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Araştırma Makalesi / Research Article

The Effect of Topical Atorvastatin on Viability of Random Pattern Skin Flaps, Experimental Study

Topikal Atorvastatinin Random Paternli Cilt Fleplerinin Yaşayabilirliğine Etkisi, Deneysel Çalışma

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Abstract

Augmentation of viability of random pattern flaps has long been an issue in plastic surgery. Up to date no agents were clinically introduced. Atorvastatins are used clinically for lipid lowering. They are also effective on flap survival with their angiogenic, anti-inflammatory, antioxidant effects with systeic administration. In this study, Atorvastatin is used topically for flap survival alleviation in Mc Farlane flap in rats. Wibstar albino type 20 male rats weighing about 180-230 grams were classified as experiment and control group. Results were evaluated by flap necrosis ratios, lymphocyte cells, neutrophile cells, capillary and granulation tissue density on the 7th postoperative day.. Flap necrosis areas were evaluated with Sasaki's paratemplate method. Tissue biopsies of 1x1 cm at the transition zone between necrotic and healthy tissue, were embedded in parafin blocks after fixation in 10% formalin. Biopsies were sliced by 4 micrometer thickness with a microtome. Cross sections were painted with hematoxylene eosin and evaluated with a light microscope. Whitneyy U test was performed for clinical and histopathological evaluation of groups ($p<0,05$). Flap viability was alleviated. Average necrosis ratios on flaps were 32.1% in the control group and 14.17% in experimental group. Capillary tissue and neutrophile cell density were found to be higher in atorvastatin group. . Granulation tissue and lymphocyte cell density were not found significantly higher.. Atorvastatins, when applied topically, are effective on flap survival. Further studies should be carried out for human clinical use.

Keywords: Flap, survival, Atorvastatin, topically , angiogenesis

Özet

Random fleplerin yaşayabilirliğinin arttırılması plastik cerrahide,süregelen araştırmalara konu olmuştur. Bir çok ajan denenmiş ancak çoğu klinik kullanıma girememiştir. Atorvastatinler lipid düşürücü olarak kullanılmaktadır. Ayrıca , sistemik uygulama ile flep yaşayabilirliğini , arttırdıkları deneysel olarak gösterilmiştir. Bu çalışmada atorvastatinin , topikal uygulama ile flep yaşayabilirliğine etkisi araştırılmıştır. 180-230 gram ağırlığında wibstor albino tipi , 20 erkek rat kontrol ve deney grubu olarak ayrılmıştır. Her bir grupta , Mc Farlane flebi eleve edilmiş , deney grubunda flep üzerine, hazırlanan atorvastatin krem uygulanırken;kontrol grubu herhangi bir tedavi almamıştır. İşlemden 7 gün sonra flep sağlıklı ve nekrotik doku geçiş zonundan 4 mm' lik kesitler halinde doku örnekleri alınarak ,hematoksilen eozin ile boyanmış ve ışık mikroskobu altında lenfosit hücre yoğunluğu, notrofil hücre yoğunluğu , kapiller dansite ve granulasyon dokusu dansitesi açısından incelenmiştir. Fleplerdeki nekroz alanları sasaki kağıdı metodu ile işaretlenmiş, işaretleme AUTOCAT 2012 programına aktarılarak fleplerdeki nekroz oranları bulunmuştur. Histolojik parametlerin ve fleplerdeki nekroz oranlarının karşılaştırılması için Mann Whitney U testi kullanılmıştır ($p<0,05$) Çalışmaya göre deney grubunda notrofil hücre yoğunluğu ve kapiller dansite artmış, granulasyon dokusu dansitesi ve lenfosit hücre yoğunluğu ise değişmemiştir. Flep nekroz oranının atorvastatin uygulanan grupta azaldığı gösterilmiştir. Flep nekroz oranı kontrol grubunda 32,1% iken deney grubunda 14,17% olarak bulunmuştur .Ek çalışmalara ihtiyaç olmakla birlikte , atorvastatin topikal olarak kullanım ile flep yaşayabilirliğini arttırmaktadır.

Anahtar Kelimeler: flep;yaşayabilirlik;atorvastatin;topikal;anjiyogenez

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1. Introduction

Augmentaion of viability of random flaps has been researched for a long time. Numerous agents were tried as a better understanding of flap physiology and loss. As there are good results with most of them, no agent was introduced clinically because of the need for systemic administration and possible side effects.

Atorvastatins are used for lowering lipids [1]. After they are proved to lower the risk of cardiac attack free of their lipid lowering effect, attentions are drawn to statins pleiotrophic effects [2]. They are thought to be effective on flap survival after showing their angiogenic effects over VEGF and IL-8 [3-4,5], anti-inflammatory[6,7,8] and antioxidant[9,10,11], endothelial vasodilatation [12,13].

Yang et al have shown that treatment of atorvastatin improved skin flap blood perfusion, vascular density and necrotic area. They have shown that this happens by angioneogenesis and VEGF mRNA expression with the use of atorvastatin [14].

In this study we studied the effect of atorvastatin's topical administration on flap survival by measuring flap necrosis ratios and comparing capillary density and granulation tissue in histopathologic samples. We also studied the effects of atorvastatin's anti-inflammatory effect on flap viability.

2. Materials and Methods

This study was performed in Research and Experiment Laboratory of Plastic Aesthetic and Reconstructive Surgery Clinic in İzmir Atatürk Training and Research Hospital . Study was approved for ethical issues by Ege University Experimental Animals Local Ethical Board. Wibstar albino type 20 male rats weighing about 180-230 grams were classified as experiment and control group. All animals were kept at between 18-20° C with 12 hours shift of light and darkness as standard. Every animal was kept alone at one cage for prevention of mutual damaging. All rats were fed with standard rat food and tap water. Anesthesia was performed with 30 mg/kg ketamine added 0.5 mg/kg basilasin through intramuscular route. Mc Farlane's rectangular 3x9 cm flap model was elevated and sutured back at the original place for both groups. Atorvastatin (Kolestor ®) Sanofi Company, İstanbul, Turkey tablet of 40 mg was dissolved in hot water and mixed with appropiate amount of lanolin and vaselin in order to make topical form[15]. Experimental group was applied 1% topical atorvastatin cream whereas control group received no treatment.

Flap necrosis areas were evaluated with Sasaki's paratemplate method on postoperative 7th day and flap viability rates were calculated. Flap dimensions and necrosis line were copied on transparent acetate paper with an acetate pen. Area of flap and necrosis were placed on milimetric scale and calculated. Their ratios were established as percentage(%). Same calculations were graphically produced with Autocad 2012 program. (figure 1a,b)

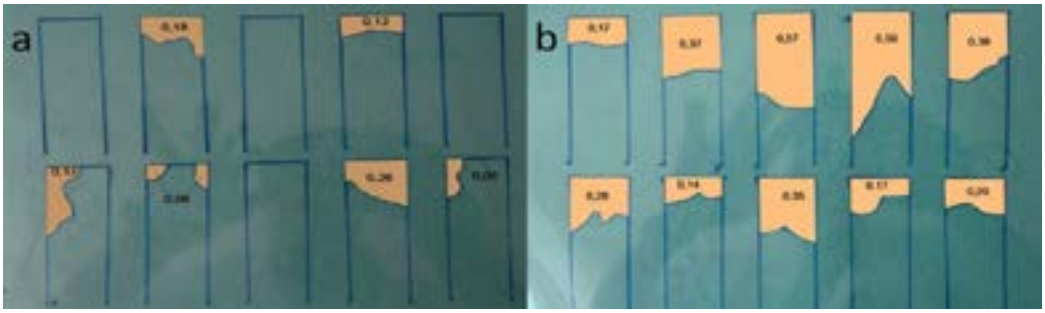


Figure 1.necrotic areas shown in autocad 2012
a)control group b) experiment group

Tissue biopsies of 1x1 cm , on the 7th postoperative day at the transition zone between necrotic and healthy tissue, were embeded in parafin blocks after fixation in 10% formalin. Biopsies were sliced by 4 micrometer thickness with a microtome. Cross sections were painted with hematoxylene eosin and evaluated with a light microscope (figure 2a,b). Evaluation was carried out on four parameters as lymphocyte density, neutrophile density, cappillary and granulation tissue density. Pathologic results were scored. (table 1) Data analysis was performed using IBM SPSS Statistics version 17.0 software (IBM Corporation, Armonk, NY, USA). Descriptive statistics were expressed as mean±SD. Whether the differences in flap necrosis ratio and histopathological density scores between control and study groups were statistically significant or not was evaluated Mann Whitney U test. A p value less than 0.05 was considered statistically significant.

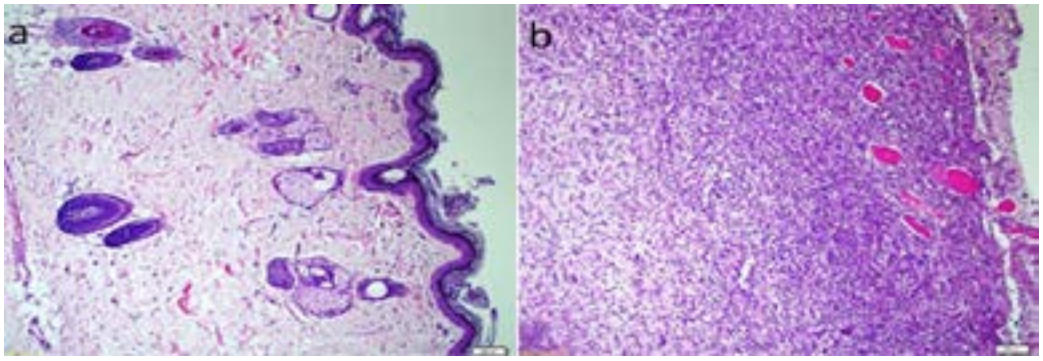


Figure 2.histopathologic tissue samples with light microscobe, hematoxylene eosin, , 40X
a)control group b) experiment group

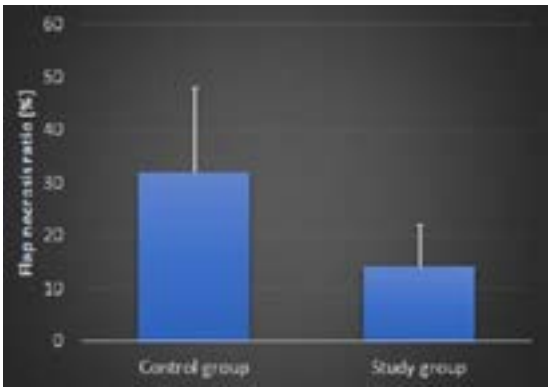
Table 1. The comparisons between control and study groups in terms of histopathological assessment

Density of	Control group	Study group	p-value †
Lymphocyte	1.40±0.52	1.70±0.48	0.189
PMNL	0.00±0.00	1.50±1.27	**0.002**
Capillary	1.10±0.32	1.90±0.74	**0.007**
Granulation	1.10±1.29	2.00±1.05	0.108

† Mann Whitney U test, p<0.05 was considered statistically significant.

3. Results

Average necrosis ratios on flaps were 32.1% in the control group and 14.17% in experimental group. Depending on this data there is statistically significant difference between necrosis ratios in control and experiment group. Flap viability is observed to be alleviated in atorvastatin applied group(p<0,05) (graphic 1)



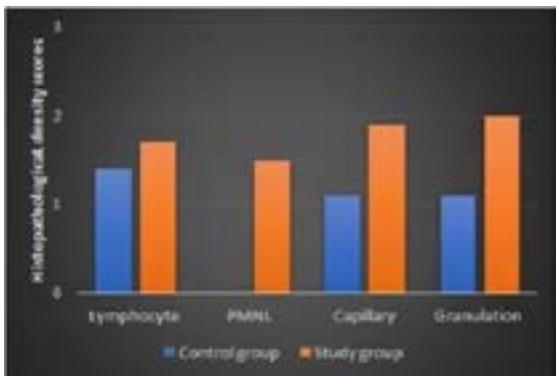
Graphic 1. Comparison of flap necrosis ratio between cases and controls. The top borders of each bar indicate the arithmetic mean of flap necrosis ratio, while the whiskers above of the bar mark plus standard deviation (i.e., +1SD).

Average lymphocyte results were calculated as 1,40±0,52 in control group and 1,70±0,48 in experiment group with scoring values of 0-3 for n=20. These results were not statistically significant($p<0,05$). Depending on this, atorvastatin cream is not shown to decrease lymphocyte density (graphic 2).

Average PMNL results were calculated as 0,00±0,00 in control group and 1,50±1,27 in experiment group with scoring values of 0-3 for n=20.These results were statistically significant($p<0,05$) (graphic 2). Depending on this, neutrophile cell density in experiment group is found to be higher than control group.

Average capillary density results were calculated as 1,10±0,32 in control group and 1,90±0,74 in experiment group with scoring values of 0-3 for n=20 . These results were statistically significant($p<0,05$). Depending on this, capillary density in experiment group is found to be higher than control group (graphic 2).

Average granulation density results were calculated as 1,10±1,29 in control group and 2,00±1,05 in experiment group with scoring values of 0-3 for n=20. These results were not statistically significant($p<0,05$). Depending on this, atorvastatin cream is not shown to make a significant difference in granulation density (graphic 2) .



Graphic 2. Comparison of histopathological density scores between cases and controls. The top borders of each bar indicate the arithmetic mean of histopathological density scores.

4. Discussion

Random pattern skin flaps, although use of microsurgery is developing, are still widely used in plastic surgery. But distal flap necrosis is an important problem and causes additional operations, elongated hospital stay, and loss of surgeon and patient motivation.

The major factor in random pattern viability is the blood flow to the flap. With better understanding of flap physiology, experimental agents affecting flap loss in different stages were succesful.

Vasodilatators like sildenafil citrate[16], calcium channel blockers like nifedipin [17] increased blood flow to the flap by dilating the vascular network. Antioxidants [18,19], anti-inflammatory agents[20], hyaluronic acid[21] decreased distal flap necrosis experimentally.

Because of their need for systemic administration and possible side effects, none of the agents could find clinical use.

The most dependable way of flap perfusion augmentation is flap delay but this requires double operations which constitutes its greatest disadvantage.

Hydrodissection [22], tens application[23], microneedling with dermaroller [24] have shown to increase flap perfusion experimentally and they also need no systemic drug use.

Studies with statins have shown that they increased proangiogenetic growth factors. Statins applied in low doses as nanomolar concentrations to human dermovascular cells have shown to increase VEGF. When applied in higher doses this increase was not observed and reported that this effect comes out in biphasic fashion[4].

Yang et al have shown systemic administration of statins have alleviated flap viability[14]. In this study, VEGF mRNA expression was significantly elevated in atorvastatin receiving group. Additionally flap perfusion was found to be higher than control group with doppler ultrasonography on postoperative 30th minute, 4th and 7th days. Capillary density in histopathologic examination was found to be higher in control group.

Significant decrease in flap ischemia with the use of atorvastatin is shown in our study. Flap loss was 32,1% in control group whereas 14,17% in experimental group with atorvastatin(graphic 1). Atorvastatin increases NO emission by direct effect on NOS in early phase and this effect comes out even at ischemic situations[2,12,13]. Elevated NO causes capillary vasodilatation. Statins also increases angiogenic affecting VEGF [3,4,5]. Flap's vascular network is reorganised with capillary dilatation by direct effect of NO and capillary proliferation by VEGF. We observed this effect in our study as increase in capillary density. Capillary density rates with histopathologic examination were found to be significantly higher in experiment group with 1,9 average whereas control group with 1,1 (Table 1) . There is only one study showing the effects of topical statins on capillary density. Toker et al applied topical atorvastatin creams of 1% and 5% concentration on diabetic rat wounds in 2009. They observed that the rates of wound healing were found to be significantly higher in the diabetic rat groups administered 1% and 5% atorvastatin compared with those administered a mixture of lanolin-vaseline and the untreated group. atorvastatin application increases capillary density significantly in both groups compared to control group with no treatment, they also concluded concentrations of 1% and 5% were not significantly different for capillary density[15].

Granulation tissue is a sign, showing that a open wound can be reconstructed, in wound healing. Main ingredients of granulation tissue are fibronectin, hyaluronic acid, macrophages embedded in loose matrix formed by collagen, fibroblasts and dense capillary network. This dense network gives granulation tissue its typical bright red colour. In our study we observed that granulation tissue density was increased in experimental group. Histopathological examination of tissues revealed average density for 1,1 for control group and 2,0 for experiment group although

this was not significantly different($p<0,05$) (table 1). Our study showed statins increased vascular component of granulation tissue.

Capillary density rates was found to be higher in experiment group (graphic 2). Extra studies can be performed to observe the effect of statins on other ingredients of extracellular matrix and cellular elements so that effect of statins on granulation tissue can be explained clearly.

Statins have anti-inflammatory effects. Mechanism for anti-inflammatory effect was explained as inhibition of cytokine synhtesis , neutrophile adhesion and ICAM 1 synthesis and shown to be dose dependent[6,7,8]. There are studies showing anti-inflammatory agents increasing flap viability[20]. Main issue is inhibition of cells and mediators responsible for postishemic reperfusion damage. In our study we observed neutrophile cells are significantly higher in experimental group with atorvastatin than the control group(graphic 4). Lymphocyte density was also higher in experiment group but that wasn't significantly different($p<0,05$) (table 1)

Atorvastatin's cream form was used for a week in our study. But studies performed for observing anti-inflammatory effects were at least two weeks long[6,7,8]. Additionally in a study with statins at MS disease, it was shown in order to observe anti-inflammatory effects, doses at least twenty times higher than lipid lowering doses should be administered[25]. In our study we think statins' anti-inflammatory effects have no role in flap viability because of short time and low doses.

Atorvastatin can be applied transdermally beacuse it doesn't need to pass from liver to get activated. Our experiment is a prestudy, in order to get into clinical use absorption differences between species should be noted. Most dependable results for transdermal application can be observed with human experiments. But human transdermal absorbtion varies greatly individually, even between body zones applied on the same individual. This also makes it harder to standardise the process. Rats show less individual differences in transdermal absorption than humans, also rat skin shows similarity to human skin. Both makes rat skin first choice for topical agent experiments[26]. Generally rat skin is twice permeable than human skin. This is explained with membrane lipophilicity, dermal and epidermal thickness, and surface hair follicle density[27].

Doses used on rats can make a prediction for human doses. But individual absorption differences makes unpredicted efficiency, which is a major disadvantage for topical use

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