PAPER DETAILS

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

Study

AUTHORS: Bilgen CAN, Okan Özgür ÖZTURAN, Meltem ORAL, Sadi BENER

PAGES: 396-402

ORIGINAL PDF URL: https://dergipark.org.tr/tr/download/article-file/1503101

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

¹Bilgen Can, ² Okan Ozgur Ozturan , ³Meltem Oral ,⁴Sadi Bener

¹Balıkesir University, Department of Plastic, Reconstructive and Aesthetic Surgery , Balıkesir, Turkey

²Özturan Plastic Reconstructive and Aesthetic Surgery Specialist, Private Clinic, Cankaya, Ankara

³Izmir Katip Celebi University, Ataturk Training and Research Hospital, Department of Plastic Reconstructive and Aesthetic Surgery Clinic, Izmir, Turkey

⁴Izmir Katip Celebi University, Atatürk Training and Research Hospital, Department of Pathology, Izmir, Turkey 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 sağılıklı 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 oranı kontrol grubunda 32,1% iken deney grubunda 14,17% olarak bulunmuştur. Ek çalışmalara ihtiyaç olmakla birlikte , atorvastatin topikal olarak kullanımı karkıları.

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

Received 11.01.2021 Accepted 31.03.2021 Online published 31.03.2021

Cite this article as:

Can B, Ozturan OO, Oral M , Bener S, The Effect of Topical Atorvastatin on Viability of Random Pattern Skin Flaps, Experimental Study, Osmangazi Journal of Medicine, 2021;43(4):396- 402 Doi: 10.20515/otd.858659

Correspondence: Bilgen CAN Balıkesir University, Department of Plastic, Reconstructive and Aesthetic

Surgery , Balıkesir, Turkey e-mail: bdenizag@gmail.com

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 antiinflammatory 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 form¹⁵. Experimental group was applied 1% topical atorvastatin cream whereas control group received no treatment.

Flap necrosis areas were evaluated with paratemplate Sasaki's 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 co	mparisons	between	control	and a	study	groups	s in t	erms o	of histo	patholog	gical	assessment
												,	

Density of	Control group	Study group	p-value †
Lymphocyte	1.40±0.52	$1.70{\pm}0.48$	0.189
PMNL	$0.00{\pm}0.00$	1.50 ± 1.27	0.002
Capillary	1.10±0.32	$1.90{\pm}0.74$	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 $1,40\pm0,52$ in control group and $1,70\pm0,48$ 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 $0,00\pm0,00$ in control group and $1,50\pm1,27$ 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 $1,10\pm0,32$ in control group and $1,90\pm0,74$ 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 $1,10\pm1,29$ in control group and $2,00\pm1,05$ 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 citrate¹⁶, calcium channel blockers like nifedipin ¹⁷ increased blood flow to the flap by dilating the vascular network. Antioxidants ^{18,19}, anti-inflammatory agents²⁰, hyaluronic acid²¹ 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 application²³, 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 fashion⁴.

Yang et al have shown systemic administration of statins have alleviated flap viability¹⁴. 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 in experimental group with 14.17% 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 density¹⁵.

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 viability²⁰. 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 than control with atorvastatin the 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 administered²⁵. 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 differences individual in transdermal absorption than humans, also rat skin shows similarity to human skin. Both makes rat skin first choice for topical agent experiments²⁶. 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

REFERENCES

- 1. Katzung BG. Basic and clinical pharmacology. Norwalk, *Appleton and Lange*; 1995
- Liao JK, Laufs U. Pleiotropic Effects of Statins. NIH Public Access. 2005;45:89-118
- 3. Dulak J, Loboda A, Jazwa A, et al. Atorvastatin Affects Several Angiogenic Mediators in Human Endothelial Cells. *Endothelium.* 2005 ; 12: 233–41
- 4. Weis M, Heeschen C, Glassford AJ, et al. Statins have biphasic effects on angiogenesis. *Circulation*. 2002;105:739-45.
- Karamouzian S, Eskandary H, Saeed A, et al. Effect of atorvastatin on angiogenesis in degenerated intervertebral disc in rat. *Spine* (Phila Pa 1976).2011;36:1824-8.
- Macin SM, Perna ER, Farías EF, et al. Atorvastatin has an important acute antiinflammatory effect in patients with acute coronary syndrome: results of a randomized, double-blind, placebo-controlled study. *Am Heart* J. 2005;149:451-7

- 7. Liu M, Wang F, Wang Y, et al. Atorvastatin improves endothelial function and cardiac performance in patients with dilated cardiomyopathy: the role of inflammation. *Cardiovasc Drugs Ther.* 2009;23:369-76
- Barsante MM, Roffê E, Yokoro CM, et al. Antiinflammatory and analgesic effects of atorvastatin in a rat model of adjuvant-induced arthritis. *Eur J Pharmacol.* 2005 15;516:282-9.
- 9. Cangemi R, Loffredo L, Carnevale R, et al. Early decrease of oxidative stress by atorvastatin in hypercholesterolaemic patients: effect on circulating vitamin E. *Eur Heart J.* 2008;29:54-62.
- Riad A, Du J, Stiehl S, et al. Low-dose treatment with atorvastatin leads to anti-oxidative and antiinflammatory effects in diabetes mellitus. *Eur J Pharmacol.* 2007;569:204-11
- 11. Hamilton PK, Hughes SM, Plumb RD, et al. Statins have beneficial effects on platelet free radical activity and intracellular distribution of

GTPases in hyperlipidaemia. *Clin Sci* (Lond). 2010;118:359-66.

- Hernández-Perera O, Pérez-Sala D, Navarro-Antolín J, et al. Effects of the 3-hydroxy-3methylglutaryl-CoA reductase inhibitors, atorvastatin and simvastatin, on the expression of endothelin-1 and endothelial nitric oxide synthase in vascular endothelial cells. J Clin Invest. 1998;101:2711-9.
- Endres M, Laufs U, Huang Z, et al. Stroke protection by 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase inhibitors mediated by endothelial nitric oxide synthase. *Proc Natl Acad* .1998;95:8880-5
- 14. S.-C. Yang Atorvastatin alleviated viability of rat ischemic flap dependent of VEGF m RNA expression Plastic & Reconstructive Surgery. 128 Supplement 2S, Third Annual European Plastic Surgery Research Council Abstract Supplement:620, August 2011
- 15. Toker S, Gulcan E, Cayc MK, et al. Topical atorvastatin in the treatment of diabetic wounds. *Am J Med Sci.* 2009;338:201-4.
- 16. Hart K, Baur D, Hodam J, et al. Short- and longterm effects of sildenafil on skin flap survival in rats. *Laryngoscope*. 2006;116:522-8.
- 17. Emery FM, Kodey TR, Bomberger RA, et al. The effect of nifedipine on skin-flap survival. *Plast Reconstr Surg.* 1990;85:61-3.
- Green CJ, Dhami L, Prasad S, Healing G, Shurey C. The effect of desferrioxamine on lipid peroxidation and survival of ischaemic island skin flaps in rats. *Br J Plast Surg.* 1989;42:565-9.
- Kargi E, Babuccu O, Hoşnuter M, et al. The effect of combined use of vitamin C, vitamin E, and ibuprofen on flap viability: an experimental study]. *Kulak Burun Bogaz Ihtis Derg.* 2005;14:116-20
- Senderoff DM, Israeli D, Zhang WX, et al. Iloprost improves survival of ischemic experimental skin flaps. *Ann Plast Surg.* 1994;32:490-5
- Shalom A, Hadad E, Friedman T, et al. Effect of hyaluronic acid on random-pattern flaps in rats. *Dermatol Surg.* 2008;34:1212-5.
- 22. Sahin C, Aysal BK, Ergun O. Is It Possible to Increase Flap Viability by Hydrostatic Dilation?: An Experimental Study in the Rat Abdominal Fasciocutaneous Flap Model. *Ann Plast Surg.* 2016;77:26-30
- 23. Özyazgan İ, Baykan H. The effect of TENS on random pattern flap survival in nicotinized rats. *Ann Plast Surg.* 2015;74:365-70
- Baris R, Kankaya Y, Ozer K, et al. The effect of microneedling with a roller device on the viability of random skin flaps in rats. *Plast Reconstr Surg.* 2013;131:1024-34
- Hamamcioğlu K, Vural O. Statins For The Treatment of Multiple Sclerosis. *Journal of Neurological Sciences*.2005;22:221-30
- 26. Takeuchi H, Mano Y, Terasaka S, Sakurai T, Furuya A, et al. Usefulness of rat skin as a substitute for human skin in the in vitro skin permeation study. *Exp Anim.* 2011;60:373-84

27. Jakasa I, Kezic S. Evaluation of in-vivo animal and in-vitro models for prediction of dermal absorption in man. *Hum Exp Toxicol*. 2008;27:281-8.

©Copyright 2021 by Osmangazi Tıp Dergisi - Available online at tip.ogu.edu.tr ©Telif Hakkı 2021 ESOGÜ Tıp Fakültesi - Makale metnine dergipark.org.tr/otd web sayfasından ulaşılabilir.