Adjuvant Therapy for Intracoronary Stents
Investigations in Atherosclerotic Swine

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Early thrombosis has complicated human stent implantation in several trials. To determine the best anticoagulation/antiplatelet therapy to maintain stent patency after percutaneous transluminal coronary angioplasty, we implanted the flexible balloon-expandable coil stent into the left anterior descending coronary artery of 28 atherosclerotic 8-month-old Hanford miniature swine. Animals were randomly assigned to one of three treatment groups: group A, aspirin (1 mg/kg/day) and dipyridamole (1 mg/kg three times a day); group B, aspirin and dipyridamole (same doses) plus Coumadin (dose required to prolong prothrombin time 1.3-1.5-fold that of normal); and group C, control. Adjuvant therapy was begun 3 days before stenting. Two pigs (one from group A and one from group B) died during implantation, both without thrombosis. Twenty-six animals survived until follow-up angiography and sacrifice at 1 month. No occlusive thrombosis of the stent occurred in survivors. Reduction of the stent lumen diameter was observed in every case at follow-up. Percent lumen reduction was 19% in group A, 26% in group B, and 24% in group C. Marked smooth muscle cell hyperplasia was seen by light and transmission electron microscopy at stent struts. Scanning electron microscopy of the luminal surface showed a variable morphology consisting of normal endothelium, adherent leukocytes, stellate periluminal cells, and occasional fibrin strands and red blood cells. Luminal narrowing was not affected by anticoagulation therapy, antiplatelet drugs, cholesterol level, or stent sizing.

We conclude that occlusive thrombosis does not complicate stent implantation in this model but that substantial luminal narrowing due in part to smooth muscle hyperplasia does occur. The significance of luminal narrowing at the stent site requires further study. (Circulation 1990;82:560-569)

The use of the intracoronary stent may solve two significant limitations of percutaneous transluminal coronary angioplasty (PTCA) — abrupt closure after dilatation and restenosis. Although the use of the percutaneously implanted metallic stent has been suggested as a possible means of ameliorating these problems in human trials, investigators have reported a 9-14% rate of abrupt closure of the stent. Because thrombosis of the prosthesis appears to be the mechanism of occlusion, Sigwart et al and Schatz et al have emphasized the importance of an aggressive antiplatelet and anticoagulation regimen after stent implantation.

Understandably, there has been a reluctance to forego anticoagulation in clinical trials. But the questions of whether anticoagulation of a patient after stent implantation is necessary or whether an antiplatelet regimen may suffice remain. We used the atherosclerotic miniature swine model subjected to intracoronary stent placement (Gianturco-Roubin stent, Cook, Inc.) to study thrombosis prevention with one of three regimens: anticoagulation and antiplatelet, antiplatelet alone, or control. In addition, we studied the morphological response of the atherosclerotic swine coronary artery to implantation of this flexible coil stent.

Methods

Animal Preparation

Thirty-two Hanford miniature swine (12-18 kg) were used. All procedures and handling of the animals were performed in such a way as to minimize
discomfort and stress on the animals. Our protocol adhered to guidelines for the handling of experimental animals set by the National Institutes of Health and was approved by the Institutional Animal Care and Use Committees of the University of Texas M.D. Anderson Hospital and Baylor College of Medicine.

**Induction of Coronary Atherosclerosis**

Animals were fed an atherogenic diet consisting of 2% cholesterol, 15% fat, and 1.5% sodium cholate. The animals were fed approximately 2% body weight of diet per day. Two weeks after the initiation of the atherogenic diet, the swine underwent endothelial abrasion of the left anterior descending coronary artery (LAD). Three days before the abrasion procedure, the animals were begun on diltiazem 2–4 mg/kg orally three times per day. Diltiazem was continued for 3 days after surgery. All animals received a perioperative injection of 250 mg i.m. ampicillin. The animals were sedated with an intramuscular injection of 20–30 mg/kg ketamine, 0.22 mg/kg acepromazine, and 0.05 mg/kg atropine. After intubation, halothane 2% and oxygen were given throughout the procedure. Ten milligrams of nifedipine (0.67 mg/kg) was administered buccally as the procedure began. Using sterile technique, the right femoral artery was surgically isolated, and a 6F hemostatic sheath (USCI) was inserted. A blood specimen was then obtained for serum cholesterol. The animal was then given 200 units/kg i.v. heparin. Every 15 minutes throughout the procedure, 5 mg/kg i.v. bretylum tosylate was administered. The LAD was cannulated with a 5.5F Visceral Cobra C3 catheter (Cook, Inc.), and angiography was performed. Before coronary abrasion, 300–400 µg i.c. nitroglycerin was administered. A 2.0–2.5-mm-diameter microdilatation probe (USCI, balloon-on-a-wire) (selected so the balloon was slightly oversized) was introduced into the LAD via the Cobra catheter and then guided to the midportion of the LAD. Three 20-second inflations were performed. During each inflation, the probe was drawn to-and-fro within the midportion of the LAD. Angiography was repeated after abrasion, and the site of abrasion was recorded for subsequent reference. The catheter and sheath were removed, the femoral artery was ligated, and the wound was repaired. The animals were observed closely during recovery. The swine remained on the atherogenic diet through stent implantation 4–5 months later and until sacrifice. The average cholesterol level was determined as the average of values of samples taken at the time of stent implantation and at follow-up.

**PTCA and Stent Implantation**

Animals were randomly assigned to one of three adjuvant therapy groups: control, antiplatelet therapy, or anticoagulant plus antiplatelet therapy. Animals assigned to the anticoagulant plus antiplatelet group underwent surgical placement of an indwelling catheter within the right external jugular vein 10 days before stent placement. Coumadin was begun 3 days after catheter placement, and daily prothrombin time determinations were made. The oral dosage of Coumadin was adjusted so a stable prothrombin time of 15–18 seconds (33–50% increase) was achieved before stent implantation. Antiplatelet therapy consisting of 1 mg/kg/day orally aspirin and 1 mg/kg orally three times per day dipyridamole was begun 3 days before stent implantation in the noncontrol animals (anticoagulant plus antiplatelet only groups). Adjuvant therapy was continued until sacrifice 1 month after stent placement. The same preanesthetic, anesthetic, and antispasm regimens used for endothelial abrasion were used for the PTCA and stent implantation procedure.

Arterial access was achieved by surgical isolation and cannulation of the left femoral artery. A blood specimen was drawn for serum cholesterol; 200 units/kg i.v. heparin was then administered. The LAD was selectively cannulated with a modified large-lumen 8F multipurpose guiding catheter (USCI). Then 400 µg i.c. nitroglycerin was administered. Coronary angiography in video and cut-film formats was performed in the left anterior oblique projection. The site of previous abrasion was identified from landmarks recorded at the time of the abrasion; in addition, luminal irregularity was often observed at this site. A 2.5- or 3.0-mm (selected so the diameter approximated the normal caliber of the vessel) angioplasty balloon catheter was inserted, positioned in the midportion of the LAD, and inflated twice for 20 seconds at 5 atm. Nitroglycerin (400 µg i.c.) was administered immediately before and after PTCA. The balloon catheter was then exchanged for a 2.5- or 3.0-mm (selected so the stent was approximately 20% greater in diameter than the normal vessel caliber) balloon-mounted, flexible coil coronary stent (constructed of 0.06-in.-diameter stainless-steel wire; Cook, Inc.) and was guided over a guidewire to the same site in the midportion of the LAD. The stent was deployed at this site by inflation of the balloon catheter to 9 atm (full expansion of the stent was usually seen at 5–6 atm) for 45 seconds. The balloon catheter was then deflated and withdrawn along with the guidewire. Another dose of 400 µg i.c. nitroglycerin was given, and coronary arteriography was repeated. Additional doses of nitroglycerin, nifedipine, or both were given if significant coronary spasm was observed distal to the stent. A final coronary angiogram in the left anterior oblique projection was then made in cut-film format. The guiding catheter and sheath were removed. The left femoral artery was ligated, and the wound was repaired. One inch of 2% nitroglycerin ointment was applied topically to the shaved flank at the conclusion of the procedure, and the animal was observed closely during recovery.

**Follow-up Angiography and Sacrifice**

Noncontrol animals remained on their adjuvant regimen for 28 days. All animals remained on the atherogenic diet during this period. At the end of this period, all animals underwent repeat angiography.
and sacrifice. The preanesthetic, anesthetic, and anti-spasm protocols used during abrasion and stent implantation were used for follow-up angiography. A blood specimen was drawn for serum cholesterol. The left carotid approach was used to perform coronary angiography. Angiograms were made in the left anterior oblique projection and recorded on cut-film format.

Under deep anesthesia, 13 animals were exsanguinated, and the hearts were taken for pathological evaluation. In 13 additional animals, sacrifice was performed by pressure fixation technique. A left thoracotomy was performed. The great vessels of the aortic arch were ligated. The ascending thoracic aorta was cannulated with an 18-gauge angiocatheter, and 0.1 M sodium cacodylate (pH 7.4) buffer was perfused into the ascending thoracic aorta and coronary arteries at a pressure of 100 mm Hg. The pulmonary artery trunk was incised to exsanguinate the animal soon after the buffer infusion was begun, and the descending thoracic aorta was simultaneously cross-clamped. Once the buffer had infused for 10 minutes, the coronary arteries were perfusion-fixed at 100 mm Hg with modified Karnovsky’s fixation (Polyscience, Bayshore, N.Y.) (2.5% glutaraldehyde buffered with 0.1 M cacodylate [pH 7.4]) for 20–30 minutes. The stented segment of LAD was excised and immersed in 2.5% glutaraldehyde for 24–48 hours. The specimens were then placed in cacodylate buffer until further processing.

Angiographic Analysis

Angiographic measurements were made as follows. The follow-up angiogram of the stented artery was projected, and the location of the most severe narrowing within the stent was identified. A manual caliper was used to designate the lumen edge at this site. The caliper dimensions were obtained with a Digisonics Echocomp computer to quantitate the linear measurement. The known diameter of the guiding catheter was used to correct this measurement to calculate stent diameter. A similar measurement was then obtained from theangiograms performed before and immediately after stent implantation at the same location within the stent. With this method, intraobserver variability was 0.15, 0.22, and 0.21 mm for stent segment diameter before implantation, after implantation, and at follow-up, respectively.

Change in percent diameter stenosis of the LAD was calculated by the formula: % Change = (Post-stent diameter − follow-up stent diameter × 100) / poststent diameter.

Histological and Morphometric Analysis

Sections for light microscopy were cut with a glass-tipped microtome and stained with toluidine blue (plastic embedded) or hematoxylin and eosin (paraffin embedded). Morphometric analyses were performed with a projecting Olympus BH microscope (×4) with drawing attachment. Projected on a digitizing board, the borders of the internal elastic lamina and lumen were traced for each section. Computer-assisted calculation of the area bounded by these borders was performed, and percent luminal area stenosis was determined. Morphometric determination of percent area luminal reduction was made at three cross-sectional sites within each specimen: proximal to the stent, intrastent, and distal to the stent.

Scanning and Transmission Electron Microscopy

After excess tissue was trimmed, 20-mm stented segments (as well as segments proximal and distal to the stent) were carefully cut longitudinally and post-fixed for 1 hour in osmium tetroxide (1% in 0.1 M cacodylate). The specimens were then dehydrated in graded ethanol baths to 100%, critical-point-dried from liquid CO₂, mounted on aluminum stubs with the luminal surfaces up, and sputter-coated with 10 nm gold/palladium alloy. The specimens were observed on the lower stage of an International Scientific Instruments DS-130 scanning electron microscope. Detailed documentation of cellular and subcellular morphology was obtained.

A 3–5-mm-long segment of the midportion of each stented artery was obtained, and the stent wire was carefully removed. Segments 5–10 mm proximal and distal to the stent were also taken. The segments were postfixed for 1 hour in osmium tetroxide (1% in 1.0 M cacodylate) and embedded in Epon or paraffin. Sections for transmission electron microscopy or scanning transmission electron microscopy were cut with an LKB ultramicrotome equipped with a diamond knife and stained with lead citrate and uranyl acetate. Conventional transmission electron microscopy was performed on a JEOL model 100 cx, Peabody, Mass., from sections of 70-nm thickness; scanning transmission electron microscopy was performed on a model DS-130, International Scientific Instruments, Inc., Pleasanton, Calif., from sections of 100-nm thickness.

Results

Procedures

Thirty-two swine were begun on the atherogenic diet. Six animals died before or during the stent implantation procedure. Five deaths were due to ventricular fibrillation. The five deaths occurred during coronary abrasion (one animal), PTCA before stenting (two animals), and stenting procedure (two animals). Thrombosis was not observed in the two animals that died during stent implantation. One animal died suddenly 3 weeks after abrasion. Thus, 26 animals were successfully stented; all 26 survived to follow-up angiography and sacrifice.

Cholesterol

Twenty-six pigs were maintained on the atherogenic diet throughout the study. Four to five months after endothelial abrasion, the serum cholesterol level was determined at the time of stent implantation. The cholesterol level was again determined 1 month later at the time of death. The average of these two serum cholesterol determinations was cal-
TABLE 1. Angiographic Data

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Group A (n=8), antiplatelet; group B (n=9), antiplatelet plus anticoagulant; group C (n=9), control.

Angiography

At follow-up, angiography revealed a patent stented LAD in all 26 animals. Table 1 lists these measurements as well as adjuvant therapy groups and calculated for each animal. Overall, the average cholesterol level was 538 mg% (range, 326–1,020 mg%). There was no significant difference in average cholesterol levels among therapy groups (Table 1).
nominal stent sizes. The data are graphically illustrated in Figure 1.

All of the stented segments showed a reduction of luminal diameter at 1 month. The average reduction in diameter was 23% (range, 5–49%). There were no significant differences in lumen reduction among treatment groups. No relation was found between stent luminal reduction and average cholesterol level or stent-to-artery ratio (stent sizing).

Histologic and Morphometric Analyses

Light microscopy revealed marked proliferation of smooth muscle cells within the intima overlying stent struts (Figure 2A). Intimal thickening also extended around the circumference of the stented arterial segment, but this thickening tended to be minor compared with that specifically overlying the struts. Each lesion in the stented segment showed a well-developed fibrous cap of circumferentially oriented spindle-shaped (smooth muscle) cells. Foam cells, variable in number, were always found at the lesion base adjacent to the strut; some arteries contained extensive foam cell infiltrates. In some of the larger lesions, a loosely organized but highly cellular connective tissue stroma was present and contained scattered foam cells. Collagen deposition was evident on Masson’s trichrome–stained sections (not shown). The internal elastic lamina typically showed discontinuity as it approached the strut region. When recognizable near the strut, the internal elastic lamina always deviated outward to lie external to the strut. The medial layer of the strut was generally atrophic. Several lesions showed neovascularization and sometimes intramural hemorrhage around struts, extending into the neointima (Figure 2B). The adventitial layer over stented segments exhibited an increase in cellularity and vascularity.

Cross sections of nonstented coronary arteries and the LAD proximal and distal to the stent revealed atherosclerotic lesions with dense accumulations of foam cells and variable numbers of smooth muscle cells (Figures 3A and 3B). Lesions generally did not have a fibrous cap. In contrast to the stented segments, intimal thickenings composed predominantly of smooth muscle cells were uncommon in these areas. The foam cell lesions were most marked in proximal and ostial coronary segments and tended to be minimal or absent in distal segments.

Morphometric analysis was done in 13 animals. Luminal percent area stenosis measured in the segments proximal to, within, and distal to the stent showed the greatest reduction of luminal area within the stent. For all animals so studied, the mean area stenosis within the stent was 39.3% (range, 23–81%). Two animals (15%) had area stenoses of more than 50%, whereas nine other animals (69%) had stenoses of 25–49%. Sections proximal and distal to the stent were more mildly narrowed. Mean area stenosis in the proximal segments was 17.9% (range, 7–62%) and in the distal segments was 5.7% (range, 2–18%). There were no significant differences among treatment groups (Figure 4).

Electron Microscopy

Scanning electron microscopy of the luminal surfaces of 30-day stented specimens from all treatment groups revealed a confluent, periluminal cell layer of endothelial-like morphology in most specimens (Figure 5A). These cells had a predominantly flattened, elongated shape with the longitudinal axis parallel to the direction of blood flow. However, there was a prevalence of pathological abnormalities at both cellular and subcellular levels (Figure 5B); these included adherent microthrombi (primarily fibrin,
with sparse erythrocytes and platelets) and leukocytes as well as periluminal cell ultrastructural abnormalities such as stellate morphology, elaborate or attenuated cell junctions, and appearance of surface microvilli, banding, and craters or pits.

Transmission electron microscopy and scanning transmission electron microscopy showed elaborate cell junctions and pinocytic vesicles in periluminal cells and confirmed the scanning electron microscopic impression of generally intact endothelium (Figure 6). With the neointimal thickening, foam cell macrophages, identified by cytoplasmic droplets, lysosomes, and absence of a basement membrane, were observed as well as numerous modified smooth muscle cells that occasionally showed lipid accumulation (Figure 7). The abundant extracellular matrix contained collagen and other components as well as occasional lipid droplets.

**Discussion**

It is unclear which animal model best approximates the human response to stenting.†2–14 Roubin and Robinson†2 used the canine model to investigate adjuvant therapy after implantation of flexible coil stents. They found that the dogs treated with an antiplatelet regimen did better than those on anticoagulation therapy or controls. Rodgers et al†3 found no stent thrombosis in either aspirin-treated or control animals in the normal (nonatherosclerotic) swine model. The absence of atherosclerosis is a probable limitation of these models.

In several respects, the atherosclerotic swine model should be an ideal test of stent patency. First, the coronary anatomy and dimensions (2.5–3.0 mm) of miniature (30–40 kg) swine approximates that of humans.†6 Second, swine develop a humanlike collag enous and partially calcified atherosclerosis.†6–9 Third, Shimakowa et al†6,17 have shown that the endothelium overlying the atherosclerotic lesions in this model is abnormal (at least in regard to release of endothelial-derived relaxing factor). Fourth, PTCA precedes stenting in this protocol, as it would in humans. Intimal injury due to angioplasty and subsequent platelet deposition has been studied in the swine model.†18–22 Finally, if coronary spasm distal to the stent and subsequent thrombosis is a potential mechanism of vessel closure, the swine’s propensity for spasm would certainly be advantageous.

The particular adjuvant regimens used in this study were chosen to simulate those used clinically. Others have noted that swine platelets and coagulation system are similar to those of humans, particularly in their response to anticoagulants and aspirin.†18–23 The “therapeutic” range of prothrombin time (15–18 seconds) was determined in a pilot study.†11 When a

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**Figure 3.** Panel A: Segment of left anterior descending coronary artery distal to stent in an atherosclerotic pig from control group. Focally thickened intima is present at a branch vessel orifice (original magnification, ×27). Bar, 500 μm. Panel B: Magnified view of atherosclerotic lesion shown in panel A. Intimal lesion contains mixed population of foam cells and smooth muscle cells. Note internal elastic lamina (arrow), medial layer (m), and orifice of small branch vessel (†) (original magnification, ×138). Bar, 100 μm. Sections stained with Verhoef-Van Giesen stain.

**Figure 4.** Bar graphs of morphometric analyses of cross sections of left anterior descending coronary artery proximal to, within, and distal to the stent shows no significant difference in luminal percent area stenosis among treatment groups.
FIGURE 5. Panel A: Scanning electron microscopy of luminal surface of segment of left anterior descending coronary artery in an atherosclerotic pig from antiplatelet treatment group. Endothelial cells are elongated in direction of blood flow, which is from left to right (original magnification, ×1,500). Bar, 10 μm. Panel B: Scanning electron microscopy of luminal surface of stented segment of left anterior descending coronary artery from an atherosclerotic pig from antiplatelet plus anticoagulant treatment group. This view shows at least two forms of cells adherent to surface. One form is round with an undulated or ruffled surface typical of adherent leukocytes (arrow). The other form is spread over the surface, tending to have a stellate shape (arrowhead). Cell type of spread cells on this micrograph cannot be distinguished; possibilities include monocytes, smooth muscle cells, or endothelial cells (original magnification, ×1,300). Bar, 10 μm.
FIGURE 6. Transmission electron microscopy of endothelial cells at luminal surface of segment of left anterior descending coronary artery from an atherosclerotic pig from control group. An intact monolayer of endothelial cells is confirmed by presence of complex interdigitating junctions (arrow) and pinocytic vesicles (original magnification, ×10,000). Bar, 1 μm.

FIGURE 7. Transmission electron microscopy of stented segment of left anterior descending coronary artery below endothelial layer. A foam cell (FC), possibly of macrophage origin, is present. Below foam cell are several smooth muscle cells (SMC) identified on the basis of myofilaments and a partially surrounding basement membrane. Several of these cells also contain lipid droplets (original magnification, ×2,900). Bar, 5 μm.
prothrombin time of more than 20 seconds was achieved, fatal hemorrhage often resulted. The aspirin dosage in this study is similar to that of clinical low-dose therapy. Lam et al.18 have observed a significant decrease in platelet deposition after angioplasty on aspirin 1 mg/kg/day in the swine model. Indeed, Badimon et al.18,19 and Steele et al.20 have extensively investigated platelet aggregation after intimal injury in this model to elucidate the mechanism of angioplasty.

In the present study, we found no occlusive thrombosis or abrupt closure in the stented coronaries of atherosclerotic swine, regardless of adjuvant therapy. Although all stents remained patent, distinct luminal narrowing of the stents was observed at 1 month follow-up. No difference in luminal reduction was seen between treated and untreated groups. All stented specimens had a similar histological appearance characterized by neointimal hyperplasia, predominantly due to marked smooth muscle cell proliferation. Scanning electron microscopy and transmission electron microscopy confirmed the presence of abundant intimal smooth muscle cells covered with a confluent monolayer of endothelial-like cells. These observations were made in all treatment groups. Morphometric analysis of a portion of each treatment group corroborates the angiographic finding that luminal narrowing (although usually not hemodynamically significant) appears to occur to a similar degree in all treatment groups.

Although angiographic or histological evidence of thrombosis was not observed in any animal regardless of treatment group, we cannot exclude the possibility that nonocclusive or laminar thrombus formation did occur early after stent placement. Because the animals were not angiographically or histologically studied until 1 month after stenting, the possibility remains that early thrombosis may have played a role in the subsequent intimal proliferation.

Comparison of follow-up angiographic measurements and morphometric analysis of individual animals did not correlate precisely in each case, probably because of the disparity between these techniques. However, the conclusion that the degree of luminal reduction within the stented segment was not affected by either treatment group or cholesterol level was drawn from both analyses. In an attempt to use a different (nonstented) segment of each coronary as its own control, we morphometrically evaluated segments proximal and distal to the stent. A striking amount of intimal hyperplasia was noted within the stented segment compared with segments proximal and distal to the device. The implication is that the stent itself induces or promotes smooth muscle cell proliferation. This comparison, however, is not pure as we cannot determine whether these sites proximal and distal to the stent were injured to the same extent by the endothelial abrasion procedure and PTCA. Progression of this artificially induced atherosclerosis compared with that of stent-induced smooth muscle proliferation remains an issue in this incompletely controlled model.

Clinical trials conducted by Sigwart et al21 with the Walstent stent and by Schatz et al22 with the Palmaz-Schatz stent noted a significant rate of thrombosis. These investigators observed that stent thrombosis tends to occur within several days of placement, usually when systemic heparin has been discontinued. Schatz et al., in their initial trial, did not pursue anticoagulation beyond 4 or 5 days of systemic heparin after stenting. Only prolonged antiplatelet therapy was used. They observed thrombosis in 11% of patients at 5–7 days after stent implantation. Since using prolonged anticoagulation with oral Coumadin in combination with antiplatelet therapy, Schatz et al.22 report that abrupt thrombosis has essentially been eliminated.

There are two possible explanations for the discrepancy between the rate of occlusive thrombosis in clinical trials of other stents and that of the present study. Perhaps the atherosclerotic swine model does not accurately simulate the human situation. Interspecies comparisons of rates of endothelialization of vascular prostheses have shown that swine endothelialize synthetic grafts more rapidly than humans.24 Within approximately 10 days, a largely nonthrombogenic cellular layer overlies the stent in swine.

An alternative is that the particular design of the flexible coil stent is less thrombogenic than the tubular mesh configuration of the Walstent or Palmaz-Schatz stent, which have undergone clinical testing in humans. A trial to compare these stents in the same model under identical circumstances would be necessary to fully investigate the relative thrombogenicities of these stents.

Conclusion

Implantation of stents within the coronary arteries of atherosclerotic swine results in a patent but partially narrowed vessel at 1 month follow-up. Adjuvant therapy does not seem to affect either patency or luminal narrowing in this model. Further investigation will be necessary to determine the factors that influence stent luminal narrowing in atherosclerotic swine.

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