Nitric Oxide Synthase Gene Therapy Rapidly Reduces Adhesion Molecule Expression and Inflammatory Cell Infiltration in Carotid Arteries of Cholesterol-Fed Rabbits

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Background—Hypercholesterolemia reduces nitric oxide bioavailability, manifested by reduced endothelium-dependent vascular relaxation, and also induces vascular adhesion molecule expression and inflammatory cell infiltration. We have previously shown that gene therapy with NO synthase in hypercholesterolemic rabbits substantially reverses the deficit in vascular relaxation. In the present study, we show that NO synthase gene therapy rapidly and substantially reduces vascular adhesion molecule expression, lipid deposition, and inflammatory cell infiltration.

Methods and Results—Thirty male New Zealand White rabbits were maintained on a 1% cholesterol diet for 11 to 13 weeks, then underwent carotid artery gene transfer with Ad.nNOS or Ad.βGal (recombinant adenoviruses expressing neuronal NO synthase or β-galactosidase, respectively), or received medium alone in a sham procedure. Arteries were harvested at 1 and 3 days after gene transfer, and the following parameters were determined by immunohistochemical and image-analysis techniques: intercellular adhesion molecule-1, vascular cell adhesion molecule-1, lipid deposition by oil red O staining, lymphocyte infiltration (CD43-positive cells), and monocyte infiltration (RAM-11-positive cells). In Ad.nNOS-treated arteries, all markers were significantly decreased relative to Ad.βGal or sham-treated arteries within 3 days after gene transfer. Ad.nNOS had a particularly striking impact on monocyte infiltration; as early as 24 hours after gene transfer, Ad.nNOS-treated arteries had 3-fold fewer monocytes than Ad.βGal- or sham-treated arteries.

Conclusions—NO synthase gene therapy rapidly ameliorates several markers of atherosclerosis in the cholesterol-fed rabbit. 

Key Words: nitric oxide • viruses • endothelium • gene therapy • atherosclerosis • cell adhesion molecules

The atherogenic process is characterized by an early deficit in NO and related biomolecules.1 Because NO inhibits many key steps in the atherogenic process (for example, platelet adhesion and aggregation;2 adhesion molecule and chemokine expression;3,4 inflammatory cell infiltration,3,5 and smooth muscle cell migration and proliferation6,7), the early NO deficit may facilitate atherosclerotic progression.

We recently developed an adenoviral vector expressing the neuronal isoform of NOS (Ad.nNOS) and showed that it expresses functional neuronal NOS (nNOS) in cultured vascular cells.8 Subsequently, we showed that in vivo gene transfer of Ad.nNOS enhanced vascular NO production by 2- to 3-fold, markedly increased the sensitivity of normal rabbit carotid arteries to acetylcholine, and substantially reversed the deficit in endothelium-dependent vascular relaxation in carotid arteries from cholesterol-fed rabbits.9 The favorable vascular effects of Ad.nNOS led us to hypothesize that it might also reduce other markers of atherosclerotic progression, such as adhesion molecule expression and inflammatory cell infiltration. Accordingly, we undertook this study to define the impact of Ad.nNOS gene transfer on these markers of atherogenesis in cholesterol-fed rabbits.

Methods

We generated Ad.nNOS and Ad.βGal, recombinant adenoviruses containing nNOS and β-galactosidase expression cassettes, respectively.9,10 All animal care and procedures were approved by the Duke University Institutional Animal Care and Use Committee and complied with NIH standards (NIH publication No. 80-23, 1985). Gene transfer to the carotid arteries was by midline cutdown, isolation of 35 mm of common carotid, and instillation of 250 μL of gene-therapy solution with 15 minutes’ dwell time.10 Segments of the vessels were used for studies of vasomotor function and NO synthase activity.9 Arterial cryosections were stained with oil red O11 or were immunostained for RAM-11, CD43, intercellular adhesion molecule-1 (ICAM-1), and vascular cell adhesion molecule-1 (VCAM-1) as described previously.10 RAM-11 and anti-rabbit CD43 antibodies were obtained commercially (Dako and Serotec, respectively), and anti-rabbit ICAM-1 and VCAM-1 monoclonal antibodies were a kind gift of Dr M. Cybulsky, University of Toronto, Toronto, Canada. Immunostaining intensity was quantified by an observer blinded to treatment group who used a computerized image-analysis system and software, as previously described.10

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2979
Results

Thirty male New Zealand White rabbits (weight, 2 to 2.5 kg each) were fed rabbit chow supplemented with 1% cholesterol for 11 to 13 weeks. Serum cholesterol levels were markedly elevated by cholesterol feeding (mean ± SE cholesterol levels: baseline, 67 ± 9 mg/dL; 4 weeks, 977 ± 40 mg/dL; 8 weeks, 1448 ± 165 mg/dL; and at gene transfer, 2137 ± 242 mg/dL). The rabbits then underwent gene transfer to both carotids with Ad.nNOS or a marker virus, Ad.βGal, or were given medium without virus in a sham procedure. Carotid arteries were harvested at 24 and 72 hours after the procedure.

In the present study, 50 arteries were examined (the remaining 10 had insufficient tissue after NO determination and vasomotor studies). These were divided into 6 groups: 24-hour harvest—Ad.nNOS (n = 6), Ad.βGal (n = 4), and sham (n = 6); 72-hour harvest—Ad.nNOS (n = 14), Ad.βGal (n = 9), and sham (n = 11).

Ad.nNOS treatment significantly reduced endothelial ICAM-1 expression at 1 and 3 days after gene transfer and VCAM-1 expression at 1 day after gene transfer relative to sham-infected and Ad.βGal-infected arteries (Figures 1 and 2). Interestingly, vessels treated with Ad.βGal showed some increase in adhesion molecule expression relative to sham-infected vessels 3 days after gene transfer. This suggests that the adenoviral vector, or possibly the β-gal transgene, induces adhesion molecule expression.

Ad.nNOS treatment significantly reduced inflammatory cell infiltration: the pan-T lymphocyte marker CD43 was significantly reduced at both 1 and 3 days after gene transfer, and the effects on monocytes (RAM-11) were even more striking (Figures 1 and 2). Relative to sham infection, Ad.βGal caused some increase in RAM-11 staining intensity at 3 days after gene transfer. RAM-11–positive cell nuclei were also counted by a blinded observer (Table). This revealed that within 24 hours of infection, Ad.nNOS-infected arteries had >3-fold fewer RAM-11–positive cells than sham- or Ad.βGal-infected arteries. There was little further reduction in RAM-11–positive cells in Ad.nNOS-infected arteries between 1 and 3 days. Finally, Ad.nNOS reduced lipid deposition at 3 days after gene transfer, as judged by oil red O staining (Figures 1 and 2).

Discussion

In addition to vasomotor deficits, arteries from hypercholesterolemic rabbits express adhesion molecules and chemo-
kines, such as monocyte chemotactic protein-1 (MCP-1), and accumulate monocytes and lipid. These processes contribute to the development of foam cell–rich vascular plaques. Available evidence from both cell-culture and whole-animal studies suggests that vascular NO deficiency may contribute to this process. For example, NO donors have been shown to inhibit hypercholesterolemia- or cytokine-induced endothelial expression of vascular selectins, adhesion molecules, and MCP-1; to inhibit adhesion, migration, and activation of inflammatory cells; and to inhibit proliferation and migration of vascular smooth muscle cells. Similarly, dietary l-arginine supplementation has been shown to improve vasomotor function and limit atherosclerotic progression in cholesterol-fed rabbits, whereas chronic NO inhibition has the opposite effect. These observations led us to hypothesize that NO synthase gene therapy would have similar favorable effects.

The impact of Ad.nNOS on monocyte infiltration develops with surprising speed. After adenoviral gene transfer, it takes ~12 hours for functional nNOS to be expressed; nevertheless, as early as 24 hours after infection, monocyte infiltration was already substantially reduced. This suggests that monocyte infiltration is highly sensitive to local vascular concentrations of NO and that monocyte turnover in atherosclerotic lesions may be more rapid than previously appreciated. It is known that NO rapidly and potently downregulates MCP-1 expression in cultured vascular cells. Moreover, it was recently shown that apolipoprotein E–null mice, ordinarily quite susceptible to atherosclerosis, are resistant to atherosclerosis if they also lack the MCP-1 receptor CCR2. These observations suggest the possibility that the antiatherogenic effect of Ad.nNOS may be mediated in part through inhibition of MCP-1 expression.

Relative to sham infection, Ad.βGal causes a modest increase in adhesion molecule expression and monocyte infiltration at 3 days. Recombinant adenovirus is known to be proinflammatory, but we chose conditions of infection that do not induce inflammation in normal vessels. Arteries from cholesterol-fed rabbits may be “primed” by chronic cholesterol exposure and are hence more susceptible to adenovirus-induced inflammation. Ad.nNOS either did not induce inflammation, or more likely, the salutary effects of NO synthase expression overcame the modest proinflammatory effect of the virus.

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