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The Effect of Local Application of Neutralizing Antibody to Interleukin-1B on the Development of Vein Graft Intimal Hyperplasia

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Objective: Intimal hyperplasia (IH) remains one of the major obstacles to long term vein graft patency. IL-1B has been demonstrated to be one of the first inflammatory cytokines expressed in the rat vein graft model of IH and may be an important initiator of the sequence of events leading to the development of IH. This study was designed to establish the role of IL-1B by demonstrating the outcome of inhibiting its effects by the use of neutralizing antibodies on the development of IH in this model.

Methods: Rat epigastric vein to femoral artery interposition grafts were treated with neutralizing antibody to IL-1B suspended in pluronic gel and harvested at the end of one week and two weeks. The amount of intimal hyperplasia was measured at the anastomotic and midgraft regions.

Results: The amount of IH was less at the anastomotic and midgraft regions of the treated grafts at the end of one week ($p<0.05$), but did not differ significantly with the untreated group at the end of two weeks.

Conclusion: Neutralizing antibody to IL-1B delivered locally retarded but did not prevent the occurrence of IH in vein grafts. The initiation of the cascade of events in the development of IH is affected in a major way , but not singularly by IL-1B.

Key words: Vein graft, intimal hyperplasia, cytokines, interleukin-1B, antibodies

Autologous saphenous vein is still the conduit of choice for most cases of infra-inguinal bypass surgery, but its initial technical success may be marred by the later development of progressive stenosis due to intimal hyperplasia (IH).[1] IH accounts for as much as fifty percent of bypass graft failures[2], and the cellular and molecular mechanisms responsible for its development have yet to be fully elucidated.[3,4,5,6,7]

In recent years, a number of investigations have been directed toward the expression of growth factors[7], cytokines[7], and early response genes[8] in relation to the development of vessel wall IH. Although much of the data are based on the study of IH in arterial models, and though the histological appearance may be similar, there is reason to believe that the process may be different in vein grafts whose composition and response to injury differ from those of arteries. Grafted veins are subjected to unique local and chronic trauma, including inappropriate graft preparation, prolonged ischemia, unfamiliar tangential and shear stresses, compliance mismatch, and elevated blood pressure.[9] IH lesions seen in human vein grafts are similar to those observed in animal vein graft models[10], and it may be more appropriate to use these in vivo vein models rather than extrapolate from in vitro arterial models.

Marin first used the rat epigastric vein-to-femoral artery interposition graft model for the study of IH.[11] Hoch[12] and Faries[13] independently developed the model further and described temporal expressions of growth factors, cytokines and cytokine gene expression in relation to the development of IH. In this particular model, it was described that interleukin 1B (IL-1B) was one of the earlier cytokines expressed that presumably triggers the subsequent cascade of events leading to the development of IH.[18] This study was designed to elucidate further the role of IL-1B by determining whether suppression of its

action by neutralizing antibodies will inhibit the growth of IH.

Methods

Animal Preparation

Thirty-seven male Sprague-Dawley rats weighing 375-400gms underwent bilateral epigastric vein to femoral artery interposition graft procedures. (Figure 1) All rats were treated and handled in accordance 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 (NIH publication No. 86-23, revised 1985). Each rat was anesthetized by an intraperitoneal injection of 40 mg/kg sodium pentobarbital. Using microsurgical techniques under 7.5x magnification, the superficial epigastric vein and common femoral artery were dissected out and isolated. A 5mm long segment of epigastric vein was excised and irrigated with heparinized saline solution. An equal segment of femoral artery was resected after attaining proximal and distal control. The epigastric vein segment was then interposed and anastomosed end-to-end onto the femoral artery using ten interrupted Dermalon (Davies & Geck) sutures. Arterial occlusion time ranged from 30 minutes to an hour. After removal of proximal and distal control, immediate restoration of blood flow was confirmed and patency ensured by standard microsurgical methods.

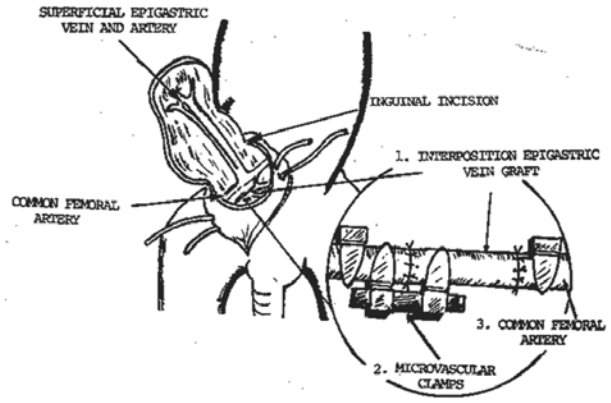

Figure 1. Rat vein graft model of intimal hyperplasia. Dissection and interposition of epigastric vein to femoral artery.

Determination of Rat Vein Graft IL-1B Content

It has been described that IL-1B expression in the vein graft peaks at 2 days after exposure to the arterial environment. The approximate levels needed to be established in order to determine the amount of neutralizing antibodies to be used. Four interposed epigastric vein grafts were harvested after two days. The specimens were homogenized and IL-1B content measured using a rat IL-1B ELISA kit (RLB00, R&D Systems). The kit is supplied with a 96 well microplate pre-coated with polyclonal antibody specific for rat IL-1B and prepared reactant solutions. 50L of tissue homogenate, standard, or control was added to each well and incubated for 2 hours at room temperature. Each well was then washed five times with 400 L wash diluted buffer solution. 100L rat IL-1B conjugate (rat IL-1B antibody conjugated to horseradish peroxidase) was then added to each well and then incubated for 2 hours. The wash cycle was repeated, followed by incubation for 30 minutes at room temperature with 100 L substrate solution (1:1 stabilized hydrogen peroxide and stabilized tetramethylbenzidine) in each well, and the reaction stopped with 100L diluted hydrochloric acid solution. An automated plate reader was used to measure optical density set to 540 nm with wavelength corrections, and IL-1B content was derived from a standard graph. Results were compared to those obtained from control uninterposed epigastric veins.

Preparation of Local Antibody Delivery Solution

Local delivery of IL-1B neutralizing antibody was through the controlled-release mechanism of the polymer F-127 pluronic gel (BASF Inc.). This route of delivery has the advantage of being able to localize the effects of the released antibody maximally on the applied target area while also being able to neutralize hemodynamically-borne IL-1B expressed from more distant sites. F-127 pluronic gel is soluble at 4ºC and solidifies on contact with tissues at 37ºC. Controlled release of substances was demonstrated as a continuous release of substance with 90% released by 2 days, and approaching 100% by the third day. Monoclonal anti-rat IL-1B antibody (MAB501, R&D Systems) was used in this study to

neutralize IL-1B activity. This antibody has a neutralization dose 50 (ND50) at approximately 10-30µg/mL in the presence of 4ng/mL rat IL-1B, and nearly 100% neutralization at 1000µg/mL. The gel-antibody solution was formulated by preparing phophate buffer of pH 7.0 (sodium phosphate dibasic [0.015 mol/L] and potassium phosphate monobasic [0.05 mol/L]). The pH was adjusted with 5N sodium hydroxide. Phosphate buffer (2.3mL) was cooled to 4ºC in a graduated test tube. Seven hundred fifty milligrams of F-127 pluronic polymer and 30mg of monoclonal IL-1B antibody previously cooled were added. The volume was completed to 3mL with previously cooled phosphate buffer solution and stored at 4ºC.

Treatment Protocol

Twenty-eight subjects underwent bilateral epigastric vein to femoral artery interposition grafting as described previously. Treatment and control sides were determined randomly for each subject. Control grafts received pluronic gel only, while the treatment vein received the gel-antibody suspension. After excision, the grafts were incubated for 30 minutes in the chilled gel or gel-antibody suspension. Subsequently, the graft was interposed onto the femoral artery, and 100µL of gel only or gel-antibody suspension was locally applied. This amount ensures delivery of 1000 g of neutralizing antibody. Following satisfactory tests of patency, the groin wounds were closed with absorbable sutures.

Specimen Harvest and Analysis

Fourteen subjects were sacrificed at the end of one week, and another fourteen sacrificed at the end of two weeks. Grafts were harvested to include 5mm proximally and distally of femoral artery. The specimens were perfusion-fixed in 10% formalin, divided longitudinally, and post-fixed in 1% osmium tetroxide and dehydrated in increasing concentrations of ethyl alcohol. The samples were then embedded in paraffin and sectioned in 1(m sections cut longitudinally and stained with hematoxylin and eosin. Vein graft intimal thickness were measured at the perianastomotic and in the midgraft regions.

Results

Rat Vein Graft IL-1B Content

ELISA assay demonstrated that at 2 days after interposition onto an arterial environment, the local concentration of IL-1B in the epigastric vein graft was 720pg/mL, whereas that in the uninterposed vein graft was 50pg/mL (Figure 2). The dose of neutralizing antibody used as set at 1000 g is more than five times that required to inhibit 100% of active IL-1B and therefore is of physiologic significance.

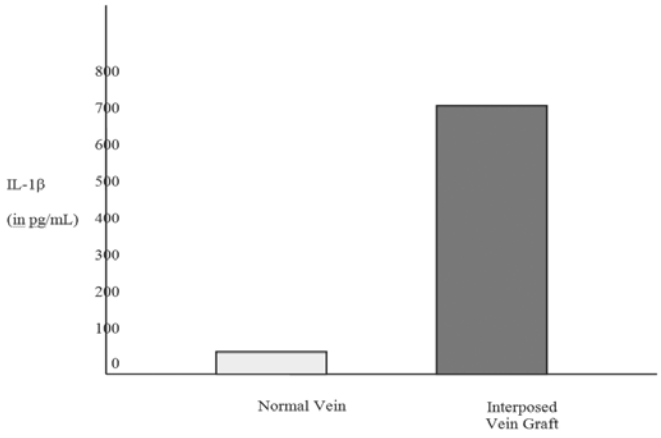

Figure 2. Interleukin 1B content in interposed and uninterposed vein grafts.

Histologic Examinations

Measures of vein graft total wall intimal thicknesses demonstrate that IH occurring at the anastomotic and midgraft regions at the end of one week were significantly less with the treated grafts. IH was maximal at the end of two weeks, however measures of IH at the anastomotic and midgraft regions for both treated and untreated grafts did not demonstrate any significant difference (Figures 3-6). Histologic examinations revealed that inflammatory cell infiltrates were present in all specimens; there were smaller numbers in the treated grafts at the end of one week, but did not attain significant differences at the end of two weeks.

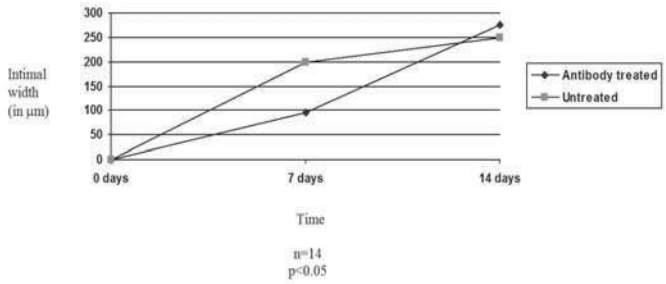

Figure 3. Total wall thickness measured at anastomotic region.

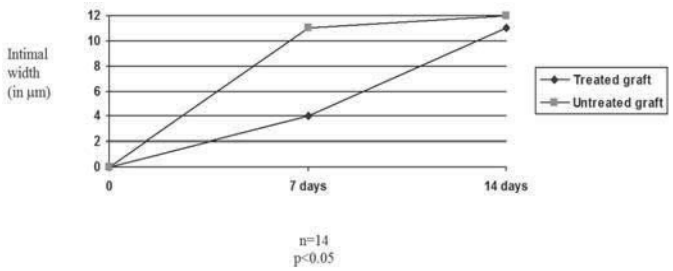

Figure 5. Maximal intimal thickness at anastomotic region.

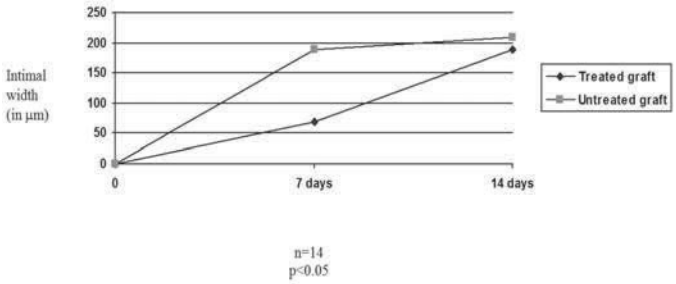

Figure 4. Total wall thickness measured at midgraft.

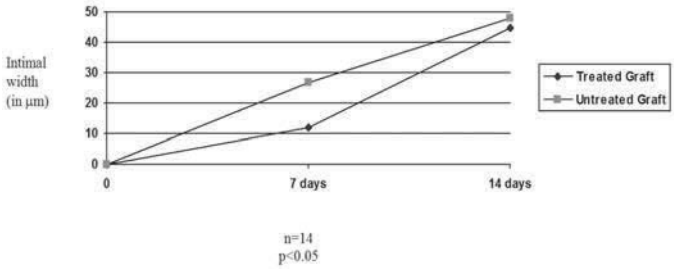

Figure 6. Maximal intimal hyperplasia at anastomotic region.

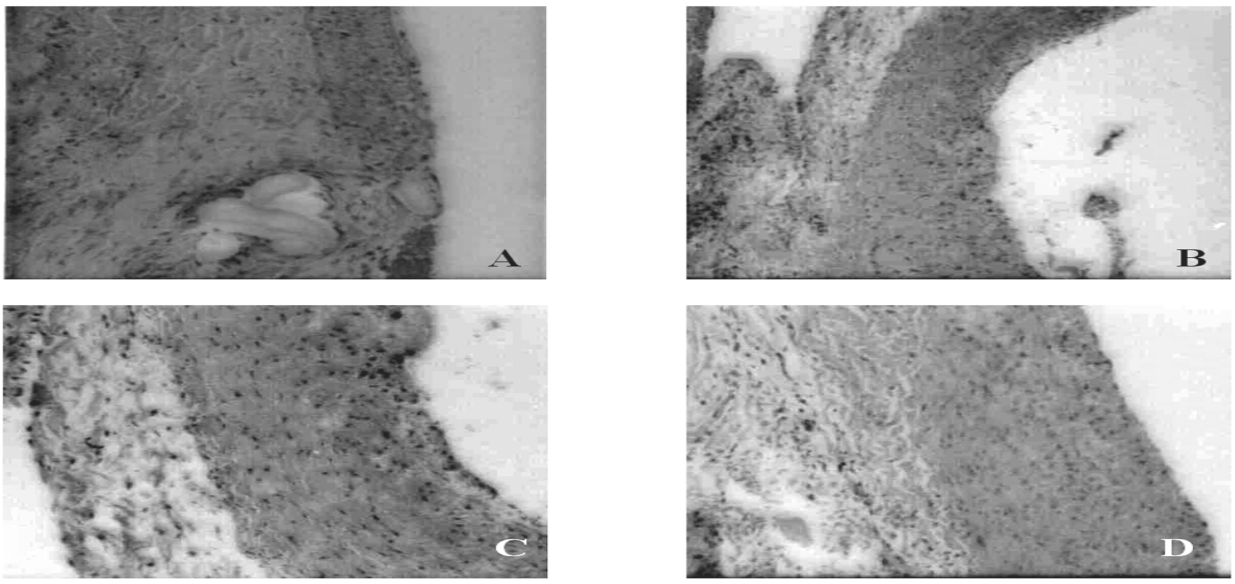

Figure 7. Histologic appearances of treated and untreated vein grafts.

A. Perianastomotic region of rat vein graft treated with neutralizing antibody to IL-1B after one week. Note the relative paucity of inflammatory cells. A suture knot is seen at the foreground.

B. Perianastomotic region of rat vein graft treated with pluronic gel only after one week. Note the greater degree of intimal hyperplasia and the numerous scattered inflammatory cells.

C & D. Perianastomotic region of rat vein graft treated and not treated with neutralizing antibody to IL-1B at the end of two weeks. The degree of intimal hyperplasia does not differ significantly.

Discussion

Although it is a universal response of a vein graft to insertion into the arterial circulation, intimal hyperplasia remains to be one of the major obstacles to long term graft patency.[14] It is considered to be the result of both the migration of vascular smooth muscle cells out of the media into the intima and the proliferation of these smooth muscle cells and deposition of an extracellular matrix.[15]

The precise initiating stimuli for IH have not been fully defined, but it appears to be the response of the vascular cells to a combination of physical, cellular and humoral factors, possibly accompanied by dysfunctional endothelial regulation. Although many different stimuli interact to produce IH, growth factors are of paramount importance in its development.[16] Platelet-derived growth factor (PDGF) was the first to be described[17], and was demonstrated to be a stimulus to vascular smooth muscle cell (VSMC) migration and proliferation in the vessel wall.[18,19,20] Fibroblast growth factor (FGF) has also been studied and found to be associated with VSMC proliferation in response to vessel wall injury.[21,22] Exogenous application of interleukin-1B (IL-1B) was shown to induce IH in coronary lesions and vasospastic responses.[23] Other growth factors such as insulin-like growth factors (IGF), transforming growth factors (TGF), and tumor necrosis factors (TNF) have all been implicated, but their exact roles are still undefined.[7]

Of these factors, those mediators of the tyrosine kinase (IGF-1, TGF-α, and IL-1B) and G-protein coupled membrane receptors may be responsible for the initial signalling event.[15] IL-1B was demonstrated to be the earliest of this group to be expressed by the vein graft in response to exposure to the arterial environment.[13] It is not only an inflammatory cytokine but also a growth factor for smooth muscle cells. However, it does not have a direct mitogenic action for smooth muscle cells, and its proliferative effects are mediated by other growth factors, mainly PDGF, which are induced and released by fibroblasts and smooth muscle cells in response to IL-1B.[23] Demonstrating the results of inhibiting the action of this early response mediator will establish its role in the development of IH.

This study demonstrated that locally-delivered neutralizing antibody to IL-1B retarded the development of IH at the end of the first week, but the inhibitory effect did not persist and was not evident by the end of the second week. Inasmuch as the inhibition was significant but not complete as evidenced by the presence of IH in all specimens, these results suggest that the initiation of the sequence of events is played in a major way but not singularly by IL-1B. Despite more than adequate amounts of antibody that can neutralize not only the locally expressed but also distally generated IL-1B, some amount of IH was still produced as a result of the interposition. It was mentioned earlier that vascular smooth muscle migration and proliferation induced by IL-1B is mediated by other growth factors such as PDGF. PDGF was originally shown to be released from aggregating platelets, and is now also known to be released from endothelial cells, macrophages, and smooth muscle cells. Other mediators therefore may be acting in concert with IL-1B to initiate the cascade of sequences responsible for the development of IH, but of all these early mediators, IL-1B most probably plays the greater role.

The study also demonstrated that antibody neutralization of IL-1B delayed the onset, but did not prevent the development of IH in these vein grafts. The local delivery of neutralizing antibodies by pluronic gel would have been completed by the end of 72 hours. The inhibitory effect was carried over four days further, as shown by the relative paucity of inflammatory cells and smaller degree of IH, but by the end of the second week, IH was just as exuberant as the treated side. The continuous exposure of the vessel wall to arterial flow and wall tension provided an unceasing signal to the vein graft to adapt to the new environment that could not be prevented by temporary inhibition of the early response mediator. The stretching forces persistently induce endothelial cell gene transcription of cytokines, transforming hemodynamic change into cellular response,[25] and once the inhibitory influence of the antibody was discontinued after the third day, the sequence of events leading to IH was still triggered, until the amount of IH in these treated grafts approximated that in the untreated group by the end of two weeks.

Conclusion

While substantiating the major role played by IL-1B as an early mediator for the development of IH in vein grafts, these results emphasize the limitation of this approach as a strategy for the prevention of IH. Given the multiple cellular and humoral influences on the development of IH in vein grafts, inhibition of a single growth factor is only likely to result in partial suppression. Multi-step inhibition or multi-modality strategies involving combinations of pharmacologic agents, growth factor antagonists, antisense inhibition, or gene therapy may be required for complete and significant inhibition of IH development. Further advances in molecular biological techniques offer enormous and exciting potentials for the better understanding of processes and the future development of effective strategies in vein graft intimal hyperplasia.

References

1. Imparato AM, Bracco A, Kim G, Zeff R. Intimal and neointimal fibrous proliferation causing failure of arterial reconstruction. Surgery 1972; 72:1007-17.
2. Stanley JC, Graham LM, Whitehouse WM, et.al. Autogenous saphenous vein as an arterial graft: clinical status. In: Stanley JC ed. Biologic and synthetic vascular prosthesis. New York: Grune & Stratton Inc. 1982; 333-49.
3. Davies AH, Magee TR, Baird RN, Sheffield E, Horrocks M. Vein compliance: a preoperative indicator of vein morphology and of veins at risk of vascular graft stenosis. Br J Surg 1992; 79: 1019-21.
4. Marin ML, Veith FJ, Panetta TF, Gordon RE, Wengester KR, Suggs WD, Sanchez L, Parides MK. Saphenous vein biopsy: a predictor of vein graft failure. J Vasc Surg 1993; 18: 407-25.
5. Underwood MJ, More R, Weeraseena N, Firmin RK, DeBono DP. The effect of surgical preparation and in vitro distension on the intrinsic fibrinolytic activity of human saphenous vein. Eur J Vasc Surg 1993; 7: 518-22.
6. Schwartz LB, O'Donohue MK, Purut CM, Mikat EM, Hagen PO, McCann RL. Myointimal thickening in experimental vein grafts is dependent on wall tension. J Vasc Surg 1992; 15: 176-86.
7. Newby AC, George SJ. Proposed roles for growth factor in mediating smooth muscle proliferation in vascular pathologies. Cardiovasc Res 1993; 27: 1173-83.
8. Foegh ML, Virmani R. Molecular biology of intimal proliferation. In: Annual of Cardiac Surgery. Yacoub M, Pepper J, eds. London: Current Science Ltd. 1994: 63-5.
9. Stark VK, Warnes TF, Hoch JR. An ultrastructural study of progressive arterial hyperplasia in rat vein grafts. J Vasc Surg 1997; 26: 94-103.
10. Adcock GD, Adcock OT, Wheeler JR, Gregory RT, Snyder SO, Gayle RG, Trivedi AN. Arterialization of reversed autologous vein grafts: qualitative light and electron microscopy of canine jugular vein graft harvested and implanted by standard or improved techniques. J Vasc Surg 1987; 6: 283-95.
11. Marin ML, Hardy AA, Gordon RE, Reemtsma K, Benvenisty AI. Immunomodulation of vascular endothelium: effects of ultraviolet B irradiation on vein allograft rejection. J Vasc Surg 1990; 11: 103-11.
12. Hoch JR, Stark VK, Hullet DA, Turnipseed WD. Wein graft intimal hyperplasia: Leukocyte and cytokine gene expression. Surgery 1994; 116: 463-71.
13. Faries PL, Marin ML, Veith FJ, Ramirez JA, Suggs WD, Parson RE, Sanchez LA, Lyon RT. Immunolocalization and temporal distribution of cytokine expression during the development of vein graft intimal hyperplasia in an experimental model. J Vasc Surg 1996; 24: 463-71.
14. Mannick JA. Improved limb salvage from modern infrainguinal artery bypass techniques. Surgery 1992; 111: 361-2.
15. Davies MG and Haagen PO. Pathophysiology of vein graft failure: a review. Eur J Vasc Endovasc Surg 1995; 9: 7-18.
16. Bryan AJ, Angelini GD. The biology of saphenous vein graft occlusion: etiology and strategies for prevention. Curr Opin Cardiol 1994; 9: 641-9.
17. Ross, R, Glomset J, Kariya B, Harker L. A platelet dependent serum factor stimulating the proliferation of arterial smooth muscle cells in vitro. Proc Natl Acad Sci USA 1974; 71: 1207-10.
18. Javien A, Bowen-Pope DF, Lindner V, Schwatrz SM, Clowes AW. Platelet derived growth factor promotes smooth muscle cell migration and intimal thickening in a rat model of balloon angioplasty. J Clin Invest 1992; 89: 507-11.
19. Bornfudt KE, Raines EW, Nakaro T, Ceraus LM, Krebs EG, Ross R. Insulin-like growth factor-1 and platelet derived growth factor induce directed migration of human arterial smooth muscle cells via signalling pathways that are distinct from those of proliferation. J Clin Invest 1994; 93: 1266-74.
20. Nabel EG, Yang Z, Liptay S, San H, Gordon D, Hauderschild C, Nable GJ. Recombinant Platelet derived growth factor B gene expression in porcine arteries induce intimal hyperplasia in vivo. J Clin Invest 1993; 91: 1822-9.
21. Edelmann ER, Nugent MA, Smith LR, Karnovsky MJ. Basic fibroblast growth factor enhances the coupling of intimal hyperplasia and proliferattion of vasa-vasorum in injured rat arteries. J Clin Invest 1992; 89: 465-73.

22. Nabel EG, Yang Z, Plantz G, Forough R, Zhau Y, Hauderschild CC, Haciag T, Nagel GJ. Recombinant FGF-1 promotes intimal hyperplasia and angiogenesis in arteries in vivo. Nature 1993; 362: 844-6.
23. Shimokawa H, Ito A, Fukumoto Y, Kadokami T, Nakaike R, Sakata M, Takayanagi T Egashira K, Takeshita A. Chronic treatment with interleukin 1B induces coronary intimal lesions and vasospastic responses in pigs in vivo. J Clin Invest 1996; 97: 769-76.
24. Jawien A, Bowen-Pope DF, Lindner V, Schwartz SM, Clowes AW: Platelet derived growth factor promotes smooth muscle cell migration and intimal thickening in a rat model of balloon angioplasty. J Clin Invest 1992; 89: 507-11.
25. Hsieh HJ, Li NQ, Frangos JA: Shear stress induced platelet derived growth factor gene expression in human endothelial cells is mediated by protein kinase C. J Cell Physiol 1992; 150: 552-8.