Effects of Mitomycin on Microvascular Anastomosis

Mitomisinin Mikrovasküler Anastomoza Etkileri

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ABSTRACT Objective: Microsurgical tissue transfers are widely used for the reconstruction of the defects resulting from the excision of the malignancies and adjuvant therapies are also commonly employed in order to improve the survival. After surgery, further need for chemotherapy and radiotherapy is not uncommon. However, these treatment modalities may have adverse effects on each other. Effects of chemotherapeutic agents on microvascular anastomosis should be known well so that treatment plan can be done. Mitomycin C is one of the most toxic chemotherapeutic agents. In this experimental study we have investigated the effects of mitomycin C on the microvascular anastomosis. **Material and Methods:** Twenty Sprague-Dawley rats were used. 10 mg/m^2 Mitomycin C was administered intraperitoneally to 10 animals in study group. The other 10 animals were used as the control group. One week later microvascular anastomoses were carried out on femoral arteries of all of the animals. **Results:** After two weeks of waiting period, animals were reoperated and patency of the anastomoses were checked, also a histologic study was carried out. Macroscopically pulsatile blood flow was observed in all but two animals in study group and one animal in control group. Histologically, no additional reaction against mitomiycin C in muscular and adventitial layers of the vessel walls was observed. We found out that there was no significant difference between the animals in control group and study group in terms of patency of the anastomoses and histological findings. **Conclusion:** Since Mitomycin C is one of the most toxic chemotherapeutic agents it is important to know that it does not affect the microvascular anastomoses adversely.

Key Words: Mitomycin; anastomosis, surgical; thrombosis

ÖZET Amaç: Günümüzde, malignitelerin eksizyonu sonucunda oluşan defektlerin rekonstrüksiyonunda mikrocerrahi yöntemleri ile doku aktarımları yaygın olarak kullanılmakta, aynı zamanda sağ kalımı arttırabilmek için adjuvan tedaviler de sık olarak uygulanmaktadır. Cerrahi sonrasında nadir olmayarak kemoterapi ve radyoterapiye ihtiyaç duyulmaktadır. Ancak, bu tedavi modalitelerinin birbirleri üzerine ters etkileri olabilir. Tedavi planının yapılabilmesi için, kemoterapotik ajanların mikrovasküler anastamoza etkilerinin iyi bir şekilde bilinmesi gerekmektedir. Mitomisin C bilinen en toksik kemoterapotik ajanlardan biridir. Bu deneysel çalışmada, bizler mitomisin C'nin mikrovaskuler anastomoza etkilerini inceledik. **Gereç ve Yöntemler:** 20 adet Sprague-Dawley cinsi sıçan kullanıldı. Çalışma grubundaki 10 adet hayvana intraperitoneal olarak 10 mg/m^2 Mitomisin C verildi. Diğer 10 adet hayvan kontrol grubu olarak kullanıldı. Bir hafta sonra tüm hayvanların femoral arterlerine mikrovasküler anastomoz tatbik edildi. **Bulgular:** İki haftalık bekleme sürecinden sonra, hayvanlar tekrar opere edildi ve anastomozlardaki açıklık kontrol edildi, ayrıca histolojik çalışmalar gerçekleştirildi. Çalışma grubundaki iki adet, kontrol grubundaki bir adet hayvan dışında makroskopik olarak pulsatil kan akımı gözlemlendi. Histolojik olarak, damar duvarının muskuler ve adventisyal tabakalarında Mitomisin C'ye karşı ekstra bir reaksiyon gözlenmedi. Kontrol grubu ve deney grubunda anastomozun açıklığı açısından histolojik olarak belirgin bir farklılık olmadığını bulduk. **Sonuç:** Mitomisin C, en toksik kemoterapotik ajanlardan biri olmasına rağmen, mikrovaskuler anastomoz üzerine olumsuz bir etki yapmadığını bilmek önemlidir.

Anahtar Kelimeler: Mitomisin; cerrahi anastomoz; tromboz

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Most of the malignancies are treated with a combination of treatment modalities to decrease the side effects of each component and also to increase the efficacy of the treatment.[1] Surgical excision of malignancies generally leads to large defects requiring microvascular free tissue transfer. Further need for chemotherapy and radiotherapy is not uncommon after or before surgical intervention. However, these treatment modalities may have adverse effects on each other necessitating the omission of one of the components.[1,2] Healing of microvascular anastomosis is an area of great interest and research. Preoperative chemotherapy may adversely affect a future free tissue transfer due to the vascular toxicity of the chemotherapeutic agents. Mitomycin C is an antibiotic obtained from *Streptomyces caespitosus*. It achieves its antitumor activity by inhibition of DNA synthesis through DNA cross linking.[3-5] It is effective against the malignancies of oral cavity, lungs, pancreas, and stomach. Systemic application via i.v route leads to severe systemic side effects, such as medullary aplasia and necrosis of subcutaneous tissues if extravasated.[6]

We have planned this study to document the effects of mitomycin C on microvascular anastomosis.

MATERIAL AND METHODS

The experimental protocol was approved by the Animal Care and Use Committee of the institution in which the study was carried out. Twenty Sprague-Dawley rats with weights ranging from 350-400 g were used in this study and the size of the study population was determined on the basis of the previous studies published in the literature. Animals were anesthetized with ketamine hydrochloride 50-100 mg/kg intramuscularly.

Animals were divided into two groups. 10 mg/m^2 mitomycin C was administered intraperitoneally to the animals in group II. Group I was the control group. After 7 days of waiting period, animals were reoperated and femoral arteries were dissected through an incision on left groin. The femoral arteries were divided sharply and end-to-end microvascular anastomosis was carried out under 10 X magnification with 9/0 nylon. Incisions were closed primarily and at two weeks postoperatively animals were reoperated and femoral artery biopsies were harvested including the anastomosis site.

All biopsy specimens were fixed in 10% formaldehyte solution and embedded in paraffin. Histologic specimens were stained with hematoxylen eosin.

RESULTS

GROSS FINDINGS

Patencies of blood flow in femoral arteries were evaluated macroscopically before harvesting femoral artery biopsies. Pulsatile blood flow was observed in all but two animals in study group and one animal in control group. Blood flow was also controlled with transsection of the femoral arteries distal to anastomosis site. Statistical analysis of the results was carried out using Fisher's test and no statistically significant difference was found between the study and control groups ($p > 0.05$).

HISTOLOGICAL FINDINGS

The two groups were compared in terms of inflammation of the vessel wall and endothelization or inflammatory reaction against the suture materials. Inflammation was classified as mild, moderate, or severe and the endothelisation as complete or incomplete. Inflammatory reaction and thickening of the vessel walls (tunica media) was noted on suture sites in both groups (Figure 1).

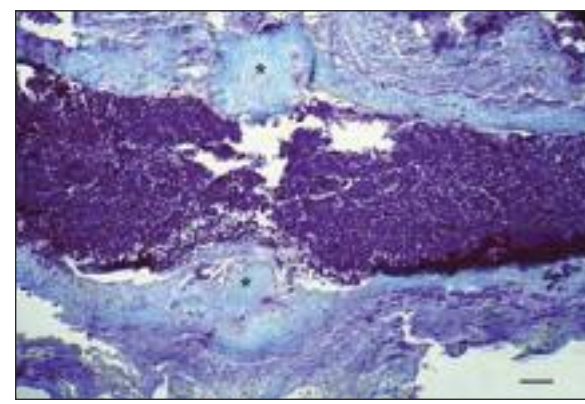

FIGURE 1: Inflammatory reaction and thickening of the tunica media on suture sites (shown with asterix) in mitomycin C treated group. (Bar 60 µm, stained with hematoxylen eosin).

Thrombus was noted in two of the animals in study group and in one of the animals in control group. Detached endothelial cells were observed in the lumen of the vessels with thrombus, and endothelial integrity was disturbed (Figure 2).

Endothelium was intact and smooth in other samples (Figure 3). The results were compared with Mann-Whitney U test. The differences between the two groups in terms of histological findings were not statistically significant ($p > 0.05$). No additional reaction against mitomiycin C was observed. in muscular or adventitial layers of the vessel walls

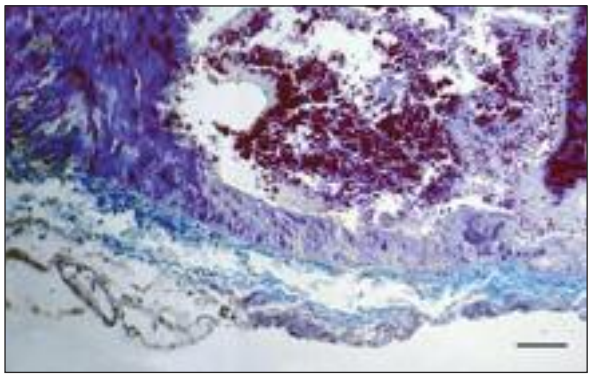

FIGURE 2: A specimen from control group. Detached endothelial cells and thrombus in the lumen of the vessel can be observed. Endothelial integrity is disturbed. (Bar 90 µm, stained with hematoxylen eosin).

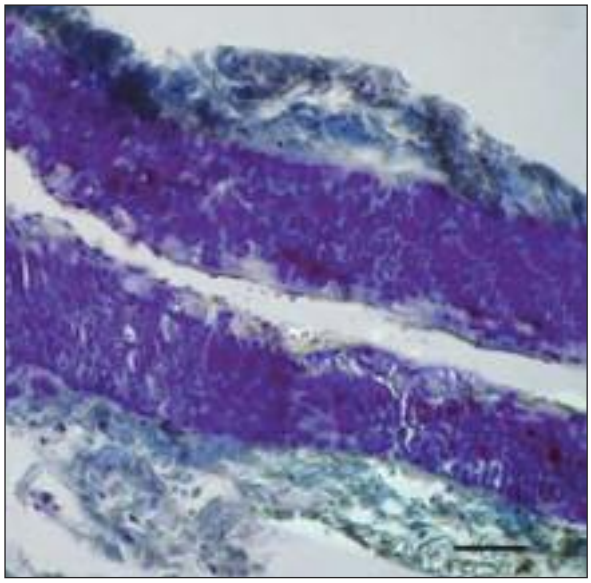

FIGURE 3: Intact endothelium of a specimen from mitomycin C treated group. (Bar 60 µm, stained with hematoxylen eosin).

DISCUSSION

Surgical treatment of the malignancies may lead to large composite defects that require a free tissue transfer for reconstruction. But adjuvant therapies like chemotherapy have been increasingly used after or before the surgical intervention either to decrease the bulk of the tumor before the operation or as a prophylactic measure against metastasis after the excision of the tumoral mass. Thus, effects of chemotherapeutics on microvascular anastomosis is a great area of research since it is critical to know if chemotherapeutic drugs lead to an increased failure rate of free flaps that are commonly used for the reconstruction of the oncologic defects. Once the evidence of increased thrombosis in anastomosis site with the use of chemotherapeutics has been found, treatment strategies will change. However, there is no study in the literature supporting this theory.

Thrombosis of the vessels is the result of two hemostatic processes; platelet aggregation and coagulation cascade. Coagulation cascade is activated in response to endothelial damage.[7] Chemotherapeutic agents have shown to be toxic to endothelium leading to endothelial swelling and this theoretically should increase the thrombus formation.[8] But this is not the case in practice. Previous reports about the effects of cisplatine and vinblastine and many other chemotherapeutics on patency of microvascular anastomosis failed to show the negative effects of these agents.[2,9-12] Gurusanthaihah et al applied intra-arterial cisplatine to the rats before microvascular anastomosis and found that intraarterial cisplatin did not affect the patency of microvascular anastomosis despite increased inflammatory changes in the vessel wall.[12] In a clinical study, Sadrian et al further documented that preoperative adjuvant chemotherapy does not lead to increased failure rates of subsequent free flap operations.[13]

Mitomycin C is one of the most toxic chemotherapeutic agents. Its well known side effects necessitate its use in combination with other agents in smaller doses to decrease its side effects. It leads to tissue necrosis when it is extravaseted[6] and it de-

lays and impairs wound healing.[3,4] It may be hypothesized that hazardous effects of mitomycin C on soft tissues may lead to an increase in thrombus formation in vessels which have already been traumatized by the manipulation during the microvascular anastomosis. Suture materials and handling with microvascular instruments induces an inflammatory change in vessel walls.[12] On the other hand mitomycin C has an antifibrotic effect that can be useful in some clinical situations. Some previous studies have shown that mitomycin C has improved the outcome when used on corneal wounds,[8] nerve repairs,[14] and keloids,[4] silicone implants[15] when used at low none cytotoxic doses. This antifibrotic effect of the mitomycin C may decrease the inflammatory response in the vessel wall.

Although mitomycin C is a locally toxic antibiotic chemotherapeutic agent; it is commonly used for its antifibroblastic effects that many other chemotherapeutics do not have. It decreases inflammation by its antifibroblastic effect. Therefore, the influence that reduces the risk of thrombus formation on anastomosis may act as a balancing factor against its toxic effects. Mitomycin C is commonly being employed one dose when used as an antifibroblastic. For this reason, the study has been planned by one dose of mitomycin.

The hypotheses above were tested in our study and we have documented that mitomycin C did not lead to an increased thrombus formation or any additional changes in the vessel wall when applied systemically.

In conclusion, even mitomycin C, which is one of the most toxic antineoplastic agents does not lead to increased thrombosis formation in microvascular anastomosis despite its hazardous effects on wound healing and soft tissues.

CONCLUSION

Mitomycin C does not affect the microvascular anastomoses adversely so it can be used safely in patients who will undergo a free flap operation.

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