Nifedipine Induces Expansive Vascular Remodeling of Carotid Arteries in Rabbit Collar Model

Nifedipin Tavşan Yaka Modelinde Karotid Arterlerin Dışa Doğru Vasküler Yeniden Modellenmesine Yol Açar

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ABSTRACT Objective: Intimal thickening is an adaptive response to injury and to stimuli acting on the vessel wall. Vascular remodeling (VR) is defined as the changes in the size and/or composition of the vessels in response to dynamic and trophic stimuli. Inappropriate VR plays a crucial role in lumen loss and pathogenesis of cardiovascular diseases. Calcium channel blockers (CCBs) have been known to have vascular protective effects. However, the precise molecular mechanisms of these effects have not been fully elucidated. The aim of this study was to investigate the effects of nifedipine on intimal thickening and pathological VR, and to examine the role of the discoidin domain receptors (DDRs), which are collagen receptors, in the VR process in the collar model. **Material and Methods:** White rabbits were randomized into two groups. The groups received vehicle or nifedipine (40 mg/kg/day, p.o.) for three weeks. After seven days, a non-occlusive silicone collar was placed around the left carotid artery. To evaluate intimal thickening and VR, the intimal area, medial and luminal perimeters were measured. Furthermore, DDR expressions were assessed immunohistochemically. **Results:** Nifedipine did not inhibit intimal thickening. The collar provoked inward VR. Neither collagen content nor DDR expressions were affected by the collar. Nifedipine constituted hypertrophic outward expansive VR by involving luminal and arterial enlargement. However nifedipine did not change either collagen ingredient or DDR expressions. **Conclusion:** Nifedipine did not inhibit intimal thickening. However, it resulted in favorable expansive VR without changing collagen contents and DDR expressions. Thus, nifedipine may help to maintain luminal patency and to prevent restenosis after balloon angioplasty.

Key Words: Calcium channel blockers; discoidin receptor; rabbits; atherosclerosis; vascular patency

ÖZET Amaç: İntimal kalınlaşma hasara ve damar duvarını etkileyen uyarılara karşı verilen adaptif bir cevaptır.Vasküler yeniden modellenme (VYM) dinamik ve trofik uyarılardan dolayı damarların büyüklük ve/veya bileşiminde oluşan değişiklikler olarak tanımlanmaktadır. Uygunsuz VYM lümen kaybında ve kardiyovasküler hastalıkların patojenezinde kritik rol oynar. Kalsiyum kanal blokörlerinin vasküler koruyucu etkileri olduğu bilinmektedir. Bununla birlikte bu etkilerin kesin moleküler mekanizmaları tam olarak aydınlatılmamıştır. Bu çalışmanın amacı nifedipinin intimal kalınlaşma ve patolojik VYM üzerindeki etkilerini araştırmak ve yaka modelinde gelişen VYM sürecinde kollajen reseptörleri olan "discoidin domain reseptörleri (DDR'ler)" nin rolünü incelemektir. **Gereç ve Yöntemler:** Beyaz tavşanlar iki gruba ayrıldı. Gruplara nifedipin (40 mg/kg/gün, p.o.) ya da taşıyıcı (plasebo) üç hafta boyunca verildi. Yedi gün sonra, karotid arter etrafına sıkıştırıcı olmayan silikon bir yaka yerleştirildi. İntimal kalınlaşma ve VYM'yi değerlendirmek için intimal alan, medya ve lümen çevreleri ölçüldü. Ayrıca DDR ekspresyonları immünohistokimyasal olarak değerlendirildi. **Bulgular:** Nifedipin intimal kalınlaşmayı inhibe etmedi. Yaka içe doğru VYM'ye neden oldu. Ancak ne kollajen içeriği ne de DDR ekpresyonları yakadan etkilenmedi. Nifedipin luminal ve arteriyel genişlemeye yol açarak hipertrofik dışa doğru genişlemeye yol açan bir VYM oluşturdu. Bununla birlikte, nifedipin kollajen içeriğini ya da DDR ekspresyonlarını değiştirmedi. **Sonuç:** Nifedipin intimal kalınlaşmayı inhibe etmedi, bununla birlikte kollajen içeriği ve DDR ekspresyonlarını değiştirmeksizin dışa doğru pozitif bir VYM oluşturdu. Bundan dolayı nifedipin lümen açıklığının sürdürülmesi ve balon anjiyoplasti sonrası restenozun önlenmesi için yardımcı olabilir.

Anahtar Kelimeler: Kalsiyum kanal blokerleri; diskoidin reseptörü; tavşanlar; ateroskleroz; damar açıkkalımı

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Intimal thickening, resulting from the accumulation of vascular smooth muscle cells, is an early step of atherosclerosis and a common cause of restenosis after balloon angioplasty.[1]

In order to elucidate of the formation of intimal thickening, various experimental models in which induction of intimal thickening can be induced either by intraluminal or by perivascular manupulation have been widely used.[1] In the collar model, which has also beenused in the present study, intimal thickening has been achieved by periadventitial placement of a nonocclusive and soft silicon collar around the carotid artery of the rabbit.[2]

Calcium-dependent processes play a central role in several different cells of the cardiovascular system, including vascular smooth muscle and endothelial cells, and also in monocytes, macrophages and platelets. Calcium channel blockers (CCBs) reduce the influx of calcium into the cells principally acting on L-type calcium channels.[3] Although the precise mechanisms have not been fully clarified, a large number of studies have demonstrated that various CCBs have been shown to retard atherogenesis in animal models and to prevent the development of early lesions in humans.[4,5]

Vascular remodeling (VR) is a dynamic process that involves any change in size and/or composition of a vessel in response to hemodynamic and humoral stimuli.[6,7] Artery size changes may be favorable (adaptive) or unfavorable (pathological) in terms of lumen size preservation.[8] Although inappropriate remodeling underlies the pathogenesis of major cardiovascular diseases such as atherosclerosis and restenosis, favorable remodeling is an adaptive mechanism which compensates for intimal thickening and involves an increase in the area comprised by the external elastic lamina (EEL) and preservation of lumen size.[6,9]

Vascular collagen matrix plays a key role in the maintenance of structural integrity, geometry and elasticity of the vessels.[10] It is generally believed that the gradual increase in collagen synthesis is directly related to constrictive remodeling and restenosis.[11,12] However, the association between collagen fiber formation and arterial remodeling remains controversial.[13-15] It was shown that some calcium channel blockers were capable of influencing the metabolism of collagens within the extracellular matrix associated with atherosclerosis.[16]

On the other hand, discoidin domain receptor tyrosine kinases 1 and 2 (DDR1 and DDR2) have been identified as novel collagen receptors.[17] DDRs bind to several types of collagens and stimulate matrix metalloproteinase (MMP) production and thereby regulate cell adhesion, proliferation and extracellular matrix remodeling.[18-20] Most of the evidence suggests that collagen turnover in the arteries are important determinants of geometric remodeling and neointima formation.[10] However, the role of DDRs in arterial remodeling process has not been well characterized.

In this context, the purpose of the current study was to investigate the potential effectiveness of nifedipine (40 mg/kg/day, p.o), the prototype of dihydropyridine CCBs, on collar-induced intimal thickening and VR, as well as to examine the possible role of DDRs histologically in this model.

MATERIAL AND METHODS

MATERIALS

Material sources were as follows: Sodium pentabarbital (Psyphac, Brussels, Belgium); silicone (MED-4011) (Nusil Silicone Technology, Anglet, France) and heparin solution (Roche, Istanbul, Turkey). Nifedipine was kindly provided by Fako İlaç Sanayii A.Ş., Istanbul, Turkey.

ANIMALS

This study was approved by the Local Ethics Committee of the Faculty of Pharmacy, Ege University, Izmir, Turkey. All procedures conformed to the recommendations of Guide for the care and use of laboratory animals (http://www.nap.edu/catalog/5140.html). White rabbits of either sex (n= 18; 1.8-2.5 kg) were divided into two groups. The first group (n= 9) received nifedipine (40 mg/kg/day, p.o). The second group (n= 9) received only the vehicle (0.5% methyl cellulose solution, 2.5 ml/kg/day,

p.o). Throughout 3-week treatment period each rabbit was kept in a separate cage and allowed to access to regular diet (standard rabbit chow and tap water *ad libitum*).

MODEL

After the seventh of treatment with nifedipine and placebo, the rabbits were anesthetized with sodium pentobarbital (30 mg/kg i.v.). Subsequently, left carotid artery was surgically accessed and dissected from the surrounding tissues. A non-occlusive, flexible silicone collar (2 cm in length) was positioned around left carotid artery, as described.[2] The contralateral carotid artery was sham-operated (i.e. it was separated from surrounding connective tissue and vagus nerve, and received a similar stretch as the left carotid artery, but was not enclosed by collar). The carotid arteries were then returned to their original positions and the incisions were sutured. After recovery from the anesthesia, all rabbits were kept in their individual cages for a further two weeks before tissue isolation.

BODY WEIGHT AND BLOOD PRESSURE MESUREMENTS

Body weight and arterial pressure (systolic and diastolic) were measured before the collar application (on day 8) and the end of the treatment period (on day 22). After insertion of 22-gauge cannula to the central ear artery in conscious rabbits, arterial pressure (mmHg) was recorded using a pressure transducer (Model 377, Harvard Apparatus, Holliston, MA, U.S.A.) and flat bed recorder (Kipp Zonen, Delft, Holland) for 15 min. Mean arterial pressure was calculated from systolic and diastolic pressure measurements.

HISTOLOGY

Morphometry

After 14 day-treatment period (placebo or nifedipine), the rabbits were anticoagulated with heparin (150 units/kg i.v.). Then they (n= 18) were sacrificed with overdose of sodium pentobarbital, and both carotid arteries were isolated and dissected. One pair of segment, each one 4 mm long, were cut from both the collared and sham-operated arteries for morphometry. Then, the segments were immediately placed in 10% neutral buffered formalin for 24 hours, dehydrated in a graded series of isopropyl alcohol (60 to 100 %), followed by toluol, before being embedded in paraffin.

The vessel tissues included in the study were processed, embedded in paraffin and 5 μm sections were prepared from each specimen. The sections were stained with hematoxylin-eosin or Masson's trichrome stain. Images of two randomly chosen transverse sections from samples stained with two different stains were recorded at x4 or x40 magnification and examined under light microscopy. The hematoxylin-eosin stained sections were used to evaluate the areas of intima, media and lumen. Masson's trichrome tissue stain is an important indicator of collagen content of the matrix. This technique marks the collagen fibrils as an increase in blue color in the matrix, and it was used to assess the increase of connective tissue. Computer assisted-image analyzer system consisting of a microscope (Olympus BX-50 equipped with a high-resolution video camera (JVC TK-890E, Japan) was used for image processing. The images were transferred to computer using the high-resolution camera and Aver TV Studio Video Capture (Version 4.21.0.0 (Software) Aver Media Technologies, Inc.). All sections were digitally photographed and measured by two morphometrists with no prior knowledge of the data. The intimal cross-sectional area with perimeters of lumen and external elastic lamina (EEL) were traced by use of the software in both sections. In each segment, the luminal and medial areas (A) were simply calculated ($A= C^2/4p$) by using perimeter of lumen and EEL (C) that is not affected by the shape of the vessel segments. Carotid artery (CA) area (area within the EEL) and CA diameter of each segment were calculated from perimeter of EEL. The residual lumen ratio defined as luminal area/(intimal + luminal) area ratio, and the index defined as intimal/medial area ratio were also calculated from these measurements. The perimeters of EEL were evaluated as CA perimeters. The medial/luminal area ratio x100 was calculated from perimeter of lumen and EEL. All morphometric measurements and calculations were performed for both randomly chosen vessel

segments and the means were determined. Examined parameters to evaluate VR were established according to previous studies.[7,9,21] The descriptors used in morphometric measurements have been shown in Figure 1.

Immunohistochemistry

DDR-1 and DDR-2 immunohistochemistry was applied to the paraffin sections by using a commercial kit (Vectastain ABC Elite Kit P-6102, Vector Laboratories Inc. Burlingame, USA) according to the manufacturer's protocol. Tissue sections from sham-operated and collared carotid arteries from each group were incubated with diluted normal blocking serum (1% BSA for 30 min) to block nonspecific binding and then, they were incubated overnight at 4°C with 1:100 diluted specific primary monoclonal antibodies for DDR-1 (anti-DDR-1, cat. # SC7553 Santa Cruz Biotechnology, Santa Cruz, CA, USA) and DDR-2 (anti-DDR-2 cat. # SC7554 Santa Cruz Biotechnology, Santa Cruz, CA, USA). On the next day, appropriate biotinylated IgG secondary antibody (Vector, Burlingame, CA, USA) was applied. The bound secondary antibody was then amplified with Vector Elite ABC® Reagent, followed by chromogenic detection of the antibody-biotin-avidin-peroxidase complexes by using 0.02% diaminobenzidine (DAB) substrate (Roche Diagnostics GmbH, Mannheim, Germany) for five minutes. The sections were counterstained with Harris-hematoxylin, cleared and mounted. Negative control samples in which an equal amount of IgG was substituted for the primary antibody were included in each assay, and were uniformly negative.

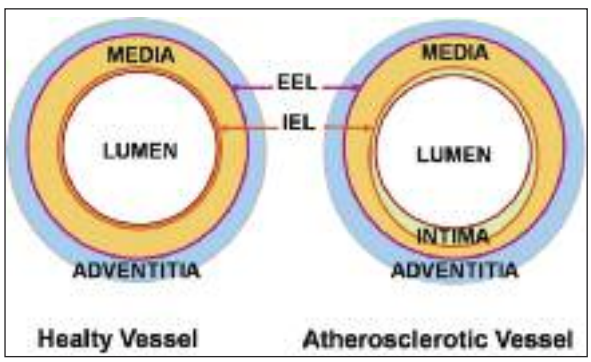

FIGURE 1: The descriptors used for morphometric measurements.
IEL: Internal elastic lamina. EEL:External elastic lamina.

STATISTICAL ANALYSIS

Statistical analyses of data were performed for drug treatments (two levels, nifedipine or placebo) as between rabbit factor; and collar (two levels, present or not) as within rabbit factor with a factorial analysis of variance (ANOVA). If there were interactions between the factors in ANOVA, the Wilcoxon signed ranks test and Mann-Whitney *U*-test were used for paired and unpaired comparisons, respectively. Shown are means±SEM.; n indicates the number of animals. Significance was accepted at $p= 0.05$. Means of all morphometric data from each segment assessment were compared.

RESULTS

SURVIVAL AND BODY WEIGHT

Only one rabbit from placebo group died during the treatment period. Nifedipine did not appear to cause any side effects. The body weights of the animals in both groups did not change during the treatment period (data not shown).

BLOOD PRESSURE

There was no significant difference in mean arterial pressure before and after collaring. Besides, nifedipin treatment did not alter mean arterial pressure (Table 1).

MORPHOMETRY

The intimal cross-sectional area and the ratio of intimal area to medial area (index) were significantly increased in collared arteries as compared to those in sham-operated arteries in the placebo group ($p< 0.05$) (Table 2, Figure 2). However, nifedipine treatment did not affect the intimal area and index (Table 2, Figure 2).

As seen in Table 1, collar placement did not alter the medial cross-sectional areas and the ratio of medial area to luminal area. Besides, the luminal area was not affected significantly by collar, but it tended to decrease. However, nifedipine treatment significantly increased luminal and medial cross-sectional areas ($p< 0.01$ and $p< 0.05$ respectively) while decreasing the ratio of the medial area to luminal area in both collared and sham-operated ($p< 0.05$) arteries (Table 2, Figure 2).

TABLE 1: Effects of collar and nifedipine (40 mg/kg/day, p.o.) treatment on mean arterial pressure (mmHg).

	Placebo (n= 8)	Nifedipine (n= 9)
Mean Arterial Pressure, mmHg		
8th day	69.64 ± 3.74	73.15 ± 2.99
22nd day	73.81 ± 5.15	72.35 ± 2.40
Significance of factors in analysis of variance:		
Collar:	n.s.	
Nifedipine:	n.s.	
Interaction:	Nifedipine by Collar	n.s.

Shown are means ± SEM. n represents the number of animals in each group.
n.s., not significant.

Although collaring significantly decreased the luminal perimeter and diameter ($p< 0.05$ and $p< 0.05$, respectively), nifedipine treatment resulted in an increase in the perimeter and diameter in both collared and sham-operated arteries ($p< 0.001$) (Table 2).

The residual lumen ratio was extremely significantly diminished in collared arteries ($p< 0.001$) (Table 2). However, nifedipine treatment significantly increased residual lumen ratio in collared arteries ($p< 0.01$) (Table 2).

The carotid artery (CA) diameter, the CA area and CA perimeter were not altered by collaring (Table 2). Nifedipine treatment significantly increased all mentioned parameters in both collared and sham-operated arteries ($p< 0.01$, $p< 0.01$ and $p< 0.01$ respectively) (Table 2).

On the other hand, as seen in Figure 3, collagen contents were not affected either by collar placement or by nifedipine treatment.

IMMUNOHISTOCHEMISTRY

Immunostaining with DDR1 and DDR2 antibodies revealed positive immunostaining for DDR1 and DDR2 in adventitia of both collared and sham-operated arteries in each group (Figure 4, 5). Nifedipine treatment affected neither DDR1 nor DDR2 expressions in collared or sham-operated arteries (Figure 4, 5).

DISCUSSION

In the present study, it has been shown that nifedipine, a dihydropyridine derivative CCB, did not prevent collar-induced intimal thickening, but involved hypertrophic outward expansive VR in rabbit carotid arteries. The effects of different CCBs on intimal thickening have been investigated in various experimental studies.[22,23] The results of these studies demonstrate that the effects of CCBs on intimal thickening are quite heterogeneous, even if

TABLE 2: Effects of collar and nifedipine (40 mg/kg/day, p.o.) on vascular remodeling parameters.

	Placebo (n= 8)		Nifedipine (n= 9)	
	Sham	Collared	Sham	Collared
Intima Area (mm^2)	0.008 ± 0.001	0.041 ± 0.008*	0.007 ± 0.001	0.048 ± 0.012*
Media Area (mm^2)	0.330 ± 0.011	0.289 ± 0.028	0.521 ± 0.083+	0.385 ± 0.056+
Index (İntima/Media)	0.024 ± 0.002	0.146 ± 0.026*	0.016 ± 0.003	0.113 ± 0.021*
Lumen Area (mm^2)	0.540 ± 0.063	0.357 ± 0.067	1.105 ± 0.139++	0.826 ± 0.116++
(Media/Lumen) x100	81.7 ± 20.1	91.6 ± 17.3	46.7 ± 3.8+	52.4 ± 10.6+
Lumen Perimeter (mm)	2.578 ± 0.154	2.080 ± 0.182*	3.667 ± 0.230+++	3.169 ± 0.202*+++
Lumen Diameter (mm)	0.820 ± 0.049	0.662 ± 0.058*	1.166 ± 0.073+++	1.008 ± 0.064*+++
Residual Lumen Ratio	0.985 ± 0.002	0.893 ± 0.015***	0.993 ± 0.011	0.947 ± 0.017**++
CA Diameter (mm)	1.047 ± 0.048	0.938 ± 0.055	1.461 ± 0.103++	1.267 ± 0.071++
CA Area (mm^2)	0.872 ± 0.078	0.705 ± 0.086	1.748 ± 0.250++	1.294 ± 0.152++
CA Perimeter (mm)	3.293 ± 0.150	2.951 ± 0.173	4.593 ± 0.325++	3.983 ± 0.223++

Shown are means± SEM. n represents the number of animals in each group.
n.s. Not significant. *$p< 0.05$, *** $p< 0.001$, sham vs. collared (ANOVA). +$p< 0.05$, ++$p< 0.01$, +++$p< 0.001$, placebo vs. nifedipine (ANOVA), CA; Carotid artery.

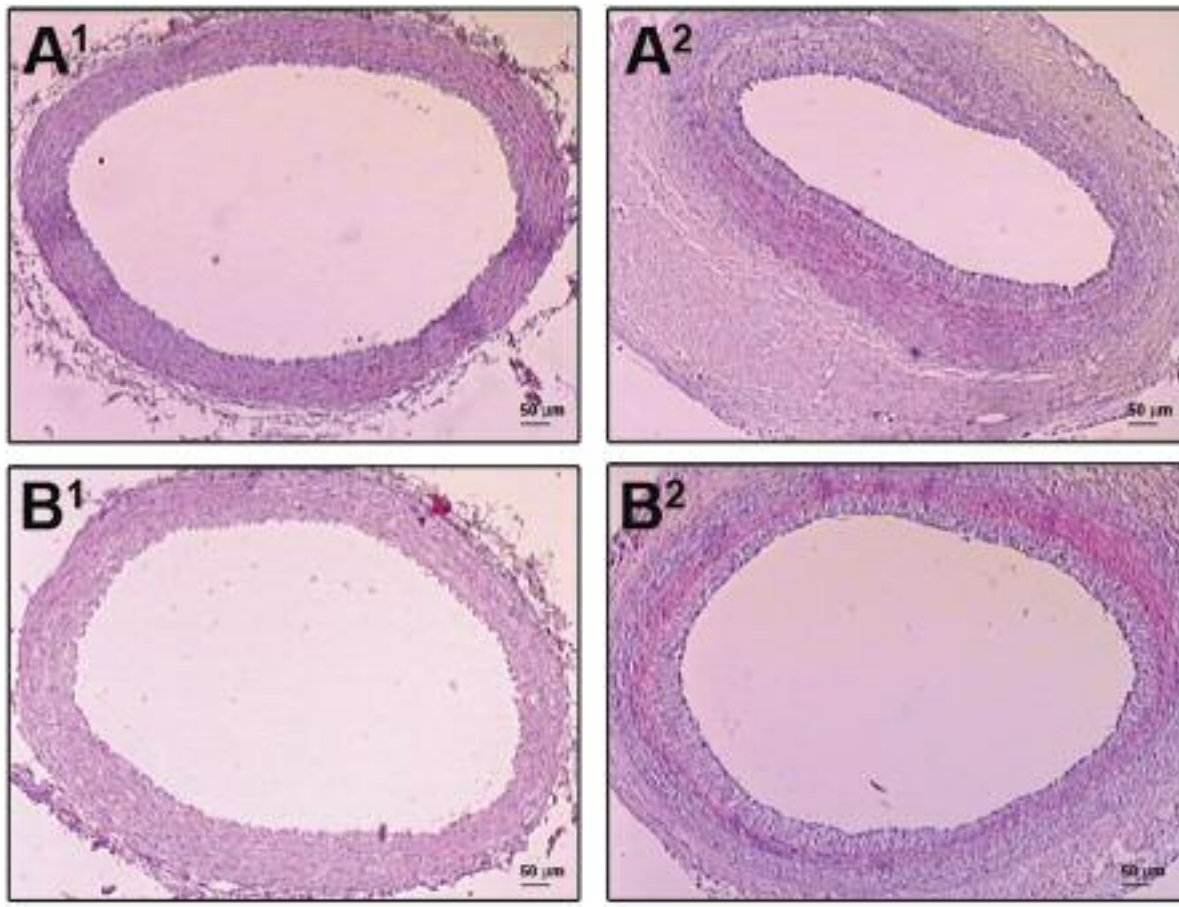

FIGURE 2: Representative photomicrographs of paraffin transverse sections of carotid arteries stained with haematoxylin-eosin. **A1)** Sham-operated artery from placebo group, **A2)** Collared artery from placebo group, **B1)** Sham-operated artery from nifedipine group, **B2)** Collared artery from nifedipine group (Original magnification x4).

they are in the same class. Previous studies of our group on collar-induced intimal thickening confirm this suggestion.[24-26]

In this study, the ineffectivenes of nifedipine on intimal thickening may suggest that the dosage of nifedipine is insufficient to decrease mean arterial pressure. However, the arterial pressure reducing effect of CCBs may be evaluated apart from the inhibitory effect on intimal thickening. Indeed, it was shown that nifedipine treatment inhibited balloon-induced intimal thickening however did not affect mean arterial pressure.[22] In this study, consistent with previous results, collar placing around the carotid artery did not affect mean arterial pressure.[27,28] Besides, nifedipine did not alter resting blood pressure in the present study.

Another possibility may be different experimental models used. Nifedipine was found to be effective in hypercholesterolemic atherosclerosis and balloon-induced intimal thickening, but it was shown to be ineffective in vein graft atherosclerosis.[22,29,30]

Although the exact mechanisms are still unclear, several explanations have been proposed to clarify the mechanism of collar-induced intimal thickening up to date. In their study, De Meyer et al.[31] suggested that obstruction of transmural fluid transport by the collar might lead to retention of toxic metabolites and cytokines within the collared vessel segment and may contribute to intimal thickening. In accordance with this proposal, our recent studies exhibited that toxic metabolites or cytokines in the segment enclosed by the collar

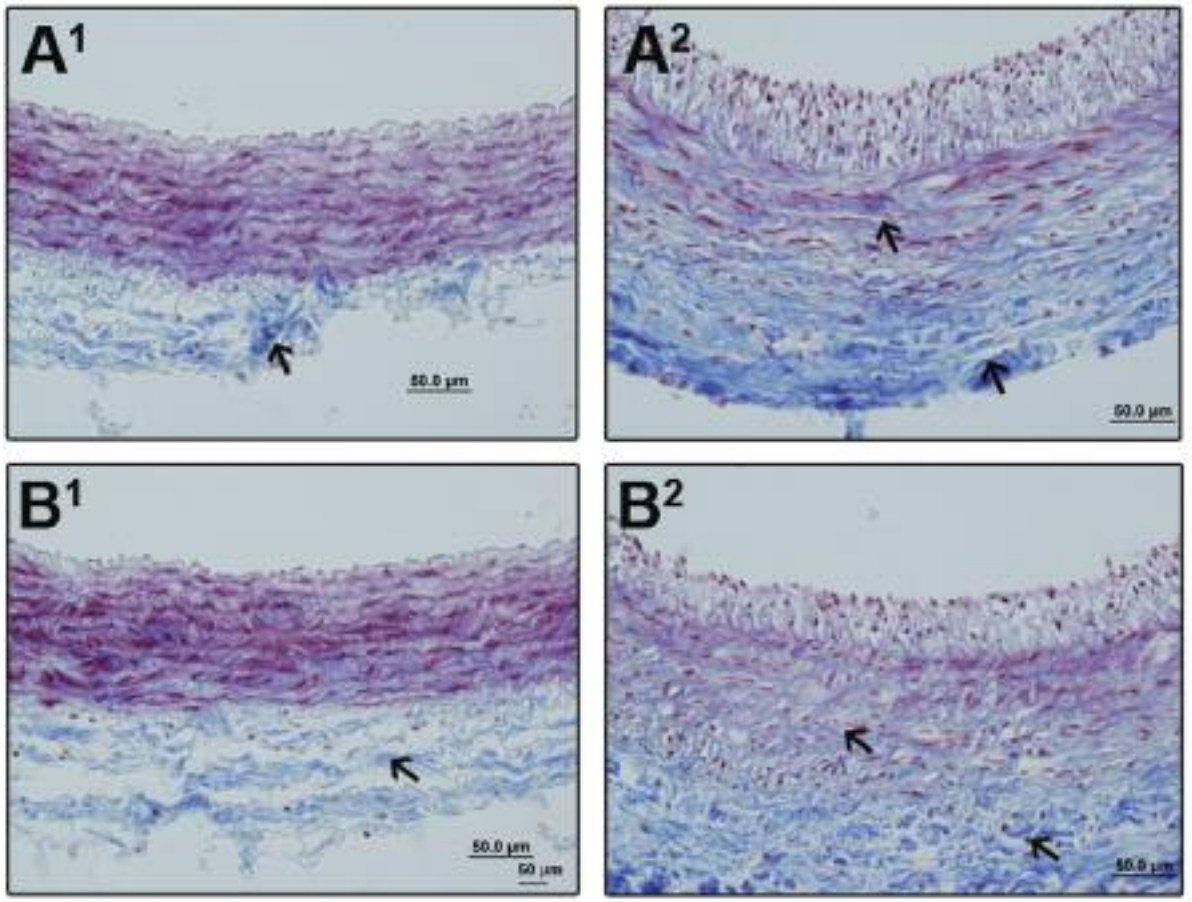

FIGURE 3: Representative photomicrographs of paraffin transverse sections of carotid arteries stained with Masson's trichrome. **A1)** Sham-operated artery from placebo group, **A2)** Collared artery from placebo group, **B1)** Sham-operated artery from nifedipine group, **B2)** Collared artery from nifedipine group. Arrows point out dark blue stained collagen (Original magnification x40).

might stimulate endothelin synthesis.[32] In this respect, the ineffectiveness of nifedipine may be caused by the inefficiency of nifedipine on cytokines, endothelin and/or adhesion molecules.[33,34]

On the other hand, this study demonstrated that the residual lumen ratio and lumen perimeter significantly decreased and the lumen area tended to be reduced due to collar-induced intimal thickening. This finding indicates that collar placement appears to provoke inward VR. Similarly, inward VR was also shown in several experimental atherosclerosis models.[21,34,35]

In our study, nifedipine treatment significantly increased the luminal area, residual lumen ratio and carotid artery area whereas there was a decreased media/lumen ratio in both sham-operated and collared arteries. These results indicate outward expansive VR. Outward expansive VR is possibly the physiological tendency of blood vessels to optimize shear stress and wall tension. Furthermore, in the present study increased carotid artery area and medial area is thought to be due to a hypertrophy in the vessel wall. Therefore, nifedipine treatment seems to provoke expansive hypertrophic VR in our study.

Recent studies emphasized the roles of matrix metalloproteinases (MMPs), in physiological and pathological VR.[6] It is known that MMPs, in particular gelatinases (MMP-2 and MMP-9), degrade the basement membrane and extracellular matrix (ECM) facilitating smooth muscle cell (SMC) migration and proliferation, and they contribute to the intimal thickening and VR process.[6,36] Similarly, we recently demonstrated that the activities of gelati-

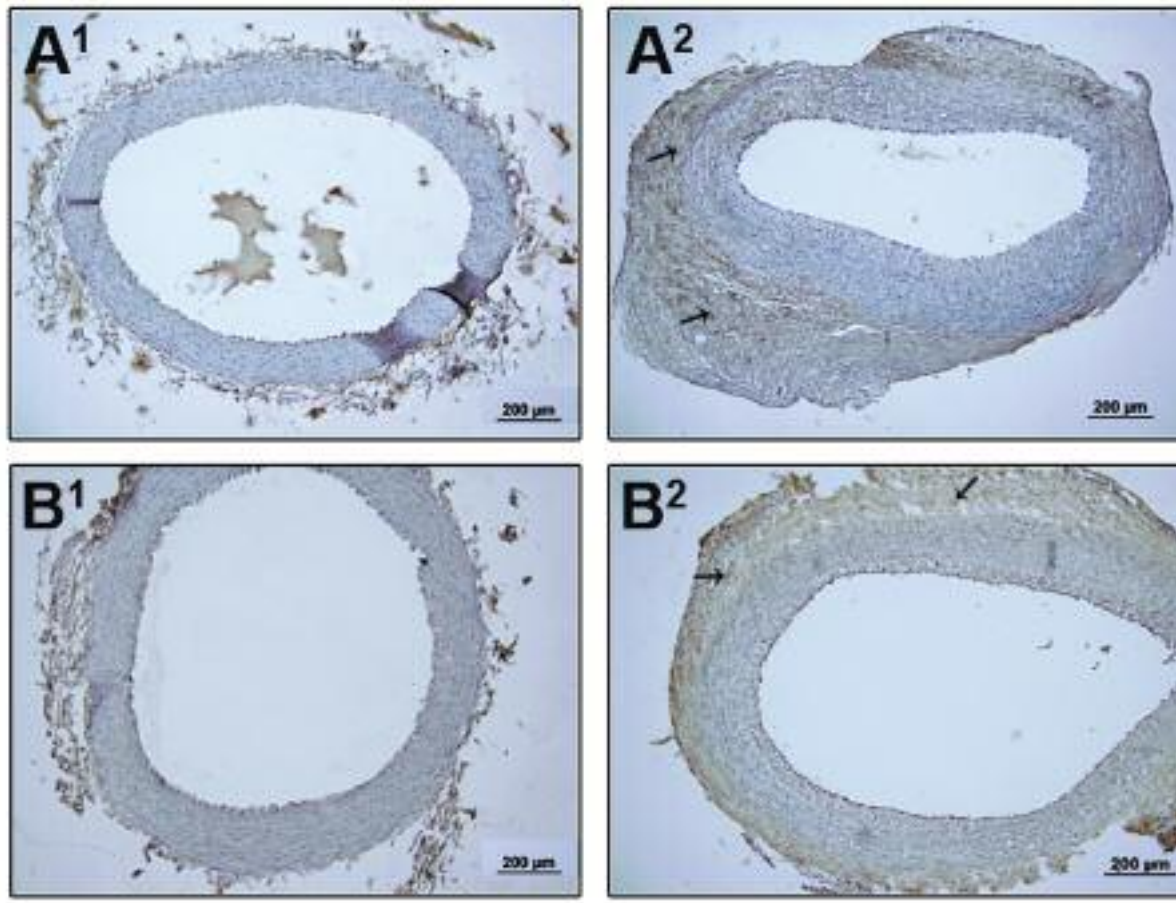

FIGURE 4: Representative photomicrographs of paraffin transverse sections of carotid arteries stained with DDR1 antibody immunohistochemically. **A1)** Sham-operated artery from placebo group, **A2)** Collared artery from placebo group, **B1)** Sham-operated artery from nifedipine group, **B2)** Collared artery from nifedipine group. Arrows show dark brown stained DDR1 positive fibroblasts in adventitia (Original magnification x4).

nases increased in collared arteries.[37] In this respect, in the present study, the constrictive VR caused by the collar seems to be a result of the increased activities of gelatinases in the collared segments.

Furthermore, it is known that both expansive and constrictive VR involve increased MMP expression and activation[6,12] Pasterkamp et al.[12] observed more prevalent gelatinase expression and activation in human atherosclerotic plaques of the expansively remodeled segments versus constrictively remodeled segments.

Recently, CCBs have been shown to increase the expression and proteolytic activity of gelatinases in vascular diseases such as essential hypertention and aneurysm.[38,39] Yue et al.[40] demonstrated that nifedipine increased MMP-2 expression in cultured rat cardiac fibroblasts. Similarly, Ikeda et al.[41] revealed that amlodipine, but not nifedipine, inhibited IL-1beta-induced MMP-1 expression in human endothelial cells.[41]Although in the present study we have not investigated the effect of nifedipine on MMP levels, expansive VR in the arteries may be a result of the increased activities of gelatinases by nifedipine treatment in this model. Furthermore, Yue et al.[40] also reported that increased MMP-2 expression and nitrite production caused by nifedipine were blunted by a nitric oxide (NO) synthase inhibitor (L-NAME) in cardiac fibroblasts. These findings suggest that nifedipine might increase MMP-2 expression through a possible NO-dependent pathway.[40] These issues need further investigation.

On the other hand, DDRs, which are non-in-

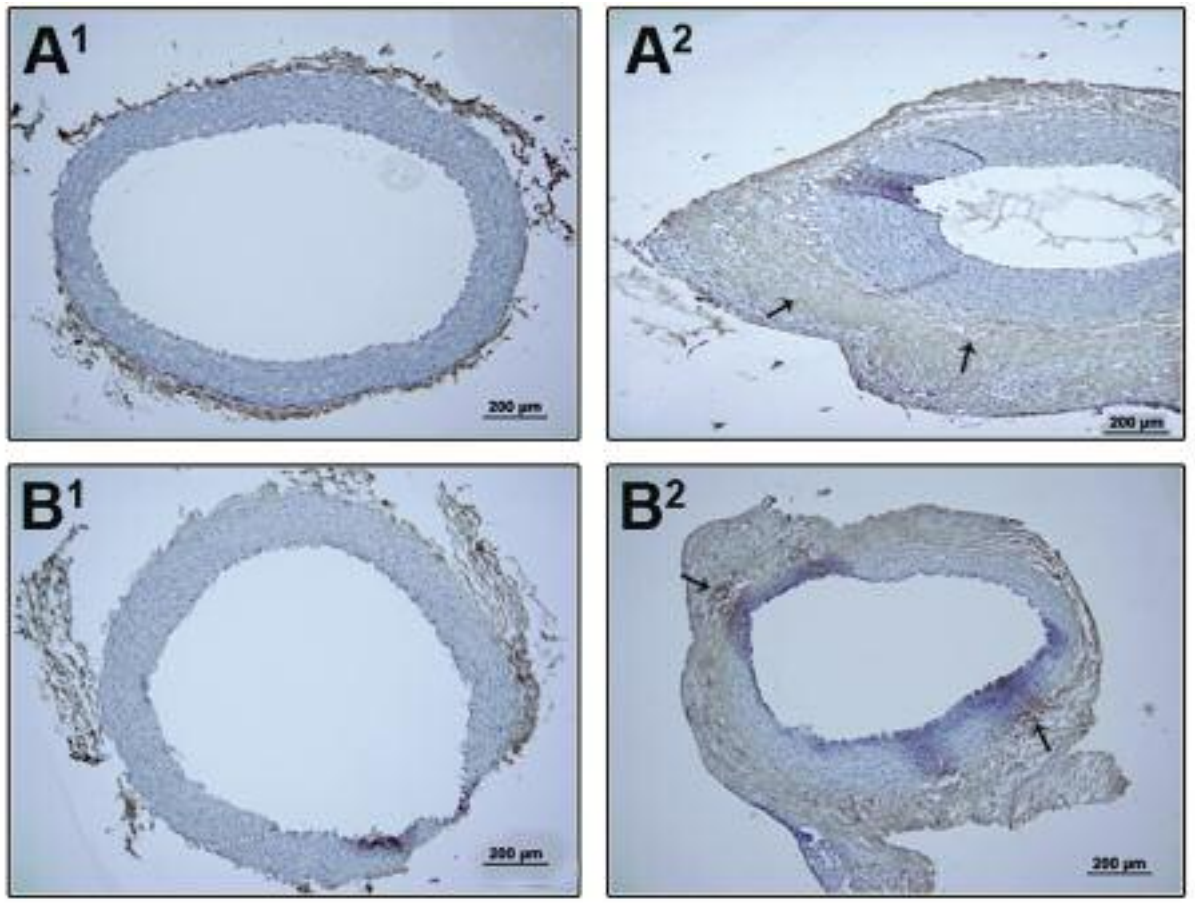

FIGURE 5: Photomicrographs of paraffin transverse sections of carotid arteries stained with DDR2 antibody immunohistochemically. **A1)** Sham-operated artery from placebo group, **A2)** Collared artery from placebo group, **B1)** Sham-operated artery from nifedipine group, **B2)** Collared artery from nifedipine group. Arrows show dark brown stained DDR2 positive fibroblasts in adventitia (Original magnification x4).

tegrin collagen receptors, are known to be implicated in neointima formation and MMP activation.[20] Hou et al.[19] demonstrated that following wire injury to the carotid artery of the DDR-null mice, MMP activity, the neointimal area and matrix accumulation were significantly lower compared to wild-type controls. Similarly, Ferri et al.[42] reported that overexpression of DDRs increased MMP activity in human SMC culture. Besides, it was observed that gelatinase activities were concomitantly reduced in DDR1 knockout aortic SMCs.[43]

In this study, immunoreactivities of DDRs were detected in the adventitia but not in intima and media in both collared and sham-operated arteries in the placebo group. Furthermore, in the adventitia of the arteries, collagen content was found in parallel with DDR immunoreactivities. It is hardly surprising that collagen, and DDRs co-localize in adventitia, since DDR receptors activated by collagen, are expressed in adventitial fibroblasts. Accordingly, nifedipine treatment did not affect the collagen matrix and DDR expression in our study. Consistent with our findings, Goto et al.[14] demonstrated that there were prominent networks of collagen bundles in the media and adventitia of the balloon-injured carotid arteries, but not in the collared carotid arteries of dogs.

The results of the present study revealed that collagen synthesis did not change and there was no role of DDRs at least in the VR process in the collar-induced intimal thickening model. However, our findings suggest that nifedipine may have the potential to protect from restenosis after balloon

angioplasty since it has the ability to prevent DDR upregulation and collagen deposition, and to induce expansive VR in the arteries.

CONCLUSION

In conclusion, the present study demonstrated that nifedipine did not inhibit collar-induced intimal thickening. However it induced outward positive VR in the arteries and preserved the lumen size. On the other hand, our studies showed that DDRs had no role in the VR process in this model. Furthermore, we showed for the first time that nifedipine did not affect DDR expression or collagen matrix in this model. These effects suggest that nifedipine may have the potential of maintaning vascular compliance and preventing restenosis after balloon angioplasty.

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