The Effect of Subcutaneously Administered Low-Molecular-Weight Heparin on Microarterial Thrombosis in the Rat

Tessa A. Hadlock, MD; Jennifer Kim, MD; Daniel G. Deschler, MD

Objective: To examine the effect of administration of a low-molecular-weight heparin derivative, enoxaparin, on the rate of arterial thrombosis in a rat model.

Study Design: Prospective, randomized, blinded study.

Methods: A standard microarterial anastomosis tuck injury was created in both femoral arteries of 25 Long Evans retired breeder rats. Thirteen animals received a subcutaneous injection of 50 IU/kg of enoxaparin 2 hours before the procedure, while 12 control animals received vehicle (isotonic sodium chloride solution) alone. Sites of injury/repair were assessed 2 hours after the procedure for anastomotic patency or thrombosis.

Results: Six (23%) of 26 vessels in the drug-treated group developed an arterial thrombosis at the site of repair, while 6 (25%) of 24 vessels in the control group developed thrombosis. There was no statistically significant difference at the 95% confidence limit between the 2 groups based on a comparison-of-proportions test.

Conclusion: The preoperative subcutaneous administration of 50 IU/kg of enoxaparin did not alter the rate of arterial thrombosis following the creation of a thrombogenic tuck injury/repair of the rat femoral artery.

Arch Facial Plast Surg. 2003;5:36-39
and arterial thrombosis in large-vessel models and clinical situations have demonstrated a benefit similar to heparin and fewer bleeding complications.13-15

The aim of this study was to examine the efficacy of enoxaparin in the prevention of thrombosis in small, 1- to 2-mm-diameter arteries following arteriotomy and intentionally thrombogenic repair. We selected an animal model shown to lead to a reproducible thrombosis rate.16 It involves the creation of a 180° arteriotomy followed by a tuck, where the distal cut edge of the vessel protrudes into the lumen, exposing the flowing blood to an intimal flap. This model examines the effect of subcutaneous administration of the drug at the recommended dose on the same-caliber vessels as those ordinarily used during microvascular free tissue transfer and mimics a common technical error in microarterial anastomosis (creation of an intimal flap within the vessel lumen), allowing more direct determination of potential effects of the drug in the microvascular reconstructive setting.

METHODS

Enoxaparin was prepared at a concentration of 40 IU/mL. Two hours before the procedure, animals received either enoxaparin (20 IU in 0.5 mL) or isotonic sodium chloride solution (0.5 mL) subcutaneously, in blinded fashion.

A total of 25 Long Evans retired breeder rats (Charles River Laboratories, Cambridge, Mass) were used in this study (average weight, 400 g). Thirteen animals received enoxaparin, and 12 received vehicle alone. Animals were housed 2 per cage and were treated according to Animal Care and Use Committee guidelines. Each animal was anesthetized with 50 mg/kg of pentobarbital. The abdomen and groin areas were then shaved, and a midline incision was made through the skin and subcutaneous tissues. Blunt dissection was used to identify the femoral pedicle, and both femoral vessels were isolated. The artery was placed into a double microvascular clamp and cleaned of excess adventitia. A 180° arteriotomy was created with microscissors, and the repair was begun.

First, a 10-0 nylon suture was placed into the distal vessel through to the lumen. It was then brought back out through the vessel wall, still on the distal side of the arteriotomy. It was then passed from within the lumen of the arteriotomy out through the proximal vessel wall so that tying it created a tuck, whereby a portion of the distal vessel edge protruded into the lumen of the artery (Figure). One or 2 additional 10-0 nylon sutures were placed on either side of the first stitch to avoid leakage at the arteriotomy site. Once hemostasis was verified following clamp removal, the same procedure was executed with the contralateral femoral artery. This resulted in a total of 26 vessels in the experimental group and 24 vessels in the control group.

Animals remained under anesthesia for the ensuing period, and each arteriotomy/repair site was assessed for patency 2 hours after the procedure. Assessment of vessel patency was accomplished by pinching the artery with a microforceps distal to the injury site, emptying the vessel for a 1-cm length with a second microforceps, then releasing the first microforceps, and observing vessel refill. Vessels lacking immediate refill were determined to be thrombosed.

RESULTS

There was no excessive bleeding during any of the surgical procedures. There was 1 anesthesia-related death in an animal that had received vehicle injection, and this animal was excluded from the analysis. Of the remaining 50 vessels operated on, there were a total of 12 thromboses, giving an overall thrombosis rate of 24%. In the enoxaparin-treated group, there were 6 thromboses in 26 repairs, yielding a 23% thrombosis rate. The control group demonstrated 6 thromboses in 24 repairs, giving a similar thrombosis rate of 25%. Based on a comparison-of-proportions test, there was no statistically significant difference in thrombosis rate between the experimental and control groups, with a 95% confidence interval.

COMMENT

Previous studies of LMWH in arterial and venous thrombosis models have shown benefit. Its beneficial role in thromboprophylaxis and acute arterial thrombotic syndromes in humans has also been established. Whether it might play a role in decreasing anastomotic thrombosis following microvascular free tissue transfer was the subject of this study.

Heparin and heparin-derived compounds exert their action by interfering with the extrinsic (tissue factor–triggered) clotting cascade.17 Tissue factor activates fac-
tor X, which in turn converts prothrombin to thrombin in the presence of factor Va, calcium, and phospholipid. Heparinoids possess anti-factor Xa properties, also referred to as tissue factor pathway inhibitor. Their pharmacologic properties are often defined in terms of “anti-Xa” units. They have also been shown to prevent platelet surface prothrombinase assembly and to inactivate platelet prothrombinase activity.

Unfractionated heparin has been shown to have a differentially greater antiplatelet effect than LMWH. This contributes to prolonged bleeding time and presumably some of the bleeding complications associated with its use. In contrast, LMWH appears to have the same inhibition of the extrinsic clotting cascade without the same degree of platelet inhibition. This makes it desirable for clinical use with the potential for fewer bleeding problems, particularly in the postoperative setting with a fresh wound.

The role for LMWH in the prevention of deep vein thrombosis in humans has been clearly established and it is now the standard of therapy at some institutions. An increasing role for this drug class is being defined for use in acute arterial thromboembolic syndromes, including unstable angina and thromboembolic stroke. It has recently been approved by the Food and Drug Administration for use in both these clinical situations. Several studies have examined the role of enoxaparin for the prevention of arterial thrombosis in the setting of peripheral vascular surgery to the distal extremity. Investigators demonstrated a higher rate of thrombosis prevention but similar rates of bleeding complications in enoxaparin-treated individuals vs patients treated with unfractionated heparin. These data suggest a potential role for the use of enoxaparin in the prevention of arterial thrombosis following microsurgical free tissue transfer.

Subcutaneously administered enoxaparin has not been extensively examined in an experimental model appropriate for conclusions regarding arterial thrombosis after microsurgical repair of 1- to 2-mm-diameter arteries. Ritter et al.18 examined its efficacy when delivered intravenously in an epigastric free flap model and found increased flap survival with fewer bleeding complications than with unfractionated heparin. These data suggest a potential role for the use of enoxaparin in the prevention of arterial thrombosis following microsurgical free tissue transfer.

Subcutaneously administered enoxaparin has not been extensively examined in an experimental model appropriate for conclusions regarding arterial thrombosis after microsurgical repair of 1- to 2-mm-diameter arteries. Ritter et al.18 examined its efficacy when delivered intravenously in an epigastric free flap model and found increased flap survival with fewer bleeding complications than with unfractionated heparin. These data suggest a potential role for the use of enoxaparin in the prevention of arterial thrombosis following microsurgical free tissue transfer.

Subcutaneously administered enoxaparin has not been extensively examined in an experimental model appropriate for conclusions regarding arterial thrombosis after microsurgical repair of 1- to 2-mm-diameter arteries. Ritter et al.18 examined its efficacy when delivered intravenously in an epigastric free flap model and found increased flap survival with fewer bleeding complications than with unfractionated heparin. These data suggest a potential role for the use of enoxaparin in the prevention of arterial thrombosis following microsurgical free tissue transfer.

Subcutaneously administered enoxaparin has not been extensively examined in an experimental model appropriate for conclusions regarding arterial thrombosis after microsurgical repair of 1- to 2-mm-diameter arteries. Ritter et al.18 examined its efficacy when delivered intravenously in an epigastric free flap model and found increased flap survival with fewer bleeding complications than with unfractionated heparin. These data suggest a potential role for the use of enoxaparin in the prevention of arterial thrombosis following microsurgical free tissue transfer.

In summary, this study did not demonstrate a significant antithrombotic effect of subcutaneously administered enoxaparin at a dose of 50 IU/kg. While the significant antithrombotic effects of this drug have been clearly demonstrated in large-vessel models and in human studies, we have been unable to corroborate these findings using a small-vessel model with subcutaneous administration at the recommended dose. With further investigation, we are hopeful that the antithrombotic effects and low bleeding complication profile of LMWH can be clearly demonstrated in a small-vessel model so that clinical trials using it for postoperative thromboprophylaxis in free tissue transfer recipients might be entertained.

Accepted for publication September 25, 2001.

This study was supported by an Investigator Development Award from the American Academy of Facial Plastic and Reconstructive Surgery, Alexandria, Va.

Corresponding author and reprints: Tessa A. Hadlock, MD, Department of Otolaryngology–Head and Neck Surgery, Massachusetts Eye and Ear Infirmary, 243 Charles St, Boston, MA 02114 (e-mail: tessa_hadlock@meei.harvard.edu).
REFERENCES


12. Monrad ES. Role of low-molecular-weight heparins in the management of patients with unstable angina pectoris and non-Q-wave acute myocardial infarction. Am J Cardiol. 2000;85:2C-9C.


Quotable

To the dismay of the videoconferencing industry, people don’t really like it—they find it flat, boring, empty and irritating. . . . Every face-to-face interaction involves an incredibly complex eye contact ballet. . . . Somehow, the brain is deciphering that complex eye contact stream to derive critical emotional information. How do lovers first connect? Their eyes meet from across the room. What do lovers do to cement their connection? They gaze into each other’s eyes. What’s the first thing a liar does to disguise his emotional miscommunication? He breaks eye contact. Somehow—and we don’t know exactly how—the brain reads eye contact duration and patterns, and pupillary dilation as well, and weaves together emotional meaning out of it that we all rely on without ever realizing how we’re picking up and assembling the cues.

Thomas Lewis, Clinical Professor of Psychiatry University of California, San Francisco