Role of the Endothelial Lining in Recurrences After Coil Embolization
Prevention of Recanalization by Endothelial Denudation

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Background and Purpose—Endovascular treatment can improve the outcome of patients treated for ruptured intracranial aneurysms as compared with surgical clipping, but angiographic recurrences are frequent. Endothelial denudation before coil embolization may prevent recanalization and improve results of endovascular treatment.

Methods—We compared angiographic and pathological results 3 months after coil occlusion of paired canine arteries (n/H11005 16), with or without previous denudation of the endothelial lining using an endovascular device. The technique was then used to denude the neck of carotid venous pouch bifurcation aneurysms before coil embolization in 8 dogs, and the angiographic evolution at 12 weeks was compared with 7 control aneurysms treated by coiling only. Qualitative scoring systems were used to compare angiographic results with time and neointimal coverage at the neck of aneurysm after necropsy. The evolution of angiographic scores was analyzed using Wilcoxon signed rank tests whereas angiographic and neointimal scores of the 2 groups were compared using the Mann–Whitney test.

Results—All arteries embolized with platinum coils recanalized, whereas most arteries (12/16 or 75%) denuded before coil embolization remained occluded at 3 and 12 weeks (P/H11021 0.001). Aneurysms treated with coils without previous denudation tended to recur, with angiographic scores significantly worse at 12 weeks as compared with T0 (P/H11005 0.015). Median angiographic and neointimal scores were significantly better at 12 weeks with endothelial denudation (P/H11005 0.011 and 0.026, respectively).

Conclusion—Endothelial denudation can prevent recanalization after coil embolization. (Stroke. 2004;35:1471-1475.)

Key Words: aneurysm ■ embolization ■ endothelium ■ animal models ■ recurrences

Coil embolization of ruptured intracranial aneurysms leads to a better clinical outcome than surgical clipping.1 Unfortunately, this endovascular approach is less definitive, as shown by a high incidence of angiographic recurrences.2 Although mechanisms responsible for clinical recurrences have not been determined, recanalization routinely occurs after coil embolization in certain animal models.3–6 Coil embolization leads to occlusion through the formation of thrombus, and this thrombus is recanalized through a cellular process that can be inhibited by in situ beta radiation.3,4 We have previously shown that the endothelial lining of the aneurysmal wall is necessary for the persistence of residual necks as well as for the development of recurrences after sponge embolization of canine experimental aneurysms.7 We have also developed canine bifurcation aneurysm models that have a tendency to recur 3 months after coil embolization.5,6 The goal of the present work was to study the effects of mechanically disrupting the endothelial layer with an endovascular device immediately before coil deposition, first in canine arteries, then in bifurcation aneurysms, in an effort to prevent recanalization and recurrences after coil embolization.

Materials and Methods

Animal Models
Protocols were approved by the Institutional Animal Care Committee in accordance with guidelines of the Canadian Council on Animal Care. All procedures were performed under general anesthesia. Twenty-seven beagles weighing 10 to 15 kg were sedated with acepromazine (0.1 mg/kg), glycopyrrolate (0.01 mg/kg), and butorphanol (0.1 mg/kg), and anesthetized with intravenous thiopental (15 mg/kg). Animals were ventilated artificially and maintained under anesthesia with 2% isoflurane. Postoperative analgesia was provided for 3 days by a 50-µg Fentanyl skin patch.

Arterial Occlusion Model
The arterial occlusion model has previously been described.3,4 Briefly, a percutaneous femoral puncture was used to reach bilateral maxillary or vertebral arteries with 2-French microcatheters (Excel- sior; Target Therapeutics). We have previously shown that single-coil occlusion of vertebral or maxillary arteries routinely leads to thrombosis by 1 hour, followed by recanalization by 2 weeks.3,4

Received November 24, 2003; final revision received January 21, 2004; accepted February 9, 2004.
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© 2004 American Heart Association, Inc.
Stroke is available at http://www.strokeaha.org DOI: 10.1161/01.STR.0000126042.76153.f7

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Angiographic and pathological findings are identical, whether arteries are maxillary or vertebral. A 3-mm-caliber 0.015 platinum coil 8 cm in length (Target Therapeutics) was implanted into a 10-mm segment of each artery, with or without de-endothelialization. Denudation of the arterial wall was accomplished using an aneurysmal neck-bridge device (ANBD). This platinum-covered nitinol device was designed to assist coiling of wide-necked aneurysms. It is composed of 3 loops 3 mm in diameter attached by a common stem. Endothelial denudation had been verified in 6 preliminary animals by en face photography of autopsy specimens stained with Evans blue as well as by sections of normal or denuded arterial segments fixed immediately for pathological confirmation that the endothelium was intact or denuded. Typically, 5 passes with rotations were performed in each arterial segment. We studied 16 arteries that were denuded and coiled, whereas 16 arteries were coiled without denudation, and 6 were denuded with the ANBD but not coiled. Six intact arteries served as controls. Angiography was performed immediately after embolization, at 1 hour, 4 weeks, and 12 weeks, immediately before euthanization. In 2 animals arteries were studied by angiography and pathology at 10 days. Multiple projections after selective injections were interpreted in a blind fashion. Occlusion was defined as the absence of antegrade blood flow through the arterial segment. Any antegrade contrast opacification was sufficient to label the artery recanalized.

**Bifurcation Aneurysm Model**

In 1 animal, the aneurysm was constructed on a Y-type bifurcation, constructed as described. Terminal bifurcation aneurysms were constructed in 15 animals after a T-type bifurcation was created between the 2 common carotid arteries as described. Bifurcation aneurysms were measured by angiography immediately before the endovascular procedure. Embolization was performed exactly as described in 7 control animals. Briefly, after a first coil of a diameter approaching the size of the aneurysm was detached, coils of decreasing diameters were introduced to pack the lesion until complete or near-complete obliteration. In 8 aneurysms submitted to denudation before coil embolization, a 12- or 16-mm ANBD was first introduced and rotated at the level of the neck of the lesion during 1 minute before retrieval. Coiling was then performed as described, but the total length of coils introduced was voluntarily decreased as compared with controls, to prevent thrombotic complications, because clot formation and aneurysmal sac occlusion tended to occur earlier during coil introduction. Transfemoral angiography was undertaken immediately after embolization, at 3 weeks, and at 3 months. Results were scored according to a previously described classification. A score of 0 indicated complete obliteration; 1, minimal residual or recurring neck (“dog ears”); 2, a more sizable recurrent neck; 3, recurrent aneurysm; 4, large saccular recurrences.

**Macroscopic Photography and Pathology**

Macroscopic and microscopic stereophotographs of cut sections of arteries and “en face” views of the neck of aneurysms were performed using a computerized imaging system (Clemex). The carotid wall was longitudinally opened to expose the luminal surface of the neck of aneurysms. Neointima formation and recanalization at the neck of aneurysms were evaluated according to a qualitative scoring system. Neointima formation was given a score of 0 when a thick neointima completely sealed the orifice; score of 1 when the neointima was similarly sealing the neck, but small areas of recanalization were seen between the neck and the wall of the aneurysm; score of 2 when a crescent of recanalization was present around the neointima covering the coil mass; score of 3 when recanalization affected the coil mass, the neointimal covering being only partial; and score of 4 when no neointima, only thrombus, covered most of the coil mass. Arteries and aneurysms were studied after formalin fixation, axial sectioning, and staining with hematoxylin-phloxine-saffron (HPS) and Movat pentachrome stain. Immunohistochemistry served to characterize cells using antibodies to smooth muscle α-actin and factor VIII. Sections of normal or denuded arteries harvested at the time of euthanization were immunostained for factor VIII for confirmation that the endothelium was effectively intact or denuded.

After each manipulation, the ANBD was inspected and rinsed in formalin, and for each animal the cytological material was pooled and collected by centrifugation. The resulting material was embedded in paraffin, sectioned and stained with HPS, and immunostained for α-actin, CD31, CD34, and factor VIII.

**Statistics**

Angiographic results of the arterial occlusion model were compared using a 2-sided χ² test with continuity correction. Independent sample t-tests were performed to compare aneurysmal diameters, volumes, and packing densities. The evolution of angiographic scores of bifurcation aneurysms with time was analyzed using Wilcoxon signed rank tests whereas angiographic and neointimal scores of the 2 groups were compared using the Mann-Whitney test.

**Results**

**Single-Coil Arterial Occlusion Model**

All arteries embolized with platinum coils recanalized (16/16), whereas most arteries (12/16 or 75%) denuded before coil embolization remained occluded at 3 and 12 weeks (P<0.001). At postmortem photography, arteries treated with coils only were recanalized, whereas most arteries treated by denudation and coiling were occluded, as shown in Figure 1e and 1f. Arteries that were denuded but not coiled, as well as intact arteries, remain patent at all times (Figure 1c and 1d).
Arteries that were immediately fixed for inspection after endothelial denudation showed inhomogeneous erosions of the intima, fading of normal endothelial ridges, and rare lacerations (Figure 1b). In some specimens studied by histopathology, the endothelial lining, as well as in rare places, the internal elastic lamina, were removed, presumably where lacerations occurred.

Arteries studied at 10 days after coil embolization showed early endothelial coverage of the clot and well-defined endothelialized channels, whereas they could not be found in arteries treated by denudation before coil embolization (Figure 2a).

Arteries treated by coil embolization only showed partial filling with neointimal tissue, but constant recanalization (Figure 2a and 2b). Pseudopolypoid endothelialized structures were sometimes found within recanalized spaces (Figure 2b). Arteries treated by denudation and coiling and studied at 3 months were filled with vascularized connective tissue, without endothelialized recanalizing spaces (Figure 3a and 3b). Arteries that were denuded but not coiled showed minimal patchy neointimal formation at 3 months. Material was recovered from the ANBD in 25% of animals. It consisted of fibrin, red blood cells, leukocytes, and rare parietal cells, some of which stained positive for factor VIII.

### Bifurcation Aneurysm Model

Initial aneurysm dimensions, angiographic results, and neointimal scores are summarized in the Table.

There was no significant difference in diameters and neck width between the 2 groups, but aneurysmal volumes tended to be larger in lesions treated by denudation, a difference that did not reach significance ($P=0.069$). The packing density (the coil/aneurysm volume ratio) was less in lesions treated by endothelial denudation ($P=0.010$).

The first animal died of carotid thrombosis that presumably started at the Y-shaped bifurcation that was inadvertently de-endothelialized by protrusion of the ANBD outside the aneurysm during manipulations. This complication could be prevented in the 7 animals with T-type bifurcations by assuring the intraaneurysmal position of the device. None of these 7 animals showed neurological deficits during follow-up.

Aneurysms treated with coils without previous denudation tended to recur, with angiographic scores significantly worse at 12 weeks as compared with $T_0$ ($P=0.015$). Lesions treated with endothelial denudation before coil embolization were stable. There was a significant difference between median angiographic scores of the 2 groups at 12 weeks ($P=0.011$).

The median neointimal score of aneurysms treated with denudation before coil deposition was 1, a significant difference as compared with lesions treated with coils only (median score $=2$; $P=0.026$) (Figure 4).

### Discussion

We have previously shown that recanalization after coil occlusion of arteries is a cellular process that can be blocked by in situ beta radiation. The process occurs within 2 weeks after occlusion but is only possible at this early stage. If the
artery is occluded 2 to 3 weeks after embolization, it remains occluded thereafter for at least 3 months.\textsuperscript{3,4} Because pathology at 3 months consistently reveals connective tissue replacement of the lumen in arteries that were not recanализed, we propose that this process can only occur within the provisional fibrin matrix that follows coil occlusion. In this model, once this fibrin matrix is replaced with a collagenous matrix, which occurs within the first weeks, recanализation is no longer possible, at least for 3 months.\textsuperscript{12}

Recanализation is associated with early endothelial invasion of the clot (Figure 2a).\textsuperscript{4} Circulating progenitor endothelial cells have been documented and their role in physiological or pathological angiogenesis is now recognized.\textsuperscript{13} After denudation of the intima, they could have been a source of endothelial cells to repopulate the clot and promote recanализation. Although we cannot exclude a role for progenitor cells, failure of recanализation after endothelial denudation suggests that these cells cannot offer the spatial and temporal continuity necessary to effectively recanализ the lumen. One interpretation of our findings is that only cells from the original endothelial layer can migrate rapidly and form a continuous nonthrombogenic sheet to offer a connecting channel throughout the organizing clot.

The vascular wall alterations caused by the device have not been exactly defined in this study. Although the goal was to remove the endothelial lining, the resulting injury was not selective, nor have we proven that denudation was homogeneous and complete. The mechanical maneuvers may have caused deeper injuries, perhaps involving the internal elastic lamina, or medial trauma and secondary stimulation of neointima formation, as found in balloon injury models. However, the arterial occlusion model was previously tested with a combination of intraluminal stents and coils and, unless the devices were radioactive, occlusion was consistently followed by recanализation despite the trauma caused by the stent deployed by balloon inflation.\textsuperscript{4}

Reduced blood flow and shear stress, which may be consequences of coil occlusion, augment endothelial cell proliferation and increase expression of platelet-derived growth factor (PDGF)-A and PDGF-B mRNA, but decrease nitric oxide synthase, responsible for production of nitric oxide, a prototype inhibitor signal from the endothelium that may be important in vessel wall quiescence.\textsuperscript{14,15} Thus, prevention of this mechanism by endothelial denudation may also explain how this procedure led to inhibition of recanализation.

Other mechanisms have to be considered such as differences in the composition of the initial fibrin–platelet clot when the coils were deployed in a denuded artery. Activated platelets trigger an inflammatory reaction with increased endothelial expression or secretion of uPA, tPA, uPAR, MT1-MMP, MCP-1, and ICAM-1,\textsuperscript{16–18} molecules that may favor recanализation or recruitment of cells involved in recanализation after coil occlusion. Denudation of the endothelial layer before coil embolization may prevent this chain of events, and thus favor collagen replacement of the fibrin clot with permanent occlusion of arteries.

We have chosen a T-shaped, rather than a Y-shaped, model because the configuration of the bifurcation permitted denudation at the neck with lesser risks of parent vessel complications. This model, which features smaller necks, also has a tendency to recur at 3 months after coil embolization, although to a lesser degree.\textsuperscript{6,9,11} Clinical recurrences may have multiple causes, but arterial recanализation at 2 weeks and angiographic recurrences observed within 3 months in canine are reproducible mechanisms that were effectively prevented by denudation. The evolution beyond 3 months has not been tested in this study, however.

There was a difference in aneurysmal volumes that did not reach statistical significance, and a significant difference in packing densities (the ratio of total coil length on aneurysmal volume) between the 2 groups. After mechanical denudation, occlusion of aneurysms occurred earlier after the introduction of a lesser length of platinum coils. We did not attempt to reach the same packing density, for fear of causing extrusion of clot and complications.

We have previously shown that clinical recurrences were significantly more frequent with increasing aneurysm size, neck size, and incomplete initial angiographic results.\textsuperscript{2} Packing density is known to decrease with increasing size of aneurysms.\textsuperscript{20} For similar aneurysm size, neck size, and initial angiographic results, the role of different packing densities on recurrences remains to be proven. However, the discrepancy observed between the 2 groups regarding both volumes and packing densities should have favored the control group. Here, angiographic results were improved with endothelial denudation despite lesser packing densities.

Complete endothelial denudation is probably difficult to accomplish in clinical practice and may increase thromboembolic complications of the procedure. The safety of the technique cannot be assessed by this experiment alone, although neurological deficits after embolic complications...
can be detected with this model. Endothelial denudation should be limited to the lesion that will be coiled to limit potential extension of the clot inside the parent artery. The exact limits of the neck of aneurysms may not always be clear, however. In large wide-necked lesions, those with increased risks of recurrences, branches and perforators may arise at or close to the denuded area.

Other potential complications are risks of perforation or rupture, particularly in lesions treated during the acute phase after subarachnoid hemorrhage. A clinical strategy could involve a dual microcatheter technique, with fundus protection by a first coil during denudation performed through a second microcatheter. Alternatively, mechanical denudation could be restricted to elective cases treated after angiographic demonstration of a recurrence.

The use of a preexisting tool was convenient but the ANBD may not be the ideal device or technique to denude a vessel with security. The fate of endothelial cells was not determined, and very little material could be recovered from ANBDs. In clinical practice, detachment of the denuding device inside the aneurysm may be considered to minimize risks of cerebral emboli.

It is too early to contemplate a clinical application. Additional work regarding safety, particularly thromboembolic complications and risks of aneurysmal rupture, is needed. Alternate methods may include chemical or physical agents that could lead to more efficient or safer denudation to prevent recanalization of aneurysms after coil embolization. It may be judicious to wait until a more definitive technique or device is selected before testing safety in preclinical experiments.

Conclusion
Endothelial denudation before coil deposition can prevent recanalization after coil embolization of canine arteries. Angiographic scores were significantly improved 3 months after endovascular treatment of experimental bifurcation aneurysms. This proof of principle may orient the future design of new devices to apply this strategy to clinical aneurysms.

Acknowledgments
This work was supported in part by grants from the Canadian Institutes of Health Research (CIHR) and the Heart and Stroke Foundation of Canada.

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Stroke. 2004;35:1471-1475; originally published online April 22, 2004;
doi: 10.1161/01.STR.0000126042.76153.f7

Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0039-2499. Online ISSN: 1524-4628

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