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Research Article

Investigating the Impact of Vascular Anastomosis on Tissue Integrity and Recovery in a Rat Limb Ischemia-Reperfusion Injury Model

Yanbo Qi[1*], Sanchin Urjin[1#], Galindev Batnasan[2#], Anar Bayarmunkh[1] and Baatarsuren Batmunkh[1*]

[1]*MNUMS, School of Medicine, Department of Surgery in Ulaanbaatar, the capital of Mongolia*

[2]*MNUMS, Institute of Bio-Medical Sciences, Laboratory animal science center in Ulaanbaatar, the capital of Mongolia*

[#]*The authors equally contributed to this work*

***Corresponding author:** Baatarsuren.B, MNUMS, School of Medicine, Department of Surgery in Ulaanbaatar, the capital of Mongolia.

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Abstract

Introduction: Ischemia-induced vascular insufficiency, particularly in peripheral artery disease (PAD), is a significant clinical issue, leading to high rates of morbidity, mortality, and limb loss. Microvascular grafting is a critical surgical intervention for severe ischemia, yet the success of grafts is often compromised by prolonged ischemia. This study investigates how different durations of ischemia impact graft patency, tissue regeneration, and complications associated with vascular grafting in a rat limb ischemia model.

Objectives: The aim of this study was to evaluate the effect of ischemic duration (3 vs. 7 days) on the outcomes of microvascular grafting, focusing on graft patency, tissue regeneration, and histological changes. The study also compared end-to-end (ETE) and end-to-side (ETS) anastomosis techniques using donor arteries preserved for either 3 or 7 days.

Results: In the ischemia-induced groups, grafts using 3-day preserved donor arteries showed significantly better outcomes, with improved tissue regeneration, endothelial cell proliferation, and graft patency. In contrast, the 7-day ischemia group exhibited poorer outcomes, including impaired graft patency, tissue necrosis, and heightened inflammatory responses due to ischemia-reperfusion injury. The end-to-end anastomosis technique using 3-day preserved vessels resulted in the best results, while the end-to-side technique showed alternative viability but with less favorable outcomes.

Conclusion: This study demonstrates that shorter ischemic durations (3 days) lead to improved graft success and tissue regeneration, while prolonged ischemia (7 days) exacerbates tissue damage and increases the risk of graft failure. Timely intervention and optimal ischemic durations are crucial for improving microvascular graft outcomes and reducing limb loss in PAD patients.

Introduction

Ischemia-induced vascular insufficiency and the subsequent risk of limb loss remain significant clinical challenges worldwide. Chronic ischemia, particularly peripheral artery disease (PAD), affects millions of individuals globally, with high rates of morbidity and mortality [1,2]. Peripheral artery disease is commonly seen in patients with comorbidities such as diabetes mellitus, hypertension, and hyperlipidemia, and it is a leading cause of limb amputations [4,11]. Approximately 10 million people in the United States alone suffer from PAD, with a notable increase in the incidence due to the aging population and increasing prevalence of diabetes [3].

For example, in the United Kingdom, individuals with diabetes face a more than 12-fold increased risk of lower-extremity amputation compared to those without diabetes [7,8,12]. Traumatic causes also contribute significantly to overall amputation rates, emphasizing the multifactorial nature of limb loss.

When blood flow is restricted due to occlusion or narrowing of blood vessels, the affected tissues become deprived of oxygen and essential nutrients [4]. The pathological consequences of ischemia include tissue necrosis, inflammation, and cellular apoptosis. In cases of severe ischemia, where the ischemic insult is prolonged or severe, these tissues may be irreversibly damaged, leading to limb loss unless effective intervention strategies are applied [5].

Among the various treatment modalities, microvascular grafting has emerged as a critical surgical intervention for patients with severe peripheral ischemia. The technique involves the anastomosis of a donor vessel to the ischemic region to restore blood flow, thereby preventing further tissue damage and facilitating regeneration [6]. Despite its success, microvascular grafting is often limited by ischemic injury, with prolonged ischemia exacerbating tissue damage and reducing graft success rates [1,3-4].

The mechanisms of ischemia involve a complex cascade of molecular events. The deprivation of oxygen leads to a cellular energy deficit, impairing mitochondrial function and activating various stress pathways [8]. These include the production of reactive oxygen species (ROS), endothelial dysfunction, and activation of inflammatory mediators such as cytokines and adhesion molecules [9]. The reperfusion of ischemic tissues often induces additional injury, commonly referred to as ischemia-reperfusion (I/R) injury. I/R injury is characterized by oxidative damage, inflammatory cell infiltration, and endothelial damage, all of which contribute to further vascular dysfunction and graft failure [10].

This study aims to assess the impact of varying ischemic durations on microvascular grafting outcomes in a rat limb ischemia model, specifically focusing on graft patency, tissue regeneration, and complications associated with prolonged ischemia. By understanding the relationship between ischemic duration and vascular graft outcomes, this research seeks to optimize surgical strategies and improve patient prognosis in the management of ischemia.

Material and Method

Animal Grouping and Study Design

A total of 70 male Wistar rats were randomly assigned to two groups. The control group (n=10) underwent direct end-to-end artery transplantation on the right femoral artery (vessel diameter: 0.3-0.5 mm). The experimental group (n=60) was subdivided into two models of blood supply insufficiency by ligating the femoral artery for either 3 or 7 days. After establishing ischemia, donor arteries-preserved for 3 or 7 days-were used to perform both end-to-end (n=15 per time point) and end-to-side transplantation (n=15 per time point). The transplantation outcomes of these two techniques were compared. All procedures were performed under the experimental protocol was approved by the Animal Ethics Review Committee of the University of Mongolian National University of Medical Sciences (MNS 6871:2020). To ensure consistency, the study environment, surgical instruments, and the rats' food and water were identical for all groups.

Anesthesia and Femoral Artery Isolation

Prior to surgery, each rat's body weight was recorded. Anesthesia was induced by intraperitoneally injecting a solution of pentobarbital sodium (0.5 ml per 100 g body weight), prepared by dissolving 500 mg pentobarbital sodium in 50 ml of 0.9% sodium chloride. A longitudinal incision was then made on the anterior aspect of the right thigh-from the greater trochanter to the lateral femoral condyle. The subcutaneous tissue was carefully dissected to expose the femoral neurovascular bundle. To minimize vasoconstriction, 2% lidocaine was applied, and the diameter of the femoral artery was measured with a digital caliper. During isolation, the connective tissue was gently separated from the vessels using forceps, ensuring that arteries, veins, and nerves were not damaged. A recommended technique involves retracting the fascia laterally with one hand while delicately separating the nerve from the vessels with the other.

Femoral Artery Ligation

In the experimental groups, the isolated femoral artery was ligated using 9-0 sutures to induce tissue hypoxia, thereby creating models with 3-day and 7-day ischemia. In contrast, the control group underwent femoral artery isolation without ligation. After isolation (and ligation, where applicable), the blood vessel and surrounding soft tissue were repositioned in their normal anatomical locations, and the wound was sutured closed.

Microvascular Grafting Procedure

Separation of Vessel Ends: The two ends of the arterial vessel were carefully separated to reduce vascular tension.

Removal of the Outer Membrane and Anastomotic Adjustment: The outer membrane of the vessel was meticulously removed to facilitate a precise anastomosis. The vessel ends were aligned to ensure that the inner membranes (endothelial layers) were accurately connected and that the surrounding muscle layers were properly matched. Vascular forceps were used to clamp the damaged ends and trim the outer membrane as needed, thereby minimizing the risk of thrombosis and protecting the vessel's inner wall.

Flushing the Lumen: After evenly trimming the vessel ends, the lumen was flushed with 0.1% heparinized saline (alternatively, 0.5% procaine or 3.8% sodium citrate solution could be used) to prevent thrombosis during grafting.

Transplantation Techniques: At 3- or 7-days post-ligation-once the blood supply insufficiency model was established-the affected arterial segment was resected. Donor arteries were then used to perform either end-to-end or end-to-side transplantation. Care was taken to ensure that the length of the transplanted vessel was not

excessive to avoid twisting, and the longitudinal axis of the donor vessel was aligned with that of the recipient to maintain optimal blood flow.

Assessment of Graft Status

Following the grafting procedures, graft patency and function were assessed using the Acland test [8-9]. This assessment provided immediate feedback on the quality of the anastomosis and the restoration of free blood flow.

Donor Vessel Preservation

Donor rat femoral arteries were dissected under microscopic guidance and cut into 1-2 cm segments. The vessels were rinsed thoroughly with 0.1% heparinized saline solution (or 0.5% procaine/3.8% sodium citrate solution) to remove residual blood and soft tissues. The cleaned segments were then stored in 50 ml of Custodial Cardioplegia solution at 40C for either 3 or 7 days until transplantation. This preservation protocol was critical for maintaining the viability and functionality of the donor vessels.

Results

The body weight of the control group rats was 227.86±21.11 g before surgery, 230.09±22.16 g after surgery, and increased to 240.24±25.77 g 21 days after vascular anastomosis ($p<0.01$). In the ischemia-induced group, the rats that underwent ETE vascular anastomosis had a preoperative body weight of 239.67±26.96 g, which decreased to 234.3±27.20 g after surgery but increased to 250.67±27.09 g after 21 days ($p<0.05$). The rats in the ETS anastomosis group had a preoperative body weight of 235.18±22.39 g, which increased to 249.62±21.12 g after 21 days ($p<0.01$) (Table 1).

Table 1: Comparison of body weight in rats from the 3-day ischemia experimental group.

		Body weight	P-value
Before Surgery	3-day ETE	239.67±26.96	0.183
	3-day ETS	235.18±22.39	0.047
During surgery	3-day ETE	234.35±27.19	0.059
	3-day ETS	233.69±21.56	0.019
At 21 Days Post-Surgery	3-day ETE	250.67±27.09	0.01
	3-day ETS	249.62±21.11	0

Note*: Data are expressed as mean values. A p-value of <0.05 was considered statistically significant.

The total surgical duration was 24.51±2.29 minutes in the control group, whereas it was 39.75±0.89 minutes for the ETE anastomosis and 46.65±8.12 minutes for the ETS anastomosis in the ischemia-induced group, requiring significantly more time compared to the control group ($p=0.001$). Intraoperative blood loss was 0.27±0.12 mL in the control group, whereas it was 0.58±0.13 mL during anastomosis and 1.12±0.61 mL during ETS anastomosis in the ischemia-induced group, with some cases reaching up to 2.35 mL ($p<0.01$) (Table 2).

Table 2: Comparison of the total duration of vascular anastomosis surgery in study groups.

Surgical time	P-value
24.56±0.72	<0.001
39.75±0.28	
46.66±2.57	

Note*: Data are expressed as mean values. A p-value of <0.05 was considered statistically significant.

In the ischemia-induced group, the stratified squamous epithelial layer was reduced, and the epithelium became thinner. However, by day 21 after vascular anastomosis, tissue regeneration was activated, leading to epithelial thickening due to squamous epithelial hyperplasia. To confirm these changes, PCNA protein was analyzed using immunohistochemical staining. Three days after ischemia induction, the number of PCNA-positive cells in the squamous epithelium significantly decreased. By day 21 after vascular anastomosis, the number of PCNA-positive cells increased, indicating enhanced wound healing in the plantar skin. Although the number of PCNA-positive cells increased, the staining intensity was weaker compared to the control group (Figures 1-3).

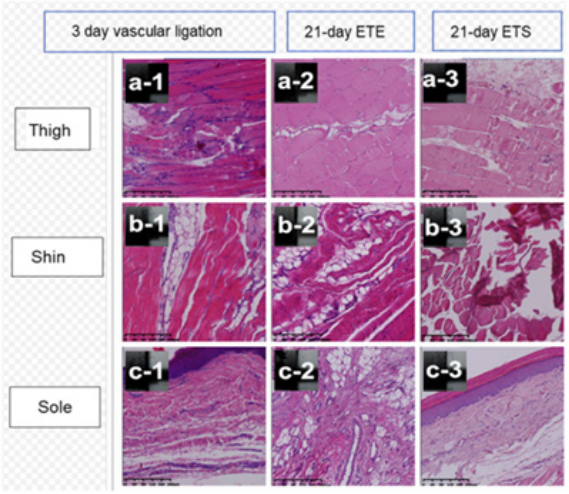

Figure 1: Histological Analysis of Thigh, Shin, and Sole Tissue 21 Days After Donor Vessel Transplantation in the Ischemia-Induced Group.

(a-1, b-1, c-1) represent tissue analysis after 3 days of arterial occlusion to establish an ischemia model.
(a-2, b-2, c-2) show tissue analysis 21 days after end-to-end (ETE) anastomosis using a donor vessel preserved for 3 days.
(a-3, b-3, c-3) depict tissue analysis 21 days after end-to-side (ETS) anastomosis using a donor vessel preserved for 3 days.
In image a-2, most of the connective tissue, muscle bundles, and cellular boundaries in the thigh tissue are well-defined, with gradually recovering damaged areas. In contrast, a-3 demonstrates a less effective recovery process compared to the ETE anastomosis.

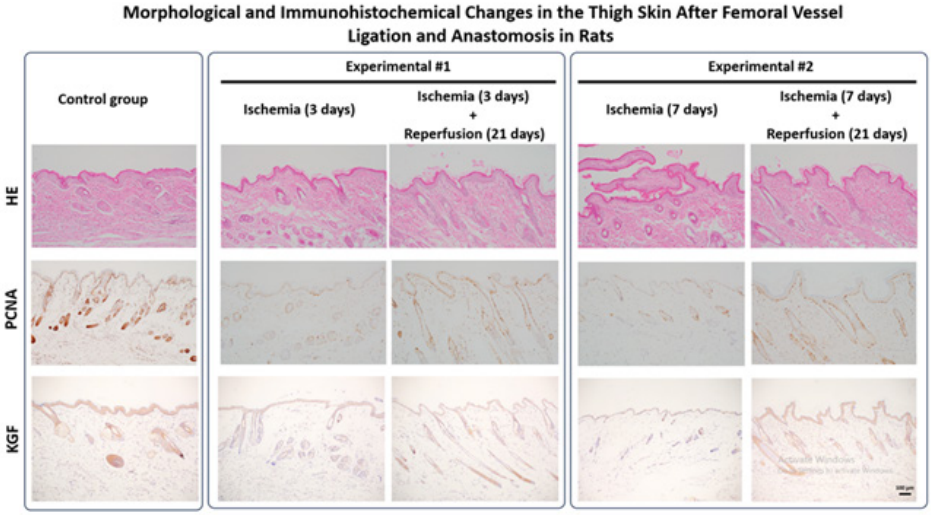

Figure 2: Morphological and Immunohistochemical Changes in the Thigh Skin After Femoral Vessel Ligation and Anastomosis in Rats.

Representative histological images show skin tissue morphology and immunohistochemical staining from control and experimental groups. The left panel represents normal skin structure in the control group. The middle panel (Experimental #1) depicts histological changes following 3 days of ischemia and subsequent 21-day reperfusion. The right panel (Experimental #2) illustrates changes after 7 days of ischemia and 21-day reperfusion.

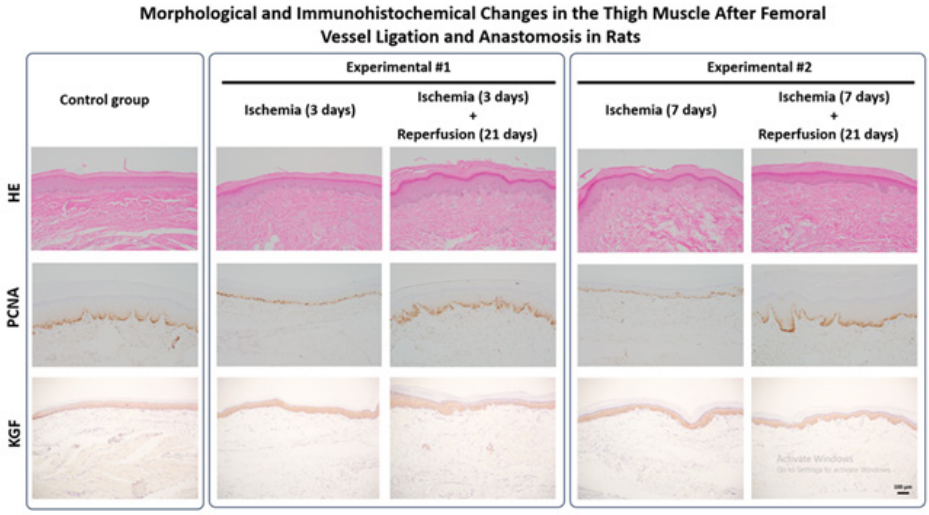

Figure 3: Morphological and Immunohistochemical Changes in the Thigh Muscle After Femoral Vessel Ligation and Anastomosis in Rats.

Muscle fiber structure appears preserved in the short ischemia group, whereas increased damage and disorganization are observed after prolonged ischemia. Reperfusion following 3-day ischemia shows better recovery compared to 7-day ischemia, where severe tissue degeneration is visible.

Discussion

This study provides valuable insight into the impact of ischemic duration on microvascular grafting outcomes. The results clearly demonstrate that prolonged ischemia (7 days) significantly impairs graft patency and tissue regeneration, likely due to the extensive tissue damage, inflammatory response, and impaired angiogenesis. These findings are consistent with previous research that has highlighted the detrimental effects of prolonged ischemia on tissue viability and graft success.

The poor graft outcomes in the 7-day ischemia group are primarily attributed to the exacerbation of ischemia-reperfusion injury. As ischemic tissues experience reperfusion after grafting, the influx of oxygen and nutrients triggers a cascade of inflammatory and oxidative processes that further compromise tissue healing. This study emphasizes the need for timely intervention in patients with ischemic limbs, as delays in treatment can result in irreversible damage to the tissue and a higher risk of graft failure.

Furthermore, the histological analysis revealed that shorter ischemic durations (3 days) allowed for more effective regeneration, as evidenced by increased endothelial cell proliferation and collagen deposition. The presence of well-formed micro vessels in the 3-day ischemia group suggests that grafts are more likely to succeed when ischemia is brief and tissue damage is limited. These findings are particularly relevant for clinical applications, where early intervention and rapid restoration of blood flow are critical to preventing limb loss.

While microvascular grafting remains an effective option for restoring perfusion to ischemic tissues, our findings suggest that optimizing the timing of grafting and minimizing ischemic durations could significantly improve outcomes. Additionally, the role of surgical technique, graft preservation, and postoperative care in influencing long-term graft survival should be further investigated to refine clinical approaches.

Conclusion

In the limb ischemia model, tissue edema, uneven cell distribution, and tissue necrosis can be seen after microvascular transplantation, which is particularly obvious in the 7-day ischemia group. Through our preoperative and 21-day postoperative histological comparison studies, in the limb tissue ischemia model, the best effect is achieved by using end-to-end anastomosis of blood vessels stored for 3 days, and end-to-side anastomosis of blood vessels can be used as an alternative therapy.

In conclusion, this study underscores the importance of ischemic duration in determining the success of microvascular grafting. The results suggest that shorter ischemic periods (3 days) lead to better graft patency, tissue regeneration, and overall surgical outcomes, while prolonged ischemia (7 days) exacerbates tissue damage, leading to increased risk of graft failure and postoperative complications. These findings have significant clinical implications, particularly for patients with peripheral artery disease and those at risk for amputation. Timely intervention and effective grafting strategies will be essential in improving patient outcomes and reducing the incidence of limb loss due to chronic ischemia.

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