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Immunohistochemical evaluation of the effects of nebivolol on intimal hyperplasia following endothelial injury

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Aim: Intimal hyperplasia is a vascular remodeling process. It is a clinical problem that forms in the vascular wall as a result of smooth muscle cell migration, proliferation, and extracellular matrix accumulation. In this study we examined the immunohistochemical evaluation of the effects of nebivolol on intimal hyperplasia in damaged endothelial tissue.

Materials and methods: The study was conducted using 21 rabbits equally divided into 3 groups: control, solvent, and nebivolol. The rabbits in the control group only underwent balloon injury of the abdominal aorta. The rabbits in the solvent group and nebivolol group underwent balloon injury and were treated with solvent and nebivolol intraperitoneally during the study. At the end of the study, the abdominal aortas were harvested. The intimal and medial areas were measured and the intima/media ratios were calculated. Tissue nitric oxide levels were determined and immunohistochemical findings were evaluated.

Results: Statistically there were no differences between the control and solvent groups with respect to the intimal and medial areas, intima/media ratios, or the tissue nitric oxide (NO) levels. The neointimal thickening was significantly less in the nebivolol group than in the control and solvent groups ($P < 0.001$). Intima/media ratio was decreased in the nebivolol group ($P < 0.001$). Tissue nitric oxide levels were greater in the nebivolol group than in the control and solvent groups ($P < 0.01$). Immunohistochemical data in the nebivolol group were significantly lower as compared with the other groups ($P < 0.05$).

Conclusion: Nebivolol may be a useful agent in early restenosis after vascular reconstructive procedures.

Key words: Intimal hyperplasia, nitric oxide, nebivolol

Endotel hasarı sonrası oluşan intimal hiperplazi üzerine nebivololün etkilerinin immünohistokimyasal değerlendirilmesi

Amaç: İntimal hiperplazi bir vasküler remodeling sürecidir. Bu vasküler duvarda düz kas migasyonu, proliferasyonu ve ekstrasellüler matriks birikimi sonucu oluşan klinik bir problemdir. Bu çalışmada hasarlanmış endotel dokusunda oluşan intimal hiperplazi üzerine nebivololün etkilerinin immünohistokimyasal değerlendirilmesi araştırdık.

Yöntem ve gereç: Çalışma, kontrol, solvent ve nebivolol şeklinde eşit 3 guruba ayrılan toplam 21 tavşan üzerinde gerçekleştirildi. Kontrol grubundaki tavşanların abdominal aortalarına sadece balon ile hasar uygulandı. Solvent ve nebivolol grubundaki tavşanlara balon hasarı uygulandı ve çalışma boyunca intraperitoneal solvent ve nebivolol verildi. Çalışma sonunda, abdominal aort kesitlerinde intimal-medial alanları ölçülerek her bir kesitteki intima/media oranları elde edildi. Doku nitrik oksit düzeyleri ölçüldü ve immünohisyokimyasal bulgular kaydedildi.

Bulgular: Kontrol ve solvent grupları arasında intimal, medial alanlar ile intima/media oranları ve doku NO düzeyleri yönünden anlamlı fark bulunmadı. intimal alan artışı nebivolol grubunda kontrol ve solvent gruplarına göre anlamlı

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derecede az iken (P < 0,001), intima/media oranı da anlamlı olarak azaldı (P < 0,001). Doku NO düzeylerinde ise nebivolol grubunda kontrol ve solvent gruplarına göre belirgin artış gözlendi (P < 0,01). Nebivolol grubundaki immünohisyokimyasal veriler diğer gruplara göre belirgin olarak azalmıştı (P < 0,05).

Sonuç: Nebivolol kullanımının vasküler girişimlerden sonra erken restenoz gelişiminin engellenmesinde yararlı bir ajan olabileceği düşüncesindeyiz.

Anahtar sözcükler: İntimal hiperplazi, nitrik oksit, nebivolol

Introduction

Arterial reconstructions can preserve the ischemia with occlusive disease. The most important problems reducing the success rate in arterial reconstructions are thrombosis, intimal thickening, and luminal narrowing that develop in the early and late periods (1). Overall, intimal hyperplasia is held responsible in approximately half of the early and late failures of reconstruction. All forms of vascular interventions cause damage to the vascular wall. Several authors have focused on the "response to injury" hypothesis in the development of intimal hyperplasia (2-4). The development of intimal thickening is a part of the physiological reparative response to induced damage by the artery or graft (2,5). It has been documented that several factors including endothelial damage, platelet adherence and activation, mitogenic factors, and smooth muscle proliferation and migration are involved in development of this response (6).

The main event in the pathological intimal hyperplasia developing in an injured artery is the replication and migration of smooth muscle cells. Macrophages and endothelial and smooth muscle cells have been shown in cell culture studies to synthesize and secrete several mitogenic factors (7,8). Among these mitogenic factors, the most significant ones are basic fibroblast growth factor (bFGF) and the platelet-derived growth factor (PDGF). The proliferation of smooth muscle cells in the media layer and their migration to the intima are induced by these 2 factors (8). Basic FGF is stored in the endothelial and smooth muscle cells. It is secreted in response to endothelial damage, and stimulates proliferation of smooth muscles in the media layer (9). On the other hand, PDGF is secreted following activation of platelets when they are adhered to and aggregated on the damaged vascular surface. Though it is nominated as platelet-derived, PDGF is also secreted by the damaged endothelial and smooth muscle cells (9-11).

The endothelial cells play critical roles in maintenance of structural and functional characteristics of the vascular wall (12). Nitric oxide (NO) synthesized by the vascular endothelium is an active compound with cytoprotective, regulatory, and cytotoxic effects (13). It inhibits cellular activation in the damaged endothelium in order to prevent adhesion of monocytes and leukocytes to the endothelium and prevents proliferation and migration of vascular smooth muscle cells (14-19). Despite the absence of in vivo studies evaluating the effects of nebivolol on the development of intimal hyperplasia, it has been shown to induce a direct vasodilator effect via activation of endothelium-derived NO, which plays a key role in regulation of the vascular tone (20). Furthermore, Kakoki et al. have proposed that nebivolol is a potent NO synthase stimulator (21).

Various pharmacological agents have been used with the purpose of preventing the development of intimal hyperplasia (5,16-18). In this study, we suggest that nebivolol, which increases NO release from the endothelium, would reduce intimal thickening. Also we studied the activity of nebivolol immunohistochemically.

Materials and methods

A modification of the balloon catheter damage model described by Hamon et al. (19) was used in this study. Following approval of the ethics committee of our faculty, the study was conducted on 21 healthy adult white male New Zealand rabbits with a mean age of 10 months and weighing 2400-2850 g. The rabbits were obtained from the Veterinary Control and Research Institute of the Ministry of Agriculture in Elazığ. During the experiments, the rabbits were kept in a sunlit room with air conditioning and the room temperature was kept at 20 ± 2 °C. Guide for the Care and Use of the Laboratory Animals was considered in all experiments carried out in this study.

Groups

The rabbits were randomized into 3 groups of 7 rabbits each.

Group I (the Controls): The 7 rabbits in this group did not receive nebivolol and only underwent the procedure of vascular balloon catheter damage.

Group II (Solvent): Two days prior to the procedure, the 7 rabbits in this group began to receive the solvent, which was used for solving the active compound, intraperitoneally at a volume equivalent to that introduced in the nebivolol group, and the administration of solvent continued for 28 days following the procedure.

Group III (Nebivolol): The 7 rabbits in this group intraperitoneally received nebivolol (Vasoxen, İ.E. Ulagay, Turkish Republic) 2.5 mg/kg/day. The administration of nebivolol was initiated 2 days prior to and was continued until 28 days after the procedure.

Preparation of the solution:

Nebivolol (Vasoxen®, Berlin-Chemie, Menarini Group, I. E. Ulagay, Germany), which is a lipophilic agent, was obtained in the form of 5-mg tablets. The solvent was prepared by mixing 80% distilled water and 20% polyethylene glycol (Merck-Schuchardt, Hohenbrunn, Germany) volumetrically. The nebivolol tablets were powdered under sterile conditions and then added to physiologic saline to obtain a mixture that included 2.5 mg nebivolol per milliliter.

Balloon catheter damage:

All rabbits were anesthetized using intramuscular administration of ketamine hydrochloride (50 mg/kg Ketalar®, Eczacıbaşı, Turkish Republic) and xylazine hydrochloride (5 mg/kg, Rompun®, Bayer, Turkish Republic). When needed, 1/3 of this dose was repeated intramuscularly. Following anesthesia, an intravenous line was inserted into the dorsal ear vein of each rabbit for intravenous administration of amoxicillin (Ampisina®, Mustafa Nevzat, Turkey) 50 mg/kg and heparin sulfate (Liquemine®, Roche, Turkish Republic) 300 IU/kg 5 min prior to surgical intervention. Then a longitudinal incision was made under sterile conditions in the right pubic area for mobilization of the right superficial femoral artery. A vertical femoral arteriotomy was performed for advancing a balloon angioplasty catheter (Cordis-Europa, Holland) with a diameter of 2.5 mm and length of 20 mm through the femoral artery in a retrograde manner to the abdominal aorta. Under fluoroscopy, the balloon of the angioplasty catheter was inflated in the infrarenal abdominal aorta using a balloon inflation device (Sedat SA, France) filled with physiologic saline until a pressure of 8 atmospheres was achieved, and was then retreated back to the femoral artery. This procedure was performed 3 times while rotating the catheter by 120°. In this way, intimal damage was induced in the vascular wall. The catheter was removed and the femoral artery was ligated at the distal and proximal sides of the arteriotomy, followed by suturing of the cutaneous incision.

Hemodynamic studies

The systolic, diastolic, and mean arterial pressures and heart rates were measured in all rabbits at both the beginning and the end of the experiment. The right middle ear artery of each rabbit was cannulated with a 24 G branula at the beginning and end of the experiment for recordings using a portable monitor (SC-600, Siemens, USA). In the treatment group, the measurements at the end of the experiment were made 24 h following the administration of the drug. To prevent the rabbits being affected by environmental factors, the hemodynamic evaluations were made using the same anesthetic methods and doses 20 min after the administration of the anesthetic agents.

Preparation of the vascular specimens

At the end of the experiment, all rabbits were anesthetized using the same method and the abdominal aorta was freed via a subxyphoid approach, and was cannulated with a 22 G branula. At the same level, the vena cava inferior was cannulated with a 22 G branula for draining the venous blood. For preserving the in vivo dimensions of the arteries until the histological examination, physiologic saline was administered at a pressure of 80 mmHg through the cannule within the aorta until clear fluid came from the vena cava inferior. Then 10% formaldehyde solution was infused through the same route at a pressure of 80 mmHg for 20 min for fixation of the vessels.

The abdominal aorta was removed to the iliac bifurcation without causing damage. A 3-cm-long segment of the aorta distal to the level of the renal artery was placed in a 10% formaldehyde solution for referral to the pathology laboratory for histochemical and immunohistochemical examinations, while the segment until the iliac bifurcation was flushed with physiologic saline and covered with aluminum foil for referral to the biochemistry laboratory.

Biochemical examinations

The preparation of the NO fraction in the aortic tissue was as follows: the weighed aortic tissues were taken into homogenization tubes and then 9 mL of Tris HCl buffer was added (0.2 mM; pH 7.5) for expressing our results as μmol/g wet tissue.

The tissue specimens were homogenized at a speed of 16,000 rpm. The homogenates were used for determination of NO levels. In several previous studies, the concentrations of endogenously synthesized NO in tissues and body fluids were determined by measuring nitrites and nitrates, as NO soon is converted to first nitrite and then to nitrate at the same site of synthesis. However, because nonspecific reactions may take place in protein-rich homogenates and solutions such as serum and plasma, we deproteinized the homogenates prior to measuring the nitrite and nitrate concentrations for preventing these nonspecific reactions. Following deproteinization**,** the amounts of nitrites and nitrates in tissues were measured using the Griess reaction. The total nitrite (nitrites + nitrates) concentration was determined using the modified cadmium reduction method. Copper-covered cadmium granules in glycine buffer (pH 9.7) were incubated for 90 min with the deproteinized specimen supernatant for reduction of the nitrates. The nitrite produced was then determined via spectrophotometric reading at a wavelength of 545 nm of the pink color that emerged by the reaction with sulphanilamide and the corresponding N-naphthylethylene diamine (NNDA) dinitrogenization

Immunohistochemical examination

From the paraffin blocks that had been prepared, 4-μm-thick slides were obtained, on which PDGF-β, bFGF, and endothelin-1 had been immunohistochemically applied using the avidin-biotin-peroxidase method. Then these 4-μm-thick slides were deparaffinized and rehydrated, followed by storing in 3% hydrogen peroxide for 5 min in order to measure the endogenous peroxidase activity. Later, they were rinsed with distilled water and kept in citrate buffer (pH 6.0) at 650 microwaves (mw) for 5 min each for PDGF-β, bFGF, and endothelin-1. Ultra V block was applied on the slides for 10 min. Then PDGF-β (Neomarkers, Fremont, CA, USA), bFGF (Chemicon International, USA), and endothelin-1 (Chamicon, Australia) antibodies were applied for 30 min in a bain-marie in a moist environment at a 37 °C. This was followed by rinsing with PBS 0.001 M (pH 7.4), incubation with avidin-biotin peroxidase, and staining with AEC chromogen. All slides were contrast-stained with Mayer Hematoxylin and mounted with ultramount medium.

The slides were scored from 0 to 3 according to the density of staining in the intimal region as follows:

0 (0): no staining

\+ (1): mild staining

++ (2): moderate staining, and

+++ (3): dense staining

Statistical Analyses

The statistical analyses were performed using SPSS 11.0 software. The data were expressed as the mean ± standard deviation. The differences between groups were analyzed using the Kruskal-Wallis H test, while the Mann-Whitney U test was used for comparing the 2 groups. Analysis of data within groups prior to and following intervention was made using the Wilcoxon test. The pathological data obtained within groups were analyzed using the chi-square (χ^2) test when the staining density was compared, and using the Mann-Whitney U test when the staining scores in the groups were compared. $P < 0.05$ was regarded as statistically significant.

Results

No statistically significant difference was found between arterial pressure (MAP) values of the groups prior to the experiment. At the end of the study, the MAP values in the nebivolol group were significantly lower as compared with both the control and solvent groups ($P < 0.01$). The MAP value in the rabbits

receiving nebivolol prior to the experiment was 82.57 ± 4.35 mmHg, while it was reduced at the end of the treatment period to 62.57 ± 3.86 mmHg (P < 0.01).

In contrast, no statistically significant difference existed between the MAP values prior to and at the end of the experiment in the control and the solvent groups (P > 0.05). Likewise, no significant difference was found between these 2 groups following the experiment (Table 1).

There was no statistically significant difference between the mean heart rates of the control, solvent, and nebivolol groups at the beginning of the study, whereas the mean heart rate at the end of the study was significantly lower in the nebivolol group than in the other groups (P < 0.01).

The mean heart rate in the nebivolol group was 211.01 ± 14.12 beats per minute at the beginning, and 182.85 ± 14.25 beats per minute at the end of the study, indicating a statistically significant decrease (P < 0.05). No significant difference was found between the heart rates prior to and at the end of the study in the control or solvent groups (Table 1).

Tissue NO levels

The mean NO levels per gram of wet tissue were 17.81 ± 2.55 μmol in the control, 16.26 ± 1.63 μmol in the solvent, and 23.44 μ 3.18 μmol in the nebivolol groups (Figure 1). There was no statistically significant difference between the control and solvent groups, while the NO levels in the nebivolol group were significantly higher (P < 0.01) than those of the other groups.

Immunohistochemical findings

The mean staining scores were 1.85 ± 0.29 in the control, 1.71 ± 0.16 in the solvent, and 0.85 ± 0.17 in the nebivolol groups (nebivolol vs. control and solvent, P < 0.05). No significant difference was found regarding the endothelin-1 staining density in the control and solvent groups, while the nebivolol group had both reduced intimal thickening and reduced intimal staining (Figure 2A, B, and C).

The PDGF-β staining in the intimal region was less dense in the nebivolol group than in the control and solvent groups (Figures 3A, B, and C). The mean staining scores were 1.56 ± 0.21 in the control group and 2.00 ± 0.33 in the solvent group, and there was no significant difference between these values. On the other hand, the mean staining score for PDGF-β was 0.71 ± 0.19 in the nebivolol group, and the staining density was significantly reduced as compared with the other groups (nebivolol vs. control and solvent, P < 0.05).

Table 1: Mean arterial pressure values, mean heart rate values, and NO levels.

Groups	Average Arterial Blood Pressure *(mmHg)*			Average Heart Rate (*beats/min*)			
	Beginning	End	P (Beginning - End)	Beginning	End	P (Beginning-End)	NO Levels (μmol)
Control (n = 7)	82.21 ± 3.48	81.57 ± 3.15	NS	209.57 ± 15.37	214.71 ± 13.87	NS	17.81 ± 2.55
Solvent (n = 7)	83.14 ± 3.97	82.71 ± 2.93	NS	211.57 ± 13.57	215.42 ± 11.87	NS	16.26 ± 1.63
Nebivolol (n = 7)	82.57 ± 4.35	62.57 ± 3.86* a	aP < 0.01	211.01 ± 14.12	182.85 ± 14.25* b	b P < 0.01	23.44 ± 3.18
P (between groups)	NS	* P < 0.01 (Nebivolol vs. Control) (Nebivolol vs. Solvent)		NS	* P < 0.01 (Nebivolol vs. Control) (Nebivolol vs. Solvent)		** P < 0.01 (Nebivolol group vs. Control group) (Nebivolol group vs. Solvent group)

* P < 0.01 (nebivolol end vs. control end and solvent end); ** P < 0.01 (nebivolol group vs. control and solvent groups);
[a] P < 0.01 (nebivolol beginning vs. nebivolol end); [b] P < 0.01 (nebivolol beginning vs. nebivolol end); NS: Not significant.

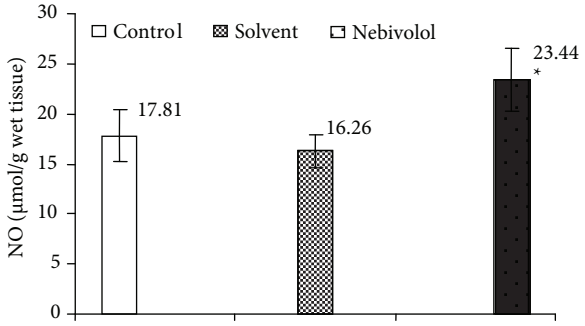

Figure 1. Tissue NO levels P < 0.01 (nebivolol vs. control and solvent).

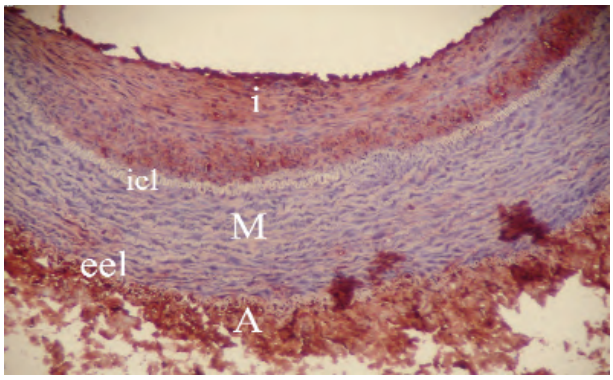

Figure 2A. Histologic section of the aorta of a rabbit from the control group ([+++] intimal immunostaining for endothelin-1, ×200).
I: Intima, M: Media, A: Adventitia
iel: internal elastic lamina, eel: external elastic lamina.

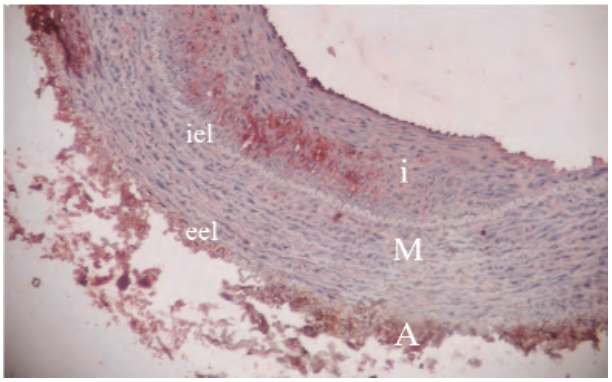

Figure 2B. Histologic section of the aorta of a rabbit from the solvent group ([+++] intimal immunostaining for endothelin-1, ×200).
I: Intima, M: Media, A: Adventitia
iel: internal elastic lamina, eel: external elastic lamina.

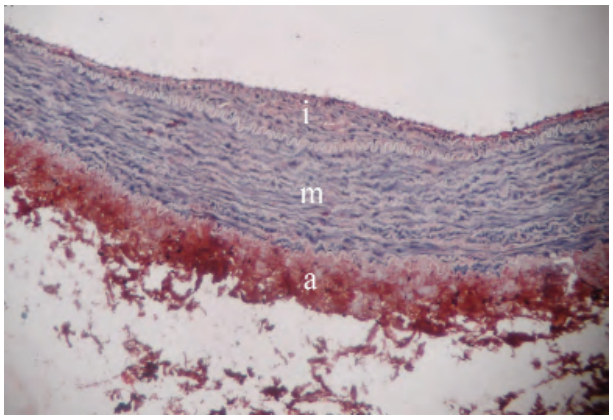

Figure 2C. Histologic section of the aorta of a rabbit from the nebivolol group ([+] intimal immunostaining for endothelin-1, ×200).
i: Intima, m: Media, a: Adventitia.

The density of staining for basic FGF in the intimal region was increased in the control and solvent groups, while it was significantly decreased in the nebivolol group (Figures 4A, B, and C). According to the scoring system, the mean scores were 2.14 ± 0.45 in the control group and 1.85 + 0.37 in the solvent group, while it was 0.85 ± 0.14 in the nebivolol group.

There was no statistically significant difference between the control and the solvent groups, while the score was significantly lower in the nebivolol group (nebivolol vs. control and solvent, P < 0.01).

Discussion

In intimal hyperplasia experiments, embolectomy, neointimal formation after balloon angioplasty catheters, and induction of tension in the wall are the most commonly used methods, particularly in rabbits (20,21). The damage caused to the endothelium by inflation of the balloon stimulates platelet adhesion and progressive smooth muscle cell proliferation in the intima of arteries (5,20). In experimental models, 3 phases have been described in the development of intimal hyperplasia, including the medial smooth

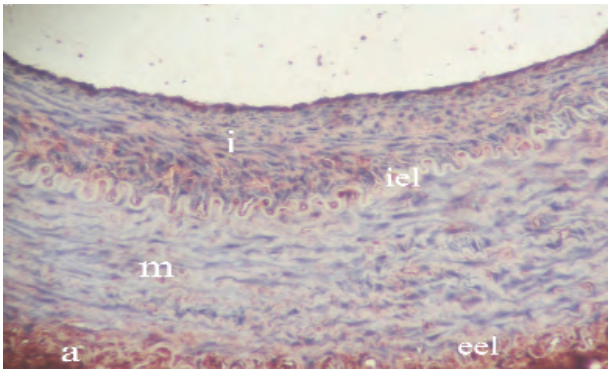

Figure 3A. Histologic section of the aorta of a rabbit from the control group ([++] intimal immunostaining for PDGF-β, ×200).
iel: internal elastic lamina, eel: external elastic lamina
i: Intima, m: Media, a: Adventitia.

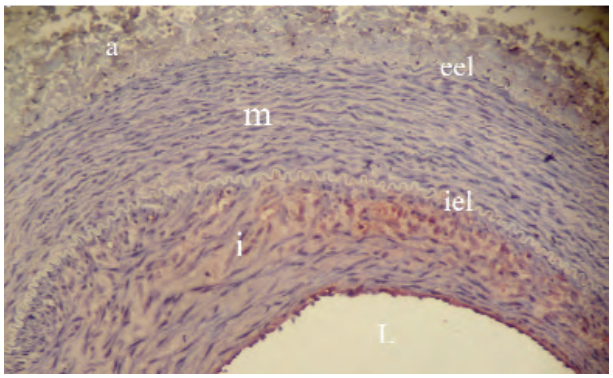

Figure 3B. Histologic section of the aorta of a rabbit from the solvent group ([++] intimal immunostaining for PDGF-β, ×200).
iel: internal elastic lamina, eel: external elastic lamina
i: Intima, m: Media, a: Adventitia.

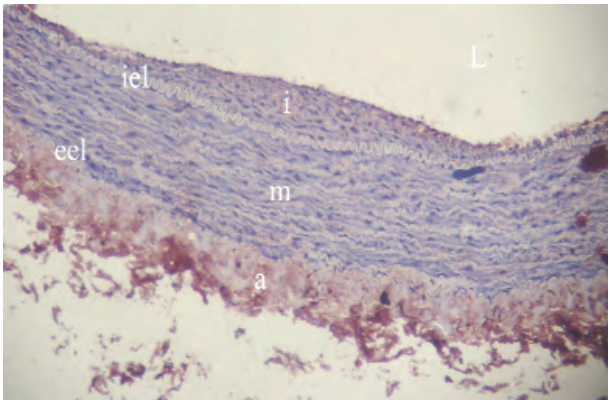

Figure 3C. Histologic section of the aorta of a rabbit from the nebivolol group ([+] intimal immunostaining for PDGF-β, ×200).
iel: internal elastic lamina, eel: external elastic lamina
i: Intima, m: Media, a: Adventitia.

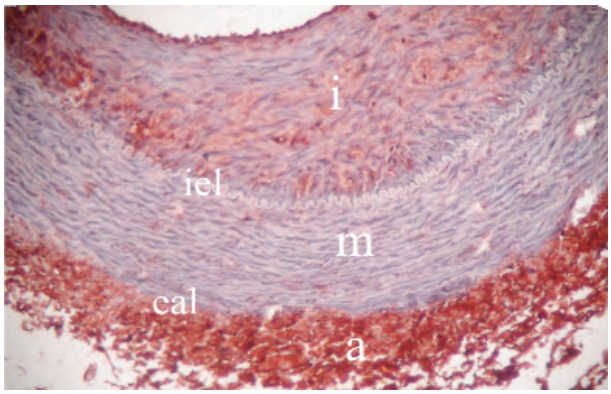

Figure 4A. Histologic section of the aorta of a rabbit from the control group ([+++] intimal immunostaining for bFGF, ×200).
iel: internal elastic lamina, eel: external elastic lamina
i: Intima, m: Media, a: Adventitia.

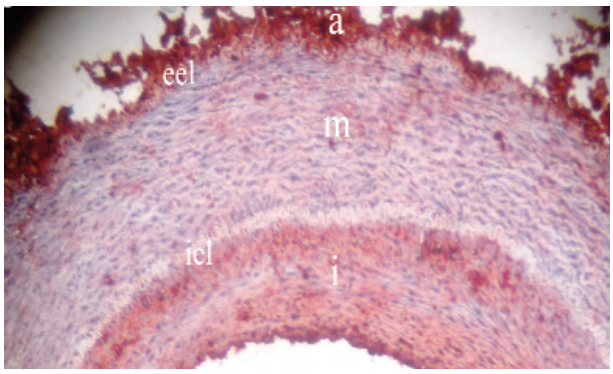

Figure 4B. Histologic section of the aorta of a rabbit from the solvent group ([+++] intimal immunostaining for bFGF, ×200).
iel: internal elastic lamina, eel: external elastic lamina
i: Intima, m: Media, a: Adventitia.

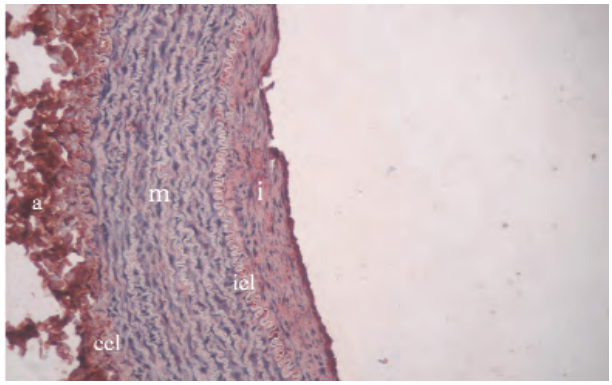

Figure 4C. Histologic section of the aorta of a rabbit from the nebivolol group ([+] intimal immunostaining for bFGF, ×200).
iel: internal elastic lamina, eel: external elastic lamina
i: Intima, m: Media, a: Adventitia

muscle cell replication observed within the first 3 days, migration of the smooth muscle cells towards the lumen that continues until the 14th day, and synthesis of the extracellular matrix that appears by the 15th day (8). In order to allow development of all phases, we based our study on histomorphologic evaluation at the end of a 28-day period.

NO is a crucial modulator of vascular damage. Indeed, NO has a number of intracellular effects that lead to vasorelaxation, endothelial regeneration, reduction of oxidation-sensitive mechanisms, inhibition of leukocyte chemotaxis, and platelet adhesion (22,23). Many commonly used drugs carry out their therapeutic actions through the production of NO and/or enhancement of NO bioactivity (24,25). For example, carvedilol, a beta-blocker currently used in the therapy of hypertension and heart failure, has been shown to possess a direct scavenging activity on oxygen radicals with consequent potent antioxidant properties (26). This agent has been more efficient than other beta-blockers against oxidation of low density lipoprotein (LDL) induced by rat vascular smooth muscle cells (VSMC) and prevented leukocyte adhesion (27). Carvedilol, but not propranolol, has been reported to be effective in reducing neutrophil accumulation in hypercholesterolemic rabbits following myocardial ischemia (28). Moreover, carvedilol exerted beneficial effects both on proliferative and early fatty lesions in cholesterol-fed rabbits (29) and endothelium dependent dilatation in patients with coronary heart disease (CHD) (30).

Nebivolol has resulted in a significant reduction of the atherosclerotic lesions elicited by the elevated dietary cholesterol and an improvement of the endothelial function and reduced the enhanced expression of inflammatory and oxidative damage markers (macrophages, adhesion molecules, and oxidative epitopes) (31). The other beta-blocker demonstrated minor effects on atherosclerotic lesion formation (32).

It has been demonstrated in experimental studies that the effective dose of nebivolol for increasing the NO levels is 2.5 mg/kg (33). We used this dose in our study. Due to the difficulty of its daily intravenous administration, and because it is lipophilic and has been used in hypertensive rats intraperitoneally, we preferred this route for administration. Traditional beta-adrenoceptor antagonists such as nebivolol reduce preload and afterload due to systemic vasodilation and improve arterial distensibility (34). In a dose of 5 mg daily, nebivolol effectively reduces systolic and diastolic blood pressure over a 24-h period. During treatment with nebivolol arterial pressure follows the natural circadian rhythm. It has been demonstrated in numerous placebo-controlled studies that exercise tolerance is not reduced during nebivolol therapy. Chronic administration to patients with left ventricular dysfunction nebivolol increases myocardial contractility (34).

In studies conducted by Hamon et al. (19) in the iliac arteries of rabbits, and by Holm et al. (18) and Chen et al. (35) in the carotid arteries of rats, orally-administered L-arginin has been shown to reduce intimal thickening profoundly. Furthermore, in another study conducted by Lee et al. (36), it has been shown that chronic NO inhalation reduces the intimal thickening that develops following arterial balloon damage.

ET-1 induces cellular hypertrophy, hyperplasia, matrix synthesis, and the release of various chemotactic and growth factors and adhesion molecules in vitro (37). In various studies it has been shown that exogenously administered ET-1 increases neointimal formations in a balloon angioplasty model in the rat carotid arteries. It has also been reported that the intimal hyperplasia can be reduced via administration of endothelin receptor antagonists (38). In human coronary artery smooth muscle and endothelial cell cultures, it has been shown that nebivolol reduces endothelin-1 secretion via reducing the preproendothelin-1 mRNA levels (39). Nebivolol increases tissue NO levels, and NO in return reduces the endothelin-1 levels. Mitsutomi et al. (40) have shown in vascular endothelium cell cultures that endogenous NO inhibits endothelin-1 synthesis via guanylate cyclase/cGMP-dependent mechanisms, and it has been also shown that exogenous NO has inhibitory effects on the synthesis of ET-1 in endothelial cells. The observations made in our study that the NO levels were higher and ET-1 immunostaining was lower in the group in which nebivolol was administered support this view.

PDGF is released following the activation of platelets in response to their adhesion and aggregation

on the damaged vascular surface (11,12). NO has been shown to inhibit platelet adhesion and aggregation. In a study conducted by Provost et al., SIN-1, a NO donor, has been shown to inhibit the mural thrombocyte adhesion to the damaged intimal region in a swine model of angioplasty. Falciani et al. (41) have demonstrated that nebivolol inhibits the aggregation of human platelets, and that this effect is achieved via increasing the NO synthesis. In a study conducted by Brehm et al. (39) in cell cultures, nebivolol has been shown to inhibit the proliferation of human coronary smooth muscle cells induced by PDGF. Likewise, the reduced immunostaining for PDGF-β in the intimal region in the group in which nebivolol was administered for 30 days as compared with the dense staining in the control and solvent groups in our study suggests that nebivolol prevents platelet adhesion and aggregation via increasing NO synthesis and indirectly reduces PDGF release (42).

Basic fibroblast growth factor (bFGF) is a complex growth factor with vasoactive activity. It induces proliferation of cells and vascular tissues via direct and indirect mechanisms. Endothelial damage is required for its proliferative activity on vascular smooth muscle cells. Edelman et al. (30) have demonstrated in a study that exogenous bFGF causes increased intimal thickening only when balloon damage is induced, and have suggested that the endothelium acts as a mechanical barrier against the effects of bFGF. Chen et al. (43) have also demonstrated that local infusion of exogenous bFGF increases the development of intimal hyperplasia and cell proliferation following vascular damage in a study conducted in endarterectomized carotid arteries of dogs.

We think that treatment that will reduce the synthesis or release of bFGF will be effective in prevention of restenotic processes.

In our reviews of the literature, we have found that Alfke et al. (44) have reported the in vitro inhibition of bFGF-induced bovine vascular smooth muscle cell migration by NO. Moreover, in another in vitro study (39), nebivolol has been shown to inhibit the bFGF-induced proliferation of human coronary smooth muscle cells.

In conclusion, balloon catheter damage causes profound intimal thickening in arteries. Nebivolol increases the vascular NO release, depresses the increment of endothelin-1 following vascular damage, reduces the releases of bFGF and PDFG, both of which are effective in proliferation and migration of smooth muscle cells, and as a consequence causes reduced intimal thickening.

Due to all of these properties, we suggest that nebivolol may be a helpful agent in preventing the development of early restenosis following vascular interventions. However, this topic needs to be investigated more in further experimental and clinical studies.

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