determination of the threshold energy dose for ultrasound-induced transdermal drug delivery. *J. Control. Release* **63**: 41–52 (2000).
12. R. E. Edens, A. al-Hakim, J. M. Weiler, D. G. Rethwisch, J. Fareed, and R. Linhardt. Gradient polyacrylamide gel electrophoresis for determination of molecular weights of heparin preparations and low-molecular weight heparin derivatives. *J. Pharm. Sci.* **81**:823–827 (1992).
13. J. Kost, S. Mitragotri, R. Gabbay, M. Pishko, and R. Langer. Transdermal extraction of glucose and other analytes using ultrasound. *Nat. Med.* **6**:347–350 (2000).
14. D. Lane and U. Lindhal. *Heparin: Chemical and Biological Properties, Clinical Applications*, CRC Press, Boca Raton, 1989.
15. L. Piazolo, J. Harenberg, R. Malsch, F. Huttner, and D. Heene. Comparison of the pharmacodynamic and pharmacokinetic profiles of two low-molecular-mass heparins in rats. *Semin. Thromb. Hemost.* **23**:109–117 (1997).