Original Article

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The Effects of Systemic IGF-I on the Arterial Anastomosis in Rats

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Abstract

Objective: In this study, we aimed to document the effects of a well-known agent — "insulin-like growth factor (IGF-I)" — on the microvascular anastomosis site.

Methods: Sixteen Sprague-Dawley rats were used in this study. The rats were classified randomly into two equally numbered groups (eight rats each): the control (Group 1) and the experiment group (Group 2). The femoral artery was dissected completely in all rats. Following division of the artery, anastomoses were conducted with microvascular techniques. Forty-five minutes after the anastomoses, an Acland milking test was performed in order to check the patency and the first surgical session was terminated. In the second stage, LONG® R3 IGF-I human (Sigma-Aldrich, St. Louis, Missouri, United States) solution was introduced to Group 2 (experimental group) intraperitoneally in doses of 2 mg/kg on the day of the surgery in addition to the third and seventh days postoperatively. On the 4th postoperative week, the patency of the anastomoses was evaluated with the Acland milking test. In addition, one centimeter of a vascular segment including the anastomosis site was excised and stained with hematoxylin-eosin. They were evaluated for edema, inflammation, vascular wall injury, intimal hyperplasia, medial atrophy, thrombus, calcification, foreign body reactions, and the endothelial proliferation.

Results: The Acland milking test showed a 100% vascular patency in both groups. A statistically significant difference was found between the experimental and control groups in terms of edema and vascular wall injury ($p<0.005$). On the other hand, inflammation, intimal hyperplasia, medial atrophy, microthrombus, and endothelialization variables did not show a statistically significant difference ($p>0.05$).

Conclusion: Under the light of the obtained data, IGF-I was effective in preventing the edema and vascular wall injury at the anastomosis site. However, the net positive clinical effect on anastomosis patency necessitates further studies.

Key words: *Anastomosis, IGF-I, microsurgery*

Introduction

Microsurgery plays a very significant role in reconstructive plastic surgery. The surgical technique and accuracy are of paramount importance for a successful outcome. Many studies have been done in order to minimize the obstacles that might emerge due to the technique and patient-related problems. There have been many pharmacological agents tested in the literature for this purpose [1,2].

The molecular structure of insulin-like

growth factor (IGF-I), which is also known as Somatomedin C, is very similar to insulin [3,4]. IGF-I is synthesized in all tissues; however, it is found mainly in the liver. It is a very significant mediator that controls cellular growth, differentiation, and transformation [3]. In addition, IGF-I shows multiple effects on the vascular system [5]. However, the microscopic effect of IGF-I on the microvascular anastomosis site has not been studied. Our aim in this study was to demonstrate the effects of IGF-I both on the microvascular anastomosis patency and on the histology of the vessel.

Materials and Methods

Sixteen Sprague-Dawley male rats were included in this study following approval by the local ethics committee. The rats whose weights varied between 350–400 gr were classified into two groups randomly, as follows: Group 1: the control group, Group 2: the experimental group. Totally, 16 femoral artery end-to-end anastomoses were performed in 16 rats.

Surgical Procedure; Session 1: The anesthesia was performed with 80 mg/kg of ketamine and 10 mg/kg of xylasine. An oblique incision above the inguinal region was made and the femoral fat pad was elevated to expose the femoral bundle. Two Acland microclamps were placed proximally and distally and a full-thickness cut was made on the femoral artery.

The vessel ends were irrigated with a serum physiologic that had 1000 IU of heparin in 50 ml. In order to prevent vasospasm, 2% of lidocaine and Papaverin solutions were used locally. An end-to-end anastomosis was performed where eight equidistant sutures were placed with a 10/0 Doğsan Dylon®. The clamps were released and after 45 minutes, a milking test was performed in order to check the patency. After being sure of the blood flow, the skin incision was closed.

IGF Application: An IGF-I recombinant analogue LONG® R3 IGF-I human (Sigma-Aldrich, St. Louis, Missouri, United States) was prepared by lipophilization in a 0.1 molar of acetic acid solution. This solution was introduced three times to Group 2 (experimental group) intraperitoneally in doses of 2 mg/kg on the day of the surgery, and on the third and seventh days postoperatively. The same doses of serum physiologic were introduced to Group 1 (control group).

Surgical procedure; Session 2: Four weeks after the first session, an anesthesia of 80 mg/kg of ketamine + 10 mg/kg of xylasine was introduced once again to explore the anastomosis. The anastomosis site was exposed, and the femoral artery was seen and freed from the surrounding tissue. The patency was evaluated with the milking test, and 1 cm of the vessel wall including the anastomosis site was excised. At the end of the procedure, the experimental animals were sacrificed with 120 mg/kg of pentobarbital sodium.

Histological Evaluation: The biopsy materials were preserved with a formalin solution and they were stained with hemotoxylin-eosin. Histological evaluation parameters were vessel wall edema, inflammation, vessel wall injury, intimal hyperplasia, medial atrophy, thrombus, endothelialization, calcification, and foreign body reactions.

Statistical Evaluation: The data obtained from the experiment were evaluated statistically with the SPSS 17 program, as well as the chi-square hypothesis test method. The data obtained were at 95% of the confidence interval, and 0.05 significance level.

Results

The milking test was performed following the second surgical procedure, and 100% vascular patency was found in both groups. A significant difference was found between the experimental and control groups in terms of edema and vascular wall injury ($p<0.05$). The unity of the vascular wall components was evaluated histologically in order to see the vascular wall injury. Marked edema was seen in five rats (62.5%) of the control group. Minimal edema was seen in one of the rats of the experimental group (12.5%). Vascular wall injury was seen in half of the control group (50%). However, no vascular injury was seen in any of the rats of the experimental group. No statistically significant differences were found among groups in terms of inflammation, intimal hyperplasia, medial atrophy, microthrombus, and endothelialization parameters ($p>0.05$) (Table 1, Figures 1–5).

Calcification was seen in one of the rats of the experimental group. A foreign body reaction was seen in all of the rats except one subject in the experimental group. These findings were not compared statistically because they were not included in the specific groups.

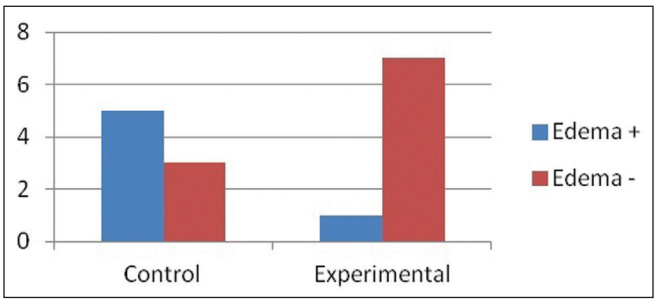

Figure 1. Comparison of edema parameter between the two groups.

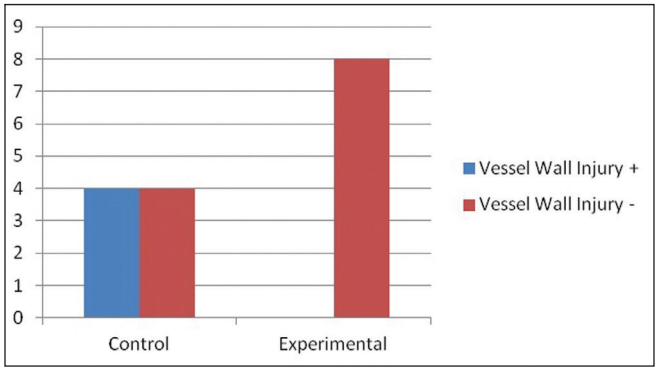

Figure 2. Comparison of vessel wall injury parameter between the two groups.

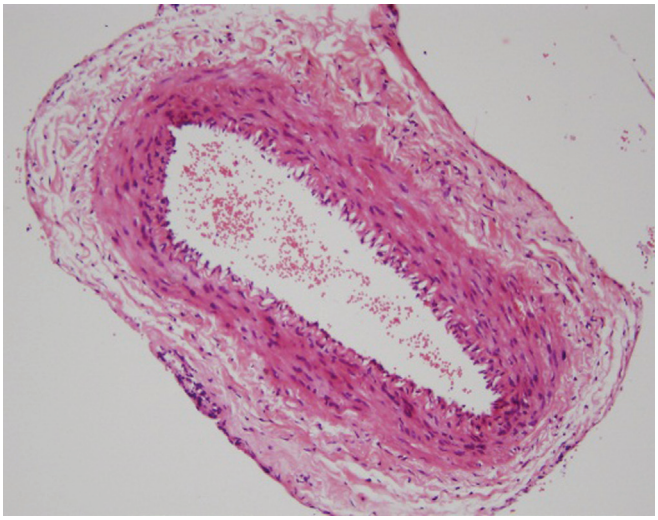

Figure 3. Endothelialization is seen without any inflammation and foreign body reaction in the experimental group (HE 100x).

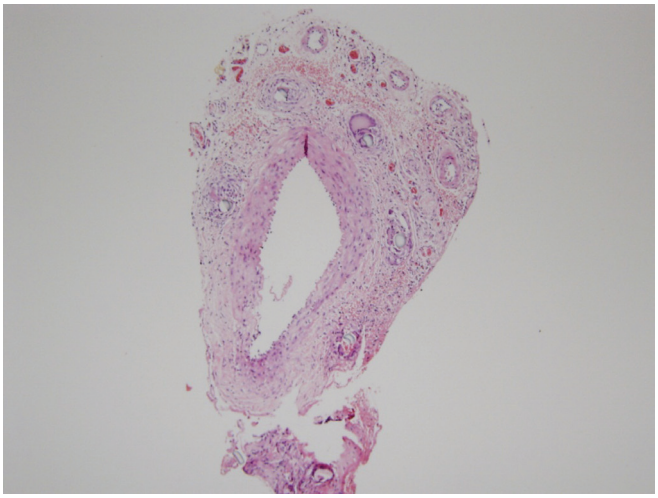

Figure 4. Endothelialization can be seen with few accompanying inflammatory cells and foreign body reaction due to the suture material in the experimental group (HE 40x).

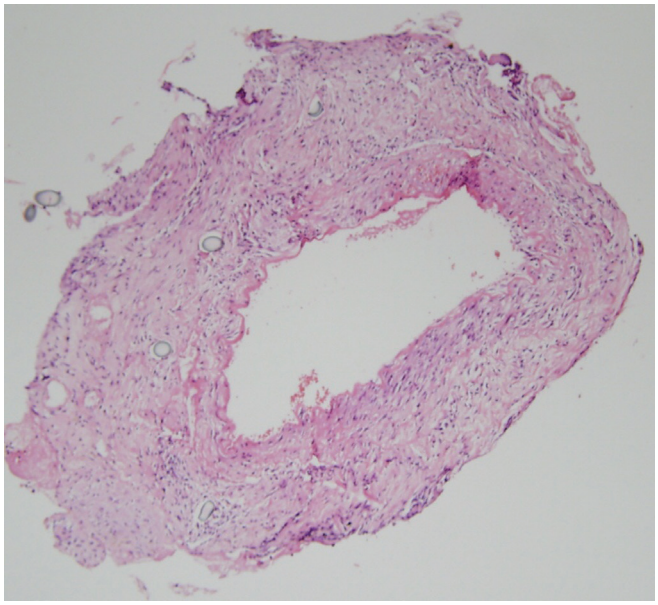

Figure 5. Vessel wall injury with edema and inflammation. No foreign body reaction was detected in the control group (HE 40x).

Discussion

The most significant factor that affects the results of the free flap transfer is the microvascular anastomosis patency. Despite the experience gained in this field, failure is still a significant problem. The failure rate has been reported as 3.6–7% in the elective procedures [6-8].

Many factors are thought to play significant roles for an anastomosis failure. The technical problems that occur during anastomoses are the most frequent reasons for the free flap losses [9]. Some of these problems can be listed as vascular wall injuries due to wrong needle and suture penetrations, endothelial tears, unequal distance between the sutures, very tight or loose sutures, back wall sutures, and the presence of adventitia in the lumen. In literature, many studies have been designed to evaluate the suturing techniques. These involve variations like interrupted, simple continued, vertical or horizontal mattress techniques, and many others [9-11]. However, the simple interrupted suture technique has still been the mainly used technique by many surgeons [10]. In our study, we preferred to use the most conventional simple interrupted suturing technique.

In spite of the effectiveness of the simple interrupted technique and it becoming a standard technique, new studies have focused on the unsuccessful

Table 1. The histological evaluation parameters at the femoral artery anastomosis site.

		Control		Experiment		p
		N	%	N	%	
Edema	Absent	3	37,5	7	87,5	0,039
	Present	5	62,5	1	12,5	
Inflammation	0	3	37,5	3	37,5	0,287
	+1	3	37,5	5	62,5	
	+2	2	25	0	0,0	
Vessel Wall Injury	Absent	4	50	8	100	0,021
	Present	4	50	0	0	
Intimal Hyperplasia	Absent	8	100	7	87,5	0,302
	Present	0	0	1	12,5	
Medial Atrophy	Normal	3	37,5	1	12,5	0,223
	Hypertrophy	0	0,0	2	25,0	
	Atrophy	5	62,5	5	62,5	
Thrombus	Absent	4	50,0	4	50,0	1,000
	Present	4	50,0	4	50,0	
Endothelialization	Absent	7	87,5	4	50,0	0,106
	Present	1	12,5	4	50,0	

anastomoses occurring despite the utilisation of this technique [9]. It is proven that the simple interrupted sutures cause endothelial loss, tunica media necrosis, and adventitia necrosis in the rabbit femoral artery [12]. Acland and Trachtenberg [13] suggested that the intimal injury is due to the mechanical trauma, and the wound irritants might prevent the endothelialization. Schubert et al. [14] suggested that the needle and the suture material itself might lead to thrombosis, and intimal hyperplasia.

The histopathological evaluation of the anastomoses has not been limited to experimental studies. Karl P. et al. [15] evaluated anastomosed vessels histopathologically in free flap cases. In his study, it was shown that the vascular lumens of the flap vessels were narrowed due to the subendothelial thickening of the intima. They found that the inflammation could cause injuries at the intima, elastic membrane, media, and the adventitia of the flap artery [15]. In our study, we observed edema, inflammation, and medial atrophy in 62.5% (five rats) of the vascular anastomoses of the control group. Vascular wall injury and thrombus were observed in 50% (four rats) of the control group. Endothelialization was seen in only one rat (12.5%). However, intimal hyperplasia was not observed in any of the subjects of the control group. The lack of intimal hyperplasia in the control group contradicts the results of the previous studies.

Many agents have been used in order to prevent the thrombosis or anastomosis success in literature. Peter et al. [16] demonstrated that low doses of aspirin might decrease the formation of thrombus significantly at the venous anastomosis on the injured femoral vessels of mice. Rothkopf et al. [17] found that dextrane increases the patency, and decreases the thrombocyte and fibrin deposits in an arterial inversion graft thrombosis model in rabbits. Ritter et al. [18] demonstrated that low molecular weight and unfractioned heparin increased the anastomosis patency, and flap perfusion. Kronen et al. [19] prepared a double blind study of an arterial inversion graft, and a crushing injury model in the rat femoral artery. They found that penthoxyphylline application (20 mg/kg/day) yielded an 84% patency in the experimental group, whereas 37% patency was detected in the control group. These studies give good examples of successful agents used for the sake

of anastomoses. However, in these studies, only the effect of the agents on the patency was evaluated, and no histopathological evaluation was done. In literature, the number of studies that compare the histopathology and the effects of the agents is limited. In a study that questions the effect of vinblastine on the healing of microvascular anastomosis, the femoral artery anastomoses were evaluated under the light microscope on the 7th, 14th, and 21st days postoperatively. No statistically significant differences were found between the two groups in terms of edema, inflammation, vessel wall injury, thrombosis, calcification, foreign body reaction, and endothelialization parameters [20]. In another study, the effect of tamoxifen on the arterial microanastomosis was evaluated by observing the perivascular infiltration, endothelial lesions, and the presence of thrombus in the lumen. Minimal inflammation was seen in both groups, but it was more in the tamoxifen application group. A prominent increase in the intimal thickness was seen in the tamoxifen group. However, it was shown that these results do not cause an intraluminal thrombosis [21]. In a study conducted by Tosun et al. [22], the objective was to show the protective effects of human recombinant erythropoietin (rHuEPO) over the unfavorable effects caused by cigarettes on the anastomosis site. The endothelial cellular healing was evaluated under the light microscope documenting the intima/media ratio, and it was found that erythropoietin might reverse the negative effects of the cigarette.

IGF-I is the most effective natural activator of the AKT signaling pathway. It stimulates the cellular growth, proliferation and it is an effective inhibitor of the programmed cellular death. IGF-I shows multiple effects on the vascular system [5]. When the vascular smooth muscle cells lose differentiation, IGF-I stimulates cellular proliferation, migration, and conducts an anti-apoptotic factor-like effect. If this aspect is evaluated, it is seen that IGF-I acts as a savior, and a proliferative factor for the vascular smooth muscle cells that cause atherosclerosis, and restenosis after an arterial injury [23]. On the other hand, IGF-I is a strong stimulator of eNOS by the phosphatidilinositole/AKT signaling pathway (IGF-I PI3K/Akt). Under physiologic circumstances, IGF-I increases the nitric oxide (NO) production in the endothelial cells, and protects the endothelial function [4]. Because of the wide spectrum of the atherogenic mechanisms inhibited by NO, it can be stated that IGF-I acts like a vascular protector, and an anti-atherogenic factor [24]. It can be concluded that IGF-I is a strong mitogen and an anti-apoptotic factor that acts on vascular cells such as vascular smooth muscle cells and endothelial cells. Also, it shows a promigratory effect [20]. For this reason, IGF-I can stimulate the migration and the proliferation of the vascular smooth muscle cells. It shows a pro-atherogenic effect by increasing the chemotactic macrophage migration, and the synthesis of cellular adherent mechanisms [3,25].

The effect of IGF-1 on tissues other than vessels has been searched in literature. In a study done by Mulholland et al. [26], authors found that IGF-1 increased the axonal growth in cultured myenteric plexus neurons and neuroblastoma cells. In addition, IGF-1 has been shown to increase axonal regeneration in the sciatic nerve following an injury [27]. Another study done by Petersen et al. demonstrated the beneficial effects of IGF-1 on the colon following an anastomosis which was related to the increased hydroxyproline [28]. Zacharakis et al. [29] again showed the positive effects of IGF-1 on the colon of the rats treated with 5- Fluorouracil. Neoangiogenesis and fibroblast activity were found to be vastly increased. Many other studies emphasized the positive effects of IGF-1 on wound healing where collagen, fibroblast and keratinocyst proliferations were stimulated together with neoangiogenesis [30,31].

In literature, the effect of systemic administration of IGF-I on intimal hyperplasia at the anastomosis site has been studied [32]. In a study done by Cittadini et al. [23], authors investigated the effects of IGF on a rat model of carotid artery injury. Following 10-day administration of IGF-I, the arterial stenosis was found to be decreased from 47% to 11%. This effect was supposed to be due to the inhibition of the neointimal hyperplasia by IGF-I without affecting the media thickness. They concluded that IGF-1 could be a potent vasculoprotective agent.

Zhu et al. [33] studied the expression of IGF-I on smooth muscle cells of transgenic mice on rat carotid arteries following an injury. They concluded that IGF-I

might be a powerful stimulus for smooth muscle cell proliferation and migration in vivo.

In spite of the various effects of IGF-I on relatively large vessels, no study has been performed on effects of the same agent on microvascular anastomosis. In our study, with the use of IGF-I, there has been a statistically significant difference between the experimental and control groups in terms of edema and vascular wall injury. However, a statistically significant difference was not found in parameters such as inflammation, intimal hyperplasia, medial atrophy, microthrombus, and endothelialization parameters. In addition, we have found that IGF-I does not have a different effect on the media. For this reason, intimal hyperplasia was also not observed. In both groups, medial atrophy accompanied a microthrombus. This could be explained by the chronic effects of the thrombus on tunica media. In cases where microthrombus has not been seen, media was evaluated as normal.

In the experimental group, four of the subjects (50%) had endothelialization, whereas it was seen in only one subject in the control group. This result was not statistically significant. However, we think that it could be significant if the number of the subjects were high. The reason for having more endothelialization in the experimental group could be due to the increased mobilization of the endothelial progenitor cells after IGF-I administration [23].

As a result, under the light of the obtained data, it was seen that IGF-I has positive histopathological effects on vascular wall injury, edema, and endothelialization. However, the effect on the anastomosis success and patency did not change. In addition, the final influence of a change in parameters such as vessel wall injury, edema and endothelialization on a clinical success necessitates further studies.

Conflict of interest statement

The authors have no conflicts of interest to declare.

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