Porous Balloon Catheters for Local Delivery: Assessment of Vascular Damage in a Rabbit Iliac Angioplasty Model

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Objectives. This experimental study assessed long-term vascular damage induced by the use of porous balloon catheters designed for local delivery.

Background. Local drug delivery using porous balloon catheters has emerged as a possible means by which compounds designed to prevent restenosis might be delivered locally at concentrations higher than achievable by systemic administration. There are, nonetheless, some concerns about the possibility of greater arterial trauma induced by the high pressure fluid jets from the delivery catheter itself, a complication that could provide additional stimulus for intimal hyperplasia. Because these concerns are based mainly on in vitro studies and on studies performed after acute experiments, further work is required to assess the long-term effects of this device on the arterial wall.

Methods. Local delivery of a saline solution was performed in 32 rabbits in one iliac artery, using an inflation pressure of 6 atm and a balloon/artery ratio of 1.3 to 1.5, followed by angioplasty in both iliac arteries. Vascular injury was assessed using tritiated thymidine incorporation at 4 days (12 rabbits) and planimetry at 30 days after the procedure (20 rabbits).

Results. Tritiated thymidine incorporation did not reveal any significant difference between the angioplasty group and the group with local delivery and angioplasty (117,921 ± 18,853 vs. 140,652 ± 23,125 cpm/mg protein, p = NS). Planimetry at 30 days was also similar in the two groups (neointimal area 0.11 ± 0.02 vs. 0.13 ± 0.02 mm²).

Conclusions. In this model the use of porous balloon catheters before angioplasty did not lead to greater intimal hyperplasia than angioplasty alone. Further experimental investigation is required to determine whether this strategy could be used to prevent postangioplasty restenosis in humans.

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Restenosis after percutaneous transluminal coronary angioplasty remains the major limitation of this procedure (1-4). In the past decade, new interventional techniques and several pharmacologic strategies aimed at reducing the risk of postangioplasty restenosis have been devised and assessed. Unfortunately, the success of these interventions has thus far been disappointing (5). One of the explanations advanced for the inability to reproduce in humans the successful pharmacologic suppression of restenosis observed in animal studies has been the lower tissue levels attainable with dosing regimens in clinical practice (6).

More recently, to overcome this problem, local drug delivery using porous balloon catheters has emerged as a possible means by which compounds designed to prevent postangioplasty intimal hyperplasia might be delivered locally at concentrations higher than achievable by systemic administration (6,7). There is, nonetheless, some concern about the possibility of greater arterial trauma induced by the high pressure fluid jets from the delivery catheter itself (8,9), a complication that could provide additional stimulus for intimal hyperplasia and thus ultimately counteract any potential beneficial effect from local drug administration. Because the concerns about greater arterial trauma are based mainly on in vitro studies and on studies of light and scanning electron microscopy performed after acute experiments, further work is required to establish to what extent these concerns are well founded. Before it is possible to evaluate potentially effective agents, more information is needed about the effects of the porous balloon catheter itself.

In this report we describe our experimental observations on the extent of vascular injury induced by the use of a porous balloon catheter in a rabbit model of intimal hyperplasia. In these studies, early changes were assessed using tritiated thymidine incorporation at 4 days, and late changes were assessed by morphometric analysis of histologic sections performed 30 days after the procedure.

Methods

Experimental model. The study protocol conformed to the guidelines of the Canadian Council on Animal Care and was reviewed by the ethical committee on animal research of our
institution. A group of 40 New Zealand white rabbits (3.0 to 3.5 kg) was chosen for the study. Under intramuscular ketamine/ xylazine anesthesia (35 and 5 mg/kg body weight, respectively), the right carotid artery was dissected free, and a 5F introducer sheath was inserted. Intraarterial heparin (100 IU/kg) was given through the side arm of the sheath. Under fluoroscopic guidance, a 4F balloon-tipped wedge pressure catheter (Arrow International Inc.) was inserted into the carotid artery and advanced into the left ventricular cavity, where it was inflated with 1.0 ml of air. The balloon-tipped catheter was then gently pulled back so that the inflated balloon could be propelled downstream into the abdominal aorta by the aortic flow. The introducer sheath was then advanced along the catheter until the sheath was positioned in the abdominal aorta, allowing easy positioning of the drug delivery catheter in one of the iliac arteries. The catheter was then deflated, and its distal tip positioned 2 to 3 cm above the aortic bifurcation. Through the proximal port of the catheter, an angiogram of the iliac arteries was performed by injecting 5.0 ml of meglumine ioxaglate-sodium ioxaglate (Hexabrix-320, Mallinckrodt Medical Inc.). The angiogram was recorded on an S-VHS videotape and reviewed on a 512-line monitor. The lumen diameter of the external iliac arteries was estimated according to the observations of Clowes et al. (10), peak DNA synthesis determined by tritiated thymidine incorporation. According to the observations of Clowes et al. (10), peak DNA synthesis after vascular injury occurs at ~4 days. For this reason, 12 animals were chosen randomly and killed for tritiated thymidine incorporation studies 4 days after vascular injury. Tritiated thymidine incorporation was also assessed in an age-matched normal control group of seven rabbits (14 iliac arteries) that had no intravascular intervention. Under intravenous sodium pentobarbital anesthesia (16 mg/kg), a 4F balloon-tipped catheter was inserted into the carotid artery and advanced to the level of the aortic bifurcation, as described earlier, and an angiogram was performed to assess vascular damage, such as stenosis or thrombosis. Iliac arteries were then dissected, and dilated segments were identified according to the videotape image reference. A central portion of the dilated segments (1 cm) was quickly recovered and kept in cold Gey's segments (1 cm) was quickly recovered and kept in cold Gey's fat, and the rabbit was killed with an intravenous sodium pentobarbital dose of 80 mg/kg. Specimens were cut into three vascular rings of similar length (3 mm), which were weighed and transferred to sterile tubes containing 0.5 ml of Roswell Park Memorial Institute (RPMI) culture medium. Vascular rings were incubated with tritiated thymidine (4 μl, specific activity 89 Ci/mmol, Amersham) at 37°C for 3 h. The rings were then washed four times with 0.5 ml of Gey's medium and subsequently incubated for 1 h at 37°C in culture medium. At the end of the incubation period, specimens were digested in 0.6 ml of 1 N sodium hydroxide at 65°C for 12 h and then neutralized with 0.72 ml of 1 N hydrogen chloride. To standardize radioactivity for every specimen, protein dosage was performed using spectrophotometry. Standard curves were obtained from a solution containing known concentrations of protein (bovine serum albumin 1 mg/ml). Radioactivity was measured by a Beckmann beta-scintillation counter, and results were expressed ascpm/mg protein.

Long-term increase in smooth muscle cell proliferation: morphometric analysis. The remaining 20 rabbits were sacrificed at 30 ± 1 days. After angiography, in situ formalin fixation at 100 mm Hg was performed, and a central portion ~1 cm was taken for histologic studies. Specimens were processed for light microscopy using Verhoff staining. Photographs 5 × 7 in. (12.7 × 17.8 cm) were taken, and planimetry was performed using a Hewlett-Packard digitizing table. Lumen area as well as areas delineated by the external elastic lamina and the internal elastic lamina were measured to determine medial and neointimal areas. The percent of neointima on total vessel area was obtained by dividing the neointimal area by the area delineated by the external elastic lamina. The percent of obstruction was calculated by dividing the neointimal area by the area delineated by the internal elastic lamina. For each specimen, planimetry was performed on three different histologic sections taken at 2- to 3-mm intervals, and the mean of the three
sections was calculated. Values were corrected for magnification factor.

Statistics. Sample size was determined to detect a 25% difference between groups, assuming an intragroup variability of 50%, alpha 0.05 and beta 0.20. For tritiated thymidine incorporation studies, the three groups of vascular rings were compared using one-way analysis of variance, and the differences between pairs of means were compared by Tukey’s honestly significant difference (HSD) test. Differences were considered significant at p < 0.05. All data are expressed as mean value ± SEM.

Results

Angiographic assessment of vascular trauma. On the angiogram performed immediately after local delivery of saline solution and bilateral angioplasty (38 rabbits), there was no evidence of vessel perforation or arterial dissection in any of the rabbits. Delayed flow was observed in one rabbit, but this was partially restored with intraarterial nitroglycerin administration. On follow-up angiogram at 4 or 30 days, according to the protocol (32 rabbits), no vascular occlusion was found, either on the angioplasty control side or on the side with local delivery followed by angioplasty. In nearly all cases, lumen irregularities could be visualized on both sides, but no angiographically significant stenosis was found, and distal flow was normal in every animal.

Assessment of smooth muscle cell proliferation after vascular injury. Subacute increase in smooth muscle cell DNA synthesis measured as tritiated thymidine incorporation. Figure 1 shows the results of tritiated thymidine incorporation in three subgroups: 1) vascular segments without any vascular intervention (n = 40 vascular rings); 2) vascular segments with angioplasty alone (n = 34); and 3) vascular segments with local delivery of normal saline solution followed by angioplasty (n = 35). Normal control iliac rings had less incorporation than the group with angioplasty only (57.284 ± 2580 vs. 117.921 ± 18,853 cpm/mg protein, p < 0.05) or the group with local delivery followed by angioplasty (57.284 ± 2580 vs. 140,652 ± 23,125 cpm/mg protein, p < 0.01). No difference could be found between the angioplasty group and the group with local delivery and angioplasty.

Long-term increase in smooth muscle cell proliferation, morphometric analysis. Microscopic examination and planimetry at 30 days did not reveal any difference between angioplasty groups. Special attention was given to the medial layer in the group with local delivery and angioplasty, but no specific healing pattern, such as healed craters or transmural disruption, was found.

Planimetry revealed no difference between groups. Measurements included the lumen and neointimal and medial areas, the percent of total vessel area with neointima and the percent of lumen obstruction (Table 1). Figure 2 shows an example of intimal hyperplasia observed 30 days after the procedure.

Discussion

This experimental study demonstrates that local delivery of saline solution into the arterial wall with a porous balloon catheter before angioplasty does not lead to measurably greater mechanical trauma nor to greater reactive intimal hyperplasia. These findings suggest that local drug delivery by this method can be attempted to prevent postangioplasty restenosis, without the method of delivery negating any beneficial effects of the drug itself.

Acute vascular trauma. Previous experimental observations have raised some concerns about the additional trauma that may be induced by porous balloon catheters (8). In those studies, light microscopy revealed endothelial denudation and nonspecific medial changes, and scanning electron microscopy showed crater formation with medial penetration corresponding to jet effects of the balloon pores. Although scanning electron microscopy was not performed in our study, an iliac angiogram could be obtained in 38 rabbits immediately after the procedure. There was no evidence of vessel perforation or arterial dissection in any of the rabbits.

Table 1. Results of Morphometric Analysis (planimetry) 30 Days After the Procedure

<table>
<thead>
<tr>
<th></th>
<th>Angioplasty Only (n = 20)</th>
<th>Local Delivery + Angioplasty (n = 20)</th>
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<tbody>
<tr>
<td>Lumen (mm²)</td>
<td>0.62 ± 0.06</td>
<td>0.68 ± 0.08</td>
</tr>
<tr>
<td>Hyperplasia (mm²)</td>
<td>0.11 ± 0.02</td>
<td>0.13 ± 0.02</td>
</tr>
<tr>
<td>Media</td>
<td>0.29 ± 0.01</td>
<td>0.28 ± 0.01</td>
</tr>
<tr>
<td>% Total vessel</td>
<td>11.3 ± 2.4</td>
<td>12.0 ± 1.9</td>
</tr>
<tr>
<td>% Obstruction</td>
<td>17.8 ± 4.1</td>
<td>18.4 ± 3.3</td>
</tr>
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Data presented are mean value ± SEM. % Obstruction = hyperplasia area/area delineated by the internal elastic lamina × 100; % Total vessel = hyperplasia area/total vessel area (delineated by external elastic lamina) × 100.
Early changes. According to the observations of Clowes et al. (10), the peak DNA synthetic activity after vascular trauma in rat carotid arteries occurs 2 to 4 days after injury. DNA synthetic activity continues at high rates for 4 to 7 days and then markedly slows from day 14 to 30. In our rabbit model, tritiated thymidine incorporation studies were undertaken 4 days after injury. Our results showed that DNA synthetic activity after delivery of normal saline solution and angioplasty was comparable to that after angioplasty alone, suggesting that trauma to the arterial wall was not increased by the use of a porous balloon catheter. The magnitude of the scintillation counts in our study was similar to that reported by Asotra et al. (11) in an experimental study on abdominal aortic angioplasty in rabbits.

Long-term changes. Morphometric analysis of histologic sections obtained 30 days after the procedure showed a similar degree of intimal hyperplasia in both groups. These findings are in accordance with the early changes observed with tritiated thymidine incorporation, suggesting again that the use of a porous balloon catheter did not produce more vascular injury than conventional angioplasty alone. The degree of intimal hyperplasia observed in this experimental study was comparable to the amount reported by other investigators using similar experimental models (12–14).

Study limitations. As in most experimental studies, the major limitation of this study is related to the animal model being used. Although the normocholesterolemic rabbit iliac angioplasty model produces considerable smooth muscle cell proliferation, it is well recognized that this response may be insufficient to create obstructive atherosclerotic lesions (15). Thus, the implications of the results of this experimental study on the clinical situation are uncertain, and extrapolation of our findings to atherosclerotic lesions in humans should be done with caution. Nevertheless, the drug delivery concept constitutes a combined mechanical and pharmacologic approach that appears safe and promising as a preventive strategy against restenosis and deserves further experimental investigation.

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References