AJNR

Swine Model of Carotid Artery Atherosclerosis: Experimental Induction by Surgical Partial Ligation and Dietary Hypercholesterolemia

A. Ishii, F. Viñuela, Y. Murayama, I. Yuki, Y.L. Nien, D.T. Yeh and H.V. Vinters

AJNR Am J Neuroradiol 2006, 27 (9) 1893-1899
http://www.ajnr.org/content/27/9/1893

ORIGINAL RESEARCH

Swine Model of Carotid Artery Atherosclerosis: Experimental Induction by Surgical Partial Ligation and Dietary Hypercholesterolemia

A. Ishii
F. Viñuela
Y. Murayama
I. Yuki
Y.L. Nien
D.T. Yeh
H.V. Vinters

INTRODUCTION: Limited availability of a large animal model of carotid atherosclerosis has limited preclinical evaluation of endovascular therapeutic devices. The present study is aimed at developing such animal models with a novel approach, emphasizing the role of hemodynamics.

METHODS: Using 18 carotid arteries from 9 miniswine, surgical partial ligation (approximately 80% stenosis) was carried out in untreated (group I; $n = 6$) and balloon-injured arteries (group II; $n = 9$). Three arteries were subjected to sham-operation for control (group III; $n = 3$). All animals were fed with a high-fat diet until sacrifice. Angiograms and histologic sections of the vessels were analyzed to evaluate both models.

RESULTS: Atherosclerotic changes were confirmed in 6 of 6 in group I and 6 of 9 arteries in group II, whereas all in group III remained intact. Three arteries in group II resulted in thrombotic occlusion. Advanced plaques with intraplaque hemorrhage and/or calcification were seen in 4 of 6 arteries in group I but none in group II. The cross-sectional area stenosis and atherosclerotic stage for plaques in group I were both significantly higher than that in groups II and III.

CONCLUSION: In this series, surgical partial ligation with concomitant dietary hyperlipidemia is an appropriate experimental technique to develop advanced atherosclerotic plaques with minimal technical complications. This model showed no evidence of such benefits when applied in balloon-injured arteries.

In the past decade, carotid artery stent placement (CAS) has emerged as a therapeutic alternative to carotid endarterectomy for patients with carotid artery stenosis.[1] Multicenter randomized clinical trials comparing CAS with endarterectomy are in progress,[2-5] and favorable initial results have been reported.[6] Nevertheless, most endovascular devices currently used for CAS were clinically introduced without having been proved beneficial in animal studies. This is largely because an appropriate large animal model of carotid artery stenosis has not been largely available.

The balloon injury approach has been a widely used experimental technique to simulate atherosclerosis-like lesions.[7] This technique has been shown to induce intimal thickening in response to mechanical intimal-to-medial injury in virtually any sites within the vascular tree in any species.[8] However, several limitations are noted in this model. Lesions usually lack advanced histologic features of "complicated atheroma," such as calcification, intraplaque hemorrhage, and necrotic cores, in contrast to lesions encountered in clinical practice.[9] Although extensive deep vascular injury with concomitant dietary hypercholesterolemia have been shown to yield more advanced features in addition to intimal thickening,[10] the high rate of acute thrombotic occlusion resulting from mechanical trauma in this model remains a major drawback in view of the high expenses encountered in working with large laboratory animals.

We took a different approach in creating a novel large animal model to overcome these limitations. Dietary hyperlipidemia[11] and experimentally induced diabetes[12] are both known to accelerate atherosclerosis in swine. Nevertheless, it has been demonstrated that carotid arteries are generally spared of lesions in both models.[11,12] Carotid arteries in swine are quite straight at the neck and do not have major branches or curvatures that disturb the laminar blood flow pattern. Numerous studies suggest that disturbed flow patterns, particularly low or oscillatory wall shear stress, increase susceptibility to atherosclerosis,[13,14] whereas vessel regions exposed to high or laminar wall shear stress remain comparatively disease-free.[15] We therefore hypothesized that the addition of hemodynamic instability to carotid arteries in hyperlipidemic swine induces advanced atherosclerotic lesions.

In this pilot study, we dynamically altered blood flow conditions in swine carotid arteries by surgical partial ligation but maintained the animals on dietary hyperlipidemia for up to 6 months. We then evaluated this model in balloon-injured arteries to determine whether the absence of intact endothelium exacerbates or attenuates evolution of atherosclerotic plaque in this model.

Methods

Animals

Eighteen common carotid arteries in 9 healthy young Yucatan minipigs (S&S Farms, Ranchita, Calif) of mixed sex with an initial weight of 20–30 kg were used in this study. Surgical partial ligation was carried out in 6 untreated (group I; $n = 6$) and 9 balloon-injured carotid arteries (group II; $n = 9$). Three arteries were subjected to sham-operation for control (group III; $n = 3$). Groups I and II were

Received September 30, 2005; accepted after revision December 28.

From the Division of Interventional Neuroradiology (A.I., F.V., Y.M., I.Y., Y.L.N.) and Departments of Pathology and Laboratory Medicine (Neuropathology) and Neurology (D.T.Y., H.V.V), UCLA Medical Center, David Geffen School of Medicine at UCLA, Los Angeles, California.

H.V.V. is supported in part by UCLA Specialized Program of Translational Research in Acute Stroke (SPOTRIAS) grant NSO44378.

Address correspondence to Akira Ishii, MD, Division of Interventional Neuroradiology, UCLA Medical Center, David Geffen School of Medicine at UCLA, 10833 Le Conte Ave, CHS, Room B7–146, Los Angeles, CA 90095-1721; e-mail: IshiiMD@gmail.com

Table 1: Subgroup allocation of experimental animals

Animal No.	Right Carotid	Left Carotid	Time of Harvest (mo)
1	Ia	IIa	3
2	Ia	IIa	3
3	IIa	Ia	3
4	Ib	III	6
5	Ib	III	6
6	III	Ib	6
7	IIa	IIa	3
8	IIb	IIb	6
9	IIb	IIb	6

further divided into 2 subgroups based on time points of sacrifice: Ia and IIa, 3 months after the surgical procedure; Ib and IIb, 6 months after the surgical procedure. Animals for group III were sacrificed at 6 months. Allocation of experimental animals is summarized in Table 1. All animals were fed with a high-fat, high-cholesterol diet (Test Diet; Purina, St. Louis, Mo) to induce hypercholesterolemia. The diet was started at least 14 days before the surgical procedure and continued for the duration of the study until the time of sacrifice. A blood sample was collected at the time of the surgical procedure after an 18-hour fast to document hyperlipidemia. After the surgical procedure, all animals were placed on aspirin, 81 mg daily, for a month to minimize the risk of thrombotic occlusion that surgical or endovascular procedures could possibly cause. Aspirin has been shown to inhibit platelet aggregation in swine.[16] A low dose (81 mg) of aspirin was chosen because aspirin administration of 100 mg daily was shown to completely inhibit platelet aggregation in swine weighing 55–97 kg.[16] Given that animals used in the present study weighed 20–30 kg, 81 mg of aspirin was considered sufficient. All animal experiments were conducted in accordance with policies set by the institutional Chancellor's Animal Research Committee and National Institutes of Health guidelines.

Group I (Subgroup Ia and Ib): Surgical Partial Ligation in Untreated Arteries

Anesthesia was induced with intramuscular tiletamine and zolazepam (Telazol) followed by orotracheal intubation. One percent to 2% isoflurane was given to maintain general anesthesia for the duration of the procedure. A 6F introducer sheath was inserted into the right or left femoral artery for the purpose of an angiogram at the end of the surgery. A midline skin incision was placed at the neck after sterilization of the surgical field. The common carotid arteries were dissected approximately 5 cm in length. A common carotid artery was tied off with 5–0 Prolene (Ethicon, Cornelia, Ga) along with a spacer (approximately 1.3 mm in diameter) placed on the external surface of the artery (Fig 1*A*). This was subsequently pulled out, leaving a tight stenosis (Fig 1*B*). Postoperative angiography was then carried out to document the degree of surgical stenosis. The surgical wound was closed layer by layer, and the groin site was manually compressed for complete hemostasis.

Postoperatively, the animals were maintained under dietary hypercholesterolemia for either 3 (subgroup Ia) or 6 months (subgroup Ib) before being sacrificed in the fashion to be described.

Group II (Subgroup IIa and IIb): Surgical Partial Ligation in Balloon-Injured Arteries

Under general anesthesia, the carotid artery was dissected and prepared for ligation after the balloon injury procedure. A 6F introducer sheath was inserted into the right or left femoral artery, followed by an intraarterial bolus injection of 100 IU/kg heparin. A 6F guiding catheter was advanced into the common carotid artery. A 6-mm angioplasty balloon was positioned into the common carotid artery over a 0.018-inch guidewire and inflated to a pressure of 10 atm. The inflated balloon catheter was moved approximately 5 cm up and down the carotid artery for 1 minute. This manipulation was repeated 3 times in the same fashion. The surgical partial ligature was then placed in the same fashion as in group I in the center of the balloon-injured site under fluoroscopic guidance. Animals were fed with a high-fat diet and sacrificed at 3 (subgroup IIa) or 6 months (subgroup IIb).

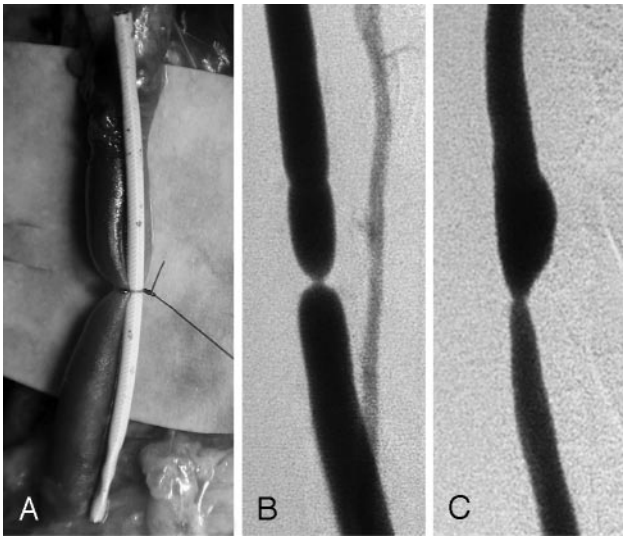

Fig 1. *A,* Intraoperative photograph showing a ligated carotid artery with a spacer on the external surface of the artery. This spacer was subsequently removed, leaving a tight stenosis, but not occlusion.

B, Angiogram showing a partially ligated artery with approximately 80% stenosis immediately after the procedure.

C, Angiogram obtained 3 months after the procedure. Note luminal narrowing proximal and extensive dilation distal to the surgically created stenosis.

Group III: Sham Operation (Control)

Under general anesthesia, the carotid artery was dissected through a midline skin incision at the neck. The surgical wound was closed layer by layer without unnecessary local manipulations. Animals were sacrificed after 6-month dietary maintenance.

Quantitative Analysis of Carotid Angiograms

All animals underwent selective carotid angiography immediately after surgery and immediately before sacrifice to document the degree of surgical stenosis and poststenotic dilation. The degree of surgical stenosis was calculated as $(1 - \text{MLD/RLD}) \times 100$, where MLD and RLD represent minimum lumen diameter and distal referenced lumen diameter, respectively. The degree of poststenotic dilation was measured as $(\text{PLD/RLD} - 1) \times 100$, where PLD stands for poststenotic dilated lumen diameter. All parameters were measured by 2 investigators with the use of a workstation equipped with an angiography machine.

Qualitative and Quantitative Tissue Analysis

All animals were euthanized with pentobarbital infusion at either 3 months (subgroup Ia and IIa) or 6 months (subgroup Ib and IIb, and group III) after the surgical procedure. Carotid arteries were perfused with 1% paraformaldehyde at physiologic diastolic pressure to fix the

arteries in situ for morphometric analysis. Standard paraffin-sectioning techniques, and staining with hematoxylin-eosin, and Van Gieson's elastic (EVG) were performed to determine the presence of atherosclerotic changes and to quantify the degree or severity of neointimal proliferation. All slides were photographed with a scale bar to allow computer-based morphometric analysis. Measurements were carried out on cross-sections including the most narrowed portion of the lumen. The cross-sectional area stenosis (%) was calculated as 1 − (actual lumen area/potential lumen area) × 100, in which potential lumen area was defined as the area within the internal elastic membrane. Immunohistochemical studies using primary antibodies to smooth muscle actin (SMA) were also used to characterize plaques. They were classified from type I (initial) to VI (complicated) using criteria set forth by the American Heart Association Committee on Vascular Lesions.[17] In brief, type I represents lesions with smooth muscle cell dominant intimal proliferation; type II, intimal proliferation with foamy macrophages; type III, small pools of extracellular lipid; type IV, core of extracellular lipid; type V, type IV plus fibrous thickening; and type VI, intraplaque hemorrhage. The plaque stage score was assigned based on these criteria as follows: intact, 0; type I, 1; type II, 2; etc; that is, a higher score represents a more advanced stage ranging from 0 to 6.

Statistical Analysis

All data are presented as mean value ± SD. Statistical analysis was carried out using SPSS 13 (SPSS, Chicago, Ill) and StatExact 4.0 (Cytel, Cambridge, Mass). Intersubgroup (subgroup Ia, Ib, etc) differences of all variables except for the plaque stage score were examined with 1-way analysis of variance and Tukey test. The plaque stage score was analyzed with Mann-Whitney test because of its discreteness. Two-way analysis of variance test was used for intergroup (groups I, II, and III) differences because each group contains 2 different time points, which may affect variables. When interactions between each variable and time points were ruled out, multiple comparison was carried out with the Tukey test. The Fisher-Freeman-Halton test was used for analysis of homogeneity of proportions, such as the rate of atherosclerotic change, total occlusion, and advanced atherosclerotic plaque. A P value <0.05 was considered statistically significant for all tests; otherwise, it was described as not significant (NS).

Results

All animal surgery was performed successfully. Various degrees of atherosclerotic plaque were successfully induced except 3 arteries for group II, which resulted in subacute thrombotic occlusion. Advanced plaques with intraplaque hemorrhage and/or calcification were commonly seen in group I; however, the most characteristic feature of lesions induced in group II was intimal thickening (Fig 2).

Plasma Lipid Levels

The mean plasma cholesterol level for each group and subgroup is shown in Table 2. No significant intersubgroup or intergroup differences were found. The mean value from all animals was 862 ± 76 mg/dL. These figures are approximately 10 times higher than those observed in swine fed with a regular diet.[18]

Angiograms

The mean degree of surgical stenosis at surgery for each group and subgroup is shown in Table 2. There was no intersub-

Table 2: Mean values of parameters for each group and subgroup

Group (Subgroup)	No. of Subjects	Time of Harvest (mo)	Partial Ligation	Balloon Injury	High-Fat Diet	Total Cholesterol (mg/dL)	Angiographic Stenosis at Surgery (%)	Poststenotic Dilatation (%)	Total Occlusion	Atherosclerotic Changes	Advanced Lesions	Plaque Stage	Cross-sectional Area Stenosis (%)
I (Ia + Ib)	6	3 or 6	Yes	No	Yes	944 ± 119	79 ± 9*	41 ± 15*	0/6	6/6*	4/6*	4.8 ± 1.6*	53.4 ± 34.8*
Ia	3	3	Yes	No	Yes	821 ± 200	83 ± 20*	48 ± 12*	0/3	3/3*	3/3*	5.7 ± 0.6*	65.8 ± 31.3
Ib	3	6	Yes	No	Yes	1067 ± 125	75 ± 6*	35 ± 16*	0/3	3/3	1/3*	4.0 ± 2.0*	41.1 ± 40.0
II (IIa + IIb)	9	3 or 6	Yes	Yes	Yes	740 ± 107	77 ± 4*	44 ± 10*	3/9	6/9	0/0	2.7 ± 1.0*	25.4 ± 16.5
IIa	5	3	Yes	Yes	Yes	730 ± 90	76 ± 5*	42 ± 12*	1/5	4/5*	0/0	2.5 ± 1.0	23.0 ± 19.0
IIb	4	6	Yes	Yes	Yes	752 ± 235	79 ± 3*	49 ± 1*	2/4	2/4*	0/0	3.0 ± 1.4	30.2 ± 14.4
III	3	6	No	No	Yes	1067 ± 200	0 ± 0	0 ± 0	0/0	0/3	0/0	0.0 ± 0.0	0.0 ± 0.0

* Indicates statistically significant difference compared with group III (control).

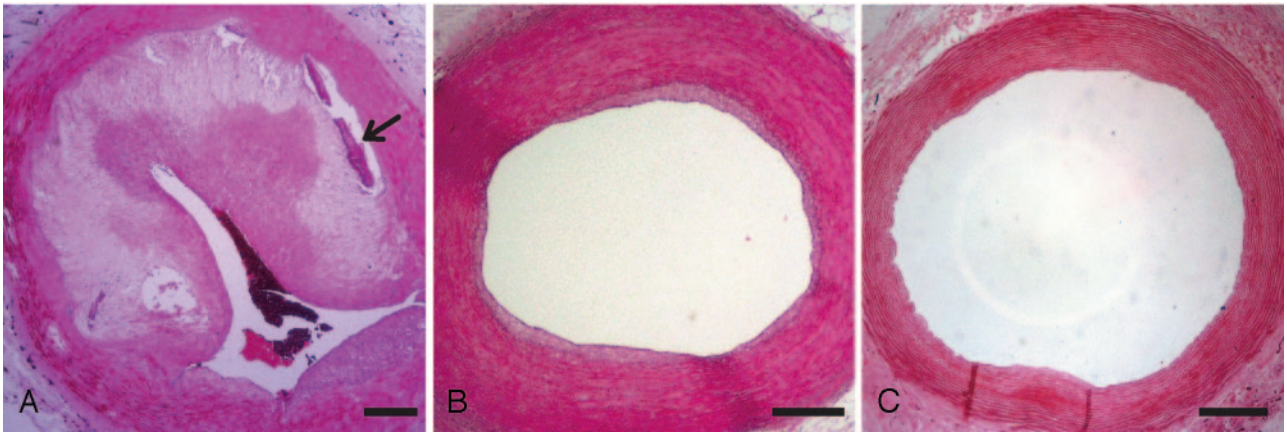

Fig 2. Representative photographs of hematoxylin-eosin-stained cross-sections for carotid arteries from each group.

A, Group I (Ib; 6 months). Eccentric advanced atherosclerotic plaque located proximal to the surgical stenosis. *Arrow* indicates calcium deposits at the bottom of the plaque. Note that the entire plaque is covered by a fibromuscular cap, suggesting the similarity to human lesions.

B, Group II (IIb; 6 months). Concentric intimal thickening. Note that in contrast with *A*, no advanced features of atherosclerosis are seen.

C, Group III (6 months). No atherosclerotic change is observed. *Scale bar*, 500 μm.

group significant difference except for group III. Final angiograms performed at the time of sacrifice showed that 3 of 9 (30%) arteries in group II (1 of 5 in subgroup IIa and 2 of 4 in subgroup IIb) were occluded, whereas all arteries in group I remained patent. The rate of total occlusion for group II was higher than that for group I, but not significantly so. All arteries in groups I and II demonstrated various degrees of poststenotic dilation (Fig 1*C*). The mean degree of poststenotic dilation of treated arteries is shown in Table 2, where 3 occluded arteries in group II were excluded because poststenotic dilation was not documented as a result of subacute vessel occlusion. No significant differences were found between the mean degree of poststenotic dilation of group I and II at either 3 months (subgroup Ia versus IIa) or 6 months (subgroup Ib versus IIb).

Qualitative Tissue Analysis

Cross-sections stained with hematoxylin-eosin demonstrated various degrees of atherosclerosis in 6 of 6 (100%) and 6 of 9 (67%) arteries for group I and II, respectively. They were more prominent proximal to the surgical stenosis. Three control arteries in group III remained intact although the animals received a high-fat diet for 6 months. Three of 9 arteries (33%) of group II were occluded by organized thrombus, most probably related to the balloon injury to the arterial intima.

All atherosclerotic lesions in group I and II contained intimal fibromuscular proliferation with large collections of foamy macrophages. Histologic features of more advanced atherosclerosis, such as foci of necrosis, calcium deposition, and/or intraplaque hemorrhage were observed in 4 of 6 (66.7%) arteries in group I (3 of 3 in subgroup Ia and 1 of 3 in subgroup Ib). None of specimens from group II (subgroups IIa and IIb) demonstrated these features.

The histologic features observed in group I were organized in layers, closely resembling human atherosclerotic plaque (Fig 3). Foamy macrophages were within the bottom of plaque, often with an overlying fibromuscular cap (Fig 3*A*, -*B*) and foci of necrosis in their central portions (Fig 3*C*). SMA staining showed immunoreactive smooth muscle cells and myofibroblasts immediately beneath the luminal aspect of the plaque (Fig 3*B*). Calcium deposition, most often found deep within plaques, was also clearly visualized (Fig 3*D*). EVG staining exhibited abundant collagen fibers and partially destroyed internal elastic membranes (Fig 3*E*). In 3 of 6 (50.0%) arteries in group I (2 of 3 in subgroup Ia and 1 of 3 in subgroup Ib), even the occurrence of intraplaque hemorrhage corresponding to the AHA criteria type 6 (ie, the most advanced [and often symptomatic] stage of atherosclerosis) was found (Fig 3*A*). Such lesions were not found in any specimens from group II. Lesions were eccentric in all (100%) arteries in group I, whereas only 2 of 9 arteries (22%) in group II showed such eccentricity. All these observations suggest that histologic features of atherosclerotic plaques in group I are quite comparable with advanced atherosclerotic lesions encountered in clinical practice.

The mean plaque stage scores for each group and subgroup are shown in Table 2 and Fig 4. Intersubgroup differences were significant between Ia versus IIa and Ia versus III. In summary, surgical partial ligation without balloon injury developed significantly more advanced plaques than that with superimposed balloon injury at 3 months.

Quantitative Tissue Analysis

The mean degree of cross-sectional area stenosis (%) is shown in Table 2 and Fig 4. Although the area stenosis for subgroups Ia and Ib seems to be higher than for IIa and IIb, respectively, no statistically significant difference was found. Although the plaque stage score for subgroup Ia was higher than that for subgroup Ib, the difference was not statistically significant. When subgroups (Ia and Ib, IIa, and IIb) are considered together, interaction between time points and subgroups was ruled out, and the mean degree of area stenosis for group I was significantly higher than that for group III. There was no statistically significant difference between groups II versus III. These observations suggest that surgical partial ligation without balloon injury developed significantly more massive plaques than in control swine whereas that with superimposed balloon injury did not.

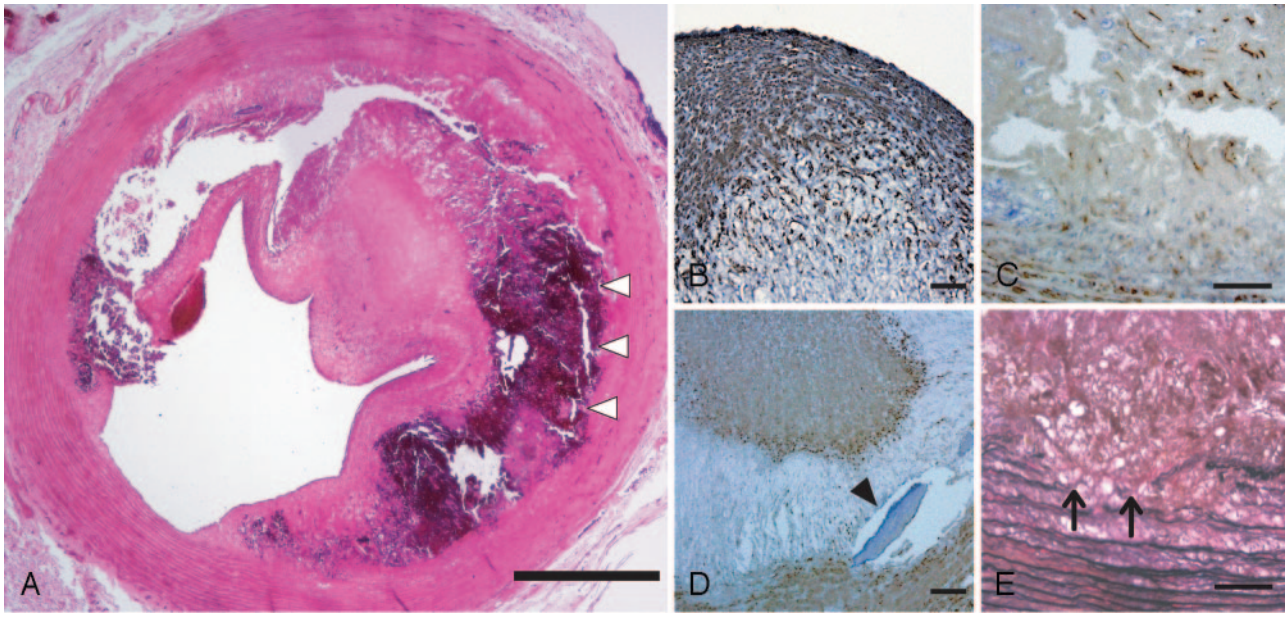

Fig 3. Histologic features of various atheromatous plaques in carotid arteries in group I. All sections are from 1 animal sacrificed at 6 months after the surgical procedure.

A, Low magnification micrograph (H-E-stained section) shows a large eccentric plaque in the portion proximal to the surgical stenosis featuring a fibromuscular cap, intra- and extracellular lipid deposition, and massive intraplaque hemorrhage (*white arrowheads*). *Scale bar*, 1 mm.

B, anti-SMA immunostaining clearly showing a fibromuscular cap covering a large collection of foamy macrophages at the bottom. Note that smooth muscle cells are scattered throughout the plaque and line its luminal surface (at the top). *Scale bar*, 100 μm.

C, Base of plaque immunostained with anti-SMA showing necrotic portion. *Scale bar*, 100 μm.

D, Anti-SMA immunostaining clearly showing calcification at the bottom of plaque (*black arrowhead*).

E, EVG-stained section of plaque shows abundant collagen deposition as well as focally defective internal elastic lamina (*arrows*). *Scale bar*, 100 μm. H-E, hematoxylin and eosin; SMA, smooth muscle actin; EVG, Van Gieson's elastic.

Discussion

Surgical Partial Ligation Model without Balloon Injury

In this series, all arteries for group I (surgical partial ligation without balloon injury) demonstrated various degrees of atherosclerosis in the portion proximal to the surgical stenosis. More importantly, 4 of 6 arteries developed advanced plaque histologically characterized by calcification and/or intraplaque hemorrhage. In contrast, all 6-month sham-operated arteries (group III) remained free of atheroma. The surgical partial ligation model without balloon injury has several advantages over currently used models.

First, no mechanical injury is necessary in this model. Maintained intact endothelium allows in vivo evaluation of endothelial function exposed to hemodynamic instability. Healthy endothelium plays a pivotal role in vascular homeostasis, not only by mediating vasomotion[19] but also by suppressing thrombosis,[20] vascular inflammation,[21] and proliferation[22] in response to blood flow dynamics. Intensive studies of the in vitro endothelial response to fluid shear stress have been performed using cultured endothelial cells plated within a flow chamber. They demonstrated that a high magnitude of wall shear stress induces a quiescent, antiproliferative, antioxidant, and antithrombotic "atheroprotective" phenotype of endothelium.[23] In disturbed flow conditions, endothelial cells monitoring flow conditions switch their gene expression profiles to "atherogenic" (ie, a thrombogenic, inflammatory, and proliferative phenotype).[23] Molecular analysis of vascular regions susceptible to plaque formation in animals have revealed that disturbed blood flow patterns prime the endothelium to respond to humoral factors, such as oxidative stress, through upregulation of the proinflammatory transcription factor nuclear factor κB.[24,25] It is thus proposed that atherosclerosis is evoked if systemic factors are superimposed onto these already-primed endothelial cells.[24,25]

Second, advanced atherosclerotic lesions can be induced with high-fat diet, with minimal complications such as thrombotic occlusion. Lesions induced in this model elicit histologic features quite comparable with those in human carotid artery: intraplaque hemorrhage, calcification, and a fibrous cap. The balloon injury model is a widely used experimental model that readily induces atherosclerosis-like lesions regardless of animal species. Recchia et al reported that high-fat diet feeding with extensive balloon injury yields advanced carotid plaques.[10] Formed lesions are comparable with those in humans except that luminal narrowing is mostly dependent on massive thrombus. Nevertheless, they also stated that 19% of carotid arteries were excluded from further studies as a result of subacute total occlusion, which remains a limitation of this method, especially in using expensive large animals. This technical complication has been reported to occur at a rate of 10%–50%,[7,10,26,27] presumably depending on the details of balloon procedures as well as anticoagulant regimens used. On the other hand, the surgical partial ligation model without balloon injury experienced no thrombotic occlusion in our series.

Third, hemodynamic force seems to play an important role as a source of atherogenesis in this model. Several lines of evidence have shown that hemodynamics play an important role in atherosclerosis. Numerous studies have shown that

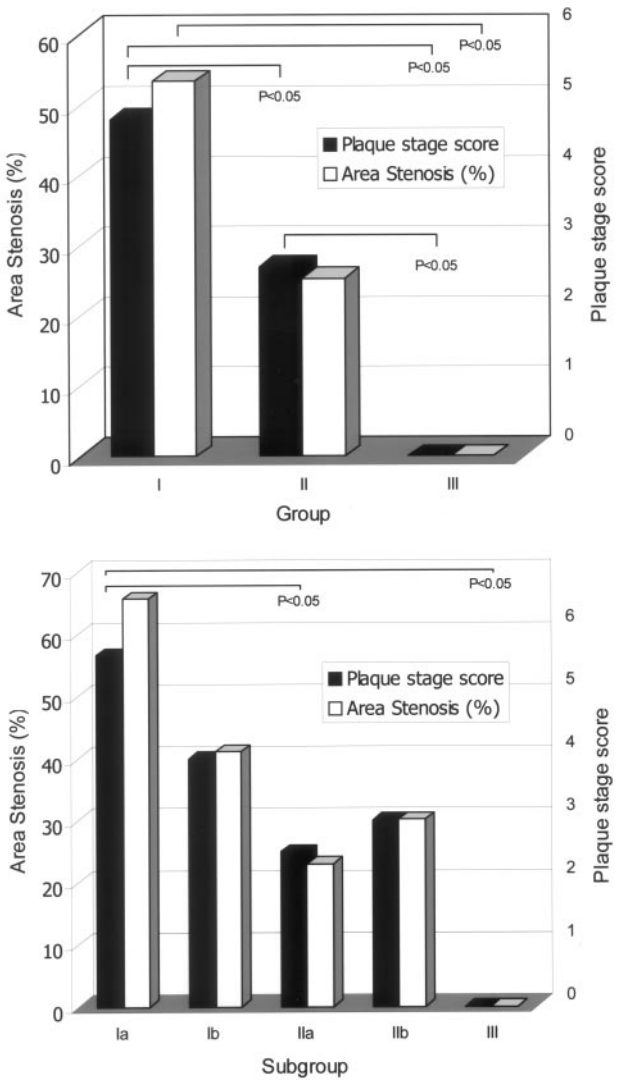

Fig 4. Bar graphs of cross-sectional area stenosis (%) and plaque stage score.

A, Area stenosis (%) and plaque stage score versus groups.

B, Area stenosis (%) and plaque stage score versus subgroups.

atherosclerosis-susceptible sites, such as the carotid bifurcation, are consistently exposed to oscillatory or low wall shear stress.[13,14] In support of these retrospective observations, Zarins et al[28] showed that the surgical creation of a tight coarctation in thoracic aorta in cynomolgus monkeys induced intimal thickening in the proximal portion of the coarctation, whereas the stenosis channel as well as the distal portion were free of lesions. Sawchuk et al[29] reported that asymmetric stenosis surgically created in thoracic aorta in hyperlipidemic swine developed intimal thickening with abundant foamy macrophages, showing the positive correlation between low wall shear stress and lesion thickness. There is no doubt that systemic risk factors for atherosclerosis, such as hypercholesterolemia, hypertension, diabetes, and smoking, also play major roles in the initiation and progression of this disease. Nevertheless, these observations (including our study) suggest that hemodynamic force is at least responsible for localization of atherosclerotic plaque within the vascular tree. Further studies focused on hemodynamics are needed in this model.

Surgical Partial Ligation with Balloon Injury

Although all arteries for group II (surgical partial ligation with balloon injury) showed atherosclerotic changes except for 3 occluded arteries, no specimens showed evidence of advanced atherosclerotic lesions observed in group I. Neointimal proliferation was the most characteristic histologic finding in group II. A high rate of total occlusion (33%) was also noted despite 1-month administration of aspirin. The absence of advanced plaque in group II should be analyzed further because both balloon injury and surgical partial ligation have been both shown to develop advanced plaque when applied individually.

Histologic composition of plaque developed by balloon injury can be affected not only by systemic conditions, such as hyperlipidemia and diabetes, but also by the details of balloon procedures. For example, regular diet feeding has been shown to yield solely intimal hyperplasia.[9] It has been demonstrated that the magnitude of intimal proliferation is affected by the degree of vessel injury caused by balloon overinflation,[30] mechanical characteristics of used balloon catheters,[31] and shear forces caused by balloon catheter withdrawal.[32] These factors may be responsible for the lack of advanced plaque in our series. In addition, intact endothelium may be essential to initiate the atherosclerotic process caused by surgical partial ligation. As described earlier, it was shown that endothelium has to be primed by disturbed flow to respond to humoral factors.[25] Further studies, including molecular approach analyses, are needed to elucidate the mechanisms underlying pathogenetic factors in this model.

In addition, a high rate of total occlusion in our series may be attributed to the anticoagulant regimen used. Although low-dose aspirin inhibits platelet aggregation in swine, higher doses of aspirin or continuous injection of heparin may be effective in preventing thrombosis. Nevertheless, more probably, reduced blood flow by surgical partial ligation may have contributed to high thrombogenicity in this model, as shown in the previous study on balloon-injured, partially ligated femoral arteries in rabbits.[33]

Limitations of the Study and Proposed Model

We acknowledge several limitations of the current study and proposed animal model. First, a relatively small number of used animals and the exploratory nature of this study may decrease its reliability, though statistical significance was found between group I versus II and group I versus III for several parameters. In addition, a high rate of thrombotic occlusion in group II may have been related to the small number of animals allocated into this group as a result of budgetary limitations. The use of a larger number of animals allocated into group II may have improved the complication rate and led to the development of advanced plaque in some animals. Second, we did not test balloon injury without surgical partial ligation because of the limited number of available animals. Although abundant literature exists on this technique, direct comparison in the same animal would help to characterize the difference between plaques formed in these 2 models. Third, a tight surgical stenosis artificially created should per se be distinguished from adjacent atherosclerotic stenosis. This surgical stenosis is mechanical rather than biological, consisting of an artificial luminal narrowing to cause hemodynamic instability. Nevertheless, this can be eliminated by the use of ab-

sorbable suture. Finally, hemodynamic measurements or molecular analysis were not presented in the current report. Further studies are forthcoming with particular attention to these aspects to elucidate mechanisms governing atherogenesis in this model.

Conclusions

Surgical partial ligation with concomitant dietary hyperlipidemia is an appropriate experimental technique to develop advanced atherosclerotic plaque in untreated carotid arteries in swine with minimal technical complications. This model showed no evidence of such benefits when applied in balloon-injured arteries.

Acknowledgments

We thank all the members of the technical staff in the Leo G. Rigler Center for their invaluable assistance in performing the experiments. We also thank James W. Sayre, PhD, for his great assistance in statistical analysis.

References

1. Roubin GS, Yadav S, Iyer SS, et al. **Carotid stent-supported angioplasty: a neurovascular intervention to prevent stroke.** *Am J Cardiol* 1996;78:8–12
2. Featherstone RL, Brown MM, Coward LJ. **International carotid stenting study: protocol for a randomised clinical trial comparing carotid stenting with endarterectomy in symptomatic carotid artery stenosis.** *Cerebrovasc Dis* 2004;18:69–74
3. **Carotid angioplasty and stenting with and without cerebral protection: clinical alert from the Endarterectomy Versus Angioplasty in Patients with Symptomatic Severe Carotid Stenosis (EVA-3S) trial.** *Stroke* 2004;35:18e-20
4. Ringleb P, Kunze A, Allenberg JR, et al. [**Evaluation of stent-protected angioplasty for therapy of symptomatic stenoses of the carotid artery. SPACE and other randomized trials**]. *Nervenarzt* 2003;74:482–88
5. Hobson RW, 2nd. **Update on the Carotid Revascularization Endarterectomy versus Stent Trial (CREST) protocol.** *J Am Coll Surg* 2002;194:S9–14
6. Yadav JS, Wholey MH, Kuntz RE, et al. **Protected carotid-artery stenting versus endarterectomy in high-risk patients.** *N Engl J Med* 2004;351:1493–501
7. Steele PM, Chesebro JH, Stanson AW, et al. **Balloon angioplasty. Natural history of the pathophysiological response to injury in a pig model.** *Circ Res* 1985;57:105–12
8. Narayanaswamy M, Wright KC, Kandarpa K. **Animal models for atherosclerosis, restenosis, and endovascular graft research.** *J Vasc Interv Radiol* 2000;11:5–17
9. Lam JY, Lacoste L, Bourassa MG. **Cilazapril and early atherosclerotic changes after balloon injury of porcine carotid arteries.** *Circulation* 1992;85:1542–47
10. Recchia D, Abendschein DR, Saffitz JE, et al. **The biologic behavior of balloon hyperinflation-induced arterial lesions in hypercholesterolemic pigs depends on the presence of foam cells.** *Arterioscler Thromb Vasc Biol* 1995;15:924–29
11. Reitman JS, Mahley RW, Fry DL. **Yucatan miniature swine as a model for diet-induced atherosclerosis.** *Atherosclerosis* 1982;43:119–32
12. Gerrity RG, Natarajan R, Nadler JL, et al. **Diabetes-induced accelerated atherosclerosis in swine.** *Diabetes* 2001;50:1654–65
13. Zarins CK, Giddens DP, Bharadvaj BK, et al. **Carotid bifurcation atherosclerosis. Quantitative correlation of plaque localization with flow velocity profiles and wall shear stress.** *Circ Res* 1983;53:502–14
14. Ku DN, Giddens DP, Zarins CK, et al. **Pulsatile flow and atherosclerosis in the human carotid bifurcation. Positive correlation between plaque location and low oscillating shear stress.** *Arteriosclerosis* 1985;5:293–302
15. Motomiya M, Karino T. **Flow patterns in the human carotid artery bifurcation.** *Stroke* 1984;15:50–56
16. Bochner F, Siebert DM, Rodgers SE, et al. **Measurement of aspirin concentrations in portal and systemic blood in pigs: effect on platelet aggregation, thromboxane and prostacyclin production.** *Thromb Haemost* 1989;61:211–16
17. Stary HC, Chandler AB, Dinsmore RE, et al. **A definition of advanced types of atherosclerotic lesions and a histological classification of atherosclerosis. A report from the Committee on Vascular Lesions of the Council on Arteriosclerosis, American Heart Association.** *Arterioscler Thromb Vasc Biol* 1995;15:1512–31
18. Reitman JS, Mahley RW. **Changes induced in the lipoproteins of Yucatan miniature swine by cholesterol feeding.** *Biochim Biophys Acta* 1979;575:446–57
19. Palmer RM, Ashton DS, Moncada S. **Vascular endothelial cells synthesize nitric oxide from L-arginine.** *Nature* 1988;333:664–66
20. Malek AM, Jackman R, Rosenberg RD, et al. **Endothelial expression of thrombomodulin is reversibly regulated by fluid shear stress.** *Circ Res* 1994;74:852–60
21. Shyy YJ, Hsieh HJ, Usami S, et al. **Fluid shear stress induces a biphasic response of human monocyte chemotactic protein 1 gene expression in vascular endothelium.** *Proc Natl Acad Sci U S A* 1994;91:4678–82
22. Kraiss LW, Geary RL, Mattsson EJ, et al. **Acute reductions in blood flow and shear stress induce platelet-derived growth factor-A expression in baboon prosthetic grafts.** *Circ Res* 1996;79:45–53
23. Malek AM, Izumo S. **Control of endothelial cell gene expression by flow.** *J Biomech* 1995;28:1515–28
24. Passerini AG, Polacek DC, Shi C, et al. **Coexisting proinflammatory and antioxidative endothelial transcription profiles in a disturbed flow region of the adult porcine aorta.** *Proc Natl Acad Sci U S A* 2004;101:2482–87
25. Hajra L, Evans AI, Chen M, et al. **The NF-kappa B signal transduction pathway in aortic endothelial cells is primed for activation in regions predisposed to atherosclerotic lesion formation.** *Proc Natl Acad Sci U S A* 2000;97:9052–57
26. Bonan R, Paiement P, Scortichini D, et al. **Coronary restenosis: evaluation of a restenosis injury index in a swine model.** *Am Heart J* 1993;126:1334–40
27. Gal D, Rongione AJ, Slovenkai GA, et al. **Atherosclerotic Yucatan microswine: an animal model with high-grade, fibrocalcific, nonfatty lesions suitable for testing catheter-based interventions.** *Am Heart J* 1990;119:291–300
28. Zarins CK, Bomberger RA, Glagov S. **Local effects of stenoses: increased flow velocity inhibits atherogenesis.** *Circulation* 1981;64:II221–27
29. Sawchuk AP, Unthank JL, Davis TE, et al. **A prospective, in vivo study of the relationship between blood flow hemodynamics and atherosclerosis in a hyperlipidemic swine model.** *J Vasc Surg* 1994;19:58–63; discussion 63–54
30. Schwartz RS, Huber KC, Murphy JG, et al. **Restenosis and the proportional neointimal response to coronary artery injury: results in a porcine model.** *J Am Coll Cardiol* 1992;19:267–74
31. Doornekamp FN, Borst C, Haudenschild CC, et al. **Fogarty and percutaneous transluminal coronary angioplasty balloon injury induce comparable damage to the arterial wall but lead to different healing responses.** *J Vasc Surg* 1996;24:843–50
32. Schwarcz TH, Dobrin PB, Mrkvicka R, et al. **Balloon embolectomy catheter-induced arterial injury: a comparison of four catheters.** *J Vasc Surg* 1990;11:382–88
33. Yamashita A, Furukoji E, Marutsuka K, et al. **Increased vascular wall thrombogenicity combined with reduced blood flow promotes occlusive thrombus formation in rabbit femoral artery.** *Arterioscler Thromb Vasc Biol* 2004;24:2420–24