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Effects of vinblastine on microvascular anastomosis healing in diabetic rats

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Objective: Diabetes mellitus and chemotherapy use are well-known factors that can affect success after microvascular repair. Although many studies have investigated each factor individually, no study exists on their combined effect. The aim of our study was to investigate the effects of preoperative vinblastine, a vinca alkaloid, on the healing of microvascular anastomosis in diabetic and non-diabetic rats.

Methods: In this study, 144 Sprague Dawley rats were assigned randomly and equally into experimental or control groups. The experimental group consisted of 72 rats which were made diabetic by alloxan injections. Diabetes was confirmed by glycosuria (>2%) and elevated plasma glucose (>300 mg/100 ml) after three days of alloxan injections. The experimental group then received a single dose of intraperitoneal vinblastine. After applying the medication, the two main groups were divided into three subgroups. For each group, we performed end-to-end femoral artery anastomosis on Day 7, 14, and 21, respectively. We further divided each subgroup equally into three smaller groups to test patency and took biopsies at Day 7, 14 and 21, respectively. Histopathologic evaluation was carried out.

Results: The comparison of patency tests and pathologic examination indicated that there was no statistically significant difference between the two groups.

Conclusion: The negative effects of diabetes and the administration of vinblastine did not significantly influence anastomosis healing.

Key words: Diabetes; microvascular anastomosis; vinblastine.

In the era of free flap, microsurgery techniques have significantly improved the potential scope of tumor resections and postoperative quality of life following major composite tissue resections.[1] Although microsurgery has advanced enormously, it may still fail due to systemic conditions which act as risk factors for microvascular anastomosis.[2] These conditions include intrinsic factors, such as systemic disease and age, as well as extrinsic factors, such as chemotherapy and radiation treatment before surgery.[3] Diabetes mellitus is known to cause microcirculation abnormalities. Experimental studies have demonstrated that the degree of intimal repair and endothelization is lower in diabetic patients with poorly controlled glucose levels and that this can lead to anastomotic failure.[4,5] However, clinical experience has shown that free tissue transfers can be done successfully in the diabetic population.[6,7]

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Reconstructive microsurgical procedures have become more common in cancer patients. Adjuvant therapies, such as chemotherapy, are usually combined with surgical approaches to improve outcomes. Vinblastine is frequently used in human cancer treatment and animal applications. Vinblastine exerts its effects on microtubules in the metaphase stage of mitosis. It causes cell death by binding tubulin molecules, an element of microtubules, and prevents their coming together. The dosage of vinblastine has been determined in pilot studies.[8] We previously studied the effects of preoperative vinblastine on healing in microvascular anastomosis.[9] Our study revealed that there was no significant difference between the vinblastine-administered group and the non-administered group in either patency test or pathologic examination.

We aimed to evaluate two different risk factors (diabetes and chemotherapy) which are believed to have an influence on microanastomosis failure. In this experimental study, the influence of intra-abdominal vinblastine on arterial microanastomosis was evaluated to determine its impact on free-tissue transfers in diabetic rats. No study has been found in the literature that evaluated the effects of chemotherapy on arterial microanastomosis healing in the diabetic population. We believed that our study can help medical professionals in the selection of appropriate reconstruction options in the diabetic population receiving preoperative chemotherapy. Additionally, we attempted to determine the appropriate timing of arterial microanastomosis following chemotherapy.

Materials and methods

The consent of the ethical board of Şişli Etfal Training and Research Hospital was obtained for the study. In this study, we used 144 Sprague Dawley male rats (weight: 250-300 g) and divided them into two main groups: the non-diabetic Group 1 (n=72) and the diabetic Group 2 (n=72). The rats were housed in separate cages under standard laboratory conditions (12 hours of light/12 hours of darkness, 22 °C, 30% humidity, food and tap water available ad libitum) throughout the experiment. Fifty mg/kg intraperitoneal Ketalar (Ketamine HCl, Eczacıbaşı, İstanbul, Turkey) was used for general anesthesia.

Rats in Group 2 were anesthetized and a single injection of alloxan monohydrate (65 mg/kg) dissolved in a 0.05 M citrate buffer (pH 4.5) saline solution was administered to the tail vein to induce diabetes just prior to injection. As alloxan can produce nonspecific kidney damage, 5-10 ml of 0.9% NaCl was given intraperitoneally immediately following the administration of alloxan, as suggested by Heimberg et al.[10] The rats, given alloxan, underwent a transient and dangerously fatal hypoglycemia period. Thus, a 50% dextrose- saline solution was administrated subcutaneously within 12 to 24 hours following the administration of alloxan.[10,11] Three days after the injection of alloxan, diabetes was confirmed with glycosuria (>2%) and elevated plasma glucose (>300 mg/100 ml). Plasma glucose levels were controlled eight weeks following the administration of alloxan, and rats showing low plasma glucose levels were excluded. Additional diabetic rats were then included in the study (Table 1).

Vinblastine, which is an alkaloid of vinca, was administrated intraperitoneally to all rats in the study as a single dose (2 mg/kg) on Day 0. Group 1 and Group 2 were each divided equally into three subgroups of 24 rats. Plasma glucose levels were assessed monthly during the study period and rats with low plasma glucose levels were excluded from the study.

End-to-end femoral artery anastomosis was performed in each subgroup, using 10/0 nylon sutures after chemotherapy on Day 7, 14 and 21, respectively. Each group of 24 rats was further divided equally into 3 smaller groups containing 8 rats. These subgroups were tested for patency, and biopsies were taken, including anastomosis, on Day 7, 14 and 21, respectively. Healing of anastomosis was evaluated pathologically in each group (Fig. 1).

All biopsies were preserved in formalin solution, and then stained with hematoxylin and eosin (H&E) and evaluated with light microscopy by the same pathologist. Evaluated parameters were inflammation, edema, thrombus, calcification, vessel wall damage, foreign body reaction on the vessel wall, and endothelization.

Pathological results of non-diabetic group and diabetic group were compared using a chi-square test.

Results

Patencies were evaluated in all groups before biopsies were taken. Patency was found to be 97.8% (70 of 72 rats) in the non-diabetic group and 98.6% (71 of 72 rats) in the diabetic group.

Table 1. Plasma glucose level of rats (mg/100 ml).

No	Glucose level		No	Glucose level		No	Glucose level	
	3rd day	3rd mo		3rd day	3rd mo		3rd day	3rd mo
1	307	314	**25**	322	324	**49**	320	315
2	312	317	**26**	305	309	**50**	318	317
3	321	302	**27**	317	316	**51**	314	326
4	335	327	**28**	314	319	**52**	305	311
5	304	331	**29**	326	320	**53**	333	325
6	317	319	**30**	321	304	**54**	317	309
7	309	315	**31**	310	325	**55**	325	322
8	314	304	**32**	315	319	**56**	341	339
9	310	330	**33**	318	317	**57**	319	314
10	321	308	**34**	304	321	**58**	302	306
11	314	315	**35**	323	319	**59**	327	334
12	306	303	**36**	337	335	**60**	315	306
13	334	331	**37**	312	309	**61**	337	331
14	312	305	**38**	306	305	**62**	322	327
15	324	321	**39**	318	324	**63**	309	319
16	317	332	**40**	331	319	**64**	317	324
17	315	327	**41**	302	313	**65**	311	319
18	321	309	**42**	316	324	**66**	331	307
19	326	304	**43**	321	320	**67**	340	338
20	307	309	**44**	307	303	**68**	327	321
21	327	319	**45**	315	320	**69**	314	319
22	324	317	**46**	311	314	**70**	323	335
23	312	308	**47**	303	317	**71**	330	306
24	330	335	**48**	327	331	**72**	309	308

Mean V±SD 3rd day 318.13 ±9.70, Mean V±SD 3rd month 349.50±20.92

No obvious changes were found in samples from either the non-diabetic or diabetic group under a light microscope. There were no differences between the two groups regarding inflammation, edema, foreign body reaction on the vessel wall, or endothelization (Figs. 2 and 3).

In the non-diabetic group (Group 1), microthrombus was present in 3 rats; one on which anastomosis was performed on Day 7 following chemotherapy and from which a biopsy was taken on Day 21, one rat on which anastomosis was performed on Day 14 following chemotherapy and from which a biopsy was taken on Day 7 (Fig. 4), and one rat on which anastomosis was performed on Day 14 following chemotherapy and from which a biopsy was taken on Day 14. In the diabetic group, microthrombus was found in one rat on which anastomosis was performed on Day 7 following chemotherapy and from which a biopsy was taken on Day 14 (Fig. 5). Three rats in the non-diabetic group presented with minimal calcification; one rat on which anastomosis was performed on Day 14 following chemotherapy and from which a biopsy was taken on Day 2, and 2 rats on which anastomoses were performed and biopsies were taken on Day 21. In the diabetic group, two rats on which anastomosis were performed 7 days following chemotherapy and from which biopsies were taken on Day 14 presented with minimal calcification. Neither microthrombus nor minimal calcification was compared statistically, as they were rare complications not specific to a particular group. However, microthrombus was seen in the late period in the non-diabetic group and the early period in the diabetic group.

The comparison of patency tests and histological examinations of endothelization indicated that there was no statistically significant difference between the two groups ($p>0.01$). Additionally, there was no significant difference between anastomosis performed on Day 7, 14, or 21 ($p>0.01$) (Table 2).

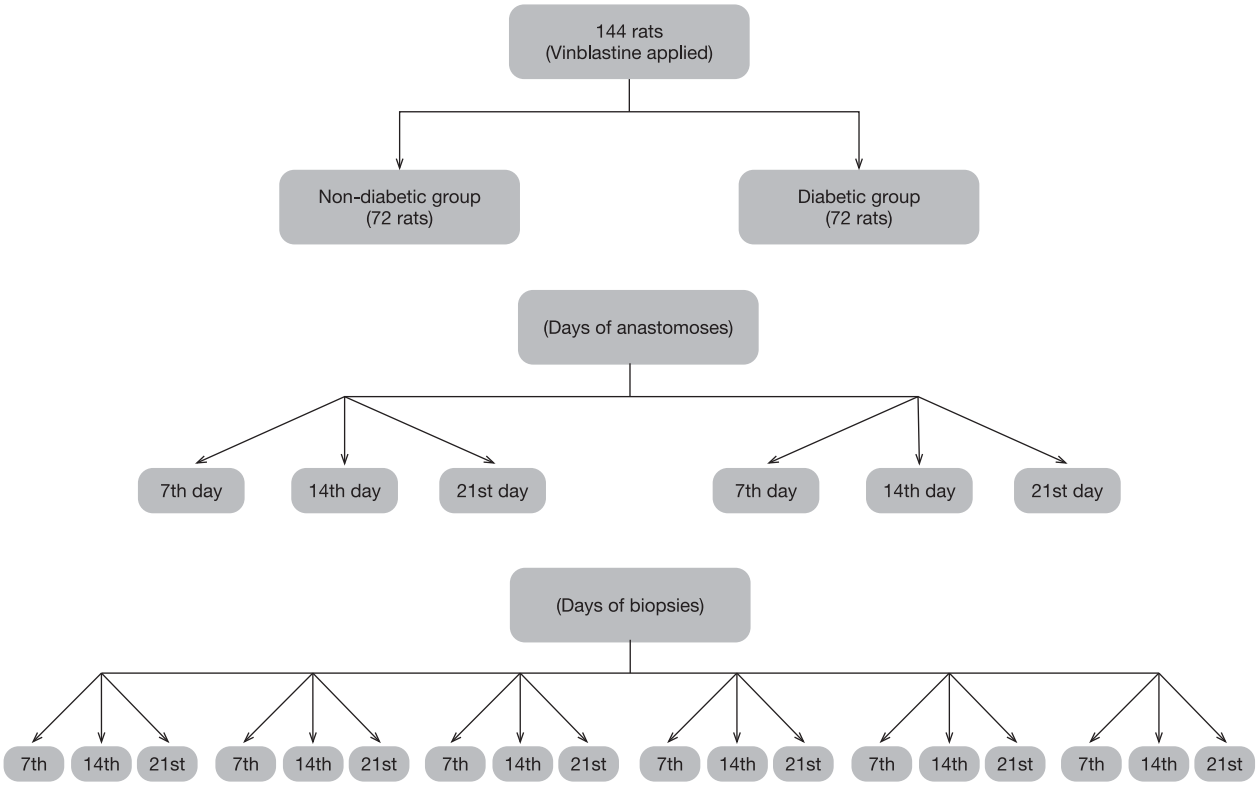

Fig. 1. Distribution of rats to groups.

Discussion

The development of microsurgical techniques has improved the potential scope of tumor resections and postoperative quality of life following major composite tissue resection. However, flap viability appears to depend on a number of independent factors associated with the patient's condition and therapeutic protocol.[6,7,12] Combining different methods in tumor patients can lead to increased risks. When chemotherapy is administered prior to surgery, it shows promise in shrinking tumors. Whenever surgical treatment and chemotherapy are combined, chemotherapy delays intervention, increases the risk of infection and the risk of the operative procedure itself, and prolongs hospitalization.[8,13] Therefore, we aimed to investigate the effects of preoperative vinblastine on healing in microvascular anastomosis. In our previous study, we observed no differences between the non-vinblastine administered group and the vinblastine administered group, in terms of patency and pathological examination.[9] As a second step, in the present study, we aimed to investigate the influence on arterial microanastomosis of two different variables: diabetes and chemotherapy.

Diabetes causes alterations in vessels. Experimental studies using rats have demonstrated that the degree of intimal repair and endothelization is lower in diabetic patients with poorly controlled glucose leves.[14] Clinical studies have not demonstrated an increase in anastomotic or flap failure rate in patients with diabetes mellitus.[2] On the other hand, hyaline deposition and vascular tumor development induced by thickening of the elastic membranes have been widely reported in diabetics, as have thickening of the tunica intima and the appearance of foam cells in the tunica intima and tunica media. In practice, free tissue transfers can be done successfully in the diabetic population; most anastomotic complications are secondary to early venous occlusion.[12,15]

Many studies on the effects of diabetes on healing in microvascular anastomosis exist, but we were unable to find any on the combined effect of diabetes and chemotherapy. We used vinblastine in our study because it is frequently used in human cancer treatment and has animal applications. Dosage has been determined in pilot studies.[8,9] Vinblastine affects

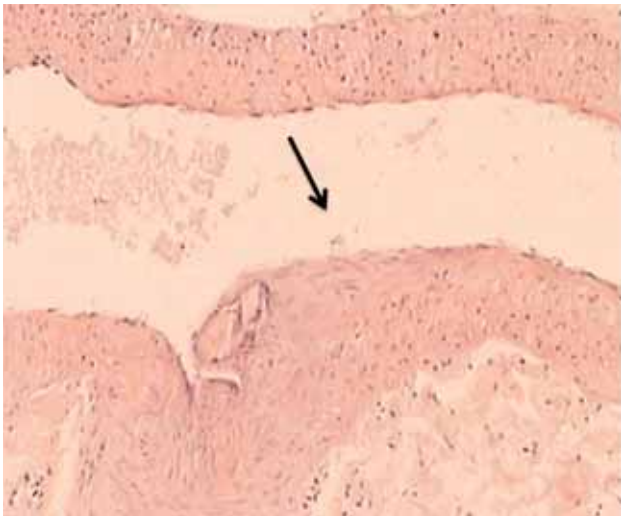

Fig. 2. Sample from the non-diabetic group wherein after chemotherapy. Anastomosis was performed on Day 7 and biopsy was performed on Day 14. The endothelization is obvious, as indicated with black arrow (H-E x100). [Color figure can be viewed in the online issue, which is available at www.aott.org.tr]

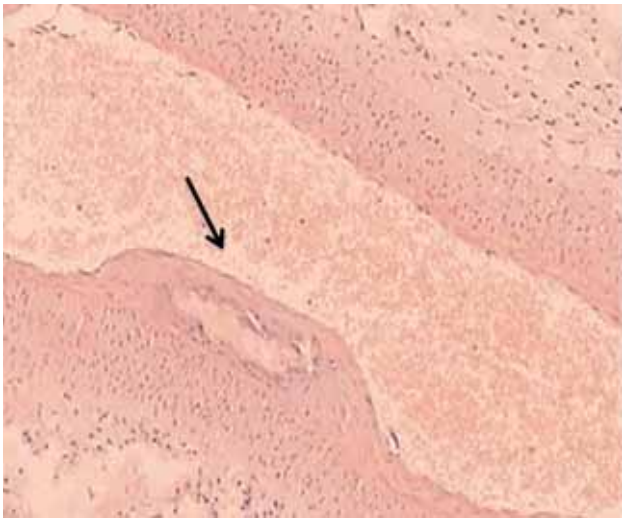

Fig. 3. Biopsy from the diabetic group. Anastomosis was performed on Day 7 following chemotherapy; biopsy was performed on Day 14. The endothelization is obvious, as indicated with black arrow (H-E x100). [Color figure can be viewed in the online issue, which is available at www.aott.org.tr]

microtubules in the metaphase stage of mitosis by binding to tubulin molecules and preventing their coming together. As a result, it causes cell death,9 After chemotherapy, white cell count drop in one week.[8,9] In our previous study, microvascular anastomosis was performed on Day 2 and all rats died between postoperative Days 3 and 5, due to the neutropenia period. We preferred the 7th, the 14th, and the 21st days for anastomosis, because dose-limiting myelosuppressive toxicity of vinblastine is seen between 5 and 9 days after administration. This dose-limiting toxicity disappeared between Days 14

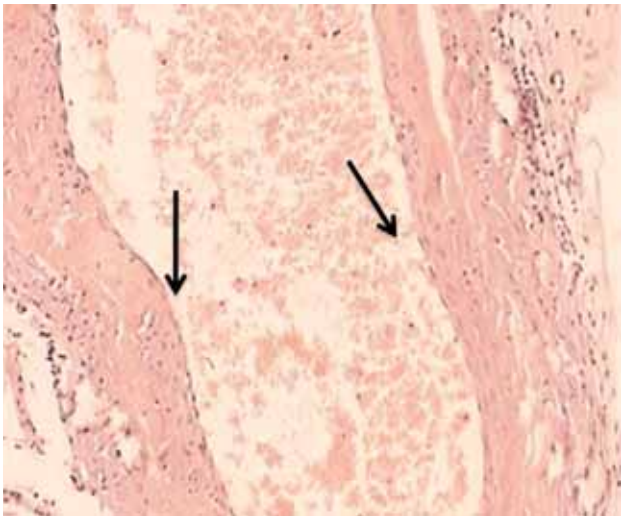

Fig. 4. Biopsy from the non-diabetic group. Anastomosis was performed on Day 14 following chemotherapy; biopsy was performed on Day 21. Endothelization (black arrows) is seen (H-E, x100). [Color figure can be viewed in the online issue, which is available at www.aott.org.tr]

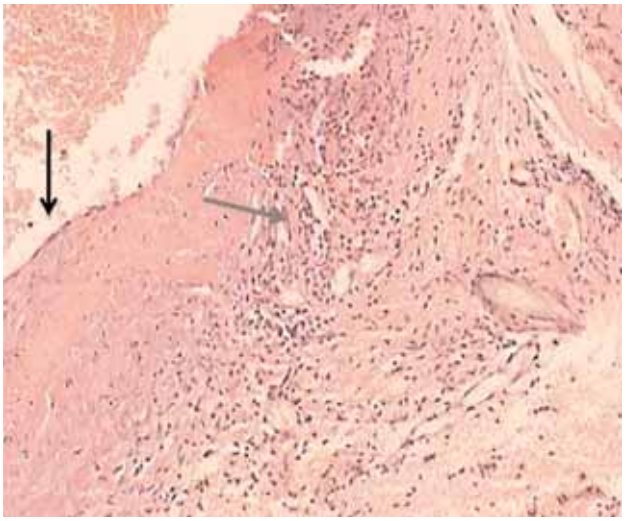

Fig. 5. Sample from the diabetic group. Anastomosis was performed on Day 14 and biopsy was performed on Day 21. Endothelization (black arrow) and foreign body reaction (grey arrow) are seen (H-E, x100). [Color figure can be viewed in the online issue, which is available at www.aott.org.tr]

Table 2. Plasma glucose level of rats (mg/100 ml).

Day		Non-diabetic / Diabetic Group (p)					
		Inflammation	Edema	Foreign body reaction	Endothelization	Microthrombus	Microcalcification
	7.	-	-	-	-	-	-
	14.	0.301	-	0.521	0.521	0.301	0.130
7.	21.	0.301	0.301	0.301	-	0.301	-
	7.	0.521	0.521	0.301	0.521	0.301	-
	14.	-	-	0.301	0.0003	0.301	-
14.	21.	-	-	-	0.521	-	0.301
	7.	0.0001	0.301	-	0.589	-	-
	14.	0.0004	-	0.521	-	-	-
21.	21.	0.301	-	-	0.614	-	0.130

and 21.[16] Furthermore, we preferred patency and pathologic examinations on Days 7, 14, and 21 because myelofibroblasts were seen in the acellular matrix, which was contained beneath a forming monolayer of endothelial cells seven to ten days after anastomosis. By Day 14 the endothelial lining was intact, and the vessel was morphologically healed by Day 21.[17] No differences between anastomosis performed on Day 7, 14 and 21 were observed. However, we speculate that there is no difference between performing arterial microanastomosis in the early or late stages following the administration of vinblastine.

A comparison of endothelization patency tests and histological examinations indicated no statistically significant difference between the two groups. Microthrombus and minimal calcification were not compared statistically because they were rarely observed. According to literature, thrombus is more common in diabetic rats. In our study, microthrombus was present in three rats in the non-diabetic group and one rat in the diabetic group. We observed microthrombus in the late period in the non-diabetic group and in the early period in the diabetic group, which is in line with the literature.[4] There were no differences between the groups, in terms of calcification.

Histological evaluations were done with H&E staining. We could not evaluate the biopsies using scanning electron microscopy (SEM). Previous SEM studies in the literature revealed lesions of the endothelium, characterized by the swelling of cells, formation of intracytoplasmatic vacuoles, and villous projections into the lumen of the vessel.[18] Gaps between the endothelium and the underlying tissue and destruction of the internal elastic lamina were also seen.[3] However, we were unable to evaluate the above mentioned parameters. In addition, the diabetic and non-diabetic rats receiving vinblastine did not have tumors or tumor-related problems, such as hypercoagulopathy. Tumor-related problems were not evaluated in our study.

In conclusion, the comparison of patency tests and the histological examination of endothelization indicated that there was no statistically significant difference regarding microvascular healing on an anastomosis site between the diabetic and non-diabetic groups. The negative effects of diabetes and the administration of vinblastine did not significantly influence anastomosis healing in this model.

Conflicts of Interest: No conflicts declared.

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