Original Article

Gene Delivery of Paraoxonase-1 Inhibits Neointimal Hyperplasia after Arterial Balloon-Injury in Rabbits Fed a High-Fat Diet

Miwa MIYOSHI[1)], Yukiko NAKANO[1)], Takemasa SAKAGUCHI[2)], Hiroshi OGI[1)], Noboru ODA[1)], Kazuyoshi SUENARI[1)], Katsuhiro KIYOTANI[1)], Ryoji OZONO[3)], Tetsuya OSHIMA[3)], Tetsuya YOSHIDA[2)], and Kazuaki CHAYAMA[1)]

Paraoxonase-1 (PON-1) is a high-density lipoprotein (HDL)–associated enzyme that hydrolyzes oxidized phospholipids, thereby preventing the oxidative modification of low-density lipoproteins (LDL). A high-fat diet reduces PON-1 activity, enhancing LDL oxidation. Thus, PON-1 is a candidate for anti-atherogenic gene therapy. In the present study, we investigated the effect of local PON-1 overexpression on the development of atherosclerotic lesions using the Sendai virus–mediated transgenic technique. One-month-old rabbits (*n*=11) were fed a high-fat diet for 8 weeks and then subjected to balloon injury of the common iliac artery and simultaneous infection with a Sendai virus vector containing the PON-1 gene (*n*=7) or enhanced green fluorescence protein (EGFP) gene as a control (*n*=4). The arteries were examined 7–10 days after the operation. Local overexpression of PON-1 almost completely eliminated the immunohistochemical signals of the lectin-like oxidized LDL receptor-1 (LOX-1), thereby inhibiting macrophage accumulation, intimal thickening (by 63% compared with control), or atherosclerotic plaque formation in the vascular lumen (by 87.5%). Decreased levels of oxidative stress in the PON-1–treated arteries were confirmed by 4-hydroxy-2-nonenal (HNE) staining. Local overexpression of PON-1 in the arteries attenuated oxidative stress, thereby inhibiting the atherosclerotic process. Delivery of the PON-1 gene may be a possible therapeutic strategy for preventing atherosclerosis. (*Hypertens Res* 2007; 30: 85–91)

***Key Words*: paraoxonase-1, atherosclerosis, gene therapy, lectin-like oxidized low-density lipoprotein receptor-1, oxidative stress**

Introduction

Paraoxonase-1 (PON-1) is a calcium-dependent esterase consisting of 354 amino acids with a molecular mass of approximately 45 kD (*1*, *2*). This enzyme is synthesized in the liver and secreted into the bloodstream. In the vessel lumen, PON-1 hydrolyzes specific oxidized cholesteryl esters and phospholipids in oxidized lipoproteins, thereby inhibiting the lipid peroxidation products from binding to the low-density lipoproteins (LDLs). It is noteworthy that PON-1 transgenic mice show a lower risk for atherosclerosis (*3*). In contrast, apolipoprotein E (apoE) and PON-1 double knockout mice face a higher risk for atherosclerosis than apoE single knockout mice (*4–6*). The PON-1–expressing vessel walls decrease the extracellular oxidative stress level of superoxide anions, as revealed by measuring the lipid peroxidase, glutathione, and LDL oxidation levels (*7*). These observations suggest that cellular and humoral increases in the PON-1 gene are beneficial for inhibiting the development of atherosclerosis.

From the [1)]Department of Medicine and Molecular Science, [2)]Department of Virology, and [3)]Department of Clinical Laboratory Medical Science, Graduate School of Biomedical Sciences, Hiroshima University, Hiroshima, Japan.

Address for Reprints: Miwa Miyoshi, M.D., Department of Medicine and Molecular Science, Graduate School of Biomedical Sciences, Hiroshima University, 1–2–3 Kasumi, Minami-ku, Hiroshima 734–8551, Japan. E-mail: miwa0081jp@yahoo.co.jp

Received June 29, 2006; Accepted in revised form September 12, 2006.

Serum PON-1 activity was reportedly reduced in apoE-deficient mice (*6*), mice fed a high-fat diet (*8*), and patients with diabetes (*9*), Alzheimer disease (*10*), or renal failure (*11*). Human serum PON-1 activity was shown to be inversely related to the risk of cardiovascular disease (*12*). PON-1 activity was reportedly increased by fenofibric acid and decreased by several statins (*13*), suggesting that the effects of those anti-hyperlipidemic agents could be mediated in part by the activation of the PON-1 gene.

These observations support the hypothesis that the enhancement of PON-1 activity may be beneficial for preventing atherosclerotic disease in humans. One strategy to increase PON-1 activity is to transduce the PON-1 gene into Kupffer cells in the liver (*14*). However, PON-1 activation may be more effective if delivered into the local atherosclerotic lesion. Since no adverse effects of the PON-1 gene have been reported, it may be a candidate for an anti-restenosis agent delivered into the coronary arteries using drug-eluting stents. Local delivery of the PON-1 gene is more applicable than intravenous administration or transgenic delivery to liver cells.

In this study, we investigated the effect of local delivery of the PON-1 gene using a Sendai virus (SeV) vector in a balloon injury model utilizing rabbits fed a high-fat diet.

Methods

SeV Vector Expressing PON-1 and Enhanced Green Fluorescent Protein

The SeV vector expressing the PON-1 gene (PON/SeV) was constructed as in the previous report (*15*). The N, P, M, F_0, HN, and L genes were lined up in the genome and the cDNA was inserted between the N and P genes. Briefly, the cDNA clone of the rat PON-1 cDNA (DDBJ/EMBL/GenBank Acc. No. BC012706) was obtained from Open Biosystems (Huntsville, USA), further amplified by polymerase chain reaction (PCR) using the primers 5′-TTGCGGCCGCAGTAAGAAAAACTTAGGGTGAAAGTTCACTTCACAATGGCGAAGCTGCTA-3′ and 5′-TTAGCGGCCGCTATTAAGCGTAGTCTGGGACGTCGTATGGGTACAGATCACAGTAAA-3′, and subcloned into the *Not*I site of pSeV18c(+). By this procedure, a hemagglutinin (HA) tag was attached at the C terminus of the PON-1 protein. An SeV vector expressing an enhanced green fluorescent protein (EGFP/SeV) was constructed in a similar manner.

PON-1 protein expression in cultured cells was confirmed by PON/SeV infection to LLC-MK_2 cells and subsequent metabolic labeling with [^{35}S]cysteine and [^{35}S]methionine, followed by immunoprecipitation and sodium dodecyl sulfate (SDS)–polyacrylamide gel electrophoresis (PAGE).

Animal Surgery

Eleven male white New Zealand rabbits aged 1 month, obtained from Kitayama Labes (Nagano, Japan), were all fed a 2% cholesterol high-fat diet for 8 weeks. The animals were then divided into two groups: a PON-1–treated group ($n=7$) and an EGFP control group ($n=4$).

The rabbits were anesthetized with intramuscular doses of ketamine (10 mg/kg) and xyladine (5 mg/kg). The right femoral artery was exposed, and a 4F sheath (Medikit, Tokyo, Japan) was placed in the right femoral artery. Subsequently, a sheath-guided 2F Fogarty balloon was advanced to the common iliac artery, whose intima was then injured by moving the balloon a couple of times. After creating the injury, the proximal site of the right common iliac artery was occluded with the balloon and 100 MOI (multiplicity of infection) of the PON/SeV or EGFP/SeV was administered into the lumen and kept for 5 min. Then the occlusion was removed and the bloodstream was restarted. After 7–10 days, the animals were sacrificed and the femoral (iliac) arteries were subjected to the analyses.

All of the animals received humane care in compliance with the animal care and use guidelines of the Institutional Animal Care and Use Committee of Hiroshima University. This investigation conformed to the principles outlined in the Declaration of Helsinki.

Total Cholesterol, High-Density Lipoprotein, LDL, Very Low-Density Lipoprotein and Triglyceride in the Rabbit Plasma

Blood samples were obtained from the rabbits through a 4F sheath placed in the femoral artery. The plasma samples were prepared by centrifugation at 3,000 rpm for 7 min. Total cholesterol (TCH), LDL, and triglyceride (TG) were measured with an automatic assay system using the cholesterol oxidase-peroxide method and glycerol kinase-glycerol-3-phosphate oxidase-peroxidase method, respectively. High-density lipoprotein (HDL) cholesterol and very low-density lipoprotein (VLDL) were measured using an enzymatic method.

Staining of Tissue Samples

The tissue samples were fixed in a 4% paraformaldehyde solution, dehydrated with ethanol, and embedded in paraffin. Cross sections of the artery were cut to a thickness of 4 μm and stained according to the standard protocols for the hematoxylin and eosin, van Gieson and oil red O–staining techniques. The specimens were examined using an Eclipse TE 200-U microscope (Nikon, Tokyo, Japan).

The intima-media thickness was measured at three points (the thickest, thinnest, and intermediate parts) of the vessel cross section by using Scion image 1.63 software. The intima/media (I/M) ratio of a section was the average of the three measurements. The oil red O–positive area was also measured using the software, and the ratio of the positive area to the negative area was calculated.

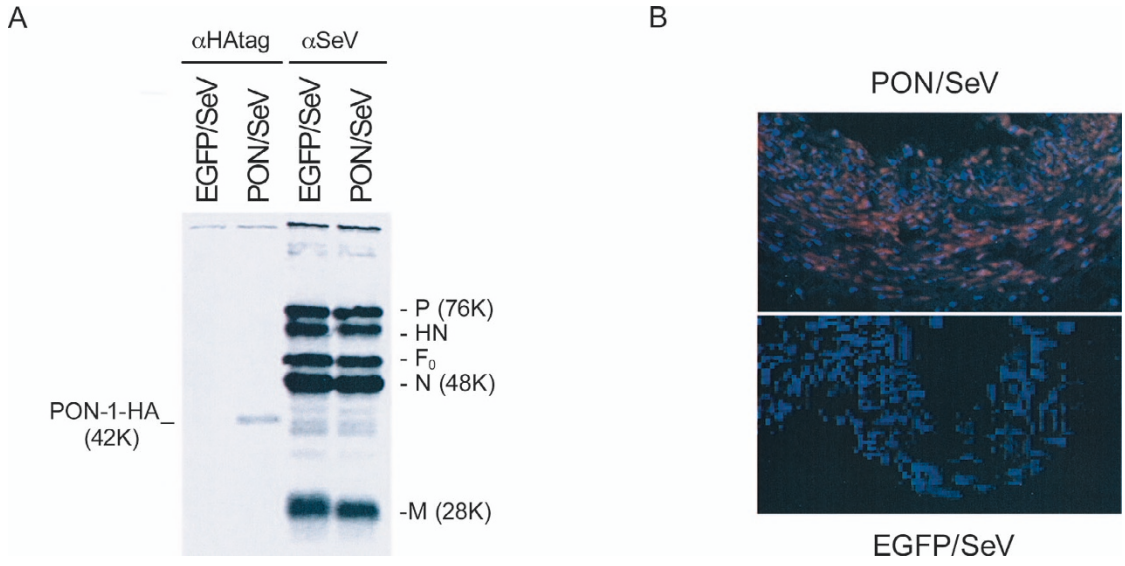

Fig. 1. *A: PON-1 expression using an SeV vector in cultured LLC-MK$_2$ cells. LLC-MK$_2$ cells were infected with PON/SeV or EGFP/SeV at an MOI of 10 and were metabolically labeled with [^{35}S]cysteine and [^{35}S]methionine for 30 min. The proteins were then immunoprecipitated with an anti–HA tag antibody or anti-SeV antibody and analyzed by SDS-PAGE and autoradiography. HA-tagged PON-1 protein expressed by PON/SeV, SeV structural proteins, P, HN, F$_0$, N, and M, are shown in the figure with their approximate molecular weights (Da). B: Immunofluorescence staining of HA-tagged PON-1 protein in rabbit arteries. PON/SeV or EGFP/SeV was administered into the lumen of the rabbit femoral arteries. After 1 week, cross sections of the arteries were stained with an anti–HA tag antibody (Alexa Fluor 568; red). The cell nuclei were stained with Hoechst 33258 (blue). Upper: a PON/SeV-infected artery. Lower: an EGFP/SeV-infected artery.*

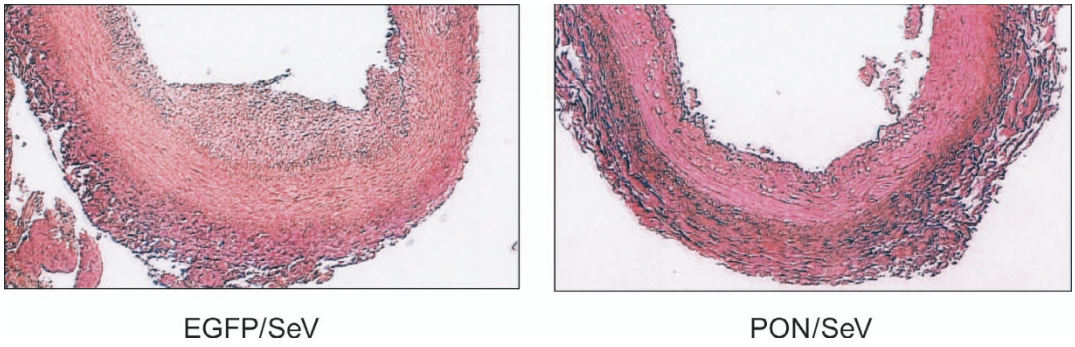

Fig. 2. *Van Gieson stain of the common iliac artery 1 week after injury and infection with EGFP/SeV or PON/SeV.*

Western Blot Analysis

The frozen artery samples were homogenized in a lysis buffer (20 mmol/l Tris-HCl, pH 7.4, 1% Triton X-100, 1% deoxycholate, 0.1% SDS, 10% glycerol, 50 mmol/l NaF, 1 mmol/l dithiothreitol [DTT], 1 mmol/l vanadate, 10 μg/ml leupeptin) with Zilconia beads at 4°C at 30 Hz for 8 min using an MM 300 homogenizer (Qiagen, Hilden, Germany) and centrifuged at 14,000 rpm at 4°C for 10 min. The protein concentrations of the supernatants were determined using a DC protein assay (Bio-Rad Laboratories, Richmond, USA). The samples were separated by SDS-PAGE and transferred onto a nylon membrane. After overnight incubation in blocking solution 0.5% skim milk in phosphate-buffered saline (PBS), the membrane was incubated with an anti–MMP-3 antibody (Cosmo Bio, Tokyo, Japan) for 1 h at room temperature. The blots were washed in PBS containing 0.03% Tween 20 and further incubated with a peroxidase-conjugated secondary antibody for 1 h. The membranes were washed and processed for chemiluminescence detection by using the ECL Plus kit (GE Healthcare Life Science, Piscataway, USA).

Immunofluorescence Staining

The iliac arteries of the treated animals were embedded in an OCT compound (Sakura Finetek USA, Torrance, USA) and frozen in liquid nitrogen. Serial sections of the arteries were cut into 4-μm slices using a cryostat, washed with PBS, fixed with 4% paraformaldehyde for 15 min at room temperature, and permeabilized with 0.5% Triton X-100 in PBS for 5 min. After blocking with 1% bovine serum albumin in PBS, the sections were incubated with primary antibodies and then were subsequently incubated with Alexa Fluor 488–conjugated anti–rabbit IgG antibody or Alexa Fluor 568–conjugated mouse IgG antibody. The specimens were examined using a DM 5000B fluorescent microscope (Leica Microsystems, Wetzlar, Germany).

The primary antibodies used were as follows: anti–HA tag mouse monoclonal antibody for the detection of the HA-tagged PON-1 protein (Cell Signaling Technology, Danvers, USA), anti-RAM11 (macrophage marker) antibody (Dako Japan, Kyoto, Japan), anti–oxidized LDL receptor (LOX-1) antibody (Santa Cruz Biotechnology, Santa Cruz, USA), and anti–4-hydroxy-2-nonenal (HNE) antibody. The cell nuclei were stained with Hoechst 33258 (Hoechst, Frankfurt, Germany).

Statistical Analysis

The Mann-Whitney test was used to compare the differences between the two groups. Differences with a p value less than 0.05 were considered significant.

Results

PON-1 Expression by SeV Vector

Immunoprecipitation with an anti–HA tag antibody revealed that a 42 kDa protein was synthesized in the PON/SeV-infected LLC-MK_2 cells (Fig. 1A). This size was compatible with that of the HA-tagged PON-1 protein. In contrast, viral structural proteins were synthesized both in the PON/SeV-infected and EGFP/SeV-infected cells. EGFP expression in EGFP/SeV-infected cells was confirmed using fluorescent microscopy and immunoblotting with an anti-EGFP antibody (data not shown). These results show the protein expression from the SeV vectors in the cultured cells.

We next confirmed PON-1 expression in rabbit tissue 2 weeks after the administration of PON/SeV or EGFP/SeV. The distribution of HA-tagged PON-1 protein was examined by immunostaining using an anti–HA tag antibody (Fig. 1B). The transduced HA-tagged PON-1 was distributed in the native and regenerated endothelium as well as in the vascular smooth muscle cells of the intima, neointima, and medial layer, but not in the adventitia. The variations in the PON-1–expressing region and expression levels among the animals were very small. The control EGFP was similarly distributed in the cross section (data not shown).

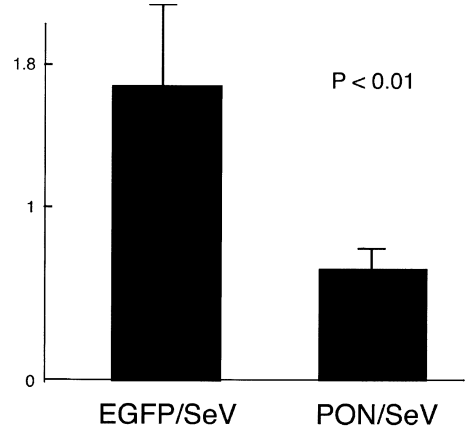

Fig. 3. *Intima/media (I/M) ratio of the arteries 1 week after injury and infection with EGFP/SeV or PON/SeV. The graph indicates the mean I/M ratios from 6 independent sections. The error bars indicate standard deviation. The* p *value was less than 0.01 with the Mann-Whitney test.*

In the SeV-mediated gene delivery system, transduced gene expression persisted for at least 2 weeks after infection. Thus, we chose to examine the effects of PON-1 administration on atherosclerosis 1–2 weeks after injury/gene delivery.

Plasma Lipids and Lipoproteins

At 3 months of age, 2 weeks after the administration of PON/SeV and EGFP/SeV, plasma was obtained from the local catheter and the levels of plasma lipids and lipoproteins were investigated. The TCH, TG, LDL, VLDL, and HDL levels were all similar between the two groups (data not shown).

Suppression of Neointima Formation by PON/SeV

The iliac arteries of the rabbits fed a high-fat diet were injured with a balloon, and then either PON/SeV or EGFP/SeV was administered. After 1–2 weeks, cross-section samples of the arteries were prepared. Van Gieson staining demonstrated intimal thickening and neointimal formation in the EGFP group arteries but not in those of the PON-1 group (Fig. 2). The normal architecture of the arteries, including the endothelial layer and elastic band, was destroyed in the EGFP group but was generally well preserved in the PON-1 group. The intimal thickening in the PON-1 group was milder compared to that in the EGFP group, suggesting that the gene delivery of the PON-1 gene inhibits neointima formation and atherosclerosis.

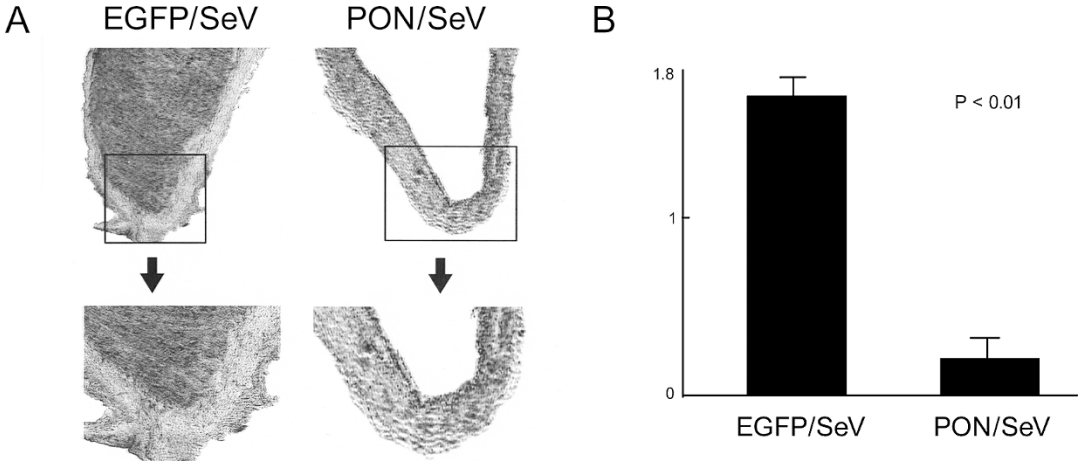

Fig. 4. *Oil red O–staining of the common iliac artery 1 week after injury and infection with EGFP/SeV or PON/SeV. A: Cross section of the artery. B: Ratio of the oil red O–stained area to the unstained area was calculated, and the average ratio from 6 independent sections is shown in the graph with an error bar corresponding to the standard deviation. The* p *value was less than 0.01 with the Mann-Whitney test.*

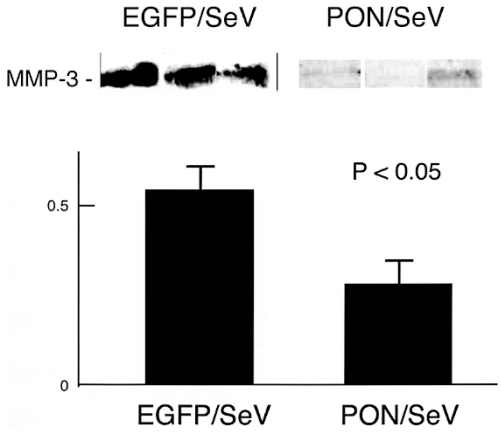

Fig. 5. *Western blot analysis of MMP-3 expression. Common iliac arteries of rabbits fed a high-fat diet were injured with a balloon and infected with PON/SeV or EGFP/SeV. After 1 week, frozen artery samples were homogenized and processed for Western blotting using an anti–MMP-3 antibody. The MMP-3 bands from 3 independent samples are shown in the figure. The intensity of each band quantified, and the average intensity from 3 independent samples is shown in the graph with an error bar corresponding to the standard deviation. The* p *value was less than 0.05 with the Mann-Whitney test.*

Inhibition of Plaque Formation by Transduced PON-1

The intima/media ratio was significantly (63.5%) lower in the PON-1 group than in the EGFP group (Fig. 3). Plaque formation, assessed by oil red O–staining, was severe in the EGFP group and attenuated in the PON-1 group (Fig. 4A). Quantification of the total area of the oil red O–stained particles revealed an 87% reduction in plaque formation in the PON-1 group compared with the EGFP group (Fig. 4B). The plaque area and neointimal area of the EGFP group were larger and the EGFP arteries had more disrupted endothelial cells and elastic bands than in the PON-1 group (data not shown).

Decrease in MMP-3 Expression by PON-1

Expression of MMP-3 (Stromelysin-1), a member of the MMP family, was investigated as a remodeling marker of the arteries. MMP-3 expression was lower in the PON-1 group than in the EGFP group (Fig. 5).

Attenuated Oxidative Stress in PON-1–Transduced Arteries

To study the mechanism underlying the anti-atherosclerotic action of PON-1 overexpression, we examined LOX-1 expression in atherosclerotic lesions. In the EGFP group, LOX-1 expression was observed in the media and smooth muscle cells (Fig. 6A). In contrast, no LOX-1 expression was observed in PON-1 group lesions, suggesting that PON-1 reduced LDL oxidation, thus preventing LOX-1 expression. Supporting the reduction in local oxidative stress, HNE staining intensity was more markedly reduced in PON-1 arteries than in EGFP arteries (Fig. 6B). HNE was diffusely distributed in the intimal and media areas in the EGFP group, whereas in the PON-1 group it was distributed only at the edges of the intimal area.

There were fewer RAM11-positive macrophages in the PON-1 lesions, indicating that macrophage infiltration was also attenuated in the PON-1–treated arteries (Fig. 6C). The distribution of RAM11-positive cells was frequently

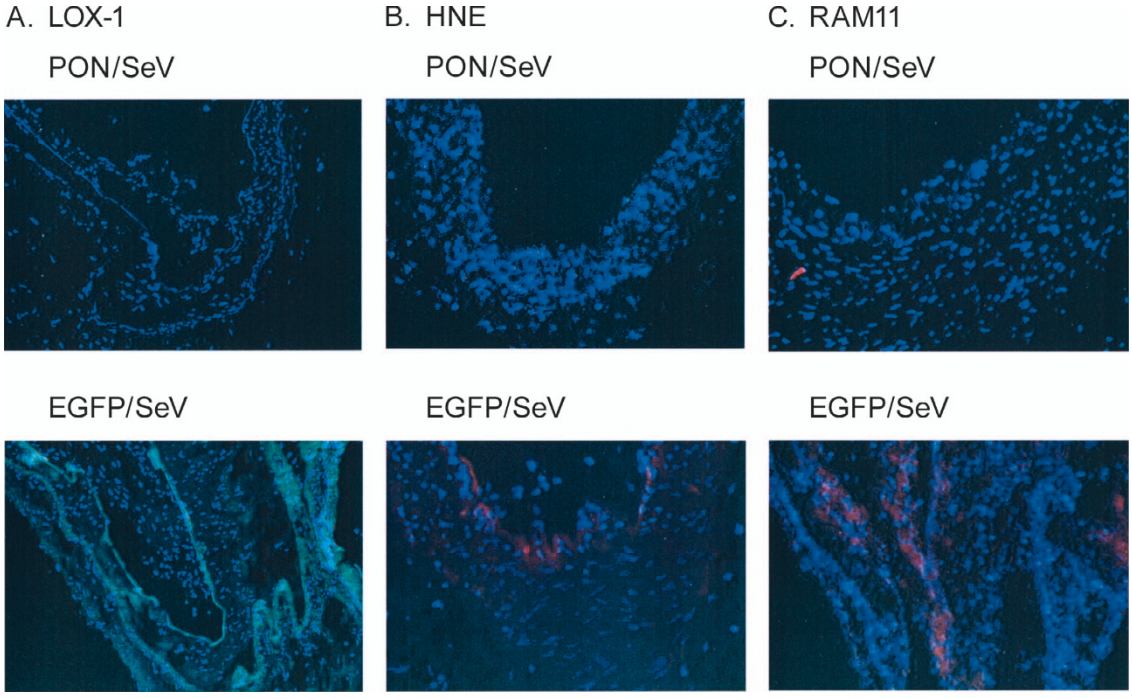

Fig. 6. *Immunofluorescence analysis of the common iliac arteries in the cross sections 1 week after injury and infection with PON/SeV or EGFP/SeV. The LOX-1 (A), HNE (B), and RAM11 (C) were stained with respective antibodies and corresponding Alexa Fluor 488 (green)– or 568 (red)–conjugated secondary antibodies. The cell nuclei were labeled with Hoechst 33258 (blue).*

observed in the areas adjacent to the oil red O–stained plaque in both groups, confirming the contribution of the macrophages to plaque formation.

Discussion

In the present study, we examined the effects of local PON-1 gene transfection in balloon-injured atherosclerotic arteries of White New Zealand rabbits. The study demonstrated, for the first time, that local PON-1 gene transfection inhibited balloon injury–induced neointimal formation in rabbits fed a high-fat diet and that prevented the progression of atherosclerosis. This effect may be due to the fact that PON-1 suppresses LDL oxidation, leading to the inhibition of the formation of lipid core of atherosclerotic lesions. Relating to this, the effect of HDLs in preventing oxidative modification of LDLs was due mainly to the presence of enzyme PON-1 in the HDLs (*5*, *16*, *17*).

Oxidized LDL (ox-LDL) activates vascular component cells, such as endothelial cells, macrophages, and smooth muscle cells, and also induces MMPs and proatherogenic genes (*18*). LOX-1 is a type II membrane glycoprotein and exists in activated vascular endothelial and smooth muscle cells as well as in macrophages. LOX-1 can be induced by ox-LDLs as well as by proinflammatory cytokines (*19*) and shear stress (*20*) in atherosclerotic lesions. LOX-1 also contributes to cell proliferation and migration through extracellular-signal–regulated kinase (ERK)1/2 activation (*21*). Thus, LOX-1 can be viewed as a mediator and biomarker of plaque formation (*22*). In the PON-1 group, LOX-1 expression was lower in the intima, media, and smooth muscle cells than in the EGFP group. These findings show that the local administration of the PON-1 gene has the advantage of preventing ox-LDL formation not only in the intima but also in the media and smooth muscle cells. *In vitro*, the ox-LDL increased MMP-3 expression in human coronary artery endothelial cells (*23*). LOX-1 upregulation activated the signaling pathways leading to the production of MMPs (*18*, *22*, *23*). MMP-3, a member of the MMP family, played a role in this remodeling and destabilized the plaque rupture in the blood vessels. In the present study, the expression of MMP-3 and remodeling of the arteries were considerably reduced in the PON-1 group.

HNE production is the most reliable marker for lipid peroxidation (*24*, *25*), and was also reduced in the PON-1 group. In light of PON-1's ability to reduce LDL oxidation *in vitro*, the anti-atherosclerotic effect of PON-1 overexpression is derived from the reduction in the ox-LDL receptors and LDL oxidation.

By using the SeV vector we could efficiently deliver the PON-1 gene to the local neointimal vessel wall. The SeV vector is a safe vector and does not pass on the infection to other animals, except for rodents. In the present study, gene transfer was localized only on the vessel wall and the virus was not

found in any other organs that we examined. Additionally, the vector had good transduction efficiency within a short time. The delivery of the PON-1 gene using the SeV vector might be a useful strategy for an anti-atherosclerotic therapy in humans.

Conclusions

This study showed that the local overexpression of the PON-1 protein inhibited neointimal hyperplasia and oxidative stress after arterial balloon injury in rabbits fed a high-fat diet. Delivery of the PON-1 gene may be a possible therapeutic strategy to prevent atherosclerosis.

References

1. James RW, Deakin SP: The importance of high density lipoproteins for paraoxonase 1 secretion, stability, and activity. *Free Radic Biol Med* 2004; **37**: 1986–1994.
2. Durrington PN, Mackness B, Mackness MI: Paraoxonase and atherosclerosis. *Arterioscler Thromb Vasc Biol* 2001; **21**: 473–480.
3. Clendenning JB, Humbert R, Green ED, Wood C, Traver D, Furlong CE: Structural organisation of the human PON1 gene. *Genomics* 1996; **35**: 586–589.
4. Shih DM, Gu L, Xia YR, Navab M, Li WF, Hama S: Mice lacking serum paraoxonase are susceptible to organophosphate toxicity and atherosclerosis. *Nature* 1998; **394**: 284–287.
5. Rozenberg O, Rosenblat M, Coleman R, Shih DM, Aviram M: Paraoxonase (PON1) deficiency is associated with increased macrophage oxidative stress: studies in PON1-knockout mice. *Free Radic Biol Med* 2003; **34**: 774–784.
6. Shih DM, Xia YR, Wang XP, Miller E: Combined serum paraoxonase knockout/apolipoprotein E knockout mice exhibit increased lipoprotein oxidation and atherosclerosis. *J Biol Chem* 2000; **275**: 17527–17535.
7. Rozenberga O, Shiha DM, Aviruma M: Paraoxonase 1 (PON1) attenuates macrophage oxidative status: studies PON1 transfected cells and in PON1 transgenic mice. *Atherosclerosis* 2005; **181**: 9–18.
8. Shih DM, Gu L, Hama S, Xia YR, Navab M: Genetic-dietary regulation of serum paraoxonase expression and its role in atherogenesis in a mouse model. *J Clin Invest* 1996; **97**: 1630–1639.
9. Ikeda Y, Suehiro T, Inoue M, Nakauchi Y, Morita T, Arii K: Serum paraoxonase activity and its relationship to diabetic complications in patients with non–insulin-dependent diabetes mellitus. *Metabolism* 1998; **47**: 598–602.
10. Paragh G, Balla P, Katona E, Seres I, Egerhazi A, Degrell I: Serum paraoxonase activity changes in patients with Alzheimer's disease and vascular dementia. *Eur Arch Psychiatry Clin Neurosci* 2002; **252**: 63–67.
11. Paragh G, Asztalos L, Seres I, *et al*: Serum paraoxonase activity changes in uremic and kidney-transplanted patients. *Nephron* 1999; **83**: 126–131.
12. Mackness MI, Durrington PN, Mackness B: The role of paraoxonase 1 activity in cardiovascular disease: potential for therapeutic intervention. *Am J Cardiovasc Drugs* 2004; **4**: 211–217.
13. Deakin S, Leviev I, Guernier S, James RW: Simvastatin modulates expression of the PON1 gene and increases serum paraoxonase: a role for sterol regulatory element–binding protein-2. *Arterioscler Thromb Vasc Biol* 2003; **23**: 2083–2089.
14. Bradshaw G, Gutierrez A, Miyake JH: Facilitated replacement of Kupffer cells expressing a paraoxonase-1 transgene is essential for ameliorating atherosclerosis in mice. *Proc Natl Acad Sci U S A* 2005; **102**: 11029–11034.
15. Sakaguchi T, Uchiyama T, Fujii Y, *et al*: Double membrane vesicles released from mammalian cells infected with Sendai virus expressing the matrix protein of vesicular stomatitis virus. *Virology* 1999; **263**: 230–243.
16. Nga CJ, Diana B, Shiha M: The paraoxonase gene family and atherosclerosis. *Free Radic Biol Med* 2005; **38**: 153–163.
17. Watson AD, Berliner JA, Hama SY, *et al*: Protective effect of high density lipoprotein associated paraoxonase: inhibition of the biological activity of minimally oxidized low density lipoprotein. *J Clin Invest* 1995; **96**: 2882–2891.
18. Kita T, Kume N, Minami M, Hayashida K: Role of oxidized LDL in atherosclerosis. *Ann NY Acad Sci* 2001; **947**: 199–205.
19. Kume N, Murase T, Masaki T, Kita T: Inducible expression of lectin-like oxidized low density lipoprotein receptor-1 in vascular endothelial cells. *Circ Res* 1998; **83**: 322–327.
20. Li D, Mehta JL: Upregulation of endothelial receptor for oxidized LDL (LOX-1) by oxidized LDL and implications in apoptosis of human coronary artery endothelial cells: evidence from use of antisense LOX-1 mRNA and chemical inhibitors. *Arterioscler Thromb Vasc Biol* 2000; **20**: 1116–11122.
21. Tanigawa H, Miura S, Matsuo Y, Fujino M: Dominant-negative lox-1 blocks homodimerization of wild-type lox-1–induced cell proliferation through extracellular signal regulated kinase 1/2 activation. *Hypertension* 2006; **48**: 294–300.
22. Vohra RS, Murphy JE, Walker JH, Ponnambalam S, Homer-Vanniasinkam S: Atherosclerosis and the lectin-like oxidized low-density lipoprotein scavenger receptor. *Trends Cardiovasc Med* 2006; **16**: 60–64.
23. Li D, Liu L, Chen H: LOX-1 mediates oxidized low-density lipoprotein–induced expression of matrix metalloproteinases in human coronary artery endothelial cells. *Circulation* 2003; **107**: 612–617.
24. Negre-Salvayre A, Vieira O, Escargueil-Blanc I: Oxidized LDL and 4-hydroxynonenal modulate tyrosine kinase receptor activity. *Mol Aspects Med* 2003; **24**: 251–261.
25. Zarkovic N: 4-Hydroxynonenal as a bioactive marker of pathophysiological processes. *Mol Aspects Med* 2003; **24**: 281–291.