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ENDOTHELIAL PERMEABILITY IN VITRO AND IN VIVO: PROTECTIVE ACTIONS OF ANP AND OMAPATRILAT IN EXPERIMENTAL ATHEROSCLEROSIS

Tomoko Ichiki, Ririko Izumi, Alessandro Cataliotti, Amy M. Larsen, Sharon M. Sandberg, and John C. Burnett Jr.

Cardiorenal Research Laboratory, Division of Cardiovascular Diseases, Mayo Clinic, Rochester, Minnesota

Abstract

Increased arterial endothelial cell permeability (ECP) is considered an initial step in atherosclerosis. Atrial natriuretic peptide (ANP) which is rapidly degraded by neprilysin (NEP) may reduce injury-induced endothelial cell leakiness. Omapatrilat represents a first in class of pharmacological agents which inhibits both NEP and angiotensin converting enzyme (ACE). We hypothesized that ANP prevents thrombin-induced increases of ECP in human aortic ECs (HAECs) and that omapatrilat would reduce aortic leakiness and atherogenesis and enhance ANP mediated vasorelaxation of isolated aortas. Thrombin induced ECP determined by I¹²⁵ albumin flux was assessed in HAECs with and without ANP pretreatment. Next we examined the effects of chronic oral administration of omapatrilat (12mg/kg/day, n=13) or placebo (n=13) for 8 weeks on aortic leakiness, atherogenesis and ANP-mediated vasorelaxation in isolated aortas in a rabbit model of atherosclerosis produced by high cholesterol diet. In HAECs, thrombin-induced increases in ECP were prevented by ANP. Omapatrilat reduced the area of increased aortic leakiness determined by Evans-blue dye and area of atheroma formation assessed by Oil-Red staining compared to placebo. In isolated arterial rings, omapatrilat enhanced vasorelaxation to ANP compared to placebo with and without the endothelium. ANP prevents thrombin-induced increases in ECP in HAECs. Chronic oral administration of omapatrilat reduces aortic leakiness and atheroma formation with enhanced endothelial independent vasorelaxation to ANP. These studies support the therapeutic potential of dual inhibition of NEP and ACE in the prevention of increased arterial ECP and atherogenesis which may be linked to the ANP/cGMP system.

Keywords

Atrial natriuretic peptides; Neprilysin inhibitor; Angiotensin converting enzyme inhibitor; Atherosclerosis; Endothelium

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Corresponding author: Tomoko Ichiki, MD, PhD, Cardiorenal Research Laboratory, Division of Cardiovascular Diseases, Mayo Clinic, 200 First Street SW, Rochester, Minnesota 55905, Tel. (507) 538-7517, Fax (507) 266-4710, ichiki.tomoko@mayo.edu.

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1. INTRODUCTION

Atrial natriuretic peptide (ANP), first discovered in the 1980s [8, 12], is an endogenous cardiac peptide which serves as a volume-regulating hormone through the guanylyl cyclase-A receptor (GC-A) and the second messenger, cyclic guanosine monophosphate (cGMP). ANP also functions as an endothelial independent vasodilator and its genetic disruption results in a murine model of hypertension [17]. In humans, a genetic variant of the ANP gene (rs5068) which is associated with higher circulating ANP is also associated with reduced prevalence of hypertension as well as metabolic syndrome and myocardial infarction [6, 18]. Further, a genetic variant of the ANP gene (rs5065) which may code for an altered molecular form of ANP is associated with increased atherosclerosis as manifested by stroke and coronary artery disease [2]. Thus, ANP goes beyond a renal natriuretic hormone and also participates in vascular homeostasis and may possess novel anti-atherogenic properties.

In endothelium, ANP increases endothelial cell permeability (ECP) under physiological conditions and therefore reduces arterial pressure through a reduction in intravascular volume independent of its natriuretic actions [7]. In contrast, ANP has potent permeability inhibiting properties in injured endothelial cells (ECs). Relevant to atherogenesis, thrombin mediated injury to ECs increases macromolecular permeability which may play one of the key roles in the progression of atherosclerosis by facilitating movement of macromolecules like lipoproteins across the endothelial barrier and contribute to plaque formation. Most importantly ANP prevents thrombin-mediated increased ECP *in vitro* cultured bovine endothelial cells [3] which may support the concept that the ANP/GC-A system could represent a potential anti-atherogenic therapeutic agent by maintaining the endothelial barrier through inhibition of EC leakiness under pro-atherogenic conditions. ANP may also contribute to preserve arterial vasorelaxation secondary to increasing intracellular cGMP in vascular smooth muscle cells (VSMCs) [19, 33] which may be impaired in the setting of atherosclerosis.

The use of ANP as a cardiovascular therapeutic has largely been restricted to acute intravenous injection due to its peptide structure and its rapid degradation by neprilysin (NEP) which is highly expressed in the kidney but also is present in ECs and VSMCs [9]. Kugiyama and colleagues reported increased NEP in atherosclerotic lesions, thus decreasing availability of circulating ANP to the vascular wall [14]. Such an increase in NEP is also accompanied by an increase in angiotensin converting enzyme (ACE). Experimental studies have shown that NEP inhibition [14, 22] or in combination with ACE inhibition [16, 31] has anti-atherogenic effects which in part have been attributed to inhibition of ANP degradation thus increasing bioavailability including increased circulating ANP and cGMP levels with GC-A receptor stimulation together with inhibition of angiotensin (ANG) II generation.

Omapatrilat (OMA) represents a first generation type of molecule that possesses two functions so as to target different enzymes and receptors. Specifically, omapatrilat is a novel small molecule that inhibits ACE to reduce ANG II generation and the AT1 receptor activation while inhibition of NEP reduces degradation of the natriuretic peptides including ANP so as to activate GC-A. Indeed the rational drug design behind dual inhibitors such as OMA and now LCZ 696, which is a combined NEP inhibitor and AT1 receptor antagonist, is not only enhance efficacy with respect to blood pressure control, but also to promote anti-proliferative, anti-fibrotic and anti-inflammatory actions through reductions in ANG II and increases in ANP.

The current studies were designed to both confirm and extend previous investigations which support the concept that ANP prevents thrombin-induced EC leakiness to macromolecules *in*

vitro. We also sought to define in a rabbit model of atherosclerosis that hypercholesterolemia results in both atheroma formation together with increased aortic leakiness and that OMA which is a dual inhibitor of NEP and ACE can reduce both atheroma formation and aortic leakiness and in isolated arterial rings enhance vasorelaxation to ANP thus serving as a novel agent to preserve aortic endothelial barrier function and prevent atherogenesis.

2. MATERIAL AND METHODS

This study was in accordance with the Animal Welfare Act, and was approved by the Mayo Clinic Animal Care and Use Committee.

2.1 ANP actions on thrombin-induced increases in ECP in human aortic endothelial cells (HAECs)

HAECs (Clonetics, San Diego, CA) were grown to confluency in culture medium (EGM-2; Clonetics, San Diego, CA) and passages 4 to 6 were used. For permeability studies, HAECs were seeded into 12 mm culture plates inserts (Millicell-HA; Millipore, Bedford, MA) containing a 0.45 μ m pore cellulose filter and grown in 24 well plates. These inserts create two chambers (inner and outer chamber) that are separated by the endothelial cell monolayer allowing diffusion of soluble substances between chambers. Changes in permeability were determined by passage of I^{125} -labeled bovine serum albumin (Phoenix Pharmaceuticals Inc., Mountain View, CA) through endothelial cell monolayer. The effects of ANP (10^{-6} M) (Phoenix Pharmaceuticals Inc., Mountain View, CA) on permeability were studied in basal conditions and after exposure of cells to thrombin (1 U/ml) (Phoenix Pharmaceuticals Inc., Mountain View, CA). ANP, thrombin and I^{125} -labeled BSA were added into the inner chamber (total volume 400 μ l), and cells were incubated at 37°C for 30 minutes and then the radioactivity in the outer chamber (total volume 600 μ l) was measured with a gamma counter.

2.2 Studies in Hypercholesterolemic Rabbits

2.2.1 Study Groups—Male New Zealand White rabbits (n=26, 2 to 4kg) were used in model of experimental atherosclerosis produced by high cholesterol diet.

We determined the actions of chronic oral dual NEP/ACE inhibitor, OMA (12mg/Kg/day, Bristol Meyer Squibb) on atherosclerosis and vascular reactivity together with aortic permeability. Specifically, rabbits were divided into 2 groups with 1% cholesterol diet, placebo-treated group (n=13) and OMA-treated group (n=13), and both dissolved in drinking water and administered once daily for 8 weeks. To demonstrate the acute effect of OMA, urine was collected after OMA or placebo administration for 24 hours at day 1, and urine volume, cGMP and ANP levels were assessed. Mean arterial pressure (MAP) was measured by catheter inserted in the central artery of the ear in both groups at baseline before treatment on day 1 and at 8 weeks. Lipid levels (total cholesterol, low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C) and triglycerides) were measured at the end of 8 weeks in both groups. At the end of the 8 weeks animals were sacrificed with overdose intravenous pentobarbital sodium (30 mg/Kg) and aortas were harvested for assessment of atheroma formation and vascular reactivity experiments (n=8 of each) while 5 rabbits from each group were used for aortic EC leakiness studies.

2.2.2 Urinary ANP and cGMP measurements—Urinary ANP was measured by radioimmunoassay to ANP as previously described [5] Urinary samples for cGMP were measured with RIA according the method of Steiner and colleagues [24].

2.2.3 Quantification of atherosclerosis extent ex vivo—Extent of atherosclerosis was examined in aorta of both groups (n=8 of each) from origin of the aortic valve to the first intercostal arteries including thoracic aorta. Atheroma formation was determined by oil red O staining [22]. After immersion fixation overnight in 10% neutral buffered formalin, the thoracic aorta was stained with oil-red O (0.3%). A single longitudinal incision along the wall opposite the arterial ostia was made, and the vessels were pinned open and photographed. The percent plaque area was determined from the values for the total area examined and the stained area by threshold analysis using true-color image analyzer software.

2.2.4 Area of vascular leakiness on rabbit aorta ex vivo—Isolation of the aorta was performed as previously reported [28]. Briefly, aortas from both group (n=5) were removed and perfused immediately with medium 199 (Gibco) and Hanks' salts solution (Gibco) to clean the aortic wall, and then incubated with Evans blue in medium 199 (0.3%) for 5 minutes. After washing the stained aorta by medium 199, aortas were fixed with buffered 3% formaldehyde and opened longitudinally and photographed for imaging analysis. The percent area of vascular leakage was assessed from the values for the total area examined and the stained area by threshold analysis using true-color image analyzer software.

2.2.5 Vascular reactivity studies ex vivo—Vascular reactivity of thoracic aorta to ANP was performed in organ chambers from both groups as previously described [22]. Rabbit thoracic aortas from each groups (n=8) were cut into eight rings (3–5 mm). Some rings were denuded of endothelium by inserting a pair of fine forceps into the lumen and gently rolling the ring back and forth on Krebs-Ringer-wetted paper. Rings with and without endothelium from each rabbit were studied in parallel. The rings were suspended between a fixed stirrup and force transducer for measurement of isometric force in organ chambers filled with aerated (95% O₂ and 5% CO₂), modified Krebs-Ringer bicarbonate buffer at 37 °C. Each ring was stretched to the optimal point on its length tension curve as determined by tension developed by 20 mM KCl at different levels of stretch (6, 8, 10, 12, and 14 g). The basal tension of the rings was 10.6 ± 0.1 g and the maximal contractile response to 60 mM KCl was 6.2 ± 0.2 g. After a 30 minute equilibration period the rings were constricted with phenylephrine (10⁻⁷ M) until a stable contraction of 4.0 ± 0.1 g was attained. The rings were then exposed to cumulative concentrations of Acetylcholine and ANP (Phoenix Pharmaceuticals Inc., CA) in half-log increments (10⁻¹¹–10⁻⁷ M).

2.3 Statistical analysis

Results are expressed as mean ± SEM. Statistical comparison between groups was made using Student's unpaired t test or one analysis of variance as appropriate. Statistical significance was defined as p<0.05.

3. RESULTS

3.1 ECP in HAECs in vitro

ANP at concentrations of 10⁻⁸ to 10⁻⁶ M had no significant effect on ECP to I¹²⁵-albumin under basal conditions. Treatment with thrombin increased ECP (p<0.05) compared to untreated cells (p<0.05, Fig. 1) and pretreatment with ANP (10⁻⁶ M) inhibited thrombin-induced increase of ECP (p<0.05, Fig. 1).

3.2 Characteristics experimental atherosclerosis with or without OMA

Initiation of OMA therapy resulted in a significant increase in urinary cGMP and ANP excretion as well as with an increase of diuresis compared to the placebo treated group, supporting the action of NEP inhibition in reducing ANP degradation (Fig. 2).

Table 1 reports blood pressure and serum lipid levels of both groups in experimental atherosclerosis after 8 weeks treatment with or without OMA. MAP was not significantly different between groups at baseline (75 ± 3 vs. 74 ± 3 mmHg, $p=ns$), however, MAP was significantly lower with OMA than in placebo groups at 8 weeks ($p<0.05$). There was no difference in total cholesterol, LDL-C, HDL-C or triglycerides levels between groups ($p=ns$ for all comparisons).

Figure 3 illustrates the area of aortic atherosclerotic plaque by oil-red O staining. OMA significantly reduced the area of plaque ($23 \pm 6\%$) compared to the placebo group ($68 \pm 8\%$) ($p<0.05$, Fig. 3).

3.3 Aortic leakiness with or without OMA

Aortic endothelial leakiness was assessed by Evans blue staining in aortas isolated from hypercholesterolemic rabbits at 8 weeks with and without OMA. Fig. 4 illustrates the area of aortic vascular leakage, shown as white area, consistent with increased aortic leakiness. The OMA group showed significantly smaller area of aortic endothelial leakage compared to the placebo group ($p<0.05$; Fig. 4).

3.4 Vascular reactivity studies *ex vivo*

Fig. 5 shows ANP mediated vasorelaxation of isolated aortic rings with (Fig. 5A) and without (Fig. 5B) endothelium from both groups. In placebo group, ANP had dose-dependent vascular relaxations in arteries both with or without endothelium. These ANP effects were significantly enhanced by OMA either with endothelium (EC_{50} ; 8.4 ± 0.07 (OMA) vs. 7.6 ± 0.1 (placebo), $p<0.05$, Fig. 5A) or without endothelium (EC_{50} ; 8.4 ± 0.08 (OMA) vs. 7.9 ± 0.01 (placebo), $p<0.05$, Fig. 5B). Enhanced vasorelaxation was observed by OMA with ANP, suggesting the mechanism(s) are independent of nitric oxide (NO) as the presence or absence of the endothelium did not modulate the response.

4. DISCUSSION

The present studies were designed to address the actions of ANP on ECP *in vitro*. We report that ANP potently inhibits thrombin-induced increases of ECP in HAECs. Importantly, these investigations also report that long-term oral administration of the dual NEP/ACE inhibitor OMA prevents hypercholesterolemic induced aortic leakiness and atherogenesis. Further, OMA also enhanced ANP mediated vasorelaxation of isolated aortic vascular rings. These actions in experimental atherosclerosis were independent of cholesterol lowering.

Atherosclerosis is a fundamental mediator of adverse outcomes secondary to cardiovascular and metabolic disease states such as hypertension, diabetes and dyslipidemia. In the initial phases of atherogenesis, dysfunctional endothelial cell function with increased ECP allows the invasion of leukocytes with lipid and pro-inflammatory cytokines into the vascular wall intima. Indeed, this endothelial cell barrier function is a key mechanism which serves to retard atherosclerosis [30].

Here we defined ANP modulation of ECP in HAECs that were activated by thrombin which increased ECP to macromolecules (i.e. I^{125} albumin). We observed no significant effect of NPs on ECP to I^{125} albumin in non-activated ECP (data not shown) that is consistent with previous reports [4, 26]. In contrast, when arterial ECs were activated by thrombin that increased ECP, ANP inhibited the increased ECP. The current findings are also consistent with the report that increased ECP induced by an inflammatory cytokine, tumor necrosis factor alpha also prevents increases in ECP which involves ANP/cGMP mediated inhibition of mitogen-activated protein kinase with the activation of p38 phosphatases [13].

Previous studies have demonstrated that NEP, which is increased in the vascular wall in experimental atherosclerosis, is widely distributed in the vascular wall and by degrading ANP may limit the vascular wall protective biological actions of these cardiovascular peptides by the NEP induced degradation [14]. Indeed, long-term NEP inhibition has been reported to decrease atheroma formation and preserve endothelium-dependent vasorelaxations [14] as well as enhance endothelial independent vasorelaxations to BNP [22] and suppress the pro-atherogenic peptide endothelin. Underscoring the anti-atherogenic properties of ANP are studies which have reported that ANP suppresses proliferation of VSMCs as well as intimal thickening in animal models [19, 33]. Further, atherosclerosis has been reported to be accelerated in GC-A^{-/-} + Apolipoprotein E^{-/-} mice [1]. Accompanying the increase of NEP in atherosclerosis is the well documented increase in ACE which has also been reported to be increased in the vascular wall in atherosclerosis and via increased generation of ANG II contribute to atherogenesis [27, 32].

In the current studies we chose to use the first of class dual NEP/ACE inhibitor OMA as to potentiate the NPs through NEP inhibition and reduce the generation of ANG II by inhibiting ACE at a dose which inhibits both enzymes including NEP as demonstrated by the increase in urinary ANP and cGMP excretion (Fig. 2). Our investigations demonstrate a reduction in the development of atheroma formation by chronic dual inhibition of NEP/ACE and that this action is independent of cholesterol lowering. In the current study it was not possible to discern the individual contributions of NEP or ACE inhibition upon the suppression of atheroma formation. Indeed it should be noted that our goal was not to assess the individual contribution of ACE inhibitor or NEP inhibitor alone but rather to assess what could be considered a strategy to target two ectoenzymes which would have favorable anti-atherogenic actions if both inhibited. Our findings are consistent with previous reports which have documented that NEPI together with ACEI inhibits atherogenesis [16, 31]. While cholesterol levels were not affected by OMA, blood pressure was lowered which could have also contributed to attenuated atherogenesis.

The importance of the barrier function of the endothelium is a fundamental one in vascular pathobiology [30]. Specifically, the vascular endothelium serves as a selective barrier that controls the movement of plasma proteins like LDL and circulating inflammatory cells across the blood vessel wall. Indeed, the development of intercellular gaps in the vascular endothelium is thought to be one of the initial conditions contributing to the development of the athermanous plaque. The mechanism of this pathological response to chemical or mechanical injury has been attributed to the reorganization of F-actin filaments followed by contraction of cells and formation of intercellular gaps [21]. Importantly, in experimental atherosclerosis produced by high cholesterol diet, simvastatin preserves endothelial barrier function with attenuation in increases in aortic permeability in response to hypercholesterolemia in association with a reduction in atherosclerosis [28]. Such a preservation of barrier function was related to a reduction in actin stress fibers that occurred in response to EC injury with thrombin as demonstrated in vitro [29].

The current study is the first to report that this first in class dual targeting molecule, OMA, prevents the increase in aortic leakiness when given chronically to a rabbit model of hypercholesteromic induced atherosclerosis. This action was associated with a reduction in atheroma formation independent of cholesterol lowering although blood pressure was reduced. While the mechanism(s) of this protective action may be multifactorial, a role for ANP and/or the natriuretic peptides in general which are degraded by NEP may participate. The initial increase in ANP and cGMP at the time of starting OMA supports such a role although a limitation is the lack of serial measurements of the ANP throughout the treatment period.

With regard to enhanced vasorelaxation produced by OMA to ANP, the mechanism(s) are independent of NO as the presence or absence of the endothelium did not modulate the response, suggesting the enhancement of GC-A mediated pathway may play a key role in VSMCs as well. Possible mechanisms for this enhanced ANP induced vasorelaxation by OMA could include reductions in ANG II which is known to oppose the actions of ANP [10], up-regulation of GC-A and reduction in phosphodiesterase activity [25]. Such an explanation would also be consistent with our observation in previous studies that NEP inhibition alone in experimental atherosclerosis enhances the vascular response to another GC-A agonist, b-type natriuretic peptide (BNP) independent of the endothelium [22].

Our study has several limitations. We used an experimental model and findings may not be similar in humans. Further studies may be needed to address other possible pathways or mechanisms to by which OMA or other similar molecules mediate endothelial protection. We need not assess the direct role of NEP versus ACE inhibition on ECP but our goal was to assess for the first time a dual NEP/ACE inhibitor.

To date, GC-A agonists ANP (carperitide), BNP (nesiritide), and the ANP molecular form urodilatin (ularitide) are being used for the treatment with heart failure. These peptides have not only natriuretic and diuretic actions but also mediate vasodilatation. In the current study, we showed that ANP also has endothelial protective effects *in vitro* in thrombin-injured ECs. We also demonstrated *in vivo* the protective actions of the dual NEP/ACE inhibitor OMA on the endothelial barrier, atherogenesis and vascular reactivity in experimental atherosclerosis. Thus, ANP and agents like OMA may have therapeutic potential in atherosclerotic diseases especially diseases such as hypertension in which one wants to achieve both blood pressure lowering as well as vascular wall protection. Clinical trials of omapatrilat in heart failure (OVERTURE) [20] and in hypertension (OCTAVE) [15] compared to ACE inhibitors showed improved clinical status in some key parameters, but its approval was declined in the US in 2000 because it had more risk for angioedema than an ACE inhibitor. The recently developed dual inhibitor of the angiotensin AT1 receptor and NEP (ARNI) designated LCZ 696 has been reported to be a potent anti-hypertensive molecule *in vivo* [11] and the first clinical trial for heart failure with preserved ejection fraction (PARAMOUNT) showed similar tolerance and risk of side effects compared to ACE inhibitor alone [23]. As such agents are developed for cardiovascular diseases, an assessment of athermanous plaque regression is warranted.

5. CONCLUSION

ANP prevents thrombin-mediated increases in EC permeability *in vitro* in cultured human arterial ECs. Chronic oral NEPI/ACEI with omapatrilat enhances endothelial independent vasorelaxation to ANP in association with a reduction in atherogenesis independent of cholesterol lowering *in vivo* and *ex vivo* experimental atherosclerosis which is linked to the inhibition of hypercholesterolemic increases in aortic leakiness. Thus, these studies provide new insights into the anti-atherogenic and vasorelaxing properties of chronic NEPI/ACEI which may involve the ANP/GC-A system.

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ABBREVIATIONS

ANP	atrial natriuretic peptide
GC-A	guanylyl cyclase-A receptor
cGMP	cyclic guanosine monophosphate
ECP	endothelial cell permeability
EC	endothelial cell
VSMCs	vascular smooth muscle cells
NEP	neprilysin
ACE	angiotensin converting enzyme
NEPI	NEP inhibition
ACEI	ACE inhibition
ANG	angiotensin
OMA	omapatrilat
HAECs	human aortic endothelial cells
MAP	mean arterial pressure
LDL-C	low density lipoprotein cholesterol
HDL-C	high density lipoprotein cholesterol
NO	nitric oxide
BNP	b-type natriuretic peptide

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ANP inhibited thrombin induced endothelial cell permeability in vitro.

Omapatrilat (dual NEP and ACE inhibitor) increased urinary cGMP and ANP excretion.

Omapatrilat reduced atheroma formation and aortic leakiness in vivo rabbit model.

Enhanced vasorelaxation by Omapatrilat with ANP.

Omapatrilat protects the endothelium in atherosclerosis via ANP/cGMP system.

Fig. 1. Effects of ANP on endothelial cell permeability to I^{125} -albumin in human aortic endothelial cells

Endothelial cell monolayers were treated with vehicle, thrombin (1 U/ml) alone or together with ANP (10^{-6} M). N = 6 in each group. * $p < 0.05$ vs. control, † $p < 0.05$ vs. thrombin.

Fig. 2. Effect of OMA on urine volume, urinary cGMP excretion and urinary ANP excretion in hypercholesterol rabbits treated with or without OMA

Urine was collected after OMA or placebo administration for 24 hours on day 1. * $p < 0.05$ vs placebo group.

Fig. 3. Percent of atherosclerotic plaque area determined by Oil red O staining in aorta from hypercholesterol rabbits treated with or without OMA
Red area represents positive staining as atherosclerotic plaques. * $p < 0.05$ vs placebo group.

Fig. 4. Percent of aortic vascular leakage area determined by Evans blue staining in aorta from hypercholesterol rabbits treated with or without OMA
Red arrow indicates areas of positive Evans blue staining (shown white) represents enhanced EC permeability. * $p < 0.05$ vs placebo group.

Fig. 5. Relaxation curves to ANP treatment on pre-constricted aortic rings from OMA (gray line) or placebo group (black line) with and without endothelium
EC₅₀; effective concentration at 50% relaxation. *p<0.05 vs placebo.

Table 1

Hemodynamic data and serum lipids after 8 weeks treatment

	Placebo (n=13)	OMA (n=13)	<i>p</i>
MAP (mmHg)	85 ± 4	72 ± 4	<0.05
Total cholesterol (mg/dl)	2047 ± 267	2177 ± 97	ns
Total triglycerides (mg/dl)	332 ± 91	412 ± 45	ns
HDL cholesterol (mg/dl)	23 ± 2	24 ± 3	ns
LDL cholesterol (mg/dl)	1274 ± 152	1171 ± 205	ns

NEPI/ACEI, Neutral endopeptidase inhibitor with angiotensin converting enzyme inhibitor; MAP, mean atrial pressure, HDL, high density lipoprotein; LDL, low density lipoprotein.