The Effect of Low-Molecular-Weight Heparin on Microvenous Thrombosis in a Rat Model

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Objective: To assess the impact of a low-molecular-weight heparin sodium, dalteparin sodium, on a thrombogenic microvenous anastomosis, using a randomized, blinded animal model.

Methods: Using male Sprague-Dawley rats, 70 IU/kg of dalteparin sodium (for the treatment group) or isotonic sodium chloride solution (for the control group) were administered subcutaneously in a blinded randomized fashion. Using microsurgical techniques, the femoral venous pedicle was isolated bilaterally. A tuck anastomosis was then performed on each side. Vessel patency was assessed periodically for 3 hours using a strip and refill test. Patency or thrombosis was confirmed by cutting the vessel proximal to the anastomosis and examining the lumen for thrombus.

Results: A total of 58 venous tuck anastomoses were performed. There was no difference in bleeding complications between the treatment and control groups. The control group had a thrombosis rate of 50%, and the treatment group had a thrombosis rate of 60%. The \( \chi^2 \) analysis does not indicate a statistical difference between these 2 groups \( (P=0.59) \).

Conclusion: Low-molecular-weight heparin, at standard therapeutic dosing, may not provide an adequate antithrombotic effect to prevent anastomotic thrombosis in free tissue transfer.

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Microvascular surgery has developed into an integral part of head and neck reconstruction. The ability to transfer a wide range of tissues such as fasciocutaneous, myocutaneous, myofascial, osseous, and osseocutaneous flaps has given surgeons the ability to address considerable lesions and the resultant defects. Free tissue transfer has reached high levels of success in terms of flap survival. Most studies report an overall survival rate of 95%. Although numerous factors contribute to flap failure, thrombosis of the microvascular anastomosis is considered to be the major cause of failure. The consequences of vessel thrombosis are severe and cause significant morbidity and even mortality.

Thrombosis of the pedicle, usually the vein, has been reported to occur in 3% to 15% of microvascular anastomoses. Thrombus formation in the high-flow arterial system is considered to be most dependent on platelet effects whereas thrombosis in the venous system is more dependent on tissue factors activating the coagulation cascade. In hopes of decreasing anastomotic thrombosis and improving overall success, there have been several pharmacologic attempts to prevent thrombus formation. Unfractionated heparin sodium, dextran, and aspirin have been evaluated; however, none of these pharmacologic agents has eliminated thrombosis without causing additional risk and morbidity. Therefore, investigations for the ideal antithrombotic agent continue. The aim of this study was to investigate the effect of a low-molecular-weight heparin on the venous anastomosis using a rat tuck model.

Methods:

After institutional review board approval, male Sprague-Dawley rats weighing 250 to 300 g were used. They were cared for under the supervision and protocols of the institution’s animal care facility. In a randomized blinded fashion, 70 IU/kg of dalteparin sodium (Fragmin; Pharmacia Corporation, Kalamazoo, Mich; 70 IU/kg is the manufacturer’s recommended dose for preventing catheter thrombosis) (for the treatment group) or isotonic sodium chloride solution (for the control group) were administered subcutaneously. After 3 hours, each rat was anesthetized with an intramuscular injection of ketamine hydrochloride (50 mg/kg) and xylazine hydrochloride (10 mg/kg). Once adequate anesthesia was achieved and confirmed with pinch testing, a transverse abdominal incision was made, and bilateral femoral neurovascular bundles were exposed. Using the

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operating microscope and microsurgical techniques, each femoral vein was isolated from the neurovascular bundle and stripped of excess adventitia. While isolated, a 2-vessel atrumatic vessel clamp was placed. A venotomy was then created by incising 180° of the vessel lumen. Isotonic sodium chloride solution was used to irrigate and clear each end of the clamped vessel.

As originally described by Stepnick et al and modified for the rat vein by Laxmeesh Nayak, MD (unpublished data, 2004), a tuck procedure was performed (Figure). Using a 10-0 nylon suture, a tuck anastomosis was created by first entering the proximal cut end of the vein and then exiting slightly more distally on the same proximal segment of the vein approximately 1 mm from the venotomy edge where the suture rested extraluminal. It was then reintroduced through the lumen of the distal segment of the venotomy, exiting through the distal segment of the vessel. A single square knot was thrown to close the venotomy and create an approximately 1-mm tucked flap that rested opposing the venous outflow. If large gaps remained at the venotomy, additional sutures were placed to close the vessel. The vessel clamps were removed, allowing the vein to refill. The same procedure was then performed on the opposite side.

Vessel patency was assessed periodically for 3 hours using a strip-and-refill test in which the vessel was gently occluded anatomically proximal to the anastomosis while a second forcep was kept in place at the proximal cut end of the vein and then exiting slightly more distally across the anastomosis, it was considered patent. If there was no refill, the vessel was considered thrombosed. Finally, patency or thrombosis was confirmed by cutting the tuck procedure.
cance and potential for having an impact on thrombosis in human microvascular surgery, we used a thrombo-
genic microvenous anastomosis tuck model. This model is excellent for creating a clinically active and reproduc-
tive thrombogenic source, and it allows for testing of phar-
macologic agents in a clinically relevant manner.

Previous work in our laboratory demonstrated no statistically significant decrease in thrombosis using the low-molecular-weight heparin enoxaparin in a microar-
terial tuck model. Explanations for this include possible
dosing below effective antithrombotic levels, inadequate time to thrombosis formation (2 hours of observ-
tion), or the mechanism of thrombosis in an arterial tuck model may be more dependent on platelet aggregation as opposed to tissue factor.

Given the potential for greater impact by tissue fac-
tors on thrombus formation in the microvenous system, investigation of low-molecular-weight heparin in a venous model remained prudent despite the initial failure to show a difference in the arterial model. Based on the previous experience, several changes were made.

Dalteparin was used instead of enoxaparin because of reported differences between low-molecular-weight heparin. Dalteparin seems to have the most optimal profile for preventing thrombosis without additional risk of bleeding. Vessels were monitored for a total of 3 hours in this study (increased from 2 hours). Also, a higher equivalent dosage of dalteparin was used rather than of enoxaparin, as in the previous experiment, to allow greater antithrombotic levels to be reached.

Despite these changes, there was not a demonstrable difference between the control and treatment groups. With the exception of 2 vessels in the treatment group, all thromboses still occurred within the first 60 minutes. The consistent results between the arterial and venous models may suggest that low-molecular-weight heparin does not provide adequate antithrombotic effects at the microvascular anastomotic site. The platelet mediated effects in thrombus formation may dominate to such a degree in all small vessels, artery and vein, that more targeted and powerful antiplatelet agents are necessary to have an impact on thrombosis rates.

Similarly, a dosing issue may be present. Other studies showing potential for low-molecular-weight heparin have typically used intravenous administration. Perhaps this allows for greater antithrombotic activity, although pharmacologically there should not be a difference. Higher dosages of low-molecular-weight heparin have been used in some previous studies and in other clinical settings such as treatment of deep vein thrombosis and myocardial ischemia. However, the dosage used in this study is approximately 2 times higher than the recommended deep vein thrombosis prevention dosage. Other models in which low-molecular-weight heparin has been effective, such as the trauma model, may have a greater degree of tissue factor activation because of the nature of the model. Similarly, various flap models that do not create a thrombogenic source may activate the coagulation cascade in a different manner compared with the tuck model.

In conclusion, this study investigated the potential antithrombotic effect of dalteparin in a microvenous tuck anastomosis model. There was not a statistically significant difference between the treatment and control groups. The treatment group did not show any signs of adverse effects; in particular, there was no increased incidence of bleeding. These data, when considered with previously published data on the effect of enoxaparin on the microarterial tuck anastomosis model, suggest that low-molecular-weight heparin may not provide an adequate antithrombotic effect to prevent anastomotic thrombosis in free tissue transfer.

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