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THE EFFECT OF ATORVASTATIN ON ANGIOGENESIS AND THE QUALITY OF PANNICULUS CARNOSUS IN RAT COMPOSITE GRAFTS

ATORVASTATİNİN SIÇAN KOMPOZİT GREFTLERİNDE ANJİYOGENEZİS VE KAS KALINLIĞI ÜZERİNE ETKİLERİ

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ABSTRACT

The grafts used for surgical reconstruction require vascularization (angiogenesis) for tissue survival. Inadequate blood supply can limit the size and the thickness of the composite grafts. As the agents with angiogenic properties can increase the survival of grafts, a study was planned to assess the effect of atorvastatin (an agent with angiogenic potential) on the survival of composite myocutaneous skin graft in rats.

Twenty-eight male Wistar rats 14 in the study group and 14 in the control group were operated by taking 2x3 cm composite myocutaneous skin grafts including the panniculus carnosus muscle. The rats in the study group were given 10 mg/kg/day atorvastatin orally and the control group was given the same amount of serum physiological. Biopsies were taken from the grafts as including 5 mm margins of normal skin on the 5^{th} and 10^{th} days. Qualitative and quantitative analysis of the biopsies were performed.

Although there was not a significant difference between the study and the control groups regarding the microvascular density counts, there was a significant decrease in the microvascular density counts of the control group between the 5^{th} and the 10^{th} days ($P<0.05$). There was a significant difference in muscle thickness in the atorvastatin group on the 5^{th} day ($p < 0.05$).

As the microvascular density counts were stable in the study group compared with the significant decrease in the control group, atorvastatin may have a role in either angiogenesis or keeping the vascular structures stable and therefore may have a role in increased tissue survival.

Keywords: Angiogenesis, Atorvastatin, Composite graft

ÖZET

Cerrahi rekonstrüksiyon için kullanılan greftlerin sağkalımı için vaskülarizasyon gerekmektedir. Greft sağkalımını arttırmak için anjiyojenik ajanlar kullanılabilmektedir. Çalışmamızda da sıçanların kompozit cilt-kas greftinin sağkalımında Atorvastatin'in etkileri incelenmiştir.

Yirmi sekiz adet Wistar cinsi sıçanın 14'ü çalışma grubunda, 14'ü kontrol grubunda olacak şekilde ayrılmış olup her birinden 2x3 cm pannikulus karnozus kasını da içeren kompozit cilt-kas greftleri alınmıştır. Çalışma grubunda yer alan sıçanlara oral yoldan her gün 10 mg/kg Atorvastatin, kontrol grubundakilere ise aynı oranda serum fizyolojik verilmiştir. Greftlerden 5. ve 10. günlerde biyopsi alınıp nitelikli ve nicelikli analizler yapılmıştır.

Kontrol grubunda, mikrovasküler yoğunluk sayımında 5. ve 10. günler arasında anlamlı bir düşüş saptanmıştır ($P<0.05$). Atorvastatin grubunda ise kas kalınlığında 5. günde anlamlı farklılık saptanmıştır ($p < 0.05$).

Çalışma grubunda mikrovasküler yoğunluk sayımı stabil iken kontrol grubunda ise anlamlı düşüş mevcuttur. Bu sonuca dayanarak Atorvastatin'in anjiyogeneziste veya vasküler yapıları stabil tutmada rolü olabileceği, bununla beraber doku sağkalımını arttırmada rolü olabileceği saptanmıştır.

Anahtar sözcükler: Anjiyogenezis, Atorvastatin, Kompozit greft

GİRİŞ

Composite graft is a tissue graft composed of more than one kind of tissue. As the skin and cartilage harvested from the ear for alar rim defects is most popular, composite grafts of skin and fat or dermis and fat are being used for different indications in reconstructive procedures.[1-3]

The drawback in using composite grafts is that they can be used in limited dimensions usually up to 1-1,5 cm. Their survival is limited because the process of imbibition and inosculation must occur from the very narrow wound edges.[4] Inadequate blood supply can limit tissue graft size and thickness. Accelerated and enhanced vascularization would provide benefit for all types of reconstructive procedures.

Many studies have been performed to try to improve composite graft survival.[5-7] Corticosteroids, dimetyhyl sulfoxide, dimethyl thiourea, melatonin, in-

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domethacin, fibroblast growth factor, chlorpromazine hydrochloride and hyperbaric oxygen therapy have been used to try to increase survival.[4]

Statins have pleiotropic effects independent of their cholesterol-lowering effects.8,9 They have strong vascular protective effects[10] and they induce a strong pro-angiogenic effect.[11] In a study evaluating the effects of atorvastatin on angiogenesis in hind limb ischemia in rats, it was found that atorvastatin strongly induced angiogenesis with increases in angiogenic cytokines and it could be considered as a potential agent for therapeutic angiogenesis.[12]

A study was planned to evaluate the effect of atorvastatin (an agent with angiogenic potential) on the survival of composite myocutaneous graft in rats. To demonstrate a functional benefit of atorvastatin, the composite myocutaneous grafts were chosen to provide a graft type with expected limited survival resulting from the increased thickness of the avascular composite graft (the inclusion of the panniculus carnosus layer).

MATERIAL AND METHODS

The study protocol was approved by the Marmara University Animal Studies Ethical Committee prior to commencement of the study. Twenty-eight male Wistar albino rats weighing between 300-325 g were used, 14 being the study the group and 14 the control group. The following surgical procedure was applied to all of the rats. The dorsum of the rats were shaved and an acetate template was used to mark out the sites of a 2x3 cm graft. The craniomedial corner was located 1 cm caudal to the scapular tip and 1 cm lateral to the spinal column over the posterior thorax. The graft including full thickness skin and panniculus carnosus muscle was harvested and after rotating the graft 180 degrees, the graft was set into the same location from which it was harvested. A continuous 4-0 suture was used to secure the grafts. A bolster tie over dressing was used. The rats were housed individually at ambient room temperature and provided with adequate water and laboratory chow postoperatively. Rats in the study group received 10 mg/kg/day atorvastatin in a 0.5 ml solution via a feeding tube per orally from the first day for 10 days. The control group received 0.5 ml serum physiologic daily via a feeding tube for 10 days to put the animals through same stress conditions. On the 5th postoperative day, 7 rats from the study group and 7 rats from the control group were selected randomly, euthanized, their dressings were removed, digital photographs of the grafts were taken and tissue orientation was marked. The tissue was harvested for histological sectioning with 5 mm circumferential border of normal skin and deep fascia and dorsal musculature were included in the harvested tissue. On the 10th postoperative day, the same procedure was applied to the remaining rats.

The qualitative and quantitative graft survival was assessed based on hematoxylin and eosin staining. To quantify angiogenesis, microvascular density counts were performed on factor VIII immunohistochemical stained samples. The qualitative appearance and the thickness of Panniculus carnosus muscle were evaluated on hematoxylin and eosin stained sections.

The results obtained were evaluated and the statistical analysis was performed using the SPSS program (version 17). One way ANOVAs test was used and the significance level was set at $p < 0.05$.

RESULTS

Variable amounts of graft taking have been noticed among the study and control groups clinically (Figure 1). Although there was higher density of blood vessels on the factor VIII-stained histological sections of study group compared to the control group (Figure 2), the difference was not statistically significant. When each group was compared between the 5th and the 10th days, there was not a significant difference between the microvessel density counts of the study group, but there was a sharp decrease in microvessel density counts of the control group which was statistically significant ($p < 0.05$) (Figure 3). Panniculus carnosus muscle was thinner, atrophic in some areas and consistent with poor health in the control group. However, the muscle was thicker and displayed normal muscle architecture in the study group (Figure 4). Quantitatively, the panniculus carnosus was significantly thicker for the grafts treated with atorvastatin compared to control group on the 5th postoperative day ($p < 0.05$) (Figure 5). No significant difference was seen in all other group comparisons.

DISCUSSION

The objective of this study was to evaluate the effect of atorvastatin on the survival of composite grafts. Graft models are pertinent to the study of angiogenesis and wound healing, since graft survival is ultimately dependent on angiogenesis. Autologous skin graft models have been used to evaluate the effects of a wide array of wound healing interventions. In this study, composite myocutaneous grafts were used to create a model with predictably poor survival rates. Because of the thick nature of the composite myocutaneous grafts, very little of the dermis survives (without intervention) and the epidermis sloughs off completely for many grafts.[13]

A thick myocutaneous graft was used to demonstrate the effect of atorvastatin on angiogenesis. Unlike flap models, the graft taking is fully dependent on production of new vessels from the graft bed. Removing panniculus carnosus could definitely improve graft survival, but the thin grafts can survive even without increased angiogenesis.[4]

Many studies have been conducted to try to improve composite graft survival. But there is no report

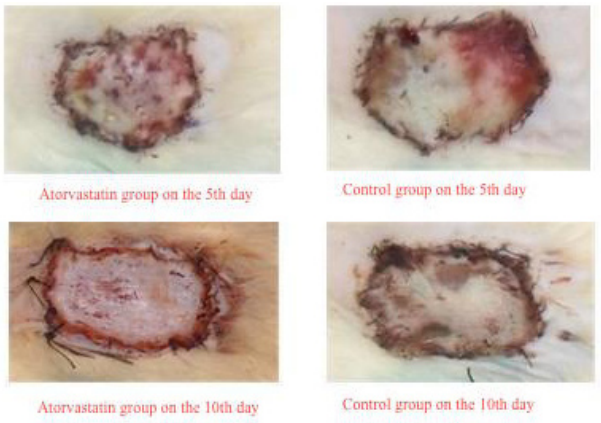

Figure 1. Graft take in Atorvastatin receiving group on the 5th day (above left), control group on the 5th day (above right), atorvastatin group on the 10th day (below left), control group on the 10th day (below right)

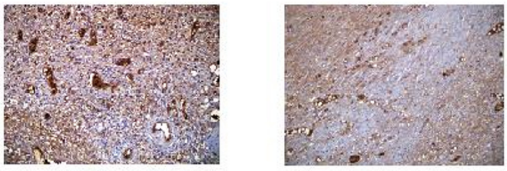

Figure 2. Histological sections (x100) showing factor VIII –stained microvessels on the 5th day; Atorvastatin receiving group (left) control group (right) arrows showing the newly formed vessels

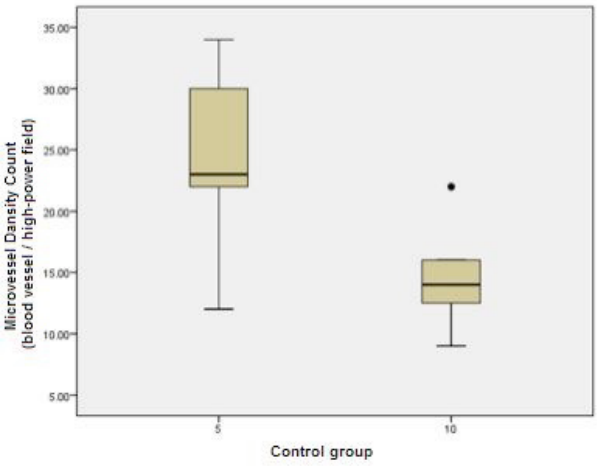

Figure 3. Comparison of Microvessel density counts of the control group statistical significance ($p < 0.05$)

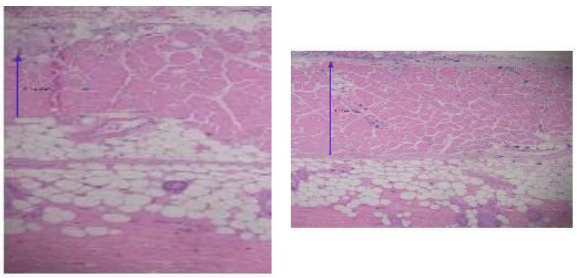

Figure 4. Hematoxylin and eosin-stained (x100) composite myocutaneous grafts on the 5th day of control group (left), atorvastatin group (right)

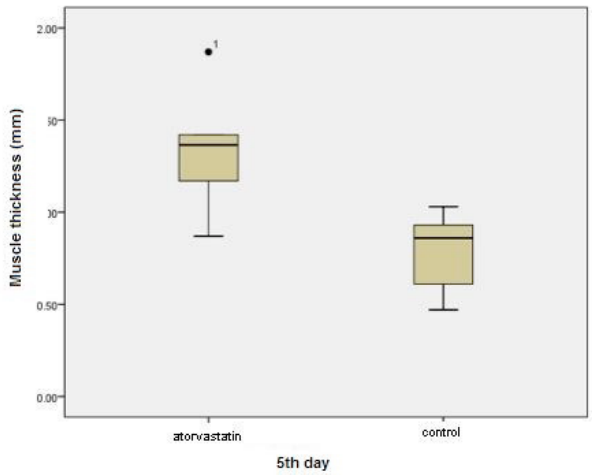

Figure 5. Panniculus carnosus muscle thickness on the 5th day statistical significance ($p < 0.05$)

about the possible benefits of atorvastatin on composite graft survival.

Statins have pleiotropic effects independent of their cholesterol-lowering effects. A large-scale clinical examination (ASCOT-LLA) showed that statins have strong vascular protective effects.[11] Statins also induce strong pro-angiogenic effects. Various studies have also been performed to demonstrate the effect of atorvastatin on angiogenesis. In 2009, Matsumura et al. published an experimental study in rats, in which the effects of atorvastatin on angiogenesis in hind limb ischemia and endothelial progenitor cell formation was evaluated. They concluded that atorvastatin strongly induced angiogenesis with increases in angiogenic cytokines, hemoxidase (HO)-1, nitric oxide synthase (eNOS) and endothelial progenitor cell (EPCs) numbers. Also in their study, they found that low-dose (10 mg/kg) atorvastatin, but not a high-dose (30 mg/kg), increased regional blood flow in ischemic hind limbs.[12] For this reason 10mg/kg/day was chosen as the atorvastatin dose that has been given to the rats in the study group.

In the present study, although there was not a significant difference between the microvascular density counts of the study and the control groups, there was a significant decrease between the 5th and the 10th day regarding the microvascular density counts of the control group ($p < 0.05$). This may be the result of vascular protective effects of atorvastatin.

On the 5th day, there was a significant difference in muscle thickness in the atorvastatin group ($p < 0.05$). The panniculus carnosus muscle within the composite myocutaneous graft was more viable histologically. In angiogenesis studies, it is not always clear that vessels are functional. The new vessels may be leaky and their network may be disordered.[14] But it is very meaningful to see more viable muscular tissue since grafted muscle is not expected to survive, because of its poor ischemic

tolerance time.[15]

This is a preliminary study and results of survival in different graft sizes could be compared. Also different atorvastatin doses could be used to evaluate the effects on angiogenesis in subsequent experiments.

CONCLUSION

Atorvastatin, a commonly used statin, has a known effect in increasing angiogenesis and keeping the vascular structures stable and therefore it may have a role in increased tissue survival. Positive benefits of atorvastatin were found in this study regarding composite graft survival. Because of its effect on augmenting vascularization, it can be studied for tissue survival in other areas of wound healing.

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REFERENCES

1. Hubbard TJ. Leave the Fat, Skip the Bolster: Thinking outside the Box in Lower Third Nasal Reconstruction. Plast Reconstr Surg Nov 2004;114:1427-35.
2. Adams DC, Ramsey ML. Grafts in dermatologic surgery: review and update on full- and split-thickness skin grafts, free cartilage grafts, and composite grafts. Dermatol Surg Aug 2005; 31(8 Pt 2):1055-67.
3. Patel IA, Hall PN. Free dermis-fat graft to correct the whistle deformity in patients with cleft lip. Br J Plast Surg Mar 2004;57(2):160-4.
4. Li EN, Menon NG, Rodriguez ED, Norkunas M, Rosenthal RE, Goldberg NH, Silverman RP. The Effect of Hyperbaric Oxygen Therapy on Composite Graft Survival. Annals of Plastic surgery Aug 2004;53(2):141-5.
5. Hartman DF,Goode RL. Pharmacologic enhancement of composite graft survival. Arch Otolaryngol Head Neck Surg 1987;113:720-3.
6. Aden KK, Biel MA. The evaluation of pharmacologic agents on composite graft survival. Arch Otolaryngol Head Neck Surg 1992;118:175-8.
7. Fann PC, Hartman DF, Goode RL. Pharmacologic and surgical enhancement of composite graft survival. Arch Otolaryngol Head and Neck Surg 1993;119:313-9.
8. Stancu C, Sima A. Statins: Mechanism of action and effects. J Cell Mol Med 2001;5:378-87.
9. Morikawa S, Takabe W, Mataki C, et al. Global analysis of RNA expression profile in human vascular cells treated with statins. J Atheroscler Thromb, 2004;11:62-72.
10. Sever PS, Dahlöf B, Poulter NR, et al. ABCOT investigators: Prevention of coronary stroke events with atorvastatin in hypertensive patient who have average or lower than average cholesterol concentrations, in the Anglo-Scandinavian cardiac outcomes trial-Lipid Lowering arm (ASCOT-JLA): a multicenter randomized controlled trail. Lancet 2003; 361; 1149-1158.
11. Dulak J, Loboda A, Jazwa A, et al. Atorvastatin affects several angiogenic mediators in human endothelial cells. Endothelium 2005;12:233-41.
12. Matsumura M, Fukuda N, Kobayashi N, et al. Effects of Atorvastatin on Angiogenesis in Hindlimb Ischemia and Endothelial Progenitor cell formation in Rats. J Atherosclerosis and Thrombosis;16(4):319-26.
13. Eckhaus AA, Fish JS, Skarja G, Semple JL, Sefton MV. A preliminary study of the effect of poly (methacrylic acid-co methyl methacrylate) beads on angiogenesis in rodent skin grafts and the quality of the panniculus carnosus. Plast Reconstr Surg Nov 2008;122(5):1361-70.
14. Jain RK. Molecular regulation of vessel maturation. Nat Med 2003;685.
15. Blaisdell FW. The pathophysiology of skeletal muscle ischemia and the reperfusion syndrome: A review. Cardiovasc Surg 2002;10:620.