Intra-Arterial Stenting in the Atherosclerotic Rabbit

Keith A. Robinson, PhD, Gary S. Roubin, MB, BS, PhD, Robert J. Siegel, MD, Alexander J. Black, MB, BS, Robert P. Apkarian, MA, and Spencer B. King III, MD

The major problem associated with percutaneous transluminal coronary angioplasty is recurrence of the stenotic lesion. Balloon catheter-mounted intracoronary stent devices may reduce restenosis by improving luminal morphology and flow characteristics. This study assessed the effects of a stainless steel wire, interdigitating coil stent on restenosis in the atherosclerotic rabbit model. Fifteen cholesterol-fed rabbits with preexisting iliac arterial lesions induced by balloon deendothelialization were instrumented in one iliac artery with a 2.0-mm diameter stent after balloon dilatation; the contralateral iliac lesion was treated by dilatation only to serve as a control. The animals were given heparin with aspirin (60 mg) and aspirin every 3rd day until death. Arteriography was repeated 4 weeks after stenting, just before death. Tissue sections from stented and control arterial segments were analyzed morphometrically. Stented arteries had a significantly larger luminal diameter at restudy, whether measured by arteriography (1.38 ± 0.19 vs. 0.94 ± 0.35 mm, p < 0.01) or from tissue sections (1.26 ± 0.18 vs. 0.81 ± 0.30 mm, p = 0.0001). Wall thickness of the stented segment was slightly, but significantly, less than the control segment (436 ± 143 vs. 532 ± 221 μm, p < 0.05). Scanning electron microscopy of five stented atherosclerotic rabbit aortas revealed regeneration of a nonthrombogenic, confluent, flow-directed endothelium by 4 weeks after placement. Intra-arterial stenting may be of benefit in the prevention of restenosis by the preservation of a larger functional lumen and by a decrease in the neointimal hyperplastic response to arterial injury. (Circulation 1988;78: 646–653)

Restenosis after percutaneous transluminal coronary angioplasty occurs in approximately 30% of patients and represents the major obstacle to long-term success of the procedure. Various pharmacological interventions have been tried, but to date none has been effective in humans. Restenosis results from proliferation of neointimal smooth muscle and fibroblastic cells under the influence of platelet, leukocyte, and vessel wall-derived mitogens. A variety of factors appear to modulate this heterogeneous response to balloon catheter injury, and these have been identified clinically. Of importance has been the finding that optimal angiographic and hemodynamic results minimize the risk of restenosis. By promoting laminar blood flow and by reducing areas of turbulence and stasis, response of the damaged intima and media to platelets and other blood elements may be reduced.

Clinical studies with a tubular mesh stent device have demonstrated improved angiographic and hemodynamic results and an apparent reduction in restenosis. A laboratory study with a different mesh stent design in atherosclerotic rabbit aortas has shown preservation of the arterial lumen in the region of the stent. In that study, atherosclerotic increases in wall thickness were restricted to the vessel wall external to the stent.

Recent studies have demonstrated the technical usefulness of a balloon catheter-mounted, coil stent device for application during percutaneous transluminal coronary angioplasty. The histological effects and long-term patency in nonatherosclerotic coronary arteries of the dog have been established. The present study was undertaken to determine the effect of this stent on arterial patency and intimal proliferation after balloon dilatation of atherosclerotic rabbit arteries.
Materials and Methods

Animal Preparation

Twenty New Zealand White rabbits (2–3 kg) of both sexes were obtained for use in this study. These were subdivided into two groups. Fifteen animals received balloon angioplasty and stent placement in one iliac artery and angioplasty alone in the contralateral artery; five animals were instrumented with stents in the infrarenal aorta for scanning electron microscopy. All experimentation and animal handling was conducted in such a manner as to minimize stress and discomfort to the animals, and the protocol was approved by the Institutional Animal Care and Use Committee of Emory University, Atlanta, Georgia. The rabbits were fed a diet of 2% cholesterol, 10% peanut oil–enriched rabbit chow for the duration of the study. One week after beginning the diet, bilateral focal endothelial denudation of the external iliac arteries was performed as follows: anesthesia was achieved with intramuscular injection of ketamine hydrochloride and xylazine, and both inguinal areas were prepared for surgery. Bilateral femoral arteriotomies were performed, and a 3F embolectomy catheter was advanced retrogradely under fluoroscopic guidance to an area within the external iliac artery, ascertained by anatomic landmarks. The balloon was inflated with 0.1–0.2-ml 70% saline and 30% meglumine diatrizoate contrast medium mixture; it was withdrawn antegradeley approximately 10 mm and deflated. This procedure was repeated five times in the same segment in each artery to ensure complete endothelial desquamation. The catheter was then removed, the femoral arteries were ligated, the incisions were closed, and 50,000 units penicillin was administered intramuscularly. This procedure resulted in the formation of focal bilateral stenoses after a 6-week maturation period (Figure 1A).

Instrumentation

Under ketamine and xylazine anesthesia and aseptic conditions, a right carotid arteriotomy was performed, and a 5F vascular sheath introduced and anchored with ligatures. Heparin (500 units) was given intra-arterially, and a 4F pediatric Swan-Ganz catheter was then advanced under fluoroscopic guidance to the abdominal aorta. Aspirin (60 mg) was then administered intra-arterially. The solution was prepared by crushing aspirin tablets, dissolving them in Ringer’s solution, and adjusting the pH to 7.4 with sodium bicarbonate. An arteriogram was taken on 35-mm cineradiographic film; a 3-ml injection of meglumine diatrizoate served as contrast medium. A 3-mm grid was positioned beneath the animal to identify the stenotic segments and for subsequent arteriographic measurements. A 0.014-in. guidewire was then introduced through the catheter, the catheter was removed, and a 2.0-mm diameter, 12-mm length balloon dilatation catheter was advanced to the sites of the stenoses. The

**FIGURE 1. Iliac arteriograms. Panel A: Before dilatation; arrowheads indicate measurement grid. Panel B: Immediately after dilatation and after stenting. Stent is visible in left iliac artery (arrowheads). Panel C: Restudy at 4 weeks.**
TABLE 1. Arteriographic Results

<table>
<thead>
<tr>
<th>No.</th>
<th>Control</th>
<th>Stented</th>
<th>Control</th>
<th>Stented</th>
<th>Control</th>
<th>Stented</th>
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<td>57</td>
<td>1.11</td>
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<td>62</td>
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<td>64</td>
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<td>1.02</td>
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<td>1.48</td>
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<td>17</td>
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<td>19</td>
<td>0.98</td>
<td>0.99</td>
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<td>1.37</td>
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<td>1.51</td>
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<tr>
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<td>28A</td>
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<td>1.33</td>
<td>1.30</td>
<td>2.17</td>
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<tr>
<td>Mean ± SD</td>
<td>0.97 ± 0.18</td>
<td>1.04 ± 0.24</td>
<td>1.07 ± 0.35</td>
<td>1.70 ± 0.26</td>
<td>0.94 ± 0.35</td>
<td>1.38 ± 0.19</td>
</tr>
</tbody>
</table>

\( p < 0.001 \quad \text{NS} \quad <0.01 \)

All values are mean; \( n = 15 \) rabbits.

*Technical radiographic problems precluded arteriographic measurements.

The balloon was inflated three times to 5, 6, and 8 atmospheres, or until a favorable angiographic result was achieved, with a 50% saline and 50% contrast mixture. One artery was then randomized to receive stent instrumentation; the balloon catheter was removed, and a 2.0-mm diameter, 15- or 20-mm length stent placement catheter (Cook) was advanced to the site selected. The balloon was inflated once to 10 atmospheres with a 50% saline and 50% contrast mixture for 45–60 seconds; it was then deflated, and negative pressure was held for 45–60 seconds. The catheter was then pushed forward slightly to disengage the stent, and withdrawn slowly, leaving the stent in place. Arterial vasoconstriction, when present, was treated with lidocaine. A postdilatation arteriogram was obtained, the catheters were removed, and the carotid artery was repaired or ligated. The surgical incision was closed, and 50,000 units penicillin was given intramuscularly. The rabbits continued to receive 60 mg aspirin by injection in an ear vein every 3rd day until death.

Restudy and Tissue Preparation

Four weeks after angioplasty, each rabbit was anesthetized with ketamine and xylazine, a left carotid arteriotomy was performed, and a 4F Swan-Ganz catheter was advanced to the abdominal aorta. A restudy arteriogram was obtained, and the animal was killed with an intra-arterial injection of 5 ml sodium pentobarbital (65 mg/ml). The treated vasculature was perfusion fixed for 15 minutes with 10% buffered formalin at 100 mm Hg of pressure through a cannula in the abdominal aorta with efflux through the inferior vena cava. The vessels were excised and immersed in formalin for at least 48 hours. The stent wires were then carefully removed, the treated segments were embedded in paraffin, and three sections were obtained from each segment. The sections were stained with hematoxylin and eosin.

Arteriography and Morphometry

Predilatation, postdilatation, and restudy arteriograms of the treated segments were measured on a Siemens projector by an experienced angiographer using digital electronic calipers and corrected to absolute luminal diameters by the 3-mm grid as an internal calibration standard. A mean luminal diameter was then calculated from at least three serial measurements for each treated segment for each time period.

For morphometric evaluation of arterial sections, three wall thickness (external lamina to lumen) and two luminal diameter measurements were obtained for each section with an ocular micrometer. Wall thickness measurements were made from areas adjacent to sites of stent wire entrenchment; one measurement was made at the thinnest portion of the wall, one at the thickest portion, and one at an area of intermediate thickening. For eccentric lumina, diameter measurements represent the smallest and largest diameters.

Scanning Electron Microscopy of Stented Aortas

Because the diameters of the iliac arteries were too small to permit cutting without extensive damage to the specimens, five rabbits underwent cholesterol feeding and balloon deendothelialization of the infrarenal aorta for scanning electron micros-
copy of stent endothelialization. This procedure did not result in the formation of aortic stenoses; some aortas showed a mild ectasia of the balloon-injured site. Stents (3.0 or 3.3 mm diameter, 20 mm length) were placed by carotid arteriotomy at these sites 6 weeks after the procedure; heparin (500 units) and aspirin (60 mg) were given intra-arterially before stenting. The animals were killed at 10 minutes, 1 day, and 1, 2, and 4 weeks after stenting. A 5-minute flush of the treated aortic segments with oxygenated, heparinized cacodylate buffer at 100 mm Hg and 37° C was performed. The vessels were then perfusion fixed in situ at 100 mm Hg with oxygenated 2.5% glutaraldehyde buffered with 0.1 M cacodylate (pH 7.4) at 37° C for 15 minutes. The segments were excised, immersed overnight in glutaraldehyde, and cut longitudinally to expose the luminal surfaces. They were then dehydrated in graded ethanol baths to 100%, critical-point dried from liquid CO₂, mounted on aluminum stubs, and sputter-coated with 10 nm gold and palladium alloy. The specimens were observed on the lower stage of an International Scientific Instruments DS-130 Scanning Electron Microscope in the secondary electron scanning mode, and a morphological description of thrombus formation and endothelial regeneration was documented.

Statistical Analysis

Comparison of immediate postdilatation and restudy lumen diameter of stented and control segments, obtained from arteriography, was performed with paired t tests. Morphometric histological measurements were analyzed by analysis of variance with repeated measures on a Vax 11/750 computer with BMDP4V statistical software. Data are pre-

Table 3. Histological Wall Thickness Results

<table>
<thead>
<tr>
<th>No.</th>
<th>Morphometric wall thickness (μm)</th>
<th>Stented</th>
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<td>62</td>
<td>540</td>
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<tr>
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<td>557</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>532 ± 221</td>
<td>436 ± 143</td>
</tr>
</tbody>
</table>

All values are mean; n = 15 rabbits.

Figure 2. Plot of arteriographic measurements at predilatation, postdilatation, and 4-week restudy (mean±SD).

Figure 3. Bar graphs of morphometric measurements from tissue sections. Panel A: Wall thickness measurements (mean±SD). Panel B: Lumen diameter measurements (mean±SD).
sented as mean ± SD; a p value of less than 0.05 was considered statistically significant.

Results

Arteriography

Table 1 shows the arteriographic results of stenting and control before dilatation, immediately after dilatation, and at a 4-week restudy. Luminal diameters before dilatation were similar (1.04 ± 0.24 mm for the stented artery and 0.97 ± 0.18 mm for the control). Diameters after dilatation were significantly different (1.70 ± 0.26 mm for the stented artery and 1.07 ± 0.35 mm for the control; p < 0.001). This difference was maintained at a 4-wk restudy (1.38 ± 0.19 mm for the stented artery and 0.94 ± 0.35 mm for the control; p < 0.01; Figure 2).

Morphometric Histology

Tables 2 and 3 show the morphometric histological results of stenting and control dilatation. Analysis of variance revealed significant overall variability in wall thickness and lumen diameter. However, lumen diameter of the stented artery measured from tissue sections was significantly greater than control (1.26 ± 0.18 mm vs. 0.81 ± 0.30 mm; p = 0.0001; Figure 3B). Wall thickness was significantly less for the stented artery compared with the control (436 ± 143 μm vs. 532 ± 221 μm; p < 0.05; Figure 3A).

Scanning Electron Microscopy

Examination of the aorta fixed 10 minutes after placement (with the coil removed) showed partial endothelial desquamation and the appearance of trenches that extended into the neointimal fibrocellular layer in which the wires had been embedded (Figure 5A). Adherent platelets and leukocytes were observed on exposed elastica and neointima.

One day after stenting, a regular array of endothelial cells and pseudoendothelial cells was observed along the exposed wires (Figure 5B). Individual cells were adherent to the metallic surface by pseudopodial extensions (Figure 5C). Scattered, adherent microthrombi and leukocytes were seen primarily in zones of stent wire entrenchment. One week after stenting, a nearly continuous endothelial and pseudoendothelial cell layer was observed on the luminal surface of the aorta, which also showed scattered microthrombi. Two weeks after stenting, the luminal cell layer was confluent and flow directed. Circumferential corrugations and intracellular banding were seen in areas overlying the stent coil (Figure 5D). Scattered adherent microthrombi and areas of exposed elastica were observed. In the 4-week stented specimen, the stent wires were embedded in a neointimal cell layer (Figure 5E). The luminal cell layer was confluent and flow-directed with occasional areas of giant cell formation and macrophage invasion.

Discussion

Despite general acceptance, the atherosclerotic rabbit is limited as a model for restenosis after angioplasty. The rabbit restenosis lesion shows a substantial foam cell component, whereas human restenosis lesions demonstrate an almost exclusively fibroblastic and myofibroblastic composition. It is often impossible in this model to distinguish the original plaque from the tissue-deposited plaque after the angioplasty treatment. Accordingly, in this permissive model of atherosclerosis, it is difficult to demonstrate an effect of interventions that might theoretically reduce intimal proliferation after bal-
FIGURE 5. Scanning electron photomicrographs of stented aortas. Large arrow indicates direction of blood flow. Panel A: Stent coil has been removed leaving remnant trench (T). Specimen was fixed 10 minutes after stent placement. Note adherent red blood cells (RBC) and white blood cells (WBC), and endothelial cells (EC) outside of trench. Panel B: At 1 day after placement, endothelial and pseudoendothelial cells (EC) are on surface of stent wire. Also, note small adherent thrombi (T). Panel C: Endothelial and pseudoendothelial cells (EC) are attached to stent wire by pseudopodial extensions (P) 1 day after placement. Panel D: At 2 weeks after placement, the luminal surface over a stent wire shows intracellular banding (arrowheads), which is suggestive of cytoskeletal stress fibers. Intercellular corrugations (C) and a prominent cell nucleus (N) are also present. Panel E: At 4 weeks after placement, stent wire is embedded in arterial wall. Inset shows adherent macrophage (M) and luminal giant cell (GC). Inset bar is 20 μm.
loon dilatation. Despite these problems, stenting produced approximately a 60% improvement in initial arteriographic dimensions, and this difference was well maintained with approximately a 50% improvement in luminal diameter at a 4-week restudy. These findings were confirmed by independent morphometry of arterial sections. In addition, histological analysis demonstrated this was in part due to reduced wall thickness in the stented arteries.

Stented iliac arteries received one more high-pressure balloon inflation (during stent placement) than controls, which may itself have influenced arterial geometry and subsequent disease progression. However, because the three initial inflations in the control and stented arteries were performed at relatively high pressures with long inflation times, it seems unlikely that one additional high-pressure inflation would have any substantial effect. There is no clinical or experimental evidence suggesting that additional balloon inflations influence late outcome with respect to restenosis.

A close relation was observed between the arteriographically measured luminal diameters at restudy and diameters obtained from arterial sections. Wall thickness, rather than vessel wall area, was measured from tissue sections because of difficulties in subtracting blank areas in the sections that were previously occupied by stent wires.

Histological examination disclosed varying degrees of atheroma accumulation both in stented and control segments. This consisted of a neointimal fibrocellular proliferation with abundant foam cells, which were predominantly internal to the internal elastic lamina, but also with foam cell infiltration in the media and focal medial calcification. Stents were firmly embedded within the arterial wall, and in cross section, wires were seen at all levels of the atheroma (i.e., atheroma accumulation occurred on both sides of the stent wires). There was some tendency for the inner portion of the atheroma to be more cellular and contain fewer foam cells than the outer portion. However, this change was noted in both stented and control segments, and no clear demarcation was seen between “neointima” that was internal to stent wires and “plaque” that was external to wires as described by Palmaz et al. This may reflect divergent cellular responses to the different stent designs.

Wall thickness of the stented artery was slightly, but significantly, less than control. Decreased turbulence of blood flow, increased blood flow rate, and decreased exposure of medial smooth muscle to platelets and leukocytes by “tacking” of intimal flaps are factors that may have contributed to this apparent inhibition of the neointimal proliferative response in stented arteries. Increasing the blood flow rate by distal arteriovenous fistula has decreased neointimal proliferation of smooth muscle cells in venous autografts in dogs.

Scanning electron microscopy revealed rapid regeneration (almost complete at 2 weeks) of an endothelial and pseudoendothelial cell layer over the stent with only minimal thrombotic consequences. The 2- and 4-week specimen contained some areas of cells exhibiting features suggestive of hemodynamic or other stress, that is, intracellular banding and intercellular corrugations (Figure 5D). It remains to be determined whether these ultrastructural features of endothelial cells in stented arteries observed with scanning electron microscopy relate to cytoskeletal stress fiber expression and whether these cells possess functional aberrations such as increased permeability or altered response to vasomotor stimuli as seen by Herman et al.

In summary, intra-arterial stenting compared with standard balloon angioplasty in the aspirin-treated atherosclerotic rabbit significantly improved initial luminal dimensions and appeared to limit the progression of atherosclerosis in this model by apparently decreasing neointimal fibrocellular hyperplasia. Further study is required to more precisely determine arterial cellular responses to stenting in models that more closely approximate human coronary artery disease and restenosis.

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References


**KEY WORDS** • arterial prosthesis • intravascular stent • restenosis • angioplasty • scanning electron microscopy
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