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Inhibitory effect of cilostazol on intimal hyperplasia and smooth muscle cell proliferation in a rabbit carotid artery anastomosis model

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ABSTRACT

Objectives: This study aims to investigate the effect of cilostazol on intimal hyperplasia and smooth muscle cell proliferation in a rabbit carotid artery anastomosis model.

Materials and methods: A total of 16 New Zealand male rabbits weighing 2 to 3 kg were used in this study. The rabbits were divided into two groups with eight in each group as Group A and Group B. A vertical neck incision was made in an appropriate position for all group rabbits and the right carotid artery was dissected. The same artery was transected and anastomosis using 8/0 polypropylene was performed with a continuous anastomosis technique. Group A was assigned as the control group and no medication was given. Cilostazol was administrated to Group B at a dose of 25 mg/kg twice a day per oral for 21 days. At the end of Day 21, the anastomosis segments of the right carotid artery and contralateral carotid artery of all rabbits were sent to the histology laboratory for analysis. The lumen diameter, lumen area, intimal area, medial area, and intima/media area ratio were estimated.

Results: In the serial sections, the mean lumen diameter of Group B was found to be significantly higher than Group A (p=0.001). The lumen area of Group B was significantly higher than Group A (p=0.001). The section series were evaluated and the area of the intima of Group B was significantly lower than Group A (p=0.001). The medial area of Group B was significantly larger than Group A (p=0.001). The intima/media area ratio was significantly higher in Group A (p=0.001).

Conclusion: Cilostazol may be useful for preventing intimal hyperplasia and smooth muscle cell proliferation after vascular surgery.

Keywords: Anastomosis, cilostazol, intimal hyperplasia, rabbit, smooth muscle cell proliferation.

Intimal hyperplasia and smooth muscle cell proliferation play an important role in restenosis after vascular interventions. Reconstruction is one of the most common interventions in the management of obstructing artery diseases. Recently, the success of this type of intervention are under expectations due to spontaneous thrombosis or restenosis.[1] After vascular reconstructive interventions, unlikely acute obstruction in which acute thrombosis is important at the late stage, intimal hyperplasia caused by smooth muscle cell migration, proliferation, and extracellular matrix (ECM) deposition are implicated in the pathophysiology of narrowing or restenosis.[2] Intimal hyperplasia is a vasoactive process characterized by vascular smooth muscle cell (VSMC) proliferation, inflammatory cell infiltration, endothelial cell injury, and increased position of the ECM. It begins with endothelial injury and ends up with partial or total restenosis in the long term. These mechanisms produce vascular lumen re-narrowing or restenosis, leading to unsuccessful vascular interventions.[3]

Cilostazol is a selective inhibitor of phosphodiesterase type 3 that increases intracellular cyclic adenosine monophosphate (cAMP) levels and activates protein kinase A, thereby inhibiting VSMC proliferation. It also significantly decrease platelet-derived growth factor (PDGF) in an experimental animal model.[4]

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Cilostazol is manufactured by Otsuka Pharmaceutical Co. Ltd. (Tokyo, Japan) under the trade name Pletal®. Cilostazol is approved for use in the United Kingdom by the National Institute for Clinical Excellence (NICE) and is licensed in the United States since 1999, by the Food and Drug Administration (FDA). It is used to treat patients suffering from intermittent claudication without rest pain and no peripheral tissue necrosis, as it improves pain-free walking distances.[5-9]

In this experimental study, we aimed to investigate the effects of cilostazol on smooth muscle cell proliferation and the formation of neointimal hyperplasia in surgical procedures in a rabbit carotid artery anastomosis model.

MATERIALS AND METHODS

In this experimental, randomized-controlled study, 16 randomly selected New Zealand-type male rabbits weighing 2 to 3 kg on average were included. During the study, all experimental animals were kept under the same conditions (in a room at a temperature of 20±2˚C with a ventilation system and receiving sunlight) and fed with rabbit feed. The rabbits were, then, divided into two equal groups. Group A (n=8) underwent right carotid artery anastomosis and received no medication, while Group B (n=8) underwent right carotid artery anastomosis and received cilostazol for 21 days following surgery. Cilostazol was administered at a dose of 25 mg/kg twice a day per oral immediately after surgery.

Surgical procedure

Before surgery, a cannula was inserted into the marginal ear vein for intravenous access. In both groups, the surgical protocol was the same: for anesthesia, 50 mg/kg of ketamine (intramuscular) and 5 mg/kg of xylazine (intramuscular) were administered. Also, we administered intravenous cefazolin (50 mg/kg) preoperatively to prevent infections. After shaving for better vision during surgery and disinfection with povidone-iodine, a right vertical neck incision was made. The right carotid artery was explored near the trachea. Proximal and distal parts of the right carotid artery were clamped with bulldog clamps after giving 100 IU/kg of heparin by ear vein, and the carotid artery was transected full layer. The artery was anastomosed in an end-to-end fashion with running monofilament sutures (8.0 polypropylene, 6.5 mm 3/8 circle [Ethicon Inc., NJ, USA]). Then, the clamps were removed to re-establish blood flow. Layers were closed in the anatomic plane, and the operation was complete. All procedures were performed with sterile instruments and surgical asepsis by a single operator using an operating microscope. All experimental animals survived the procedure and were followed up for 21 days without any complications. While study animals (Group B) received cilostazol daily per oral, control subjects received normal food and water. At the end of Day 21, all animals were anesthetized using the same protocol. The right anastomosed and the left non-anastomosed carotid artery segments were removed and sent to the histology laboratory for examination. The arterial segments were kept in a 10% buffered formaldehyde solution and sent to the histology laboratory for analysis. All rabbits were sacrificed using 150 mg/kg pentothal at the end of the procedure.

Histological examination

After fixation of the vessels in the 10% buffered formaldehyde solution, they were embedded in paraffin. Serial cross-sections in 5-µm thickness were obtained by cutting the paraffin blocks at the level of the anastomosis with a rotary microtome (Leica RM, 2135; Leica Biosystems Nussloch GmbH, Nußloch, Germany). The arterial specimens were stained using hematoxylin-eosin and Masson's trichrome. The sections of anastomosed and the corresponding contralateral sides were evaluated under a light microscope (Olympus BH-2, Tokyo, Japan). Photomicrographs were taken with a high-resolution JVC TK-890E, video camera (JVC Kenwood Corporation, Yokohama, Kanagawa, Japan). Obtained images were assessed with a digital imaging analysis program (UTHSCSA; Image tool version 3.0 University of Texas, TX, USA). The images were analyzed via a digital image analysis program and lumen diameter, lumen area, intimal area, medial area, and intima/media area ratio were estimated. Serial cross-sections taken from paraffin tissues were captured and transferred to a computer environment. Intimal and medial areas were measured, and the sections were three-dimensioned via Reconstruct version 1.0.9.9 software (JC Fiala; developed by J.C. Fiala and K.M. Harris at Boston University, MA, USA).

Statistical analysis

Statistical analysis was performed using the SPSS version 15.0 software (SPSS Inc., Chicago, IL, USA). Descriptive data were presented in mean ± standard deviation (SD) or number and frequency, where applicable. The Mann-Whitney U test was used for the comparison between the two groups and the Kruskal-Wallis analysis of variance (ANOVA) test was used for the comparison of the differences between the groups. A p value of <0.05 was considered statistically significant.

RESULTS

Histopathological evaluation

All the animals survived throughout the study, and none of them exhibited neurological deficits or wound infection. Arterial anastomosis was patent in all animals in both groups at the end of the study.

In the histological sections of Group A, the right carotid artery was compared with the left carotid artery. The right carotid artery lumen was found narrowed and its smooth circular shape was impaired (Figure 1a, b). Smooth muscle cell proliferation, disorganized cellular arrangement, intensive connective tissue increase, and development of intimal hyperplasia were observed in the intimal area (Figure 1c).

The lumen of the right carotid artery of Group B was larger and its geometrical shape was more proper than the lumen of the right carotid artery of Group A. Consequently, the vascular lumen of Group B was larger and smoother (Figure 1b-f). When the right carotid arteries of both groups were compared, intimal hyperplasia and medial hypertrophy were much more in Group A (Figure 1c-h).

Histomorphometric measurements

Luminal diameter

The mean luminal diameter was 490,067±50,972 µm in Group A and 716.018±24.797 µm in Group B. It was significantly larger in Group B (p=0.001) (Table 1).

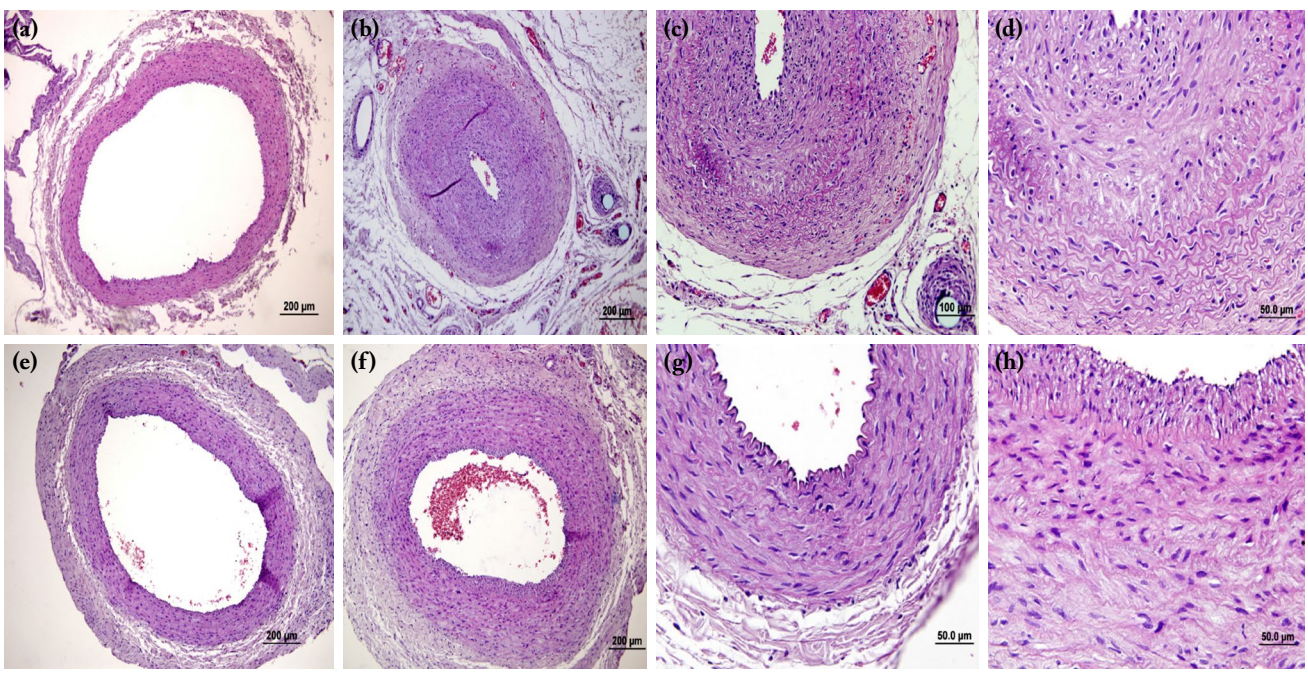

Figure 1. Histological sections of groups. **(a)** Group A, the histological section of the left carotid artery (H&E, ×40). **(b)** Group A, the histological section of the right carotid artery (H&E, ×40). **(c)** Group A, intimal hyperplasia in the right carotid artery (H&E, ×40). **(d)** Group A, media hypertrophy in the right carotid artery (H&E, ×20). **(e)** Group B, the histological section of the left carotid artery (H&E, ×40). **(f)** Group B, the histological section of the right carotid artery (H&E, ×40). **(g)** Group B, intimal hyperplasia in the right carotid artery (H&E, ×10). **(h)** Group B, media hypertrophy in the right carotid artery (H&E, ×40).

Table 1
Comparison of mean lumen diameters

	Anastomosis not performed	Anastomosis performed	
Group	Mean lumen diameter (μm²)±SE	Mean lumen diameter (μm²)±SE	*p*
Group A	874.932±22.003	490.067±50.972	0.001
Group B	828.585±21.966	716.018±24.797	0.009

SE: Standard error.

Table 2
Comparison of mean luminal areas

	Anastomosis not performed	Anastomosis performed	
Group	Mean lumen diameter (μm²)±SE	Mean lumen diameter (μm²)±SE	*p*
Group A	564474.278±67707.555	144087.608±28545.057	0.001
Group B	495509.965±44001.575	366638.070±62509.091	0.115

SE: Standard error.

Table 3
Comparison of mean intimal areas

	Anastomosis not performed	Anastomosis performed	
Group	Mean lumen diameter (μm²)±SE	Mean lumen diameter (μm²)±SE	*p*
Group A	16427.127±2713.660	181500.733±16731.850	0.001
Group B	13684.608±2014.059	51268.378±6535.621	0.001

SE: Standard error.

Table 4
Comparison of intima/media area ratio

	Anastomosis not performed	Anastomosis performed	
Group	Mean lumen diameter (μm²)±SE	Mean lumen diameter (μm²)±SE	*p*
Group A	0.016±0.002	0.295±0.028	0.001
Group B	0.015±0.002	0.061±0.011	0.001

SE: Standard error.

Luminal area

The mean luminal area was 144.087,608±28.545,057 μm² in Group A and 366.638.070±62.509.091 μm² in Group B. It was significantly lower in Group A (p=0.001) (Table 2).

Intimal area

The mean intimal area was 181.500,733±16.731,850 μm² in Group A and 51.268.378±6.535.621 μm² in Group B. It was significantly larger in Group A (p=0.001) (Table 3).

Intima/media area ratio

The intima/media area ratio was significantly lower in Group B (p=0.001) (Table 4).

DISCUSSION

Intimal hyperplasia is a normal adaptive response of arteries against hemodynamic stress and also is an exaggerated healing process after arterial injuries such as bypass grafting, endarterectomy, and balloon angioplasty with or without stenting. Neointimal hyperplasia develops through a complex process including platelet aggregation, leukocyte chemotaxis, VSMC proliferation and migration, ECM alterations, and endothelial cell proliferation.[10]

The intimal response that develops after arterial damage is observed in three stages. Smooth muscle cell proliferation begins in the first 24 hours. After endothelium damage develops, the damaged area is coated with platelets. Following adhesion, platelets release vasoactive and thrombotic factors in their granules (serotonin, adenosine diphosphate, fibrinogen, and Von Willebrand factor) and release growth factors (PDGF, transforming growth factor, and epidermal growth factor). Mitogenic growth factors initiate the proliferation of smooth muscle cells. Proliferated smooth muscle cells in the media layer migrate to the intima and lead to intimal hyperplasia. On Days 3 and 14, these smooth muscle cells migrate to the intima, and neointima and neointimal hyperplasia develop. In the third stage, smooth muscle cells create a layer that results in the narrowing of the vessel lumen rapidly.[11,12]

Cilostazol has many pharmacological effects including vasodilation, inhibition of platelet activation and aggregation, thrombosis inhibition, increased blood flow to the limbs, improvement in serum lipids with the reduction of triglycerides and elevation of high-density lipoprotein cholesterol, and VSMC growth inhibition.[13] Owing to these effects, cilostazol is used to reduce the risk of restenosis and repeat revascularization after percutaneous coronary interventions.[14]

Cilostazol is used for the treatment of peripheral arterial occlusive disease by oral delivery.[15] Systemic administration of cilostazol at 30 mg/kg per oral twice per day was reported to inhibit neointimal formation in balloon-injured rat carotid arteries by 32%.[16]

In a study, Yamamoto et al.[17] showed that locally applied cilostazol inhibited neointimal hyperplasia and medial thickening in a vein graft model. A 1-cm segment of the right femoral vein was harvested and transplanted into the abdominal aorta in an end-to-end fashion. In the cilostazol-treated group, rats with the anastomotic stricture received a topical application of 20 mg of cilostazol dissolved in 200 µL of dimethyl sulfoxide containing 25% Pluronic® gel (Letco Medical, Decatur, AL, USA) around the interposed graft. The rats in the control group received the dimethyl sulfoxide Pluronic® gel without cilostazol. The effectiveness of cilostazol applied locally to implanted vein grafts was demonstrated in suppressing neointimal hyperplasia in this rat model.

Bilateral reversed jugular vein interposition grafts of the common carotid artery were performed in 12 Beagle dogs. Starting from seven days before surgery, either cilostazol (30 mg/day; n=6) or a placebo (n=6) was given orally twice daily. Vein grafts were harvested at Week 1 or Week 4. At Week 1 after implantation, the cilostazol group showed significantly less cell proliferation than the placebo group. At Week 4 after implantation, the intimal and medial thickness was significantly thinner in the cilostazol group than in the placebo group.[18]

Cilostazol is an agent with a pleiotropic mechanism of action and multiple beneficial effects through a combination of vasodilation, platelet inhibition, antiproliferative effect, and lipid-lowering properties. Based on these properties, cilostazol has shown promising effects in the management of atherosclerotic vascular disease in coronary, cerebrovascular, and peripheral arteries.[19]

The primary limitation of our study was the lack of molecular data. In the future, we plan to perform a study on a higher budget and include immunohistochemistry data and oxidative stress parameters.

In conclusion, our study results showed that reduction in the lumen area and diameter after anastomosis were significantly improved in the cilostazol group compared to the control group. The area of intima and intima/media ratio was smaller in the cilostazol group compared to those in the control group, and the difference was statically significant. The medial area of the cilostazol group was significantly higher than the control group. Based on these findings, cilostazol may be useful for

preventing intimal hyperplasia and smooth muscle cell proliferation after vascular surgery.

Ethics Committee Approval: The study protocol was approved by the Dokuz Eylül University Faculty of Medicine Ethics Committee (date: 16.12.2011, no: 69/2011). The study was conducted in accordance with the principles of the Declaration of Helsinki.

Data Sharing Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author Contributions: Idea/concept, design, critical review: A.C.E.; Data collection and/or processing, writing the article, control/supervision: Ç.B.; Literature review, analysis and/or interpretation, writing the article: U.K.

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