

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

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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.

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SUCCESS RATES for free tissue transfer have steadily increased over the last 2 decades to levels of greater than 95% at most large centers. However, because flap loss is a devastating and potentially fatal complication, continued efforts to prevent loss are required. Microvascular thrombosis at the site of vascular anastomosis remains the most common cause of graft failure, and efforts are ongoing to decrease the incidence of this phenomenon. Current management strategies are focused on pharmacologic thrombosis prevention, as well as prompt recognition of its occurrence, so that surgical reexploration and thrombectomy can be performed for graft salvage.

Methods of preventing thrombosis are greatly preferred to surgical thrombectomy. The most critical factor in thrombosis prevention is meticulous surgical technique, with attention to pedicle orientation, position, and quality of the microanastomotic repair.¹ In addition to meticulous technique, many surgeons administer perioperative antithrombotic agents as an adjunct to decrease the risk

of thrombosis. However, the administration of antithrombotic agents is associated with increased bleeding time, hemorrhage, wound hematoma, and systemic complications.² Pulmonary edema, renal failure, hemodilution, and other adverse hemodynamic phenomena have also been reported with antithrombotic therapy.

The search is ongoing for antithrombotic agents that decrease vessel thrombogenicity to a satisfactory degree but with acceptable bleeding time. Fractionated low-molecular-weight derivatives of heparin (LMWHs) have been shown to maintain the antithrombotic properties of heparin, but have a less deleterious effect on overall bleeding time.³⁻⁹ One particular LMWH, enoxaparin, has been shown to lead to decreased venous thrombosis in patients at risk for deep vein thrombosis.¹⁰ It has also been shown to decrease progression of conditions associated with arterial thrombosis, including acute coronary syndromes and thromboembolic stroke.^{11,12} Human and animal studies involving administration of enoxaparin for prevention of venous

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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.

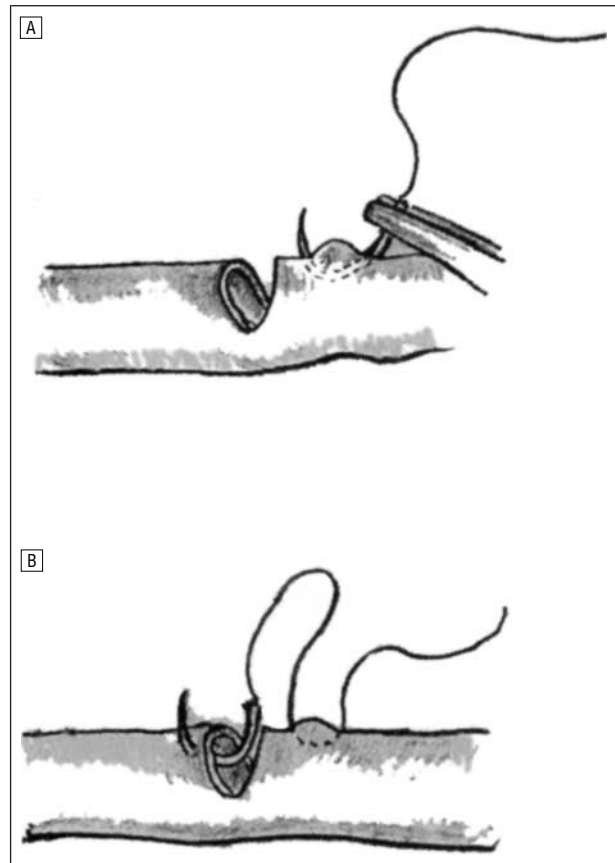
A total of 25 Long Evans retired breeder rats (Charles River Laboratories, Cambridge, Mass) were used in this study (average weight, 400 g). Thirteen animals received enoxaparin, and 12 received vehicle alone. Animals were housed 2 per cage and were treated according to Animal Care and Use Committee 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



Schematic illustration of the tuck arteriotomy and repair method. A, Following 50% arteriotomy, a suture is passed through the distal segment of the vessel and brought back out. B, The proximal vessel segment is then sutured with a transmural stitch from inside the lumen, creating a full-thickness flap of vessel wall partially obstructing the lumen.

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 al¹⁸ 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. Korompilias et al¹⁹ examined the effect of route of administration on efficacy and found that topical administration of high-dose enoxaparin decreased thrombosis rate in small-caliber vessels after crush and anastomosis, but systemic administration alone did not. The work of Zhang and Wieslander⁷ and Zhang et al⁸ using intravenously administered LMWH demonstrated a beneficial early antithrombotic effect in small-caliber arteries and veins using an intimaectomy model and reinforced the lack of prolonged bleeding time compared with conventional heparin therapy.

The results of the present study, like those of Korompilias et al,¹⁹ do not demonstrate a beneficial antithrombotic effect of systemically administered enoxaparin. Possible explanations for this finding include that a concentration below effective antithrombotic levels was used, or that time to thrombosis formation (2 hours) was inadequate to show a decreased rate

of thrombosis formation. This second possibility is unlikely, however, because most thrombotic events following microsurgical vascular anastomosis occur within the first several hours postoperatively. It could also be that the mechanism of thrombus formation in this tuck model is less dependent on tissue factor and more closely related to platelet aggregation, therefore decreasing the efficacy of LMWH, which more strongly affects tissue factor. This would be consistent with findings of a prior study that demonstrated fibrin-platelet clots within vessels undergoing similar tuck-type injuries.¹⁶ It is well established that platelet-mediated events dominate in small-vessel thrombosis, whereas in large blood vessels, the extrinsic clotting cascade governs thrombus formation. It may be that the more powerful antiplatelet effect of unfractionated heparin is critical in the prevention of microvascular thrombosis, and that LMWH will not provide adequate antithrombotic effects in free tissue transfer.

The tuck model for the study of microarterial thrombosis after microsurgical vessel repair is relatively new. Technical issues related to surgical technique, degree of arteriotomy, and extent of intimal flap created may have prevented the demonstration of clear differences between groups. Of note, developers of this model found a higher overall thrombosis rate (72%) than we found in our control animals, indicating some variation in technique.¹⁶

Future studies that may help resolve some of the issues raised by the negative findings in this comparative study include further use of this tuck model with administration of drugs with better-defined antithrombotic effects. In addition, higher concentrations of enoxaparin may be examined. Further, a histologic analysis of type of clot might be carried out to discern thrombosis by platelet aggregation from that caused by extrinsic clotting cascade.

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.

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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.

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