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Aminoguanidine induces constrictive vascular remodeling and inhibits smooth muscle cell death after balloon injury

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Abstract

We examined the effects of aminoguanidine, an inhibitor of inducible nitric oxide synthase, in the rat model of balloon injury. Arteries were assessed by histomorphometry, and vascular smooth muscle cell death and proliferation were examined 24 h and 14 days after balloon injury by in situ terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) of fragmented DNA and expression of proliferating cell nuclear antigen, respectively. Aminoguanidine decreased the luminal area 14 days after balloon injury (0.19 ± 0.04 mm^2 vs. 0.35 ± 0.02 mm^2; $P < 0.005$), and this effect was attributable to reduction of the total vessel area, i.e., constrictive vascular remodeling (0.42 ± 0.03 mm^2 vs. 0.55 ± 0.03 mm^2; $P < 0.005$). At 24 h after injury, the percentage of TUNEL-positive cells in the medial layer was reduced by aminoguanidine ($2.0 \pm 1.0\%$ vs. $17.3 \pm 5.4\%$; $P < 0.05$), and the percentage of proliferating cells was increased ($18.4 \pm 5.5\%$ vs. $4.9 \pm 2.2\%$; $P < 0.05$). Aminoguanidine did not influence the density of VSMC nuclei in the injured artery wall, systemic blood pressure or endothelium-dependent vasorelaxation. We conclude, that in the rat model of balloon injury, aminoguanidine induces luminal loss by constrictive vascular remodeling in association with reduced early VSMC death and increased proliferation.

Keywords: Aminoguanidine; Nitric oxide (NO); Vascular remodeling; Apoptosis; Smooth muscle cell, vascular death; Restenosis

1. Introduction

Development of restenosis represents the major limitation to the therapeutic success after percutaneous transluminal coronary angioplasty, and both neointimal formation and constrictive vascular remodeling (i.e., reduction in total vessel area; arterial 'shrinkage') contributes to this pathophysiological response (Gibbons and Dzau, 1994; Schwartz et al., 1995). Balloon injury is characterized by initial endothelial denudation, with loss of endothelial nitric oxide (NO) synthase, which during physiological conditions is responsible for continuous low output production of NO that maintains vasodilator tone, inhibits platelet activation and leukocyte adhesion, and regulates vascular smooth muscle cell proliferation and migration (Moncada et al., 1991). In addition, balloon injury is associated with an early cytokine-stimulated upregulation of inducible NO synthase in VSMCs at the site of vessel injury (Joly et al., 1992; Hansson et al., 1994; Bosmans et al., 1996; Yan and Hansson, 1998). Expression of this high output NO synthase isoform has generally been implicated in host autotoxicity, e.g., during septic shock (Nathan, 1992), but it was recently suggested that inducible NO synthase induction in response to balloon injury may be a protective phenomenon, that compensates for the loss of vascular endothelium (Yan et al., 1996).

Apoptosis, i.e., genetically programmed cell death, plays an important role in the regulation of vessel wall cellularity in the course of lesion formation after balloon injury (Bochaton-Piallat et al., 1995; Isner et al., 1995; Perlman et al., 1997; Malik et al., 1998), but the mediators governing apoptosis and other forms of cell death in this pathophysiological setting have not been defined. High levels of NO can induce VSMC apoptosis in vitro (Nishio et al., 1996), and a correlation between cytokine-mediated inducible NO synthase expression and apoptosis of isolated VSMCs has been reported (Geng et al., 1996). Furthermore, transfection of inducible NO synthase gene was recently shown to induce apoptosis in VSMCs (Iwashina et

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al., 1998). We hypothesized that upregulation of inducible NO synthase in the vascular wall after balloon injury might influence lesion formation by mechanism involving apoptosis. Aminoguanidine is a selective inhibitor of inducible NO synthase (Misko et al., 1993; Wolff and Lubeskie, 1995; Scott et al., 1996), and the purpose of the present study was therefore to investigate the effect of this agent on vascular remodeling and regulation of VSMC death and proliferation in balloon-injured rat carotid arteries.

2. Material and methods

2.1. Rat model of balloon injury

Male Sprague–Dawley rats (3 months old; average weight 300 g) were anaesthetized by intraperitoneal sodium pentobarbital (50 mg/kg), and the left carotid artery was subjected to concentric vessel wall damage and endothelial denudation as previously described (Clowes et al., 1983). Briefly, a Fogarty F2 balloon catheter (Baxter Healthcare) was introduced through the left external carotid artery and passed down to the level of the aorta. The catheter was sufficiently inflated with saline to generate slight resistance, and then withdrawn to the entry point. This procedure was repeated three times, whereupon the external carotid artery was ligated after removal of the catheter. Immediately after balloon injury, rats were randomly assigned to two experimental groups, receiving either daily intraperitoneal injection of aminoguanidine hemisulphate (50 mg/kg) dissolved in vehicle (0.9% NaCl), or intraperitoneal vehicle (control group). Femoral artery blood pressure was measured before balloon injury and after 14 days. Animals were killed under sodium barbital anaesthesia at the indicated times after injury. The investigation conformed with the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH publication No 85-23, revised 1996).

2.2. Assessment of neointimal formation and vascular remodeling

After 24 h or 14 days, the rats were perfusion fixed under constant pressure (100 mmHg) with 4% (wt/vol) phosphate-buffered formaldehyde and the injured left and unmanipulated right common carotid arteries were carefully removed for histological analysis. The vessel segments were embedded in paraffin, and 3–5 μm thick sections were cut and stained with van Gieson Orcein. Morphometric analysis were performed with computerized semiautomatic image analysis (Leitz Texture analyzing system). The slides were projected with a light microscope to a high resolution computer screen, and the following areas were calculated: luminal area (area circumscribed by the intima border), neointimal area (area between the lumen and the internal elastic lamina), medial area (area between the internal and the external elastic lamina), total vessel area (area circumscribed by the external elastic lamina), and intimal-to-medial ratio (I/M ratio; neointimal area divided by medial area).

2.3. In situ detection of VSMC death

Dying VSMCs were detected in isolated balloon-injured and noninjured carotid vessels by terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick-end labeling (TUNEL) essentially as previously described (Gavrieli et al., 1992). This technique provides a rather sensitive albeit nonspecific method for detection of DNA fragmentation in apoptotic nuclei (Grasl-Kraup et al., 1995; Kockx et al., 1998). In brief, paraffin sections from arteries removed 24 h or 14 days after balloon injury were dewaxed, rehydrated, and incubated with 20 μg/ml proteinkinase K (Pharmacia Biotech) for 1 h, followed by inactivation of endogenous peroxidase with 3% hydrogen peroxide for 5 min. Fragmented DNA was nick end-labeled with terminal deoxynucleotidyl transferase (TdT; 22.5 U/section [Pharmacia Biotech]) in TdT buffer (30 mM Tris–HCl, 140 mM sodium cacodylate, 1 mM cobalt chloride) together with biotinylated dUTP (0.2 nmol/section [Sigma]), and stained with streptavidin-conjugated peroxidase (Dako). After TUNEL, the sections were counterstained with Mayer's hematoxylin, and mounted with Kaiser glycerol gelatine. For each tissue, positive controls were provided by pretreatment with DNAase I (100 U/ml) (Worthington Biochemical), and by a section of rat small intestine, respectively (Gavrieli et al., 1992). A negative control was incubated in the absence of the terminal deoxynucleotidyl transferase. Cell counting was performed under a light microscope, and the cells with a clear nuclear labeling were defined as TUNEL-positive. The number of TUNEL-positive nuclei and the total number of nuclei

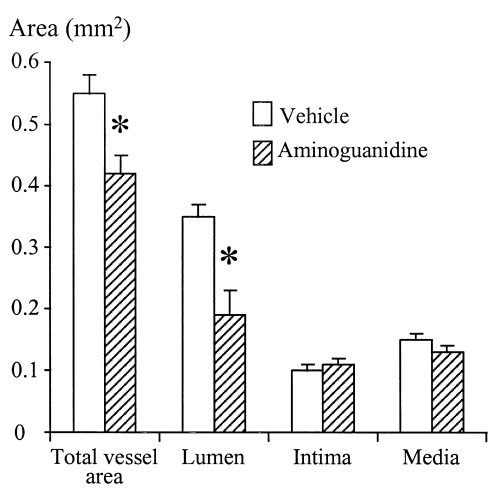

Fig. 1. Morphometric analysis of rat carotid arteries 14 days after balloon injury. Animals were treated with either aminoguanidine (50 mg kg^{-1} day^{-1}, $n = 7$) or vehicle ($n = 9$). Columns are means, bars = S.E.M. *$P < 0.005$ vs. treatment with vehicle alone.

within each vessel layer were counted, and cell death was expressed as the TUNEL index (100 × [number of TUNEL-positive nuclei/total number of nuclei]).

2.4. Immuncytochemical detection of proliferating VSMCs

The proliferative profile in the carotid arterial segments was evaluated by immunocytochemical detection of proliferating cell nuclear antigen, which is considered to be a valid indicator of cell replication in this model (Zeymer et al., 1992). Paraffin sections from carotid arteries isolated 24 h or 14 days after balloon injury were dewaxed, rehydrated, and boiled for 2 × 5 min with antigen retrieval buffer in a microwave oven. Endogenous peroxidase was blocked for 8 min with 3% hydrogen peroxide. Sections

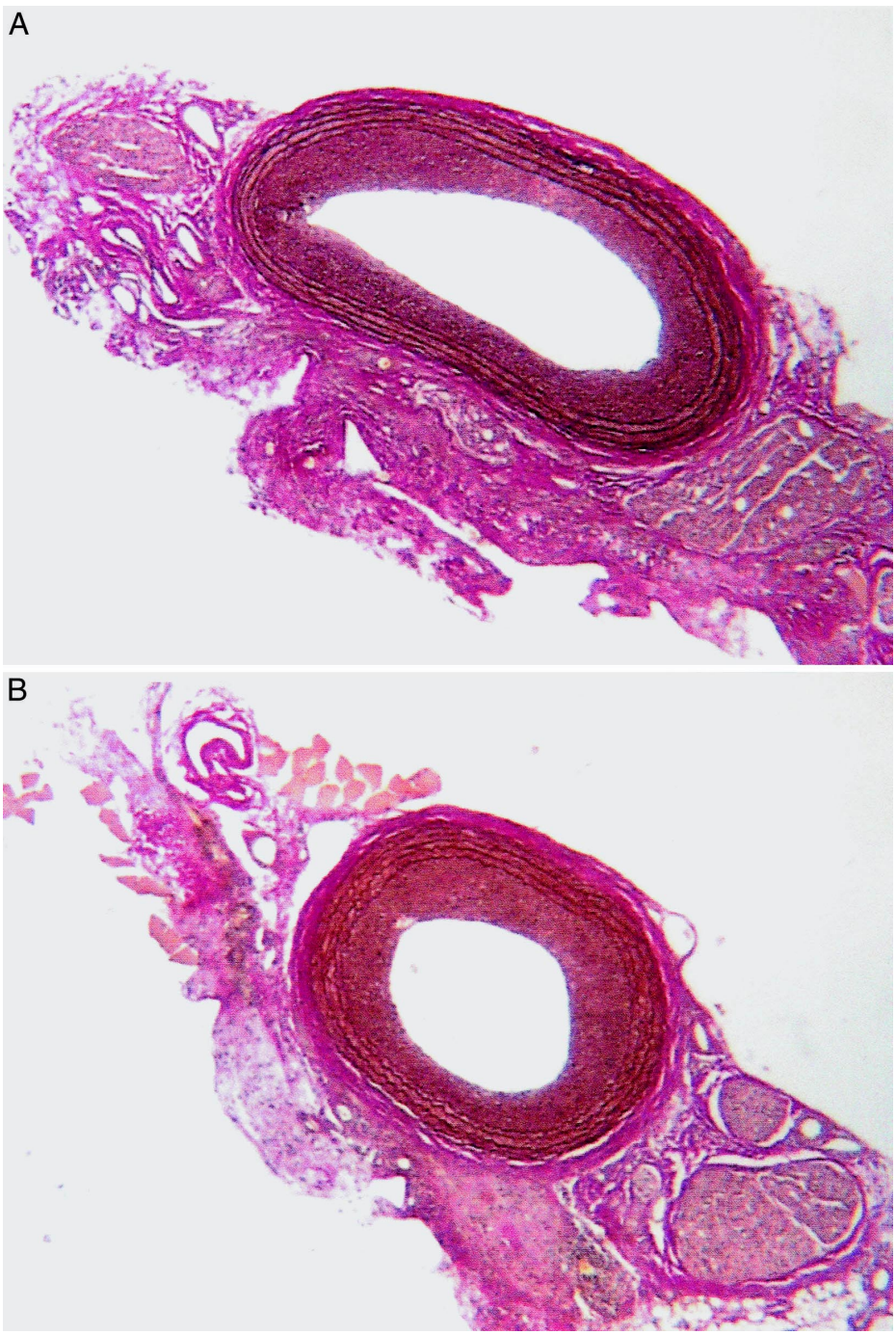

Fig. 2. Representative van Gieson Orcein-stained cross-sections of carotid arteries 14 days after balloon injury from rats treated with vehicle (A) and rats treated with aminoguanidine (50 mg kg^{-1} day^{-1}; B) (×25). The luminal area was more reduced in rats treated with aminoguanidine, and this effect was attributable to a decrease in the total vessel area.

were incubated with a monoclonal anti-proliferating cell nuclear antigen antibody (Dako clone PC10, 1:100 dilution) for 25 min. A biotinylated rabbit anti mouse secondary antibody was then applied for 25 min, followed by streptavidin peroxidase (Dako) for 25 min. Immune detection was visualized with a standard peroxydase enzyme substrate (aminoethyl carbazole) for 15 min. Sections were counterstained with Mayer's hematoxylin, and mounted with Kaiser glycerol gelatine. The number of proliferating cell nuclear antigen-positive nuclei and the total number of nuclei within each vessel layer were counted, and proliferation was expressed as the proliferating cell nuclear antigen-labeling index ($100 \times$ [number of proliferating cell nuclear antigen-positive nuclei/total number of nuclei]). Moreover, the number of VSMCs per cross-sectional area (nuclei/mm^2) in vessels removed 24 h or 14 days after balloon injury was determined by counting the total number of nuclei in the neointima and medial layer and dividing these numbers by the respective areas determined by morphometry.

2.5. Measurement of blood pressure and endothelial NO synthase-dependent vasorelaxation

Femoral blood pressure was measured in anaestesized rats before balloon injury and after 14 days. In addition, unmanipulated right common carotid arteries were carefully removed from animals killed 14 days after balloon injury (and not subjected to perfusion fixation), and 2 mm long segments were mounted in an isometric myograph containing Krebs–Henseleit solution (KHS; composition in mM: NaCl 118, KCl 4.75, $CaCl_2 \cdot H_2O$ 2.54, $MgSO_4 \cdot 7H_2O$ 1.19, $NaHCO_3$ 12,5, glucose 10) at 37°C, gassed with 5% CO_2–95%O_2. The vessels were equilibrated for 1 h and set to a normalized internal circumferential diameter estimated to be 0.9 times the circumference they would maintain if relaxed and exposed to a transmural pressure of 100 mmHg (Mulvany and Halpern, 1977). After normalization, the vessel segments were maximally activated 3 times at 5–10 min intervals with K-KHS (composition as for KHS, but with an equimolar exchange of NaCl for KCl). When the vascular tone had returned to resting level, the vessels were contracted with noradrenaline (1 mM) and the response to cumulative concentrations of acetylcholine (1 nM–1 mM) was determined. Each dose–response curve was characterized by determining the maximal response to acetylcholine (E_{max}), and the concentration of the drug evoking 50% relaxation of contraction to noradrenaline (EC_{50} value).

2.6. Statistical analysis

All data are expressed as mean ± S.E.M. A two-sided two-sample *t*-test was used for comparison of independent observations, and a paired *t* test was used to examine differences between paired observations. Statistical significance was defined as $P < 0.05$.

3. Results

3.1. Effects on neointimal formation and vascular remodeling

In the first series of experiments, 16 rats were subjected to balloon injury. The animals received either aminoguanidine ($n = 7$) or vehicle ($n = 9$), and there were no postprocedure deaths. As shown in Figs. 1 and 2A–B, morphometric analysis of balloon-injured carotid arteries isolated 14 days after injury disclosed that the average luminal area in balloon-injured vessels was 46% lower in animals treated with aminoguanidine than in rats receiving vehicle (0.19 ± 0.04 mm^2 vs. 0.35 ± 0.02 mm^2; $P < 0.005$), and this effect was attributable to a decrease in the total vessel area, i.e., constrictive vascular remodeling (0.42 ± 0.03 mm^2 vs. 0.55 ± 0.03 mm^2; $P < 0.005$). Intimal and medial areas were not significantly altered by aminoguanidine, and the I/M ratio was also of a comparable magnitude in the two treatment groups (0.82 ± 0.08 vs. 0.65 ± 0.08; NS).

3.2. Effects on VSMC death

The TUNEL assay disclosed evidence of extensive cell death in the vessel wall following balloon injury, whereas sections from uninjured arteries did not exhibit any detectable TUNEL-positive staining. As shown in Figs. 3 and 4A–B, TUNEL-positive cells were localised to the medial layer in rats killed 24 h after injury ($n = 9$ in each treatment group), and the medial TUNEL index was significantly reduced by aminoguanidine (2.0 ± 1.0% vs. $17.3 \pm$

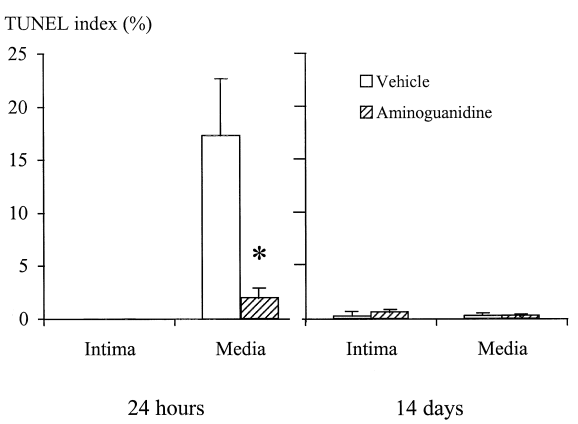

Fig. 3. Percentage of terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick-end labeling (TUNEL)-positive cells in different layers of rat carotid arteries 24 h ($n = 9$ in each treatment group) and 14 days ($n = 10$ in aminoguanidine group, $n = 8$ in vehicle group) after balloon injury. Cross sections were stained by TUNEL, and the TUNEL index was calculated as the fraction of the total number of nuclei within each vessel layer that was TUNEL-positive. Columns are means, bars = S.E.M. * $P < 0.05$ vs. treatment with vehicle alone.

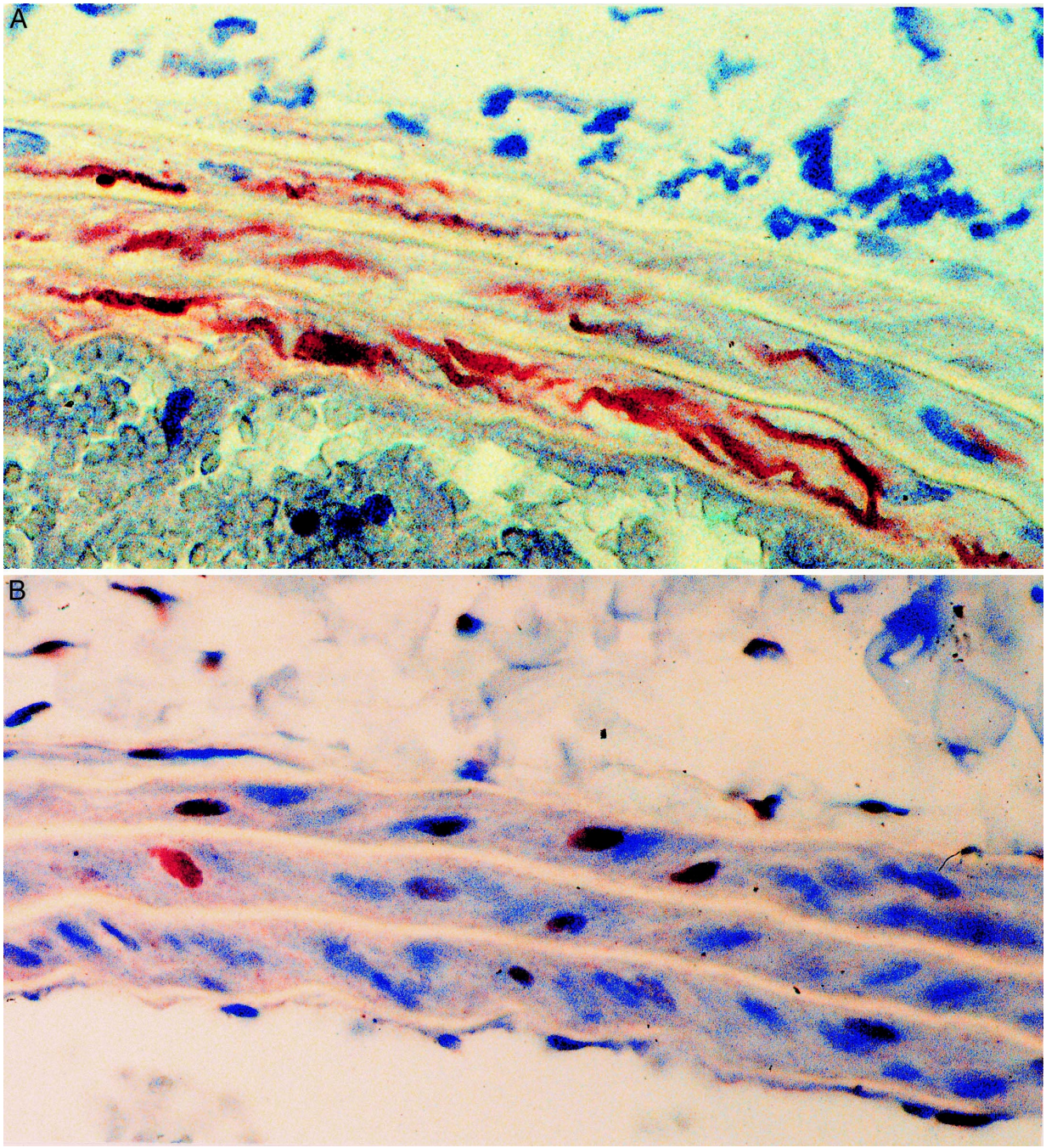

Fig. 4. Representative in situ terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick-end labeling (TUNEL) of cells in rat carotid arteries 24 h after balloon injury (×250). (A) Rat treated with vehicle, and (B) rat treated with aminoguanidine (50 mg kg^{-1} day^{-1}). Note the magnitude of TUNEL-positive cells (red-brown staining) in the medial layer in (A) as compared to the few apoptotic cells nuclei in (B).

5.4%; $P < 0.05$). At 14 days after injury, the percentage of TUNEL-positive nuclei had declined, and at this time point, the medial TUNEL index was not significantly different in rats treated with aminoguanidine ($n = 10$) or vehicle ($n = 8$), respectively. In the neointimal layer, a very low level of TUNEL-positive staining was apparent 24 h and 14 days after balloon injury, and was not significantly altered by aminoguanidine. To verify the specificity of TUNEL, sections of rat small intestine were stained by identical methods, and TUNEL-positive signals were detected in the uppermost nuclei at the luminal edge of the crypts (Gavrieli et al., 1992). In addition, sections treated with DNAase I showed intense staining of all nuclei, and no staining was found in negative control sections incubated without terminal deoxynucleotidyl transferase (not shown).

3.3. Effects on VSMC proliferation and cell density

Immunohistochemical detection of proliferating cell nuclear antigen disclosed a predominant staining of cells in the medial layer 24 h following balloon injury, and in the neointimal layer 14 days hereafter (Figs. 5 and 6A–B). Treatment with aminoguanidine increased the proliferating cell nuclear antigen-labeling index in the medial layer 24 h ($18.4 \pm 5{,}5\%$ vs. $4.9 \pm 2.2\%$; $P < 0.05$), whereas the percentage of proliferating cell nuclear antigen-positive cells in the neointimal layer 14 days after injury was similar in the two treatment groups. In arteries removed 24 h after injury (where intimal cells were absent), AG did not significantly influence the VSMC density in the medial layer (1143 ± 353 vs. 1257 ± 264 nuclei/mm^2; NS), and administration of the agent also failed to affect the VSMC density in the medial layer (2281 ± 71 vs. 2352 ± 345 nuclei/mm^2; NS) and neointimal layer (3878 ± 353 vs. 3320 ± 262 nuclei/mm^2; NS) after 14 days. Proliferating cell nuclear antigen protein was not detectable by immunochemistry in uninjured arteries (not shown).

3.4. Effects on blood pressure and in vitro functional responses

The mean arterial blood pressure before balloon injury and after 14 days was not significantly different in animals treated with aminoguanidine (86 ± 3 mmHg vs. 89 ± 6 mmHg; NS) or vehicle (89 ± 7 mmHg vs. 83 ± 3 mmHg; NS). Moreover, as shown in Fig. 7, administration of aminoguanidine failed to influence eNOS-dependent vasorelaxation to acetylcholine in noninjured carotid segments as indicated by E_{max} (84.3 ± 2.9 vs. $78.9 \pm 3.1\%$; NS) and EC_{50} ($-\log$ M) values (6.0 ± 0.3 vs. 5.9 ± 0.3; NS).

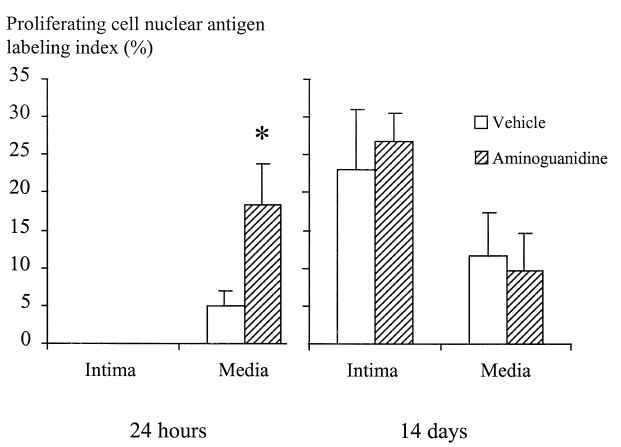

Fig. 5. Percentage of proliferating cells as determined by expression of proliferating cell nuclear antigen in different layers of rat carotid arteries 24 h and 14 days after balloon injury (same animals as in Fig. 3). Cross-sections were studied by proliferating cell nuclear antigen immunohistochemistry, and the proliferating cell nuclear antigen labeling index was calculated as the fraction of the total number of nuclei within each vessel layer that was proliferating cell nuclear antigen-positive. Columns are means, bars = S.E.M. *$P < 0.05$ vs. treatment with vehicle alone.

4. Discussion

The current study demonstrates that inhibition of inducible NO synthase with aminoguanidine increases luminal loss in balloon-injured rat carotid arteries by mechanisms involving constrictive vascular remodeling (i.e., decreased total vessel area) and altered profiles of VSMC death and proliferation in the vessel wall. Although neointimal formation was previously thought to be the dominant pathophysiological process in development of restenosis after balloon injury, constrictive vascular remodeling has more recently been shown to play an important role both in humans after percutaneous transluminal angioplasty (Mintz et al., 1996), and in larger animal models of arterial injury (e.g., coronary injury models in pigs) (Andersen et al., 1996). The response of the rat carotid artery to balloon injury is the best-studied model of neointimal formation, and vascular remodeling has generally not been investigated in this model (Schwartz et al., 1995). However, the present study shows for the first time that inducible NO synthase inhibition with aminoguanidine can induce constrictive remodeling in the rat model, and our results hereby add to recent evidence suggesting that upregulation of inducible NO synthase after balloon injury may represent a protective mechanism aimed at reestablishing the favourable regulatory effects of NO in the absence of a functional vascular endothelium (Yan et al., 1996).

Increased expression and activity of inducible NO synthase in vascular smooth muscle cells located in the forming neointima has conclusively been demonstrated in rat carotid arteries within 24 h after balloon injury (Joly et al., 1992; Hansson et al., 1994; Yan and Hansson, 1998), and has also been observed after incubation of these vessels with inflammatory cytokines (Joly et al., 1992). In addition, vascular expression of inducible NO synthase after balloon injury is tightly regulated by other growth factors and factors of the coagulation and fibrinolytic system, e.g., it is inhibited by transforming growth factor-β1 and thrombin, and enhanced by calcitonin gene-related peptide and fibroblast growth factor, respectively (Schini and Vanhoutte, 1993). In several inflammatory states, e.g., endotoxaemic shock, diabetes, myocarditis and cardiac allograft rejection, inducible NO synthase expression has been suggested to augment tissue damage, probably by extensive generation of secondary cytotoxic radicals derived from NO (e.g., peroxynitrite) (Nathan, 1992), and inhibition of inducible NO synthase with aminoguanidine may have protective effects in experimental models of these conditions (Corbett et al., 1992; Woorall et al., 1995; Ishiyama et al., 1997). However, induction of inducible NO synthase in the vascular wall after balloon injury was recently suggested to inhibit platelet adhesion and restore blood

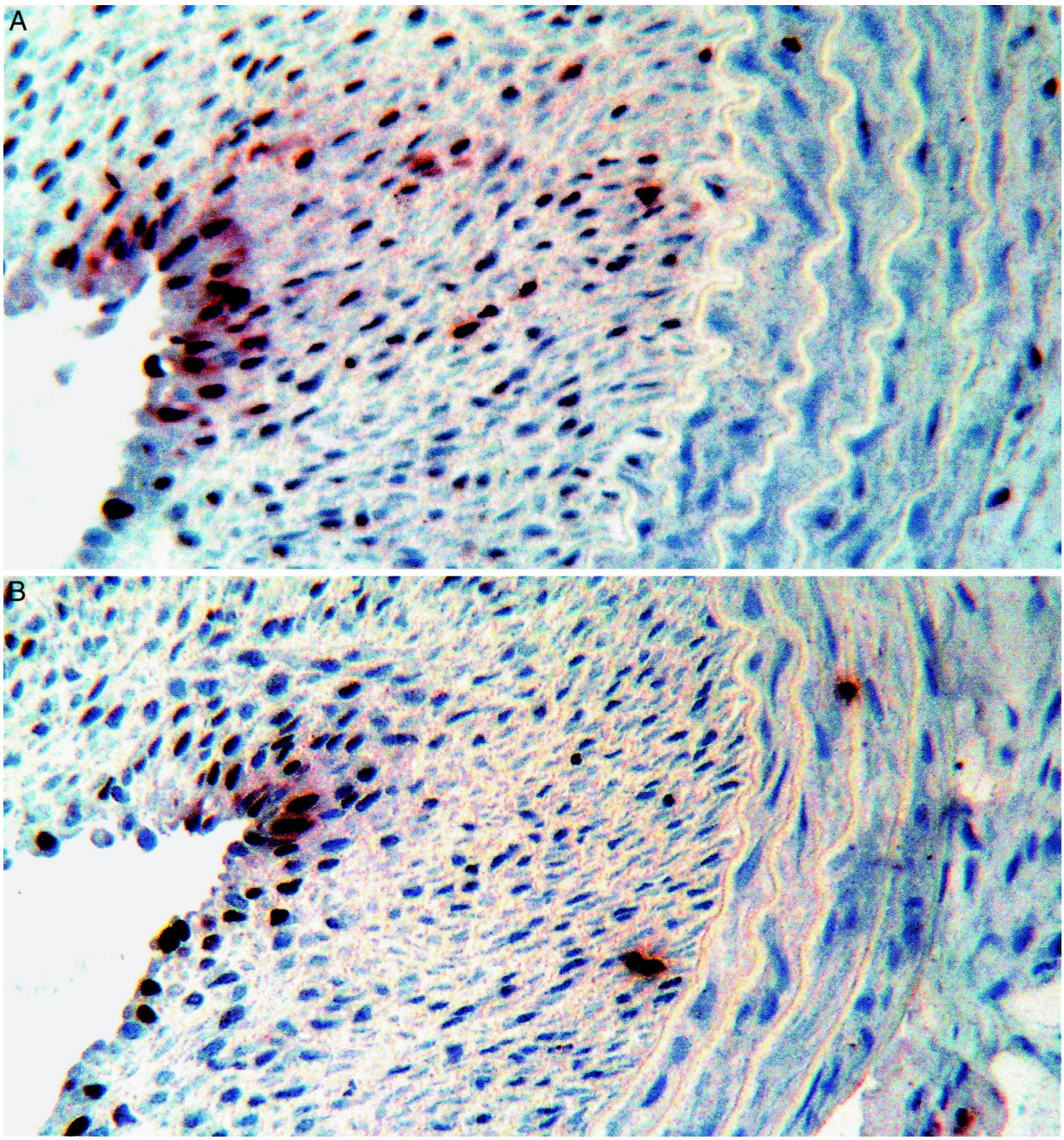

Fig. 6. Representative proliferating cell nuclear antigen immunostaining rat carotid arteries 14 days after balloon injury (×500). (A) Rat treated with vehicle, and (B) rat treated with aminoguanidine (50 mg kg^{-1} day^{-1}). No differences were observed in proliferating cell nuclear antigen immunostaining between the two treatment groups.

flow in the rat model (Yan et al., 1996), and we now extend these observations by providing evidence that inducible NO synthase inhibition with aminoguanidine induces constrictive vascular remodeling by a mechanism involving decreased VSMC death in the vascular wall.

The molecular mechanisms underlying vascular remodeling involve at least four processes, i.e., cell death, cell proliferation, cell migration, and alterations of the extracellular matrix (Gibbons and Dzau, 1994). All nucleated cells possess genetic programmes for suicide, and this process, described as apoptosis, may be activated by a variety of internal and external stimuli for purposes such as defence, development, aging, and homeostasis (Vaux and Strasser, 1996). Apoptosis is accompanied by rapid intranucleosomal DNA cleavage into multiples of approximately 180 bp, and detection of the DNA breaking points (nicks) by TUNEL has facilitated identification of apoptosis (Gavrieli et al., 1992). However, this technique does not provide a specific marker of apoptosis, and TUNEL may also occur in accidental cell death (i.e., necrosis or oncosis), and even

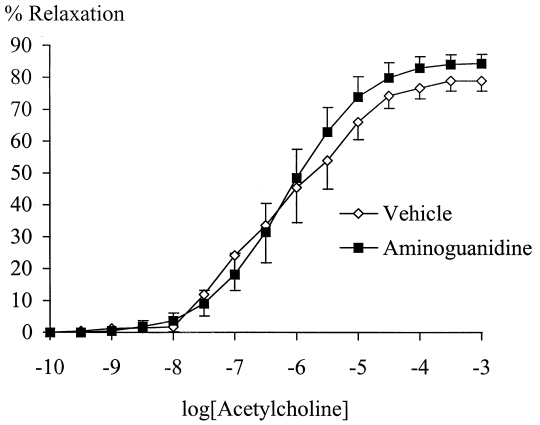

Fig. 7. Endothelium-dependent acetylcholine-induced relaxation (percentage of contraction to noradrenaline) in unmanipulated right common carotid arteries from rats treated with aminoguanidine ($n = 10$) or vehicle ($n = 8$). Bars = S.E.M. There was no difference in the relaxation to acetylcholine between the two treatment groups.

in nonapoptotic nuclei with aboundant RNA transcription and splicing (Grasl-Kraup et al., 1995; Kockx et al., 1998). Apoptosis was recently established as an important feature of the vascular response to balloon injury in rats (Bochaton-Piallat et al., 1995; Perlman et al., 1997), pigs (Malik et al., 1998), and humans (Isner et al., 1995). NO may induce apoptosis of vascular smooth muscle cells in vitro (Nishio et al., 1996), and inducible NO synthase has been implicated in cytokine-induced apoptosis of vascular smooth muscle cells (Geng et al., 1996). Furthermore, an association between inducible NO synthase expression and apoptosis of cardiac myocytes has been observed during cardiac allograft rejection (Szabolcs et al., 1996) and myocardial infarction (Suzuki et al., 1996). The present data show an impressive VSMC death response in the medial layer 24 h following balloon injury, and in accord with the characteristics of apoptosis, the high frequency of TUNEL-positive dying cells was not associated with signs of inflammation in the vessel wall (Fig. 4A–B). However, TUNEL of seeming normal nuclei and even in the cytoplasma of vascular smooth muscle cells was observed, and the extent to which nonapoptotic mechanisms may have contributed to TUNEL in the present model of arterial injury remains to be defined. Aminoguanidine significantly reduced the number of TUNEL-positive nuclei in the medial layer after 24 h, and this finding suggests that increased NO levels caused by inducible NO synthase upregulation participate in the mechanisms leading to apoptosis of vascular smooth muscle cells after balloon injury. On the other hand, administration of aminoguanidine was associated with enhanced cell replication at the balloon-injured site after 24 h as measured by PNCA-labeling. This discordance of aminoguanidine-mediated effects (i.e., decreased VSMC death and increased proliferation) was expected to produce an increase in tissue cell mass. We did not, however, observe any significant alterations in the cross sectional densities of VSMC nuclei after 24 h or 14 days, and how these responses can be reconsiled with the finding of geometric constrictive remodeling and unchanged neointimal area remains to be determined. However, our study represents a static view at two selected time points, and we did not attempt to provide a complete picture of the quantitative rates of VSMC death and proliferation over the life of the experimental lesions. Indeed, studies in other cell systems have shown that apoptosis may be completed so rapidly that the cells disappear in an hour or less (Vaux and Strasser, 1996). Moreover, vascular remodeling is likely to involve other processes, e.g., cell migration and modulation of the extracellular matrix, that were not directly assessed in the current experiments (Gibbons and Dzau, 1994).

NO synthase isoforms synthesize NO from one of the guanidino nitrogens of L-arginine, and these enzymes are competitively inhibited by analogues of L-arginine (Moncada et al., 1991). The guanidino-substituted L-arginine analogues (e.g., N^G-nitro-L-arginine) are nonselective NOS inhibitors, and their application in vivo is limited by hypertension resulting from inhibition of endothelial NO synthase. Moreover, use of a nonspecific NO synthase inhibitor can cause other effects (e.g., reduced endothelium-dependent vasorelaxation and altered function of platelets and leukocytes) that may confound actions caused by selective inducible NO synthase inhibition. Aminoguanidine contains the guanidino nitrogen groups linked to hydrazine, and this compound is considered to be a highly selective inhibitor of inducible NO synthase (Corbett et al., 1992; Misko et al., 1993; Wolff and Lubeskie, 1995; Woorall et al., 1995; Scott et al., 1996; Ishiyama et al., 1997). In agreement with this contention, we found that aminoguanidine did not significantly increase blood pressure or attenuate endothelium-dependent vasorelaxation in uninjured carotid arteries, indicating no substantial inhibition of endothelial NO synthase. Aminoguanidine concentrations were not assessed in the current study, but serum levels of approximately 100 μM have been observed in rats given 50 mg/kg aminoguanidine once daily (Baylin et al., 1975). Administration of an identical dose of aminoguanidine in our study is therefore likely to have provided plasma levels well above IC_{50} values previously reported for inhibition of inducible NO synthase (5–50 μM) (Misko et al., 1993; Wolff and Lubeskie, 1995), and it is notable, that a comparable dose of aminoguanidine can inhibit inducible NO synthase-dependent vascular hyporeactivity in the rat model of endotoxaemic shock (Wu et al., 1995).

The present results should be cautiously interpreted, since vascular NO levels were not measured, and aminoguanidine can have other effects, e.g., reduction of hyperglycemia-induced generation of advanced nonenzymatic glycosylation products and collagen cross-linkages, inhibition of biogenic amine synthesis, and redox-active properties, that may have contributed to our observations

(Giardino et al., 1998). In addition, our assessment of the densities of dying and proliferating cells in the vessel wall was made without use of stereological methods, and there are, for example, no data available regarding the duration of TUNEL positivity once VSMCs are committed to the apoptotic pathway. Finally, the rat model cannot adequately simulate the complexity of restenosis development after percutaneous transluminal angioplasty in humans, and studies in other models (e.g., pigs) are required for a more reliable extrapolation of the results to the clinical setting. In summary, we have shown that aminoguanidine has the capacity to increase luminal compromise in balloon-injured rat carotid arteries by induction of constrictive vascular remodeling in association with an early reduction of VSMC death and increased proliferation at the site of injury. These results suggest a favourable effect of inducible NO synthase activity on vascular remodeling, and we speculate that inducible NO synthase-mediated apoptosis of vascular smooth muscle cells after balloon injury may represent a compensatory mechanism aimed at preservation of vessel patency.

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