Animal Model That Mimics Atherosclerotic Plaque Rupture

Mark D. Rekhter, Gary W. Hicks, David W. Brammer, Carolyn W. Work, Jong-Seung Kim, David Gordon, Joan A. Keiser, Michael J. Ryan

Abstract—Atherosclerotic plaque rupture is the main cause of coronary thrombosis and myocardial infarcts. Currently, there is no animal model of plaque disruption. We have developed a rabbit model in which an atherosclerotic plaque can be ruptured at will after an inflatable balloon becomes embedded into the plaque. Furthermore, the pressure needed to inflate the plaque-covered balloon may be an index of overall plaque mechanical strength. The thoracic aorta of hypercholesterolemic rabbits underwent mechanical removal of endothelial cells, and then a specially designed balloon catheter was introduced into the lumen of the thoracic aorta. As early as 1 month after catheter placement, atherosclerotic plaque formed around the indwelling balloon. The plaques were reminiscent of human atherosclerotic lesions, in terms of cellular composition, patterns of lipid accumulation, and growth characteristics. Intraplaque balloons were inflated both ex vivo and in vivo, leading to plaque fissuring. The ex vivo strategy is designed to measure the mechanical strength of the surrounding plaque, while the in vivo scenario permits an analysis of the plaque rupture consequences, eg, thrombosis. In addition, our model allows local delivery of various substances into the plaque. The model can be used to study the pathogenesis of plaque instability and to design plaque stabilization therapy. (Circ Res. 1998;83:705-713.)

Key Words: atherosclerosis ■ catheter ■ plaque ■ rupture ■ thrombus

The abrupt closure of an artery by an occlusive thrombus is the main cause of myocardial infarcts and other thrombotic sequelae of atherosclerosis. This thrombosis is often associated with a fissure that develops in the underlying atherosclerotic plaque. Therapy that prevents plaque rupture may significantly decrease the incidence of heart attack and stroke, while treatment that reduces blood clot formation associated with plaque disruption may reduce mortality after such catastrophic events. Currently, there is no specific therapy for plaque rupture. Research on the mechanisms of plaque destabilization and development of “stabilization therapy” have been hampered by lack of an appropriate animal model. Our aim was to develop an animal model of atherosclerosis, in which the plaque could be ruptured at will under controlled conditions.

Atherosclerotic plaque rupture occurs as a result of interactions between external mechanical triggers and vulnerable regions of the plaque, when forces acting on the plaque exceed its tensile strength. The exact nature of these external forces is still unknown, thus making it difficult to design specific treatments to prevent plaque rupture. However, plaque tissue properties undoubtedly determine the mechanical strength/vulnerability of plaques and may be realistic targets for therapeutic intervention. Therefore, we focused on designing a model to evaluate plaque mechanical strength/vulnerability characteristics. The admittedly important problem of clinically relevant triggers of plaque disruption remains to be addressed.

Our experimental approach was based on the hypothesis that an atherosclerotic plaque can be ruptured at will if an inflatable balloon is embedded into the plaque. Furthermore, the pressure needed to inflate the plaque-covered balloon may be an index of overall plaque mechanical strength. We have been able to demonstrate that balloons can be inflated either ex vivo or in vivo. Our ex vivo experiments were designed to measure mechanical strength of the surrounding plaque, while the in vivo scenario permits an analysis of plaque rupture sequelae (eg, thrombosis).

Materials and Methods
Reproducible plaque formation on the balloon surface is essential. Our preliminary results (data not shown) revealed 3 key elements necessary and sufficient for the growth of balloon-associated plaques: (1) smooth muscle cell (SMC) migration from media into intima; (2) stable direct contact between arterial wall and balloon surface, enabling vessel-derived cells to adhere to the balloon surface; and (3) balloon surface properties that facilitate SMC migration and proliferation. Described below are the experimental procedures that meet the requirements for balloon-associated plaque formation (specific catheter preparation, vessel preparation, and placement of the catheter into the vessel). Also described are the balloon inflation procedures used to cause plaque disruption.

Diet
All procedures that used animals were conducted in compliance with state and federal laws, as well as guidelines established by the
Parke-Davis Animal Care and Use Committee. Our preliminary observations (data not shown) indicated that cholesterol feeding is not crucial for the induction of balloon-associated tissue growth. However, cholesterol feeding results in plaque lipid accumulation, a feature reminiscent of human atherosclerotic lesions. Male New Zealand White rabbits (Covance, Denver, Pa, weighing 2.5 to 3.0 kg) were meal-fed a rabbit chow diet supplemented with 0.5% cholesterol, 3% peanut oil, and 3% coconut oil. The diet was started 1 week before surgery and continued until the end of the experiments. Plasma cholesterol was determined using the Abbot VP analyzer. At the end of the experiment (5 weeks on the diet), the average plasma cholesterol level was 721.6 ± 116.7 mg/dL.

Catheter and Balloon Surface Preparation
For lesions to form, the balloon must have constant direct contact with the injured arterial wall, facilitating SMC migration from the artery to the balloon surface. To assure direct contact, we used a custom-designed catheter (NuMed Inc) with a C-shaped tip and an expandable latex balloon situated on the curved segment (Figure 1A). Because the width of the curvature slightly exceeded the diameter of the artery, the balloon was firmly pressed against the arterial wall (Figure 1B). The catheter had 3 ports and channels: 1 to accommodate a guide wire, a second for balloon inflation, and a third to deliver biologically active agents locally into the lesion (Figure 1A).

To provide a substrate that supports cell migration and growth, a small segment of vein was placed over the balloon (Figure 2A). Rabbits were anesthetized by the concurrent intramuscular injection of xylazine, ketamine, and atropine in doses of 2, 35, and 0.062 mg/kg, respectively. Anesthesia was maintained with isoflurane gas (0.25% to 0.5%) using a mask technique. A skin incision was made on the neck, and the left jugular vein was surgically exposed. The vein segment was isolated by 2 ties, frozen using a metal applicator cooled in liquid nitrogen, and then allowed to thaw. It has been previously shown that freezing effectively kills all the cells yet preserves the extracellular matrix composition, thus providing a good substrate for further SMC and endothelial cell migration and proliferation.11 The C-shaped indwelling catheter was inserted into the isolated vein segment through a small incision and advanced, so the vein segment covered the balloon as well as the infusion outlet, a feature reminiscent of human atherosclerotic lesions. The catheter had 3 ports and channels: 1 to accommodate a guide wire, a second for balloon inflation, and a third to deliver biologically active agents locally into the lesion (Figure 1A).

Catheter Placement
To induce SMC migration, the thoracic aorta was injured with an embolectomy balloon catheter (Figure 2B). First, a guide catheter (4.1F cerebral catheter, Cook Inc) was advanced through a carotid arteriotomy to the descendent thoracic aorta under fluoroscopic guidance; then a guide wire (0.014-inch Sceptor “EnTre-Style,” Scimed Life Systems, Inc) was passed into the aorta through the guide catheter. The guide catheter was then removed, leaving the guide wire in place. Next a 4F embolectomy catheter (Baxter) was introduced over the guide wire with the tip placed at the level of diaphragm. The balloon was inflated and then pulled back to remove endothelial cells in the thoracic aorta. This process was repeated 3 times, and the catheter was removed.

Finally, the embolectomy catheter with the vein-covered balloon was passed into the injured segment of thoracic aorta over the guide wire (Figure 2C). Under fluoroscopic guidance, the balloon was placed at the level of the fifth rib (Figure 1B). The wire was...
removed, the proximal end of the catheter implanted in a subcutaneous pocket on the neck, the incision closed, and the animal allowed to recover.

Four weeks after catheter placement, the balloon was firmly attached to the aortic wall and completely plaque covered in 15 of 17 animals. Six rabbits were perfusion fixed with either methacarn (methanol:chloroform:acetic acid) or 10% neutral-buffered formalin to characterize makeup of intact balloon-associated atherosclerotic lesions. Seven rabbits were used for ex vivo plaque rupture, and 2 rabbits were used for in vivo plaque rupture experiments (see below).

**Plaque Rupture**

*Ex Vivo Experiments*

Ex vivo plaque rupture was performed to measure the mechanical strength of plaques 1 month after implantation of the vein-covered balloons. Rabbits were anesthetized by concurrent intramuscular injection of xylazine, ketamine, and butorphanol in doses of 7.5, 52, and 0.15 mg/kg, respectively, and then perfused with saline via an abdominal aortic catheter to remove the blood. The thoracic aorta was opened longitudinally, and the segment of aorta containing the plaque was excised as 1 block with attached catheter. The aortic segment was then pinned to a corkboard, so the plaque was fully exposed on end. The catheter was filled with distilled water and attached to an HPLC pump (Waters 600 Multisolvent Delivery System, Waters Corporation). To remove air bubbles, the water was sparged with helium. Pressure within the plaque-covered balloon was measured using a test gauge and electronic pressure transducer. The analog signal from the pressure transducer was both recorded on a strip chart and captured for data analysis by an analog-to-digital converter sampling at a rate of 250 samples per second. For each pressure measurement, the interballoon volume was calculated as a function of time. The balloon was inflated by infusion of water at a constant rate (0.5 mL/min) using the HPLC pump, and the pressure within the intraplaque balloon was increased until the plaque ruptured. The moment of visible plaque rupture was coordinated with a sudden drop of pressure on a recorded pressure-time curve. This pressure was considered the “rupturing” pressure and an index of overall mechanical plaque strength. As a control, we recorded pressure-volume curves for 5 balloons without plaques. A video camera (DXC 960 MD, Sony Corp) and laser videodisk recorder (LVR-300N, Sony Corp) that collected 30 video frames a second were used to record balloon inflations. Digital images were obtained using a frame grabber (LG3, Scion Corp) and computer (model 8100, Apple Computer Inc) attached to the laser videodisk recorder.

*Procurement of Ex Vivo Ruptured Plaques for Histology*

Plaque fissures often have very complex geometry and are not always easily recognizable on the two-dimensional tissue sections. Moreover, artificial cracks and other tissue displacements may occur during specimen preparation. To label original irregular contours of the plaques, we used the Davidson Marking System (Bradley Products). This system is designed for permanent laboratory marking of tissue in clinical pathology. A marking dye is resistant to the effects of tissue fixation, processing, embedding, and sectioning and is clearly visible in the light microscope. Thus, after ex vivo rupture, plaques were incubated with green tissue-marking dye for 5 minutes, blotted, washed in PBS, fixed in methacarn and processed for histological analysis.

*In Vivo Experiments*

To demonstrate the feasibility of using the model to study consequences of plaque rupture, plaques were disrupted in vivo in 2 rabbits. Four weeks after initial placement of the indwelling catheter, rabbits were anesthetized as described above and the port used to inflate the balloon was exteriorized through a small surgical incision. The intraplaque balloon was then inflated in vivo. Three hours later, rabbits were killed, perfused with PBS, and then fixed by perfusion with 10% neutral-buffered formalin.

**Histology, Electron Microscopy, and Immunocytochemistry**

To macroscopically visualize neutral lipids, formalin-fixed samples of intact plaques were stained en block with Sudan IV. Plaques were then cut into 3 to 4 pieces. One piece was frozen, a second processed and paraffin embedded, and a third prepared for transmission electron microscopy (TEM). Formalin-fixed samples of plaques ruptured in vivo were either paraffin embedded or processed for scanning electron microscopy (SEM). All methacarn-fixed specimens were paraffin embedded and cut into 5-μm cross-sections.

To characterize the general architecture of the atherosclerotic plaques, cross-sections underwent hematoxylin and eosin or Movat pentachrome staining. Neutral lipids were visualized on frozen sections using oil red O staining. Specimens for SEM and TEM were prepared as previously described. Cellular composition, cell proliferation, collagen synthesis, and presence of tissue factor were characterized immunocytochemically. Primary antibodies were as follows: an anti–smooth muscle α-actin antibody (Boehringer Mannheim) to identify SMCs, RAM11 (DAKO) to identify macrophages, anti–von Willebrand factor (vWF) (Antibodies) to identify endothelial cells, L11/135 (Serotec) to identify T cells, anti–type I procollagen antibody SPLD8 (Developmental Studies Hybridoma Bank, University of Iowa) to visualize cells actively synthesizing type I collagen, anti–proliferating cell nuclear antigen (PCNA) antibody PC10 (DACO) to detect proliferating cells, monoclonal (No. 4511) and polyclonal (No. 4513) antibodies to identify tissue factor (American Diagnostica). Standard immunoperoxidase phosphatase or immunoperoxidase techniques were applied as previously described.

**Results**

**Structure of the Balloon-Associated Lesions**

One month after catheter placement, the vein-wrapped balloon was firmly attached to aortic wall and completely covered with fibrotic tissue (Figure 3A). Microscopically, the lesion consisted of an empty core region previously occupied by the balloon, a fibrous cap–like area that separated the empty core from the lumen of the aorta, and a shoulder area that connected the cap with the thickened aortic intima. Both the cap and shoulders contained collagen-rich remnants of the vein, surrounded by newly grown tissue in the peripheral (periluminal) part of the lesion. Pentachrome staining revealed the presence of collagen, elastin, and glycosaminoglycans within the plaque (Figure 3B). Thus, the balloon-associated plaque became an integral part of the thickened aortic intima.

Immunocytochemical analysis demonstrated that the plaques contained α-actin–positive SMCs, RAM11–positive macrophages, L11/135–positive T cells, vWF–positive endothelial cells, and some cells that did not express either of the above cell type–specific markers. SMCs predominately occupied the peripheral part of the plaque (Figure 3C). The luminal surface of the plaque was covered by endothelial cells. In all plaques, the shoulder region consistently contained macrophages (Figure 3D) and microvessels (Figure 3F). Approximately 30% of the shoulder macrophages contained numerous cytoplasmic vacuoles (“foam” cell appearance, as shown on Figure 3E). Macrophages often formed a ring encircling the central part of the plaque previously occupied by the balloon, and some macrophages appeared to be multinucleated giant cells. T cells were scattered throughout the plaque, but most colocalized with plaque microvessels (Figure 3G). PCNA immunostaining showed focal cell pro-
Figure 3. Characterization of unruptured balloon-associated lesions. A, Macroscopic en face view of the plaque. The red product of Sudan IV staining shows preferential accumulation of neutral lipids in the plaque shoulders. B, Microscopic anatomy of the lesion. The asterisk shows the space in the center of the plaque, previously occupied by the balloon catheter (removed during specimen preparation); the arrow, a fibrous cap; and arrowheads, plaque shoulders. The yellow ring surrounding the empty core represents vein remnants. The nick at the top of the cap area was introduced during tissue processing to remove the catheter. Movat pentachrome staining, 4× objective. C, α-Actin-positive smooth muscle cells (red product of immunoalkaline phosphatase reaction) in peripheral part of the lesion; 4× objective. D, Macrophages (red product of immunoalkaline phosphatase reaction, anti-RAM11 staining) localized mostly in the shoulders and base of the lesion; 4× objective. E, “Foamy” RAM11-positive macrophages in the shoulder area; 100× objective. F, Plaque vascularization (anti-vWF staining). Capillaries (red product of immunoalkaline phosphatase reaction) are located in the shoulder region; 25× objective. G, T Cells (red product of immunoalkaline phosphatase reaction) often found around plaque capillaries (shown by asterisks); 100× objective. H and I, Proliferating cell nuclear antigen–positive proliferating cells with labeled nuclei (black product of immunoperoxidase reaction, shown by arrows) located in both shoulder (H) and fibrous cap (I) regions. The asterisk denotes the space in the center of the plaque, previously occupied by the balloon. Methyl green nuclear counterstain; 25× objective. J and K, Type I procollagen staining (red product of immunoalkaline phosphatase reaction, shown by arrows) indicating ongoing collagen production in both shoulder (J) and fibrous cap (K) regions. The asterisk denotes the space in the center of the plaque, previously occupied by the balloon; 25× objective. L, Neutral lipid accumulation predominantly in the plaque shoulders. Oil red O staining; 50× objective. M, Tissue factor (red product of immunoalkaline phosphatase reaction, shown by arrows) present in experimental lesions; 25× objective. N, RAM11 immunostaining (red product of immunoalkaline phosphatase reaction, shown by arrows) performed on a serial section, demonstrating that tissue factor is preferentially associated with macrophages; 25× objective. O, Green dye delivered into the lesion via infusion port. En face view demonstrates uniform label distribution along the length of the lesion. Macroscopic photo. Lum indicates aortic lumen.
liferation both in macrophage-rich and SMC areas of the lesion (Figure 3H and 3I). Application of an anti–type I procollagen antibody revealed numerous collagen-producing cells on the periphery of the lesion, consistent with localization of SMCs (Figure 3J and 3K).

Balloon-associated plaques accumulated neutral lipids. Lipid accumulation occurred predominantly in the plaque shoulders (Figure 3L), while the fibrous cap area was usually spared. Lipids were deposited both intracellularly and extracellularly. Although intracellular lipid droplets were found in both macrophages and SMCs, the vast majority of lipid accumulation was associated with macrophages (data not shown).

Immunostaining with the antibodies against rabbit tissue factor consistently demonstrated the presence of this protein in balloon-associated lesions (Figure 3M). Tissue factor was always found in macrophage-rich areas (Figure 3N) and often was associated with plaque microvessels (data not shown).

The injection of surgical margin dye into the infusion port exteriorized on the neck demonstrated successful delivery of a marker into the space surrounding the balloon. The dye also leaked into the periphery of plaque tissue and was uniformly distributed around the lesion (Figure 3O).

**Figure 4.** Ex vivo plaque rupture. A through C, Video recording of the plaque during balloon inflation: A, beginning; B, progression; C, disruption. D, Ex vivo ruptured plaque incubated with a green dye and then processed for histology. The real plaque fissure has green margins (shown by arrow), while the artificial tissue crack (shown by arrowhead) is not labeled. Hematoxylin and eosin; 25× objective. E and F, Macrophages (red product of immunoalkaline phosphatase reaction,) colocalized with the site of plaque rupture, delineated by green margins (shown by arrow). E shows a specimen fixed after catheter removal, a process that caused the plaque tissue to contract. F exemplifies the situation when a catheter was extracted after fixation via an artificially introduced nick at the top of a fibrous cap. lum indicates aortic lumen. The asterisk denotes the space in the center of the plaque previously occupied by the balloon; 4× objective. G, A fissure, typically either spread between macrophages (right side of the panel) or demarcating macrophage-rich area (left side of the panel). RAM11-positive macrophages (red product of immunoalkaline phosphatase reaction) are shown by arrows; 25× objective. H, Disrupted region (arrowhead), which is often smooth muscle cell depleted. α-Actin–positive smooth muscle cells (red product of immunoalkaline phosphatase reaction) are shown by arrows; 25× objective. I, Fissure separating macrophage-rich area (red product of immunoalkaline phosphatase reaction, shown by arrow) and acellular area (arrowhead); 25× objective. D, E, and F represent 3 different experiments (ie, 3 rabbits). Higher-power images (G through I) belong to the lesion shown in F.

**Plaque Rupture**

Balloon-associated atherosclerotic plaques were ruptured by inflation of the balloon both in vivo and ex vivo. Our initial emphasis has been on ex vivo rupture. This approach enables direct observation of rupturing dynamics both spatially and temporally.

In ex vivo plaque rupture experiments, gradual balloon inflation first led to the formation of a “hump” on the lesion surface followed by rupture (Figure 4A through 4C). A representative time-pressure trace obtained from a 1-month-old plaque-covered balloon is illustrated in Figure 5A. In this experiment, degassed water was infused into the plaque-covered balloon at a constant rate (0.5 mL/min) as detailed in “Methods.” Pressure within the balloon rose from 0 to 6 atm during a 15-second infusion. When the interplaque pressure reached 6 atm, there was a notable inflection in the time-
pressure trace. This abrupt pressure fall coincided with the balloon opening and plaque fracture observed visually.

The pressure-volume curve illustrated in Figure 5B is derived from the data in Figure 5A. For each interballoon pressure, an interballoon volume was calculated. Pressure-volume curves obtained from a plaque-covered balloon and a balloon alone are shown in B and D, respectively.

Figure 5. Biomechanics of plaque rupture. Examples of a representative pressure-time trace obtained from a plaque-covered balloon and a balloon alone are shown in A and C, respectively. Traces represent digitized samples acquired from an electronic pressure transducer at a rate of 250 Hz. Balloons were inflated by infusion of water at a rate of 0.5 mL/min. For each interballoon pressure, an interballoon volume was calculated. Pressure-volume curves obtained from a plaque-covered balloon and a balloon alone are shown in B and D, respectively.

The balloon itself had material properties that clearly differed from the plaque that covered the balloon. Interballoon pressure reached a pressure of <1 atm over a 15-second infusion (Figure 5C). Furthermore, the balloon alone had a volume-pressure curve slope (Figure 5D) that was very flat compared with that of the plaque-covered balloon (Figure 5B), indicating that plaque elasticity differs from that of the balloon alone.

The rapid fall in interballoon pressure coincident with plaque fracture is a reproducible phenomenon that can be used as a “signature” of plaque rupture. We found that 1-month-old plaque-covered balloons opened at a pressure of 4.3±0.8 atm (n=6), while balloons alone opened at a significantly lower pressure of 1.3±0.1 atm (n=4). Thus, we have been able to demonstrate that the pressure needed to inflate a plaque-covered balloon differs significantly from that of a balloon alone and can be used as an end point of plaque rupture.

Future experiments are needed to determine the sensitivity of these end points to external factors and drug treatments that have been proposed to influence plaque stability. For example, it has been reported that elevated cholesterol predisposes patients to plaque rupture and cholesterol lowering decreases the frequency of fatal coronary events.\textsuperscript{16,17} Given these clinical observations, we have initiated studies to determine the influence of plasma cholesterol on plaque strength. Preliminary results indicate progressive plaque weakening in rabbits fed a high-cholesterol diet for 3 months, while plaque strength is maintained in rabbits fed standard chow (manuscript in preparation).

Ex vivo ruptured plaques were incubated with a surgical margin permanent dye to label the plaque fissure sites. Areas of experimental tissue disruption were stained green using this technique. Artificial cracks and other tissue displacements that occurred during specimen preparation were not stained (Figure 4D). A combination of such en block labeling with immunostaining performed on tissue sections provides a convenient methodology to address specific questions about the spatial association between potential culprit cells/molecules and rupture (ie, the weakest) plaque regions. Figure 4E and 4F shows that rupture occurred in a macrophage-rich shoulder area. We have observed two major patterns of spatial association between a fissure and macrophages (Figure 4G): (1) the fissure “snaking” among macrophages and (2) the fissure separating a macrophage-rich area and an area containing no macrophages. Disrupted regions were often SMC depleted (Figure 4H) or appeared to be acellular (Figure 4I).

To demonstrate the immediate consequences of plaque rupture, the intraplaque balloon was inflated in vivo. In these experiments, rabbits were anesthetized, the balloon inflation port was exposed through a small incision, and the plaque was ruptured by inflation of the balloon. Three hours after plaque rupture, the animals were killed. Gross examination revealed the presence of concise red thrombus covering about 15% of the plaque surface, as well as scattered intraplaque hemorrhages (Figure 6A). Microscopic analysis proved precise colocalization between thrombus and site of plaque rupture (Figure 6B). The deep portion of the thrombus was directly associated with foam cells at the edge of disrupted plaque tissue (Figure 6C), while the luminal surface contained aggregates of platelets, red blood cells, and a fibrin meshwork (Figure 6D).

**Discussion**

**Features of the Balloon-Associated Plaques**

We have demonstrated that fibrotic lesions can be developed around an expandable balloon. Arterial lesions induced by indwelling plastic tubing have been described previously,\textsuperscript{18,19} however, the tubing itself was not always embedded into the lesions. The critical difference between our model and models previously described is that the balloon catheter becomes an integral part of the lesion. Therefore, the behavior of the lesion may be controlled by balloon inflation and local infusion of biologically active materials into the lesion.

Importantly, the balloon-associated lesion in our model is reminiscent of human atherosclerotic plaques in terms of architecture (presence of an acellular core and a fibrous cap), cellular composition (SMCs, macrophages, T cells), growth characteristics (cell proliferation, collagen synthesis), and patterns of lipid accumulation.\textsuperscript{14,15,20–22} Moreover, neangiogenesis, an important feature of advanced human lesions,\textsuperscript{23}...
relevant to plaque rupture, was observed. Neoangiogenesis has not been found previously in rabbit or smaller animal models of atherosclerosis. Our model is the only one that consistently produces plaque microvessels in a defined location. However, it should be noted that the described histological findings are typical but not necessarily specific for atherosclerotic plaques and might be interpreted as a foreign body response of the injured artery in a hyperlipemic animal.

The main feature of our model is the ability to rupture the plaque by inflation of the intraplaque balloon and thereby directly measure the mechanical characteristics of a plaque tissue. We are aware of only 1 attempt to design an animal model of plaque rupture. Abela et al. using the technique initially developed by Constantinides and Chakravarti, induced fibrolipid lesions in the rabbit aorta by combining mechanical injury with hypercholesterolemia and then challenged the rabbits with a combination of a proteolytic procoagulant viper venom and histamine. Although this treatment triggered thrombus formation, no associated plaque fissures were demonstrated. Furthermore, the development of suitable lesions took over eight month. In our model, advanced atherosclerotic plaques are formed in a relatively short time (1 month), plaque disruption can be easily documented, and cause-effect relationships between plaque fissuring and thrombus formation are unequivocally established.

Morphological features of disrupted lesions in our model are also suggestive of human atherosclerotic plaque in that the fissures are localized in the shoulder region, associated with the presence of macrophage and SMC depletion, and associated with thrombus formation after in vivo disruption.

Technical Potential
Our model provides unique technical opportunities by virtue of the fact that the “inside” of the plaque can be accessed without plaque alterations. In our pilot studies, a dye was injected into the plaque through a remote catheter port, providing proof of the concept that bioactive substances may be locally delivered into the plaque. More specific experiments are required for detailed characterization of this local intraplaque delivery method.

We suggest that the delivery of genes, genetically modified cells, proteins, and other substances (eg, lipids) may allow for atherosclerotic plaque tissue engineering to study the influence of specific molecules or cells on plaque stability. A local drug delivery may be used to test the efficacy of compounds designed to stabilize plaques, including drugs with poor pharmacological activity.

Figure 6. In vivo plaque rupture. A, En face view of the plaque 3 hours after in vivo balloon inflation. A red thrombus (shown by arrow) is formed on the plaque surface. Macroscopic photo. B, Microscopic view of the cross-section of the same specimen. A thrombus (shown by arrow) is associated with plaque rupture. Disrupted flaps of a fibrous cap are shown by arrowheads. lum indicates aortic lumen. The asterisk denotes the space in the center of the plaque, previously occupied by the balloon. Movat pentachrome staining; 4× objective. C, A fragment of B demonstrates that the edge of the thrombus (Thr) is directly associated with foam cells (shown by arrows); 25× objective. D, Surface of rupture-induced thrombus containing platelets (shown by arrows), red blood cells (shown by asterisks), and fibrin meshwork (shown by arrowheads). Scanning electron microscopy; magnification×12,000.
biocompatibility. Another exciting potential of the accessible intraplaque space may be the placement of various probes and arrays (eg, temperature, ultrasound) to monitor changes in plaque composition and mechanical properties after treatment. Finally, using the catheter infusion port, samples of tissue fluids may be taken to monitor secretion of various proteins during plaque formation and plaque healing after disruption.

**Suggested Applications**

The field of atherosclerotic plaque instability has evolved mostly from retrospective clinical and pathological observations. There are many hypotheses relevant to both plaque rupture pathogenesis and treatment, the discussion of which is beyond the scope of this methodological report. Our model may provide a valuable tool to test these hypotheses under controlled experimental conditions, as well as to generate new ones.

The focus of our model is on the mechanical properties of atherosclerotic plaques. Therefore, evaluation of breaking pressure and stress-strain relationships are viewed as the major end points. Since we have demonstrated the presence of relevant cell types (macrophages, T cells, SMCs, capillary endothelial cells), extracellular matrix components, and lipids in balloon-associated lesions, I strategy would be to correlate biology of these cells (invasion, proliferation, death, lipid accumulation, gene expression), balance between matrix degradation and synthesis, and tissue mechanical properties; in other words, to study “the biology of fibrous cap strength.” Another paradigm would be to analyze the dynamics and mechanisms of thrombosis after plaque disruption, because we have documented the presence of tissue factor in experimental lesions and demonstrated thrombus formation after in vivo plaque rupture. We also hope that formation of nonocclusive thrombi after plaque disruption may serve as a tool for analysis of the rupture-related asymptomatic lesion growth suggested in angiographic and pathological human studies. Respectively, our model can be used to evaluate treatment strategies designed to stabilize vulnerable plaques (primary prevention), to diminish thrombosis after disruption, and to promote the healing of ruptured plaques (secondary prevention).

Animal models are essential for development of diagnostic imaging techniques. Although our model would not be optimal for examination of events that lead to plaque rupture, it may be helpful in accurate imaging and identification of fibrous cap fissures, as well as serial examinations of events after rupture, ie, thrombus formation and organization.

**Limitations**

The presence of an inflatable balloon within atherosclerotic plaque literally comprises the core of our model, enabling plaque “eruption,” mechanical measurements, and local delivery. However, the same feature produces limitations of the model. First, the balloon catheter cannot be miniaturized indefinitely. Therefore, only animal species with fairly large arteries can be used. Second, tissue disruption by virtue of balloon inflation does not permit analysis of specific triggers that may be clinically relevant to plaque rupture. Development of alternative plaque-rupture models emphasizing the triggering event would complement our model, which is well suited to evaluate plaque mechanical properties.

Thus, we suggest that our new animal model may provide valuable information about the pathogenesis of atherosclerotic plaque rupture. It may also serve as a powerful tool in evaluating novel “plaque stabilization” treatments aiming at primary and secondary prevention of heart attack and stroke, as well as development of diagnostic imaging modalities.

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