

Original
Article

Effect of Tadalafil on Neointimal Hyperplasia in a Rabbit Carotid Artery Anastomosis Model

Mehmet Guzeloglu,¹ Koray Aykut,¹ Gökhan Albayrak,¹ Soner Atmaca, MD,²
Suleyman Oktar,³ Alper Bagriyanik,² and Eyup Hazan¹

Purpose: Intimal thickening, which results from the response to arterial damage caused by therapeutic interventions or other reasons, is usually called as neointima. Neointimal hyperplasia is a main step in the pathogenesis of late-term restenosis, which is developed after vascular interventions. Reduction in nitric oxide (NO)/cyclic guanosine monophosphate (cGMP) signaling plays a substantial role in the pathogenesis of neointima formation. Phosphodiesterase V is detected in the peripheral coronary and pulmonary vascular smooth muscle cells and in the cardiac tissue. Based on the effects of phosphodiesterase V inhibitors on vascular smooth muscle cells, in the present study, the effect of tadalafil, a new member of phosphodiesterase V inhibitors, on neointimal hyperplasia was investigated in the rabbit carotid artery anastomosis model.

Materials and Methods: Fourteen male New Zealand white rabbits weighing between 2.5-3 kg, were used. The rabbits were randomly divided into two equal groups; tadalafil group received oral tadalafil (2 mg/kg/day), and PBS group received sterile PBS solution (normal saline; 2 mg/kg/day) for 28 days after the surgery. The right carotid arteries of all rabbits were anastomosed in an end-to-end fashion using 8/0 polypropylene suture. The rabbits were sacrificed at the end of the postoperative period of 28 days. After sacrificing, firstly anastomosis segment on the right carotid artery and secondly a part of the left carotid artery (as control) of each rabbit were removed. Morphometric examination of tissue sections was performed under a light microscope connected to an image capture system.

Results: There was a significant difference between the right and left carotid arteries in terms of intimal area and intima/media ratio both in tadalafil and PBS groups ($p < 0.001$ for each). Intimal area and intima/media ratio were increased in the right carotid arteries compared to the left carotid arteries ($p < 0.001$ for each). Besides, when the right carotid arteries of both groups were compared using covariance analysis, it was observed that intimal area and intima/media ratio in the anastomosis site were significantly reduced with tadalafil treatment ($p < 0.001$).

Conclusion: The present study was promising in terms of tadalafil use as a new agent for the prevention of neointimal hyperplasia, which is the leading cause of late-term graft failure in vascular surgery.

Keywords: Neointima, tadalafil, nitric oxide, cyclic guanosine monophosphate signaling, rabbit

¹Izmir University Faculty of Medicine, Department of Cardiovascular Surgery, Izmir, Turkey

²Dokuz Eylul University Faculty of Medicine, Department of Histology and Embryology, Izmir, Turkey

³Mustafa Kemal University Faculty of Medicine, Department of Pharmacology, Hatay, Turkey

Received: April 25, 2012; Accepted: July 6, 2012

Corresponding author: Mehmet Guzeloglu. Department of Cardiovascular Surgery, Izmir University Medical School, Karsiyaka, Izmir, Turkey

Email: mehmetguzeloglu@hotmail.com

©2013 The Editorial Committee of *Annals of Thoracic and Cardiovascular Surgery*. All rights reserved.

Introduction

The term neointima is frequently used for intimal thickening, which results from response to arterial damage caused by either therapeutic interventions or other reasons. Neointimal hyperplasia is a main factor in the pathogenesis of restenosis, which occurs following arteriosclerosis, vascular damage and angioplasty, and of the late-term graft failure in vascular surgery.¹⁻⁴⁾ Neointimal hyperplasia is developed in three steps: destruction of extracellular matrix (ESM), proliferation of vascular smooth muscle cells (VSMC), and their migration from media to intima.

Reduction in nitric oxide (NO)/cyclic guanosine monophosphate (cGMP) signaling plays an important role in the pathogenesis of neointima formation.⁵⁾ Experimental studies have demonstrated that neointimal proliferation is reduced by endothelial NO synthase gene transfer.⁶⁾ It has also been demonstrated that YC-1, a benzyl indazole derivative that stimulates soluble guanylyl cyclase (sGC) activity and cGMP production, reduces neointimal hyperplasia after balloon angioplasty in the rat carotid artery.⁷⁾

Phosphodiesterase V (PDE5) catalyzes cGMP destruction in smooth muscle cells and consequently reduces intracellular cGMP concentration. PDE5 inhibitors are routinely used in the treatment of erectile dysfunction. PDE5 has been detected in the peripheral coronary and pulmonary vascular smooth muscle cells and in the cardiac tissue.⁸⁾ Among this group, FDA approved the use of sildenafil in the treatment of patients with pulmonary hypertension. Studies on sildenafil, a PDE5 inhibitor, have reported its antiproliferative effect on human pulmonary arterial smooth muscle cells.⁹⁻¹¹⁾ However, clinical studies on the use of this group of drugs in Reynaud's phenomenon, essential hypertension and stroke have been continuing.¹²⁾

Tadalafil is the new member of PDE5 inhibitors with the longest half-life; thus, easy to use as a single dose.⁸⁾ In the present study, based on the effects of PDE5 inhibitors on vascular smooth muscle cells, the effect of tadalafil on neointimal hyperplasia was investigated in rabbit carotid artery anastomosis model.

Materials and Methods

Preparation of animals

The present study was approved by the Local Ethics Committee of Dokuz Eylul University, Faculty of Medicine. All animals received humane care in compliance

with the Principles of Laboratory Animal Care formulated by the National Society for Medical Research and the Guide for the Care and Use of Laboratory Animals. In the present study, 14 male New Zealand white rabbits weighing between 2.7–3.2 kg were used. The rabbits were randomly divided into two equal groups: tadalafil group received oral tadalafil (2 mg/kg/day) for 28 days after the surgery, and PBS group received sterile PBS solution (normal saline; 2 mg/kg/day) for 28 days after the surgery. Rabbits that died before the 28-day postoperative period were excluded. The rabbits were kept in separate cages during the postoperative period. The rabbits were fed ad libitum with standard rabbit chow and allowed free to access tap water. The drug treatment was well-tolerated by the rabbits. Neither the body weights nor the survival periods of the rabbits in each group were affected by the treatment protocol (data not shown).

Application of carotid artery anastomosis model

All surgical procedures were performed by the same surgical team using 3.5x surgical loupe (Designs for Vision Inc., Ronkonkoma, New York, USA). Prior to the surgery, all rabbits were anesthetized with intramuscular xylazine (3 mg/kg) and ketamine (50 mg/kg) mixture. The right carotid artery was explored through an oblique incision performed on the right side of the neck. Rabbits were heparinized with 100 IU/kg heparin. Three minutes after heparinization, the proximal and distal parts of the artery were controlled and a full-thickness transection of the right carotid artery was performed. Then, both ends of the right carotid artery was anastomosed in an end-to-end fashion using 8/0 polypropylene suture, the layers were closed to recreate the anatomic plane, and the surgical procedure was finalized. All rabbits were administered xylazine for postoperative analgesia.

The rabbits were sacrificed on the postoperative 28th day using a high-dose pentothal (150 mg/kg). Immediately after sacrificing, firstly anastomosis segment on the right carotid artery and secondly a part of the left carotid artery (as control) of each rabbit were removed. All samples were examined under a light microscope (Olympus BH2, Tokyo, Japan). Tissues were taken for histological examination.

Histological examination

Morphometry

The obtained tissues were kept in the tubes containing 10% formalin for 24–48 hrs. Then, they were embedded in paraffin and serial sections of 5 µm thickness were

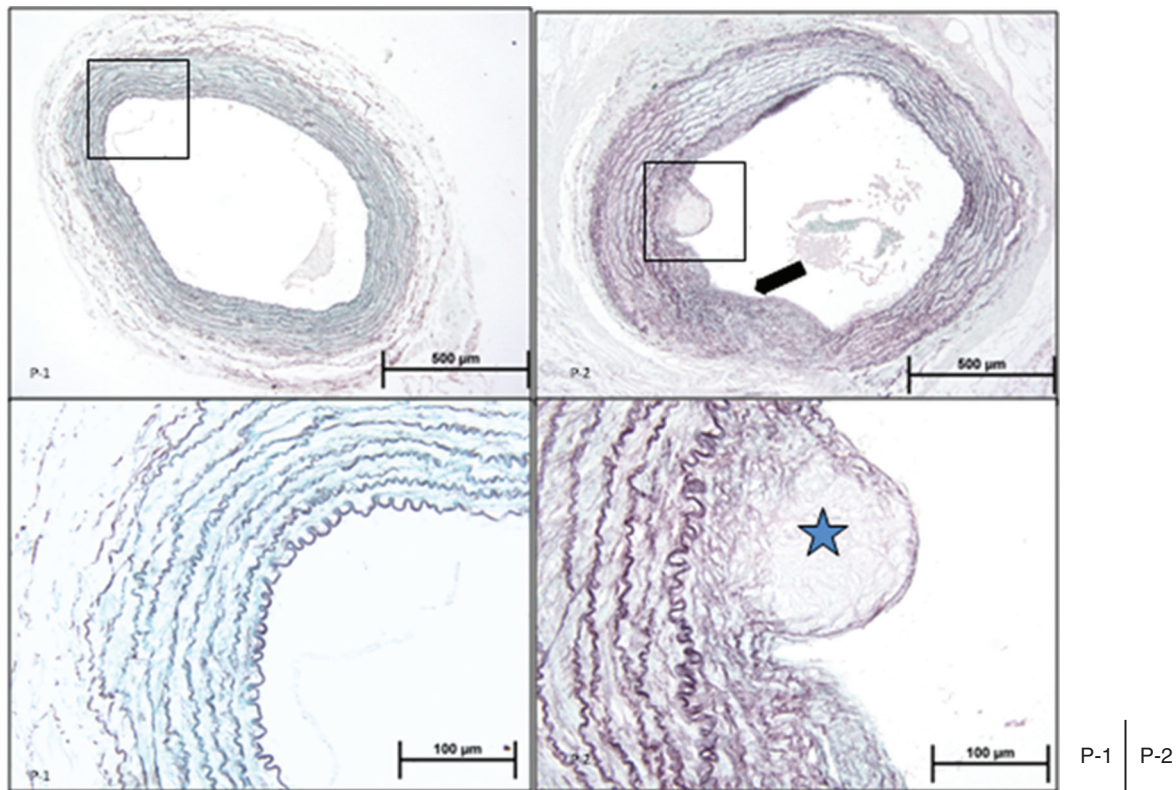


Fig. 1 Carotid artery views of the PBS group; (P-1), Cross-sectional image of the left carotid artery; (P-2), Cross-sectional image of the right carotid artery (staining with orcein light green).

mounted onto glass slides. After staining with orcein light green, the sections were mounted in Entellan mounting medium. Ten samples for each artery were examined under a light microscope (Olympus BH2, Tokyo, Japan) connected to an image capture system and the images were transferred to the computer using a digital video camera (Olympus DP71, Tokyo, Japan). Multiple fields (up to 5) were selected at maximal intimal thickness from each section of each sample in a systematic random pattern and images were analyzed using Image Tool software version 3.00 (Uthsca). Intima, media and lumen areas (μm^2) were measured and the intima/media ratio (index values) was calculated.

Statistical analysis

Data were analyzed using the Statistical Package for the Social Sciences version 17.0 (SPSS Inc., Chicago, Illinois, USA). Based on the assumption that the data were normally distributed, the repeated-measures analysis of variance (ANOVA) was used. For the changes in which interaction was important, for the intergroup comparisons independent paired t-test was used for the left carotid arteries, whereas convergence readiness was

used for the right carotid arteries. For the intragroup comparisons between the left and right carotid arteries, t-test was used. A p value <0.05 was considered significant.

Results

Histological examination

Intimal thickening at the anastomotic sites occurred in both groups in different ratios. In the PBS group, no intimal hyperplastic tissue was observed in the left carotid arteries, whereas massive intimal hyperplastic tissue was observed in the right carotid arteries (**Fig. 1**). In the tadalafil group, no intimal hyperplastic tissue was observed in the left carotid arteries; however, less massive intimal hyperplastic tissue was observed in the right carotid arteries compared to that of the PBS group (**Fig. 2**).

There was a significant difference between the right and left carotid arteries in terms of intimal area both in tadalafil and PBS groups ($p < 0.001$ for each). Intimal area was increased in the right carotid arteries compared to the left carotid arteries; however, this increase was four times higher in the PBS group than the tadalafil

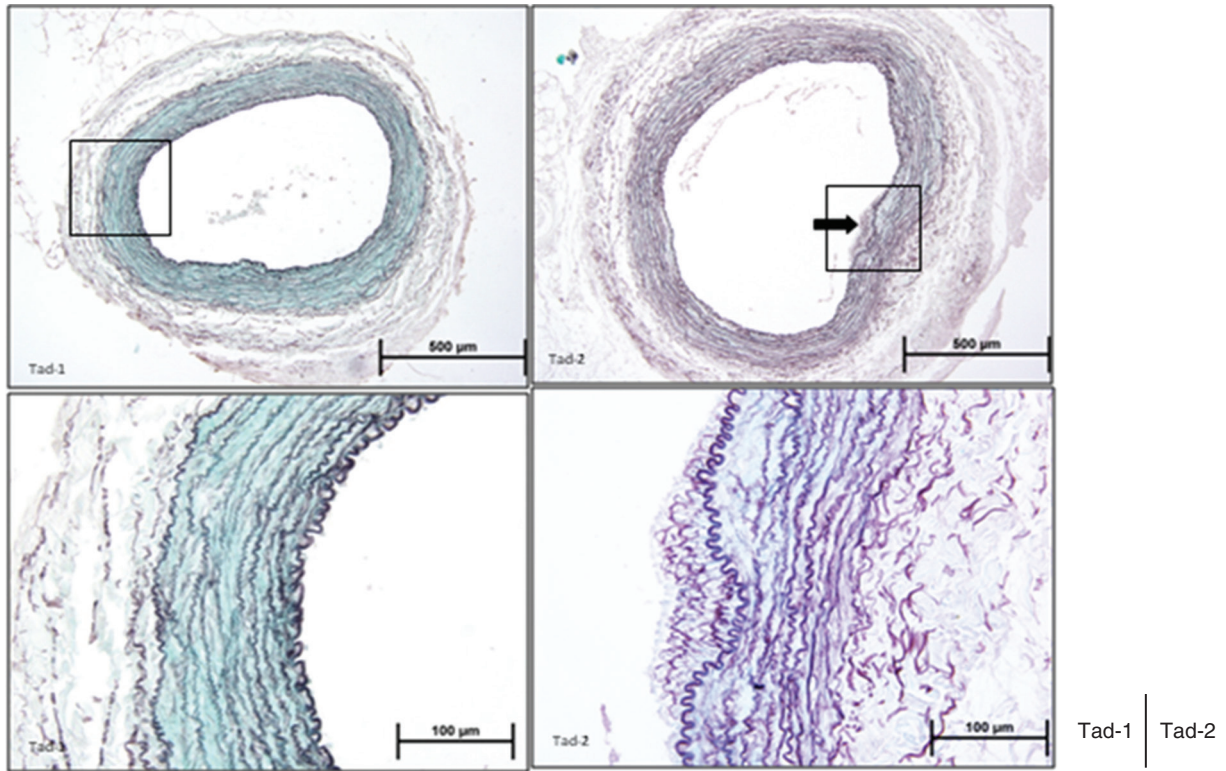


Fig. 2 Carotid artery views of the tadalafil group; (Tad-1), Cross-sectional image of the left carotid artery; (Tad-2), Cross-sectional image of the right carotid artery (staining with orcein light green).

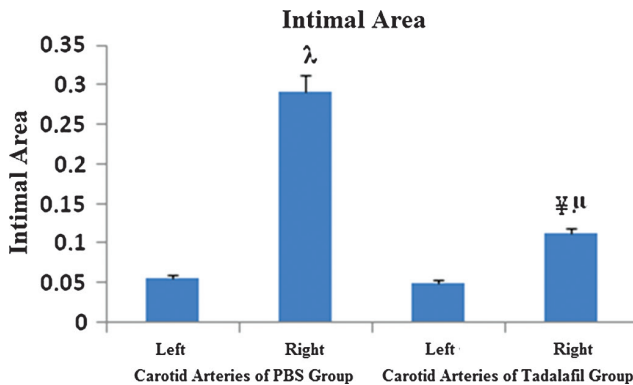


Fig. 3 Intimal area values in the PBS and tadalafil groups
^λ Left versus right carotid arteries in the PBS group ($p < 0.001$)
[¥] Left versus right carotid arteries in the tadalafil group ($p < 0.001$)
^μ Intergroup comparison for right carotid arteries ($p < 0.001$)

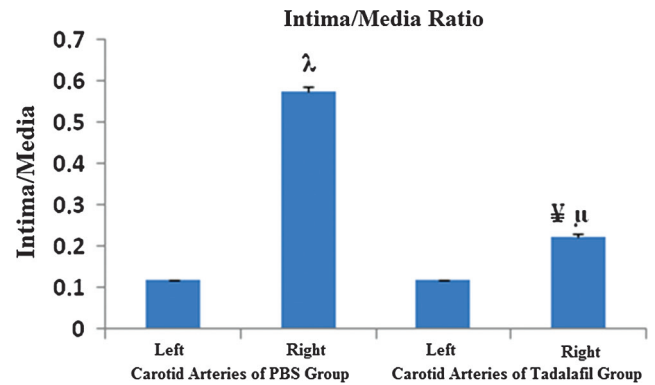


Fig. 4 Intima/Media ratios in the PBS and tadalafil groups
^λ Left versus right carotid arteries in the PBS group ($p < 0.001$)
[¥] Left versus right carotid arteries in the tadalafil group ($p < 0.001$)
^μ Intergroup comparison for right carotid arteries ($p < 0.001$)

group. When the right carotid arteries of both groups were compared by covariance analysis, it was observed that intimal area in the anastomosis site was significantly decreased with the tadalafil treatment ($p < 0.001$; **Fig. 3**).

When the groups were examined in terms of intima/media ratio, a statistically significant difference was observed between the right and left carotid arteries in favor of increment ($p < 0.001$ for each). As a consequence, tadalafil

treatment caused a significant decrease in intima/media ratio in the anastomosis site ($p = 0.001$; **Fig. 4**).

There was no significant difference both between the tadalafil and PBS groups and the right and left carotid arteries in terms of lumen area and media area ($p > 0.05$). There was also no significant difference between the left carotid arteries of tadalafil and PBS groups according to intimal area and intima/media ratio.

Discussion

Neointimal hyperplasia is an exaggerated healing process occurring in the vessel walls following an injury. Restenosis due to neointimal hyperplasia limits the success of many vascular interventions such as bypass grafting, endarterectomy, and balloon angioplasty with or without stenting. Injury-induced neointimal hyperplasia develops through a complex process including platelet aggregation, leukocyte chemotaxis, VSMC proliferation and migration, extracellular matrix (ECM) changes, and endothelial cell proliferation.^{13–15} In order to improve vascular damage, effective potential treatments on these mechanisms, which play a role in the pathophysiology of neointimal hyperplasia, have been investigated.

NO-based therapies have been investigated since the role of NO in the pathophysiology of neointimal hyperplasia has been propounded. NO-based therapies have been recommended to take place among pharmacological approaches in the vascular surgery due to their potential clinical effects on neointimal hyperplasia formation.¹⁴ Agents or mechanisms, of which potential effects on neointimal hyperplasia are being investigated, include Nutlin-3, a non-genotoxic activator of the p53 pathway,¹⁶ cinaciguat, the novel soluble guanylate cyclase activator,¹⁷ gene therapy,¹⁸ trichostatin A, a histone deacetylase inhibitor,¹⁹ estrogen,²⁰ and abciximab, a monoclonal antibody raised against alpha IIb Beta3 integrin.²¹

PDE inhibitors as well are among the agents that are being investigated. The PDE superfamily plays a role in the hydrolysis of the second messenger molecules, it catalyzes cyclic adenosine and cGMP to inactive nucleotide monophosphates. The PDE superfamily comprises 11 different family members classified based on their specificity for the cyclic nucleotides, inhibitors, and cloning and sequencing properties.²²

Cilostazol, a PDE3 inhibitor, 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.²³ Owing to these effects, cilostazol is used to reduce the risk for restenosis and repeat revascularization after percutaneous coronary interventions.²⁴ Similarly, coated balloons and stents with various agents are used as well.^{25,26}

Sildenafil, vardenafil and tadalafil are three PDE5 inhibitors which were approved worldwide for the treatment of male erectile dysfunction. Sildenafil and tadalafil were also approved in the treatment of pulmonary arterial hypertension. Many clinical trials have investigated the effects of PDE5 inhibitors on the treatment of additional indications.²² Interest in the effects of PDE5 inhibitors on cardiovascular field, particularly on heart and arteries, has been gradually increasing.^{27,28}

Experimental studies conducted with PDE5 inhibitors have demonstrated that these drugs cause substantial decrease in TGF- β 1 levels by increasing cGMP levels.²⁹ The primary effect of cGMP is to inhibit proliferation and migration of VSMC and to change oxidative and apoptotic signals, and thus, to reduce neointimal hyperplasia.^{30,31} Studies have demonstrated the relation between TGF- β 1 and neointimal hyperplasia.^{32,33} In a study, it has been reported that YC-1, which is a partial PDE5 inhibitor, reduces neointimal hyperplasia after balloon angioplasty in rat carotid artery.⁷ Vardenafil, which is also a selective PDE5 inhibitor, has been shown to be effective in decreasing neointimal hyperplasia after carotid endarterectomy in rats.²⁹ In the present study as well, the effect of tadalafil, a PDE5 inhibitors, on neointimal hyperplasia process was investigated. Various animal models including mouse, rat, rabbit, and pig are being used while investigating potential therapeutic approaches for neointimal hyperplasia. It has been reported that mouse is the most suitable animal for the initial testing of drugs due to both economical and practical reasons. Vein grafting is also appropriate in rabbits to test pharmaceuticals, especially when diabetes, hypertension or hypercholesterolemia complicates the disease. When clinically relevant, vein grafting may also be performed in pigs and a well-characterized model of neointimal formation (similar to the clinical situation) can be obtained within the graft.³⁴ In the present study, male New Zealand white rabbits were used.

Tadalafil differs from the other PDE5 inhibitors, such as vardenafil and sildenafil, due to its pharmacokinetic profile including maximum plasma level achieved 2 hours

after oral administration, half-life of 17.5 hours, and efficacy lasting for 36 hours.³⁵⁾

Similar to the previous studies,³⁶⁾ in the present study, neointimal hyperplasia was observed at the anastomosis site on the postoperative 28th day in rabbit carotid artery anastomosis model. However, a remarkable decrease in neointimal hyperplasia at the anastomotic site was obtained with tadalafil treatment after postoperative period of 28 days. This finding was similar to the results in the literature regarding the effects of the drugs on cGMP signaling in neointimal hyperplasia created by various experimental models.^{7,29)}

In conclusion, the present study showed that tadalafil, a new member of PDE5 inhibitor family with the longest half-life, inhibited neointimal hyperplasia in rabbit carotid artery anastomosis model. Tadalafil may be a promising drug to enhance vascular patency and to prevent neointimal hyperplasia after revascularization procedures.

Disclosure Statement

The authors have no conflict of interest about the subject of the manuscript.

References

- 1) Merzkirch C, Davies N, Zilla P. Engineering of vascular ingrowth matrices: are protein domains an alternative to peptides? *Anat Rec* 2001; **263**: 379-87.
- 2) Mills JL. Mechanisms of vein graft failure: the location, distribution, and characteristics of lesions that predispose to graft failure. *Semin Vasc Surg* 1993; **6**: 78-91.
- 3) Donaldson MC, Mannick JA, Whittemore AD. Causes of primary graft failure after in situ saphenous vein bypass grafting. *J Vasc Surg* 1992; **15**: 113-8; discussion 118-20.
- 4) De Meyer GR, Bult H. Mechanisms of neointima formation—lessons from experimental models. *Vasc Med* 1997; **2**: 179-89.
- 5) Melichar VO, Behr-Roussel D, Zabel U, et al. Reduced cGMP signaling associated with neointimal proliferation and vascular dysfunction in late-stage atherosclerosis. *Proc Natl Acad Sci U S A* 2004; **101**: 16671-6.
- 6) Janssens S, Flaherty D, Nong Z, et al. Human endothelial nitric oxide synthase gene transfer inhibits vascular smooth muscle cell proliferation and neointima formation after balloon injury in rats. *Circulation* 1998; **97**: 1274-81.
- 7) Tulis DA, Durante W, Peyton KJ, et al. YC-1, a benzyl indazole derivative, stimulates vascular cGMP and inhibits neointima formation. *Biochem Biophys Res Commun* 2000; **279**: 646-52.
- 8) Ghiadoni L, Versari D, Taddei S. Phosphodiesterase 5 inhibition in essential hypertension. *Curr Hypertens Rep* 2008; **10**: 52-7.
- 9) Tantini B, Manes A, Fiumana E, et al. Antiproliferative effect of sildenafil on human pulmonary artery smooth muscle cells. *Basic Res Cardiol* 2005; **100**: 131-8.
- 10) Wharton J, Strange JW, Møller GM, et al. Antiproliferative effects of phosphodiesterase type 5 inhibition in human pulmonary artery cells. *Am J Respir Crit Care Med* 2005; **172**: 105-13.
- 11) Li B, Yang L, Shen J, et al. The antiproliferative effect of sildenafil on pulmonary artery smooth muscle cells is mediated via upregulation of mitogen-activated protein kinase phosphatase-1 and degradation of extracellular signal-regulated kinase 1/2 phosphorylation. *Anesth Analg* 2007; **105**: 1034-41, table of contents.
- 12) Sharma R. Novel phosphodiesterase-5 inhibitors: current indications and future directions. *Indian J Med Sci* 2007; **61**: 667-79.
- 13) Mehta D, Izzat MB, Bryan AJ, et al. Towards the prevention of vein graft failure. *Int J Cardiol* 1997; **62**: 55-63.
- 14) Ahanchi SS, Tsihlis ND, Kibbe MR. The role of nitric oxide in the pathophysiology of intimal hyperplasia. *J Vasc Surg* 2007; **45**: 64-73.
- 15) Curcio A, Torella D, Indolfi C. Mechanisms of smooth muscle cell proliferation and endothelial regeneration after vascular injury and stenting: approach to therapy. *Circ J* 2011; **75**: 1287-96.
- 16) Hashimoto T, Ichiki T, Ikeda J, et al. Inhibition of MDM2 attenuates neointimal hyperplasia via suppression of vascular proliferation and inflammation. *Cardiovasc Res* 2011; **91**: 711-9.
- 17) Hirschberg K, Tarcea V, Páli S, et al. Cinaciguat prevents neointima formation after arterial injury by decreasing vascular smooth muscle cell migration and proliferation. *Int J Cardiol* 2012 Feb 20.
- 18) Jacob T, Hingorani A, Ascher E. p53 gene therapy modulates signal transduction in the apoptotic and cell cycle pathways downregulating neointimal hyperplasia. *Vasc Endovascular Surg* 2012; **46**: 45-53.
- 19) Kee HJ, Kwon JS, Shin S, et al. Trichostatin A prevents neointimal hyperplasia via activation of Krüppel like factor 4. *Vascul Pharmacol* 2011; **55**: 127-34.
- 20) Nyalala JO, Brown AT, Luo S, et al. Estrogen replacement attenuates exaggerated neointimal hyperplasia following carotid endarterectomy in rats. *Vasc Endovascular Surg* 2011; **45**: 720-6.
- 21) Wu CH, Chen YC, Hsiao G, et al. Mechanisms involved in the inhibition of neointimal hyperplasia by abciximab in a rat model of balloon angioplasty. *Thromb Res* 2001; **101**: 127-38.
- 22) Bell AS, Palmer MJ. Novel phosphodiesterase type 5 modulators: a patent survey (2008 - 2010). *Expert*

- Opin Ther Pat 2011; **21**: 1631-41.
- 23) Weintraub WS. The vascular effects of cilostazol. *Can J Cardiol* 2006; **22**: 56B-60B.
 - 24) Biondi-Zoccai GG, Lotrionte M, Anselmino M, et al. Systematic review and meta-analysis of randomized clinical trials appraising the impact of cilostazol after percutaneous coronary intervention. *Am Heart J* 2008; **155**: 1081-9.
 - 25) Cremers B, Toner JL, Schwartz LB, et al. Inhibition of neointimal hyperplasia with a novel zotarolimus coated balloon catheter. *Clin Res Cardiol* 2012; **101**: 469-76.
 - 26) Kavanagh CA, Rochev YA, Gallagher WM, et al. Local drug delivery in restenosis injury: thermoresponsive co-polymers as potential drug delivery systems. *Pharmacol Ther* 2004; **102**: 1-15.
 - 27) Kass DA, Champion HC, Beavo JA. Phosphodiesterase type 5: expanding roles in cardiovascular regulation. *Circ Res* 2007; **101**: 1084-95.
 - 28) Reffelmann T, Kloner RA. Phosphodiesterase 5 inhibitors: are they cardioprotective? *Cardiovasc Res* 2009; **83**: 204-12.
 - 29) Hirschberg K, Radovits T, Loganathan S, et al. Selective phosphodiesterase-5 inhibition reduces neointimal hyperplasia in rat carotid arteries after surgical endarterectomy. *J Thorac Cardiovasc Surg* 2009; **137**: 1508-14.
 - 30) Münzel T, Feil R, Mülsch A, et al. Physiology and pathophysiology of vascular signaling controlled by guanosine 3',5'-cyclic monophosphate-dependent protein kinase [corrected]. *Circulation* 2003; **108**: 2172-83.
 - 31) Yu SM, Hung LM, Lin CC. cGMP-elevating agents suppress proliferation of vascular smooth muscle cells by inhibiting the activation of epidermal growth factor signaling pathway. *Circulation* 1997; **95**: 1269-77.
 - 32) Fu K, Corbley MJ, Sun L, et al. SM16, an orally active TGF-beta type I receptor inhibitor prevents myofibroblast induction and vascular fibrosis in the rat carotid injury model. *Arterioscler Thromb Vasc Biol* 2008; **28**: 665-71.
 - 33) Wolf YG, Rasmussen LM, Ruoslahti E. Antibodies against transforming growth factor-beta 1 suppress intimal hyperplasia in a rat model. *J Clin Invest* 1994; **93**: 1172-8.
 - 34) Thomas AC. Animal models for studying vein graft failure and therapeutic interventions. *Curr Opin Pharmacol* 2012; **12**: 121-6.
 - 35) Rosen RC, Kostis JB: Overview of phosphodiesterase 5 inhibition in erectile dysfunction. *Am J Cardiol* 2003; **92**: 9M-18M.
 - 36) Güzeloglu M, Gül M, Reel B, et al. The effects of zoledronic acid on neointimal hyperplasia: a rabbit carotid anastomosis model. *Anadolu Kardiyol Derg* 2011; **11**: 93-100.