Original article

Microsurgical training in vein anastomoses: the use of systemic heparin in a rat model

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Abstract. *Objective:* To investigate whether the use of systemic heparin could be useful for vein microvascular anastomoses in microsurgical training on rats. *Materials and Methods:* Design: Femoral end-to-end venous anastomoses were performed on both thighs of 40 Wistar rats by two microsurgery trainees from October 2018 to February 2019 (80 anastomoses in total). We divided the rats into 2 groups of 20 specimens (40 femoral end-to-end anastomoses) each: group A received no heparin administration; group B received subcutaneous systemic heparin administration before starting dissection. We compared both vein patency after the procedures. *Results:* Patency tests showed no difference between the two groups after 5 minutes. At the delayed test after 120 minutes, vein patency was significantly better in the systemic heparin group (85,0% vs 55,0%). Even though both trainees judged practicing on both groups very instructive, they found useful performing anastomoses when heparin was administrated. *Conclusion:* We suggest including the use of systemic heparin in microsurgery training programs, especially for the beginners. Systemic heparin administration in rat models is educative for trainees. (www.actabiomedica.it)

Key words: heparin, vein, microsurgery, rat, femoral, end-to-end

Introduction

Microsurgical training is very challenging. It ranges from simple exercises on plastic tubes to very complex ones, as microvascular free flaps and organ isotransplantations (1). Exercising on rats is the most used model in microsurgical training.

After presurgical planning, the exercise requires an accurate dissection. Microvascular anastomosis is the final stage. Hence, end-to-end anastomosis of femoral vessels is often the first exercise learners face in a rat model.

Unlike clinical practice, anastomosis thrombosis is frequent in rat models, especially in veins (2). This is due to peripheral vasoconstriction, small vessel caliber (approximately 1 mm), low blood pressure. Anastomosis remains patent for a few minutes, but after a few hours thrombosis is often revealed. This can be frustrating; trainees can easily feel hopeless and despaired.

To avoid blood clots formation, systemic subcutaneous heparin administration after microsurgical anastomoses has been described in rat models (3–5).

We investigated the use of systemic heparin in microsurgical training rat models, and its eventual advantages in daily training.

Materials and methods

Surgical training was performed according to the guiding principles for research involving animals and the European legislation. Surgical training was approved by the local ethics committee (protocol number: 1036/2016-PR, IF295-21). The present investigation consists in a prospective analysis of microsurgery training sessions performed from October 2018 to February 2019 in one center for experimental research, in accordance with the approval of the ethics committee.

All procedures were performed by two trainees at skill stage 3 according to Komatsu et al (1).

- Microsurgeon 1, MP, 28 years old, orthopedics residency program, right handed;
- Microsurgeon 2, MS, 30 years old, plastic surgery residency program, right handed.

Approval of the ethics committee for animal experimentation was obtained. Femoral end-to-end venous anastomoses were performed on two groups of more than 5 weeks age Wistar female rats.

- group A: didn't receive systemic heparin administration;
- group B: received systemic heparin before starting procedures.

The study was carried out under the control and supervision of the scientific project manager (RDV) and senior surgeons tutors. General anesthesia was performed by the veterinarian by intraperitoneal injection of ketamine. Systemic sodic heparin (PharmaTex Italy, Milan) was administered subcutaneously on the abdominal wall according to rat weight (25000 I.U./kg) 30 seconds before the first skin incision. Femoral vein anastomoses were performed according to the standard technique.[2] Procedures were performed under general anesthesia. Throughout the whole experiment, a veterinarian was in charge of keeping the animal properly anesthetized, hydrated and warmed. Diathermocoagulation was never used. Collateral branches were ligated to completely skeletonize the main vessel.

A background was placed under the skeletonized vessel, then the vessel was clamped.

Anastomoses were performed using a single stitch technique (nylon, Ethilon suture 11-0). In both groups A and B, microsurgeon 1 performed one femoral vein anastomoses, while microsurgeon 2 performed the vein anastomoses on the other side. Each trainee performed the same number of procedures on each side.

After finishing the suture, the trainee had to check the suture and then only turn it over to the tutor if it was considered perfect. Total suture time was measured from vessel section to suture handoff. Dissection time was measured from skin incision to vessel section.

The final patency test was performed by a senior surgeon, using standard patency empty/refill technique as described by Adam WP Jr et al. (6) Trainees did not know the outcome of the test.

Two assessments were performed:

- early (after 5 minutes);
- delayed (after 120 minutes).

After the first patency assessment the skin was closed using 4-0 silk suture by the senior surgeon, and was open again just to test the patency for the second time, and then sutured again.

Surgery was not performed between the first and the second patency test, meanwhile the rat was maintained anesthetized, hydrated and warmed. After the second assessment, the anastomosis was performed on the opposite side by the other microsurgeon, and then tested for two times.

The veterinarian would suspend the experiment and cancel the results from that animal, he/she found clinical signs of systemic failure in the animal during the experiment.

The rat was sacrificed at the end of the experiment.

Outcomes

Primary outcome was to assess if the mean long term patency (after 120 minutes) was improved or not by heparin administration.

Secondary outcomes were short term patency assessment (after 5 minutes), dissection and anastomosis duration time. Failures' features (side, bilateral) were registered.

Any advantages or disadvantages from the use of heparin were discussed among the trainees and senior reference tutors.

Sample size calculation statistical analysis

The sample size (n) has been calculated according to the formula:

$$n = \frac{z^2 p\ (1-p)}{e^2}$$

Where: z = 1.96 for a confidence level (α) of 95%, p = proportion (incidence of thrombosis in venous anastomosis), e = margin of error.

Because no similar study has ever been performed, we did not know the true incidence of thrombosis with or without heparin administration 120 minutes after the anastomosis.

Before performing the study, we observed that about half of the end-to-end venous anastomoses after two hours manifested suboptimal flow (p=50%). Considering that the patency of the anastomosis depends on many factors, both related to the systemic condition of the animal and to the microsurgical technique, we considered a margin of error of 11%. The sample size is equal to 80.

The study was performed in 40 laboratory animals, with 80 end-to-end venous anastomoses.

The Fisher exact test was used for categorical variables. Wilcoxon test was performed for two dependent continuous variables. Mann–Whitney U-test was used to compare two independent continuous variables. A value of $p<0.05$ was identified as statistical significant. Data were reported as mean ± standard deviation (SD) for continuous variables and as percentage and frequency for categorical variables. Statistical. analysis was performed using the SPSS v.20.0 software (SPSS Inc.; Chicago, IL).

Results

Forty Wistar female rats (mean weight 0,275 kg ±0,180) were included:

- group A (n=20 rats; n=40 anastomoses) not receiving systemic heparin;
- group B (n=20 rats; n=40 anastomoses) receiving systemic heparin.

At the delayed test (120 mins): 22 patent anastomoses were found in group A (55,0%). and 34 in group B (85,0%) ($p < 0.00001$); no differences were found in dissection and suturing time comparing group A and B (Table 1). The patency at 5 minutes was similar in both groups, 37 patent anastomoses in groups A (92,5%) and 38 (95,0%) in group B ($p>0.05$) (Table 1).

Failures have been resumed in Table 2.Regarding the primary outcome, there was bilateral failure in 4 rats, for a total of 8 failed anastomoses. Ten cases of unilateral failures are in addition. There were no significant differences according to Fisher's test.

Trainees felt that the surgical exercise performed using heparin was more instructive and very useful for the management of bleeding. Trainees found dissection slightly more difficult if heparin was administered, but operative times were not different in the two groups (Table 1).

Table 1. Patency and surgical duration time.

	Patent vessels (after 120 minutes)	Patent vessels (after 5 minutes)	dissection time (minutes) mean ± SD	Suturing time (minutes) mean ± SD
Group A (no heparin)	22 (55.0%)	37 (92.5%)	14 ± 7	25 ± 8
Group B (heparin)	34 (85.0%)	38 (95,0%)	13 ± 8	27 ± 10
p value	p < 0.00001	p>0.05	p>0.05	p>0.05

Table 2. Failures.

	Group A			
	Microsurgeon 1	**Microsurgeon 2**	**Bilateral failure**	**Monolateral, side of failure (first side or second side sutured)**
Failure after 120 minutes	9	9	4	First side 4, second side 6
Failure after 5 minutes	2	1	0	First side 2, second side 1
total	11	10	9	

	Group B			
	Microsurgeon 1	**Microsurgeon 2**	**Bilateral failure**	**Monolateral, side of failure (first side or second side sutured)**
Failure after 120 minutes	3	3	1	First side 2, second side 2
Failure after 5 minutes	1	1	0	First side 2, second side 0
total	4	4	2	

Discussion

There is no consensus in literature about whether to use heparin in rat models, with evidences in favour,(5, 7, 8) and against it (3).

Yousef et al. observed no effectiveness of postoperative heparin administration in thrombosis prevention after microsurgical vascular repair compared to placebo (7 days post-operative assessment)(3).

Nevertheless, some studies encourage the use of systemic heparin administration in rat free flap models, considering their long-term results (after 7 days). (7, 8)

Otherwise, we investigated the short-term effects (after 120 minutes) of systemic heparin administration in microsurgical daily training rat models.

Microsurgical training is very challenging and continuous practice is required, in particular for what concerns veins (2).

The use of systemic heparin significantly increases time of anastomosis patency. Thrombosis of the anastomosis at 120 minutes could not depend by microsurgeon skills but could occur because of dehydration and cooling of the femoral region, and consequent peripheral vasoconstriction.

Systemic heparin is fundamental to reduce the risk of thrombosis caused by independent and causal factors and, subsequently, to improve the microsurgical trainees' satisfaction. This is proved by excellent results obtained in group B (92,5% self-assessed excellent results). Both trainees were satisfied about the surgical experience they have done.

On the other hand, the use of systemic heparin makes the procedure more challenging, mimicking a real-life surgery and forcing the trainees to perform all the steps of the standard anastomosis technique. In our opinion its use during basic microsurgical exercises with daily evaluation is useful in the dissection time:

- it can improve self-confidence in trainees during bleeding management;
- it is fundamental to perform effective collateral ligatures;
- it can emulate operations in non-coagulated patients.

We suggest using systemic heparin in particular at the first stage of training, to improve dissection and anastomosis skills. Alternating both kind of exercises, with and without systemic heparin, is desirable.

Conclusion

Training in microvascular anastomosis is difficult and can be learnt by practicing on animal models. The delayed patency test (after 120 minutes) improves considerably with heparin use. It could be useful in all venous anastomosis exercises such as groin free flap and renal isotransplantation. Heparin will promote microsurgical trainees to take up and refine microsurgery techniques, helping not to be discouraged, and certify them for doing similar surgeries in human patients.

Conflicts of Interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article.

Ethics Approval: All animals were housed in proper facilities, 2 in each cage, and all animals received care in compliance with the American, European, or any other Convention on Animal Care. The protocol of the study (protocol number: 1036/2016-PR, IF295-21) was approved by the institutional Ethics Committee.

Source of Funding: The authors report no source of funding. No benefits in any form have been received or will be received from a commercial party related directly to the subject of this article

Acknowledgments: none.

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Received: 15 October 2022
Accepted: 26 January 2023
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