Experimental Thromboprophylaxis with Low Molecular Weight Heparin After Microsurgical Revascularization

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Abstract There is great variability among microsurgeons as regards the use of prophylactic anticoagulant after revascularization and this is probably due to lack of comparative data. Also, there has been much debate regarding the benefit of antithrombotic therapies versus the risk of complications such as systemic bleeding and hematoma formation. To evaluate the effectiveness of postoperative low molecular weight heparin (LMWH) as a prophylactic anticoagulant therapy after microsurgical repair of the femoral artery and vein in rats. Randomized, blinded study. The femoral artery and vein of 40 Sprague Dawley rats were sectioned and repaired with microsurgical sutures under general anesthesia. They were randomly divided into 2 groups: Group (A) in which the 20 rats were injected with Enoxaparin subcutaneously at a dose 1.5 mg/kg once daily for 3 successive days; Group (B), the control group, in which 20 rats were injected with isotonic sodium chloride 0.9 % subcutaneously in a blinded fashion. After 7 days, the femoral vessels were re-explored and patency of the femoral vessels was assessed with empty-and-refill test. There were a total of 12 vascular thrombosis among 74 microsurgical repair in both groups with percentage of 16.22 % including, 5 arterial anastomosis and 7 venous anastomosis. The incidence of thrombosis in the treatment group (A) was 18.4 % while the incidence of thrombosis in the

control group (B) was 13.8 %. This difference was not statistically significant using Fisher exact test. Postoperative administration of LMWH did not provide the desired protection against thrombosis after microsurgical vascular repair.

Keywords Antithrombotic therapy · Microsurgery · Prophylaxis

Introduction

Replantation is considered an important source of challenge in reconstructive microsurgery with a considerable risk of thrombosis (7–32 %) and eventual failure [1]. The risk of thrombosis is greatest within the first 48 h after microsurgical revascularization [2]. Platelet aggregation is the underlying cause of arterial thrombosis whereas venous thrombosis is primarily the result of fibrin clotting [3]. Although early diagnosis and revision of a thrombosed anastomosis has been shown to be a reasonable choice for salvage but prevention remains of primary importance [4]. Also, endogenous injured tissues are implicated in the genesis of anastomotic thrombi, therefore anticoagulant drug therapy attempts to positively impact this risk [5].

To avoid the risk of thrombosis, many surgeons started to use prophylactic anticoagulant therapy after replantation in an attempt to avoid this potential complication. The antithrombotic agents that usually used are aspirin, heparin, low-molecular-weight heparin and dextran. However, the administration of anticoagulants is associated with increased bleeding time, hemorrhage, wound hematoma, and systemic complications [6].

Low-molecular-weight heparin (LMWH) is a class of anticoagulant medications that was developed to avoid the potentially serious side effects, and relative unpredictability of

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heparin use. It has been used for many years for the prevention and treatment of venous thromboembolism. The effectiveness of LMWH as an anticoagulant is clearly documented. Also, in different clinical situations such as acute coronary syndromes, including acute myocardial infarction, heart surgery, vascular surgery, coronary and peripheral percutaneous revascularization (angioplasty or atherectomy, with or without stenting) and acute stroke, controlled studies confirmed the expected superiority of LMWH in the prevention of arterial thrombosis [7]. However, there is great variability among microsurgeons as regards the use of prophylactic anticoagulant after revascularization and this is probably due to lack of comparative data that demonstrates its superiority [8]. There has been much debate regarding the benefit of anti-thrombotic therapies versus the risk of complications such as systemic bleeding and hematoma formation with subsequent compression of the anastomotic site [9].

The purpose of this study is to evaluate the effectiveness of postoperative LMWH as a prophylactic anticoagulant therapy after microsurgical repair of the femoral artery and vein in rats.

Methods

After approval of the ethics commit for animal experimentation of the University of Pavia, 40 Sprague Dawley rats were used with weight ranging from 225 to 250 g and were housed one in each cage with adjusted suitable environment as regard temperature, humidity and ventilation with 12-h light and dark cycles. They also had free access to standard rat food and water. They were randomly divided into 2 groups: treatment group (A) in which the 20 rats were injected with enoxaparin (Clexane; Sanofi aventis) subcutaneously at a dose 1.5 mg/kg once daily for three successive days with the first dose was given after vascular division; group (B), the control group, in which 20 rats were injected with isotonic sodium chloride 0.9 % subcutaneously for three successive days in a blinded fashion.

Surgical Procedure

After general anesthesia using intramuscular injection of a combination of tiletamine hydrochloride, and zolazepam hydrochloride (Zoletil, Vibrac) with a dose of 10 mg/kg, the left groin area was shaved and the rat was stabilized in supine position on a woody tray with the use of elastic bands attached to forelimbs and hind-limbs. Under aseptic condition (disinfection with Povidone-iodine and alcohol 70 % and draping with sterile towels), left oblique inguinal incision about 3 cm long was performed through the skin, then gentle dissection was continued through the subcutaneous tissue until identification and isolation of the femoral artery and vein were done. A double vascular clamp was applied to the femoral vessels

and complete division was performed using micro-scissor in a perpendicular direction. The vessel cut ends were prepared with excision of adventitia and dilatation of the lumen and end-to-end microscopic anastomosis was performed using 9–0 prolene suture (Fig. 1). Then the anastomosis was assessed for patency after removal of the clamp with empty-and-refill test which was accomplished by occluding the artery with a microforceps distal to the anastomosis, emptying the vessel for a 1-cm length with a second microforceps, then releasing the first microforceps, and observing vessel refill. Vessels lacking refill within 20–30 min after repair were determined to be thrombosed and were excluded from the study.

After 7 days, the rat was re-operated with the same previous surgical procedure under general anesthesia and the femoral vessels were re-explored. The patency of the femoral vessels was assessed with empty-and-refill test. Thrombosed vessels were also identified by inspection (Fig. 2) (absent expansile pulsation with collapsed lumen) and cut sections of the vessel were performed to confirm the diagnosis of thrombosis. Any local complications were reported. Then the rat was sacrificed with intravenous injection of a lethal dose of Zoletil (200 mg/kg).

Statistical Analysis

Statistical analysis was performed using Chi-squared test to confirm that 2 groups do not differ significantly as regard the number. Fisher's exact test was used to evaluate the differences in thrombosis outcome between the 2 groups with a value of $P < 0.05$ is considered to be statistically significant.

Results

Six microsurgical anastomosis were excluded due to negative empty-and-refill test after clamp release (2 in group A and 4 in

Fig. 1 Microsurgical repair of the femoral artery and vein with good patency

Fig. 2 Picture of femoral artery thrombosis with absent pulsation and visible thrombus inside the lumen

group B). The two groups had shown to be indifferent significantly using Chi-squared test with X^2 value equals 0.18 and P value equals 0.398.

There were a total of 12 vascular thrombosis among 74 microsurgical repair in both groups with percentage of 16.22 % including, 5 arterial anastomosis and 7 venous anastomosis (Table 1).

The incidence of thrombosis in the treatment group (A) was 18.4 % while the incidence of thrombosis in the control group (B) was 13.8 %. This difference is not statistically significant using Fisher exact test with P value is 0.754957.

There were a total of 5 arterial thrombosis among 34 arterial anastomosis with percentage of 17.7 % among the two groups with 2 thromboses in the treatment group (A) with a percentage of 11.77 % and 3 thromboses in the control group (B) with percentage of 17.6 %. The thrombosis percentage was less in group (A) compared to group (B), however, this difference was statistically insignificant with the two-tailed P value equals 1.00.

Also, there were a total of 7 venous thrombosis among 40 venous anastomosis with percentage of 17.5 % among the two groups with 5 thromboses in the treatment group (A) with a percentage of 23.8 % and 2 thromboses in the control group (B) with percentage of 10.5 %. The thrombosis percentage was less in the control group (B) compared to the treatment group (A), however, this difference was statistically insignificant with the two-tailed P value equals 0.4124.

Table 1 Frequency of thrombosed and patent vessels in both the treatment group (A) and the control group (B)

	Thrombosed		Patent		Total
	Artery	Vein	Artery	Vein	
Treatment group (A)	2	5	15	16	38
Control Group (B)	3	2	14	17	36
Total	5	7	29	33	74

Hematoma formation was observed in one rat in the treatment group (A) and was associated with both arterial and venous thrombosis (Fig. 3).

Discussion

The anticoagulant therapy is used widely as a common prophylactic measure to guard against vascular thrombosis after microsurgical reconstruction [10–12]. However, no agreement exists about which type of anticoagulants, the timing of administration or for how long it should be used after microsurgical repair, so many surgeons use their own regimen of prophylactic antithrombotic agents depending on their practical experience rather than on a rigid scientific recommendation [13]. In a prospective multicenter study of 493 free flaps, Khouri et al. [14] reported that only the administration of subcutaneous LMWH had shown a statistically significant association with a decreased rate of vascular thrombosis. However, this study included 23 different surgeons with variable surgical techniques and used the antithrombotic drug therapy in different protocols, depending on the occurrence of either intraoperative or postoperative thrombosis so this would invalidate such conclusions of the study due to the presence of such confounding factors [13]. Also a small minority of surgeons advocates no prophylactic antithrombotic therapy. Veravuthipakorn et al. [15] reported their results on 40 microvascular cases (22 free flaps and 18 replantations) in which no anticoagulants were used. Their survival rates were 91 % for replantation and 95 % for free flaps, which compared favorably with other reported results.

Low molecular weight heparin (LMWH) is derived from the unfractionated heparin through deaminative hydrolysis of standard heparin. It is believed to exert the same strong inhibitory effect on active factor X with weak antithrombin activity. Consequently, LMWH is as effective as the standard heparin in the prevention of vascular thrombosis with less hemorrhagic side effects [16].

Fig. 3 Picture of visible hematoma on the treatment group (a) rat where LMWH was administrated

Our experimental study is considered to be the first to investigate the efficacy of LMWH in the prevention of thrombosis after microsurgical revascularization with postoperative dosing protocol as that usually used in the emergency replantation surgery. All previous experimental studies that were performed to investigate the efficacy of LMWH as thromboprophylactic agent, had been done with preoperative dosing protocol as that usually used in elective flap surgery.

The role of preoperative LMWH as a prophylactic antithrombotic drug agent after microsurgical repair is a point of debate in the literature. Ritter et al. [17] had found improved microsurgical anastomotic patency with LMWH in epigastric free flaps of 66 rats. Also, Malm et al. [18] had shown that the administration of LMWH (dalteparin) was effective to prevent arterial thrombosis after microsurgical repair of the injured carotid artery in rats without significant bleeding side effect. In addition, Zhang et al. [19] had demonstrated that patency was significantly increased in the LMWH (Fragmin) group compared to the control group after arteriotomy of central arteries of the rabbits' ears. On the other side, Hadlock et al. [20] had found that preoperative administration of LMWH (enoxaparin) did not affect significantly the incidence of arterial thrombosis compared to the control group after 50 % arteriotomy of rat femoral artery with thrombogenic repair. Also, Emerick et al. [21] had performed a total of 58 venous tuck microsurgical anastomoses of the femoral vein and found that the control group in which saline was administered, had a thrombosis rate of 50 %, while the treatment group in which 70 IU/kg of LMWH (dalteparin sodium) was administered, had a thrombosis rate of 60 %.

In our study, the postoperative administration of LMWH did not provide the desired protection against thrombosis after microsurgical vascular repair in the treatment group compared to the control one. This may point to the decreased effectiveness of LMWH as prophylactic antithrombotic at microsurgical anastomotic site. This might be due to fundamental contribution of the platelet aggregation to the thrombus formation at the anastomotic site in the small arteries and veins [21] that perhaps makes the anti-platelet agents more effective as a prophylactic antithrombotic therapy or it could be combined with LMWH in order to have a more convenient effectiveness.

Although bleeding complications are infrequent with the used low dose of LMWH due to weak antithrombin activity with its small molecular size, hematoma had been reported which was associated with both arterial and venous thrombosis. We think that hematoma might occur in case of improper hemostasis before LMWH administration, exceeding the low prophylactic dose and concurrent administration of other antithrombotic agents.

The currently available data in the literature are not adequate to provide a strong evidence-based protocol of postoperative antithrombotic therapy after microsurgery. Further research for the ideal antithrombotic agent or combination

therapy with their mode and timing of administration, should be continued to avoid thrombosis at the site of anastomotic repair without serious bleeding complication and hematoma formation.

Compliance with Ethical Standards

Conflict of Interest Authors declare that they have no conflict of interest

Ethical Approval The study was approved by the Ethics Committee of Animal Experimentation (Comitato Etico per la Sperimentazione Animale) of the University of Pavia, Italy on 15/10/2013 (No. 3/2013) and all procedures performed were in accordance with the ethical standards. All applicable international, national, and institutional guidelines for the care and use of animals were followed.

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