Adenovirus-Mediated Human Tissue Kallikrein Gene Delivery Inhibits Neointima Formation Induced by Interruption of Blood Flow in Mice

Costanza Emanueli, Maria Bonaria Salis, Julie Chao, Lee Chao, Jun Agata, Kuei-Fu Lin, Antonella Munàò, Stefania Straino, Alessandra Minasi, Maurizio C. Capogrossi, Paolo Madeddu

Abstract—Tissue kallikrein cleaves kininogen to produce vasoactive kinin peptides. Binding of kinins to bradykinin B2 receptors on vascular endothelial cells stimulates the release of nitric oxide and prostacyclin, thus activating the cGMP and cAMP pathways. In this study, we evaluated the effects of adenovirus-mediated human tissue kallikrein gene (Ad.CMV-cHK) delivery in a mouse model of arterial remodeling induced by permanent alteration in shear stress conditions. Mice underwent ligature of the left common carotid artery and were injected intravenously with saline or 1.8 \times 10^9 plaque-forming units of Ad.CMV-cHK or control virus (Ad.CMV-LacZ). Fourteen days after surgery, morphometric analysis revealed that Ad.CMV-cHK reduced neointima formation by 52% (P<0.05) compared with Ad.CMV-LacZ. Expression of human tissue kallikrein (HK) mRNA was detected in mouse carotid artery, aorta, kidney, heart, and liver, and recombinant HK was present in the urine and plasma of mice receiving HK gene. Kallikrein gene transfer resulted in increases in urinary kinin, cGMP, and cAMP levels. The protective action of Ad.CMV-cHK on neointima formation was significantly reduced (P<0.05) in mice with knockout of the kinin B2 receptor gene compared with wild-type control mice (J129Sv mice). In contrast, the effect of Ad.CMV-cHK was amplified (P<0.05) in transgenic mice overexpressing human B2 receptor compared with wild-type control mice (c57/B6 mice). Thus, the inhibitory effect of recombinant kallikrein on structural alterations caused by the interruption of blood flow appears to be mediated by the B2 receptor. These results provide new insight into the role of the tissue kallikrein-kinin system in vascular remodeling and suggest the application of HK gene therapy to treat restenosis and atherosclerosis. (Arterioscler Thromb Vasc Biol. 2000;20:1459-1466.)

Key Words: human tissue kallikrein ■ gene delivery ■ neointima formation ■ bradykinin B2 receptors ■ mice, transgenic and knockout

R estenosis is one of the major complications of percutaneous transluminal angioplasty and can be regarded as a combination of neointima (NI) formation and arterial remodeling triggered by vascular injury. Vascular remodeling also occurs as an adaptive phenomenon in response to chronic hemodynamic alterations aimed at maintaining a predetermined level of shear stress by permanent modifications in vascular geometry. In the endothelium is considered a critical mediator of the flow-dependent remodeling process. In fact, vascular endothelial cells (VECs), acting as sensors of intraluminal mechanical forces, release growth factors and vasoactive substances able to induce cell proliferation, migration, and death as well as matrix deposition. The presence of a local kallikrein-kinin system in the vasculature is firmly established, and evidence is now emerging regarding the possible participation of this system in vascular remodeling. Tissue kallikrein is a serine protease that cleaves low molecular weight kininogen to produce kinin peptides. Kinins stimulate the release of nitric oxide (NO) and prostacyclin (PGI2) through the activation of bradykinin (BK) B2 receptors expressed by VECs. NO and PGI2 exert antiproliferative and antimigratory effects on vascular smooth muscle cells (VSMCs) by increasing intracellular cGMP and cAMP, respectively.

The contribution of kinins in the protective effect of angiotensin-converting enzyme (ACE) inhibitors against arterial thickening caused by balloon injury was demonstrated in rats by the use of a B2 receptor antagonist. Recently, adenovirus-mediated human tissue kallikrein (HK) gene delivery proved to be an efficient strategy to increase local or circulating kinin levels for a limited period of time. By this mechanism, HK gene therapy reportedly prevents the develop-

Received August 16, 1999; revision accepted February 25, 2000.

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Arterioscler Thromb Vasc Biol. is available at http://www.atvbaha.org
ompent of hypertension, cardiac hypertrophy, and renal failure and suppresses NI formation in balloon-injured rat arteries. Whether the beneficial effect of HK gene therapy can be extended to other models of vascular remodeling remains unknown. Recently, Kumar, Lindner, and colleagues have shown that NI formation can be induced in the mouse carotid artery by disrupting local blood flow, a maneuver that causes permanent changes in shear stress conditions. One important feature of this approach is that vascular endothelium is not removed, thus underlining the importance of substances that are specifically released by or targeted to VECs. In addition, availability of a murine model of vascular remodeling is essential to exploit the informative potential of genome manipulation, which is usually carried out in mice.

The aim of the present study was to explore the potential beneficial effects of HK gene delivery on NI formation caused by ligation of the common carotid artery. The effectiveness of gene therapy was tested in wild-type mice as well as in genetically manipulated mice lacking the B2 receptor gene (B2<sup>-/-</sup> mice) or carrying an exogenous transgene encoding for the human B2 receptor (HB2, transgenic mice).

**Methods**

**Preparation of Replication-Deficient Adenovirus Vector Carrying the HK Gene**

An adenovirus vector containing the HK gene (Ad.CMV-cHK) was generated as previously described. The expression of HK cDNA under the control of the cytomegalovirus (CMV) enhancer/promoter, followed by a bovine growth hormone poly(A) signal sequence. An adenovirus harboring the β-galactosidase gene under the control of the CMV enhancer/promoter (Ad.CMV-LacZ) was also prepared as described.

**Animals**

All procedures complied with the standards for care and use of animal subjects as stated in the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, National Academy of Sciences, Bethesda, Md.). Experiments were carried out in male mice (aged 2 to 3 months). Swiss mice were purchased from Charles River (Varese, Italy). B2<sup>-/-</sup> mice, kindly provided by Dr Fred Hess (Merck Laboratories, Rahway, NJ), were generated by gene targeting and homologous recombination on a 11295v genetic background. Wild-type 11295v mice (Jackson Laboratory, Bar Harbor, Maine) served as controls for B2<sup>-/-</sup> mice. HB2 transgenic mice were developed on a c57/B16 background. In a separate set of experiments, B2<sup>-/-</sup>, HB2, and their respective wild-type control mice underwent carotid artery ligation and were injected via the left femoral vein with sterile saline (vehicle) or 1.8 × 10<sup>9</sup> pfu Ad.CMV-cHK or Ad.CMV-LacZ. Fourteen days after surgery, mice were euthanized for morphometric analysis. Each group consisted of at least 7 mice.

**Expression of HK**

Evidence of successful infection by systemic Ad.CMV-cHK delivery was obtained by measuring HK mRNA levels in tissues and recombinant protein in plasma. Swiss mice (n = 3) were injected with recombinant protein in plasma. Swiss mice (n = 6) or Ad.CMV-LacZ (n = 5). Three days later, blood was withdrawn from the hearts of anesthetized mice. Carotid artery, thoracic aorta, kidney, heart, and liver were isolated and removed for total RNA extraction by the Trizol method (BRL). Reverse transcription-polymerase chain reaction (RT-PCR) was performed with the use of specific oligonucleotide probes for HK (5′ primer, 5′-AAC ACA GCC CAG TTT GT-3′; 3′ primer, 5′-CTT CAC ATA AGA CAG CA-3′; and internal probe, 5′-GACCTAAATCTTGCC-3′) was performed as described. Expression of mouse β-actin mRNA in tissues was used as an internal control. Circulating HK levels were determined by ELISA with use of an antibody that recognizes only the active moiety of HK.

**Measurements of HK, Kinin, cGMP, and cAMP Levels in Urine**

Twenty-four-hour urine collections were obtained under basal conditions and 3, 7, and 14 days after intravenous injection of Ad.CMV-cHK or Ad.CMV-LacZ (n = 6 mice for each treatment) from Swiss mice placed in metabolic cages. Urinary HK levels were determined by ELISA. Urinary levels of cGMP and cAMP were determined by an enzyme immunoassay (Biotrak). Urine samples for kinin measurements were collected in ethanol to prevent enzymatic degradation. Kinins were measured by radioimmunoassay (Phenix), after extraction with Sep-Pak C18 columns (Waters).

**Morphometric Analysis**

Fourteen days after carotid ligation or sham operation, the mice were anesthetized and perfusion-fixed at a constant pressure (100 mm Hg) via the left ventricle with 4% paraformaldehyde in 0.1 mol/L phosphate buffer (pH 7.3). The whole left common carotid artery was excised and placed for 24 hours in 4% paraformaldehyde. Vessels were then processed for paraffin embedding. Five serial sections (200 μm apart) of 3-μm thickness were cut, starting from 1 mm below the carotid bifurcation and proceeding to the aortic arch. Sections were stained with hematoxylin-eosin. Morphometric analysis was performed in a blind fashion using a dedicated software package (KS300, Zeiss). The areas enclosed by the external elastic lamina (EEL) and internal elastic lamina (IEL) and the length of the IEL from EEL to the IEL were measured. IEL was taken as an index of inward remodeling; EEL, as a measure of vascular constitution. The area of the media was calculated by subtracting the area delimited by the IEL from the area delimited by the EEL. The total number of cells in the NI and media was evaluated at a magnification of ×1000 with use of a calibrated grid. Cellular density in the NI and media was calculated by dividing total cell count by the respective area. For each carotid artery, the values obtained from the 5 sections were averaged.

**Statistical Analysis**

Data are expressed as mean ± SEM. Multivariate repeated measures ANOVA was performed to test for interaction between time and the grouping factor. In multiple comparisons among independent groups in which ANOVA and the F test indicated significant differences, the statistical value was determined according to the method of Bonferroni. Differences within and between groups were determined by
paired or unpaired Student t test, respectively. A value of $P<0.05$ was considered statistically significant.

**Results**

**Expression of HK After Gene Delivery**

The expression of HK mRNA in mice after kallikrein gene delivery was detected by RT-PCR, followed by Southern blot analysis with the use of 3 oligonucleotides specific for HK. Figure 1 shows that HK mRNA can be detected in the aorta, carotid artery, heart, liver, and kidney but that the RT-PCR products from mice receiving Ad.CMV-LacZ did not hybridize to the HK gene probe (top panel). Similar levels of β-actin mRNA were detected in tissues of the experimental and control groups, verifying the quality of RNA in these samples (bottom panel). Three days after intravenous injection of Ad.CMV-cHK, circulating levels of immunoreactive HK averaged 145±20 ng/mL. In addition, as shown in Figure 2A, immunoreactive HK was detected in the urine, peaking at 7 days after injection. In contrast, immunoreactive HK levels in plasma and urine were undetectable in mice injected with Ad.CMV-LacZ (see Figure 2).

**Urinary Kinin, cGMP, and cAMP Levels After Gene Delivery**

Urinary kinin, cAMP, and cGMP levels were not altered by Ad.CMV-LacZ (Figure 2B through 2D). In contrast, mice treated with Ad.CMV-cHK showed a 3.8-fold increase in
immunoreactive kinin levels at 7 days after injection (Figure 2B). Urinary cAMP and cGMP increased by 5.3- and 2.5-fold, respectively (Figure 2C and 2D).

**Morphometric Analysis**

Consistent with previous studies performed in FVB mice, we found that also in Swiss mice disruption of carotid blood flow causes a reduction in vessel lumen area that is due to a combination of NI formation (NI area 41 817 ± 5381 μm²), medial hyperplasia (37 445 ± 5552 versus 23 820 ± 5368 μm²) in sham-operated mice, P < 0.05, and reduction in IEL and EEL lengths (1095 ± 143 versus 1318 ± 137 μm and 1162 ± 92 versus 1404 ± 137 μm, respectively; P < 0.05 for both comparisons). NI was maximally represented close to the ligation site and decreased in thickness in the direction of the aortic arch.

In Ad.CMV-LacZ–injected mice, the vascular remodeling response to carotid ligation was comparable qualitatively and quantitatively to that observed in mice given saline. In contrast, as shown in Figure 3, Ad.CMV-cHK delivery attenuated NI formation (21 059 ± 3417 versus 43 885 ± 2778 μm² in Ad.CMV-LacZ–treated mice, P < 0.05) without affecting the medial area. As a consequence, the NI/media ratio was reduced by 52% (0.59 ± 0.18 versus 1.28 ± 0.06 in Ad.CMV-LacZ–treated mice, P < 0.01). The lengths of the IEL and EEL were unaffected by Ad.CMV-cHK delivery (972 ± 59 and 1064 ± 72 μm versus 1072 ± 133 and 1158 ± 111 μm in Ad.CMV-LacZ–treated mice, respectively; P = NS for both comparisons).

Chronic administration of the B₁ or the B₂ receptor antagonists did not change the vascular remodeling response to carotid ligation in Ad.CMV-LacZ–treated mice (data not shown). However, when these agents were tested in Ad.CMV-cHK–treated mice, we found that the B₂ antagonist icatibant completely abrogated the protective effect of HK gene delivery against NI formation, whereas the B₁ receptor antagonist DALBK was ineffective (Figure 3).

As shown in Figure 4, HK gene transfer decreased NI total cell count (101 ± 20 versus 268 ± 65 cells in the cross section Ad.CMV-LacZ group, P < 0.01) and increased NI cell density (22 ± 2 versus 6 ± 1 cells/mm² in the Ad.CMV-LacZ group, P < 0.001). In contrast, HK gene delivery produced borderline changes in medial total cell count (149 ± 14 versus 109 ± 10 cells cross section in the Ad.CMV-LacZ group, P = 0.06) and in medial cell density (7 ± 1 versus 5 ± 1 cells/mm² in the Ad.CMV-LacZ group, P = 0.09). Icatibant contrasted the effect of kallikrein on NI cell count and density (Figure 4A) without affecting these parameters in the media (Figure 4B). The B₁ antagonist DALBK did not alter the effects of HK gene delivery on cellular count and density.

Figure 5 shows the typical pattern of vascular remodeling caused by ligation of the carotid artery in Swiss mice treated with Ad.CMV-LacZ (panel B), Ad.CMV-cHK (panel C), or Ad.CMV-cHK plus icatibant (panel D).

In Ad.CMV-LacZ–treated Swiss mice, L-NAME did not alter the remodeling response to carotid ligation. Evaluation of the effect of NO synthase inhibition after HK gene transfer was precluded by a dramatic reduction in the survival rate in the group given L-NAME in combination with Ad.CMV-cHK. In fact, all 12 mice that entered this treatment showed a progressive deterioration of general conditions and died within 7 days.

**Effect of Ad.CMV-cHK Delivery on Vascular Remodeling in B₂<sup>−/−</sup> and HB₂ Transgenic Mice**

Morphometric analysis of carotid arteries did not detect any difference between B₂<sup>−/−</sup> and HB₂ transgenic mice and their respective wild-type controls, J129Sv and c57/B16, in the absence of vascular injury as well as after artery ligation (data not shown). However, genetically manipulated animals dif-
ferred from controls regarding the effectiveness of HK in preventing NI formation. In fact, as shown in Figure 6A (left bars), the protective effect of Ad.CMV-cHK delivery was reduced in the B2−/− strain compared with the wild-type J129Sv strain, leading to a greater NI/media area ratio in knockout mice (Figure 6C, left bars). Conversely, inhibition of NI formation by Ad.CMV-cHK was potentiated in HB2 transgenic mice compared with their wild-type controls, c57/Bl6 (Figure 6, right bars).

**Hemodynamic Measurements**

As shown in the Table, no change in systolic blood pressure was observed in Swiss mice after ligation of the common carotid artery and injection of Ad.CMV-LacZ. A tendency of the heart rate to increase was observed in both groups, but this change did not reach statistical significance (P<0.08).

**Discussion**

To the best of our knowledge, this is the first study demonstrating a protective role of adenovirus-mediated HK gene delivery against vascular remodeling in the mouse. Another novel discovery is that insertion or deletion of the BK B2 receptor gene respectively enhances or precludes the protective action of the tissue kallikrein gene on NI formation, thus demonstrating the essential role of B2 receptor signaling in this gene therapy approach.

Injury to the arterial wall induces the synthesis of gene products that stimulate smooth muscle cell migration and proliferation, thus leading to intimal hyperplasia. It has been hypothesized that the suppression of tissue kallikrein gene expression in the damaged vessel may contribute to the pathogenesis of restenosis after angioplasty.19 This idea is supported by the observation that kinins, generated from kininogen by tissue kallikrein, inhibit vascular cell proliferation.19 On the basis of this assumption, HK gene delivery has been successfully used to suppress NI formation in the rat balloon-injured carotid artery, an effect that appears to be mediated by activation of the kinin B2 receptor.

A large body of evidence now indicates that a chronic decrease in shear stress can also produce vascular wall alterations similar to those caused by mechanical injury in normal and in atherosclerotic vessels.27–29 Therefore, we thought it would be worthwhile to evaluate the efficacy of HK gene delivery in preventing the vascular remodeling caused by permanent alteration in shear stress conditions. To this aim, we exploited the mouse model recently established by Kumar and Lindner,20 in which disruption...
of blood flow triggers NI formation together with reduction in vessel diameter. In this setting, the proliferative response of VSMCs is thought to be stimulated by the increase in arterial wall tension that occurs proximal to the ligation.27,28

Under basal conditions, we found no difference between B2/2 and wild-type control mice regarding the vascular structure of the carotid artery; neither B2 receptor gene knockout altered vascular remodeling after ligation. Thus, the congenital absence of the B2 receptor neither results in structural vascular abnormalities nor affects the remodeling response to vascular injury. Similarly, NI formation was not worsened by B2 receptor antagonism in Swiss mice injected with Ad.CMV-LacZ. However, a functional B2 receptor appears to be essential for the vascular protection exerted by HK gene delivery. In fact, the suppression of NI formation by Ad.CMV-cHK was abrogated by the B2 receptor antagonist icatibant. These data are in line with the results obtained by Farhy et al15 in the rat balloon-injury model by the use of an ACE inhibitor. In fact, icatibant, which by itself did not worsen NI formation, partially prevented the protective effect of ACE inhibition. Altogether, these results indicate that kinins are indeed able to contrast vascular remodeling, provided that their levels are augmented through pharmacological or genetic interventions.

This assumption, together with the identification of the receptor implicated in the action of Ad.CMV-cHK, was further challenged by the use of murine models in which the endogenous B2 receptor gene was either deleted or added to the human B2 receptor gene. This approach has obvious advantages over the classic use of receptor antagonists, including the possibility of recognizing a correlation between biological effects of HK and the number of copies of the B2 receptor gene. A gene dosage effect was found. In fact, we have shown that the protective action of Ad.CMV-cHK is virtually absent in B2−/− and enhanced in HB2 transgenic mice, thus demonstrating the essential role of the B2 receptor in the beneficial effect of HK gene delivery.

The HK gene was expressed in the vasculature, liver, kidney, and heart after intravenous injection of Ad.CMV-cHK into mice. The secreted nature of the gene product is indicated by the presence of active HK in the circulation and urine. Therefore, suppression of NI formation by Ad.CMV-cHK may be attributable to the effect of recombinant protein either locally expressed or circulating in the blood stream. The protection exerted by Ad.CMV-cHK delivery appears to be limited to a reduction in NI formation, whereas medial hyperplasia or vessel shrinking remained unaffected. The finding that after Ad.CMV-HK treatment total cell count was decreased in the NI and unchanged in the media suggests that HK could act by inhibiting VSMC proliferation. An antiproliferative role of Ad.CMV-HK on cultured VSMCs has been previously shown by Murakami et al.19 In vitro experiments also indicate that the binding of kinins to aortic VSMC receptors stimulates PGI2 formation, with increased cAMP levels and subsequent inhibition of VSMC proliferation.30

The mechanism by which Ad.CMV-HK transfer inhibits vascular growth in vivo may also involve the induction and/or

![Figure 5. Representative hematoxylin-eosin-stained carotid transverse sections taken 1 mm apart from the carotid bifurcation of sham-operated Swiss mice (A, magnification ×100) or ligated left carotid arteries of Swiss mice treated with Ad.CMV-LacZ (B, magnification ×400), Ad.CMV-cHK alone (C, magnification ×400), or Ad.CMV-cHK in combination with icatibant (D, magnification ×400).](http://atvb.ahajournals.org/)

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activation of NO synthase. The binding of kinins to vascular endothelial B2 receptor increases NO release from VECs. C GMP, a potent inhibitor of VSMC proliferation and migration.7,8,32 Accordingly, previous studies showed that kinin-stimulated NO release contributes to the beneficial effect of ACE inhibitors in balloon injury-induced remodeling by reducing VSMC migration from the tunica media to the NI.15,33 As stated above, cell count in the media was unchanged by Ad.CMV-cHK treatment. We would expect that if HK blocked VSMC migration to NI without affecting the proliferation rate, the total number of cells would be increased in the tunica media. Because this was not the case, it is likely that VSMC migration and proliferation were inhibited by Ad.CMV-cHK treatment. Further studies that use markers of cellular turnover may help dissect the importance of these 2 mechanisms. The finding that NI cell density was augmented in the Ad.CMV-HK group favors a suppressive role of HK against matrix accumulation.

Although activation of cAMP and cGMP pathways by HK is well documented in the present study, investigation of the role of NO was precluded by the fact that L-NAME dramatically reduced the survival rate in mice given the HK gene, possibly because of severe bronchoconstriction and hypertension.34,35 L-NAME by itself did not affect remodeling in animals treated with Ad.CMV-LacZ. This is in apparent discordance with studies of vascular remodeling in endothelial NO synthase knockout mice, which show increased wall thickness after carotid ligation, compared with wild-type control mice.9 However, it should be noted that only the external carotid artery was ligated in endothelial NO synthase knockout mice,9 whereas in our experimental setting, blood flow was completely interrupted by ligature of the common carotid artery. Abrogation of blood flow may have reduced the basal release of NO from vascular endothelium to such an extent that no further decrease would be expected with NO synthase inhibition.

Although carotid occlusion could disturb blood flow to the brain and baroreceptor function, this had no consequence on systemic blood pressure. Failure to detect changes in blood pressure could be due to the fact that adaptive adjustments may have already occurred at the time the first measurements were performed. In addition, because of an efficient collateral flow, ligature of 1 carotid artery may be not sufficient to induce brain ischemia in mice. Disturbance in baroreceptor function is supported by the tachycardia observed after surgery.

In conclusion, we have demonstrated the feasibility and efficacy of adenovirus-mediated HK gene transfer in a mouse model of vascular remodeling. These results underline the importance of the kallikrein-kinin system in vascular biology and open a new avenue for gene therapy in vascular diseases.

Figure 6. Effects of systemic delivery of Ad.CMV-cHK in carotid- ligated kinin B2 receptor gene knockout mice (B2−/−, n = 8; hatched bars) and their wild-type controls (J129, n = 8; solid bars) and in transgenic mice harboring the human B2 receptor (HB2trans, n = 7; shaded bars) and their wild-type controls (c57/B16, n = 9; open bars). NI area (A), medial area (B), and ratio between NI and medial areas (C) are plotted. *P<0.05 vs respective wild-type controls.

SBP and HR Measured Under Basal Conditions and at Different Time Points After Injection of 1.8×10⁹ pfu Ad.CMV-cHK or Ad.CMV-LacZ in Femoral Veins of Swiss Mice

<table>
<thead>
<tr>
<th>Groups</th>
<th>Basal</th>
<th>3 d</th>
<th>7 d</th>
<th>14 d</th>
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<tbody>
<tr>
<td></td>
<td>SBP, mm Hg</td>
<td>HR, bpm</td>
<td>SBP, mm Hg</td>
<td>HR, bpm</td>
</tr>
<tr>
<td>HK (n=6)</td>
<td>116±5</td>
<td>571±32</td>
<td>112±4</td>
<td>640±32</td>
</tr>
<tr>
<td>LacZ (n=5)</td>
<td>118±2</td>
<td>475±13</td>
<td>121±4</td>
<td>580±19</td>
</tr>
</tbody>
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Values are mean±SEM. SBP indicates systolic blood pressure; HR, heart rate; HK, Ad.CMV-cHK–injected group; and LacZ, Ad.CMV-LacZ–injected group.
Acknowledgments
We gratefully acknowledge Telethon-Ohlus for financial support (grant A.105) and National Institutes of Health grants HL-29397 and HL-52196. In addition, we would like to thank Dr Renzo Filippetti, Vittorio Lelii, and Leandro Travaglini from the Università Cattolica del Sacro Cuore (Rome, Italy) for their assistance in the animal care.

References
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Arterioscler Thromb Vasc Biol. 2000;20:1459-1466
doi: 10.1161/01.ATV.20.6.1459
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1079-5642. Online ISSN: 1524-4636

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