Role of the Endothelial Lining in Persistence of Residual Lesions and Growth of Recurrences After Endovascular Treatment of Experimental Aneurysms

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Background and Purpose—We sought to investigate the role of the endothelial lining in aneurysmal persistence and recurrence after endovascular treatment of aneurysms.

Methods—Bilateral venous pouch canine carotid aneurysms were studied by angiography and pathology 1, 2, and 3 weeks after intraoperative collagen sponge embolization, coated or not coated with fibrinogen (n = 15). In 12 dogs the endothelial lining of one aneurysm was removed before embolization, and results were compared with a control aneurysm after bilateral sponge embolization. In 4 animals embolization with a sponge covered with endothelium was compared with treatment with a sponge covered with adventitia. In 4 animals an inverted venous pouch aneurysm embolized with an adventitia-covered sponge was compared with a normal aneurysm embolized with an endothelialized sponge. In 3 animals inverted venous pouch aneurysms were embolized with a sponge covered or not covered with endothelium. Control aneurysms (n = 3 or 4 each) included untreated normal, inverted, and de-endothelialized venous pouch aneurysms. Angiographic results at 3 weeks were compared by Wilcoxon’s test.

Results—Endothelialization of the clot that forms on the sponge was complete at 1 week, forming clefts that developed into recurrences. Reversed or de-endothelialized aneurysms spontaneously thrombosed, while normal venous pouch aneurysms remained patent for at least 3 weeks. The addition of fibrinogen, endothelium, or adventitia to the sponges did not prevent recurrences, which occurred routinely after embolization of endothelialized aneurysm. De-endothelialization of the aneurysmal wall improved angiographic results at 3 weeks (P = 0.02), while reversing the venous pouch before embolization led to complete healing (P = 0.003).

Conclusions—The endothelial lining is essential to the persistence of residual lesions. Early endothelial invasion of the clot leads to recanalization and recurrences after embolization of aneurysms. This observation provides new opportunities to improve results of endovascular treatment of aneurysms. (Stroke. 2002;33:850-855.)

Key Words: cerebral aneurysm ■ endovascular therapy ■ dogs

Intracranial aneurysms are increasingly treated by less invasive endovascular methods, mainly with Guglielmi detachable coils. One important drawback of endosaccular coiling of aneurysms is the higher incidence of residual and recurrent lesions compared with surgical clipping. Healing mechanisms or their failures are poorly understood in human aneurysms treated by coil embolization. They can be studied with the use of animal models. We have developed a lateral wall venous pouch aneurysm model that uses intraoperative collagen sponge embolization. When this model is applied to pigs, aneurysms routinely thrombose; a thick neo-intima forms at the neck, and within 3 weeks the lesion is permanently cured. When the same model is used in dogs, residual necks are frequent and persistent, and recurrences occur early. We have previously shown that vascular smooth muscle cell grafts, recovered from femoral explants, can improve the angiographic outcome and thicken the neo-intima at the neck of treated canine lesions. The neo-intima, a nonspecific vascular response to injury or foreign bodies, is composed of mesenchymal cells and extracellular matrix covered by a single layer of endothelial cells. It is fundamental to vascular healing. The endothelium itself, the biologically active inner lining of blood vessels, ensures, among many functions, a living nonthrombogenic surface that rapidly regenerates after injury. As a way to improve long-term results of endovascular treatment of aneurysms, some investigators have suggested stimulation of endothelialization, eg, with protein coatings or vascular endothelial growth factor. We propose that endothelialization after embolization may be premature. We hypothesized that the endothelium prevents
thrombosis and subsequent neointimal cell colonization and healing at the interface between the embolic agent and the aneurysmal wall. Furthermore, if thrombosis did occur, early recanalization of the clot and the formation of nonthrombogenic endothelialized clefs that remain connected to the parent artery at the neck of aneurysms might be responsible for recanalization and recurrences. We tested this hypothesis in our canine model by studying angiographic results and pathological findings after collagen sponge embolization of experimental aneurysms deprived or not of the endothelial layer. We also tested the effects of the addition of an endothelial or an adventitial layer to the surface of the sponges used for embolization as well as the effects of substituting a thrombogenic surface (the adventitia) in place of endothelium by inversion of the venous pouch before aneurysm construction. The present study supports the hypothesis that the endothelium plays an important role in the persistence of residual lesions and in aneurysmal recanalization after embolization. This hypothesis provides new opportunities to improve long-term results of endovascular treatment of aneurysms.

Materials and Methods

Experimental Design

We have previously reported a canine lateral wall aneurysm model that reproduces in vivo the problems of residual necks and recurrences after embolization. This model involves intraoperative embolization with collagen sponges with or without fibrinogen. Two symmetrical aneurysms permit direct comparisons within the same animal. We have modified the model and studied 5 different groups of animals, as illustrated in Figure 1. We first studied early pathological changes at 1, 2, and 3 weeks in 15 dogs (group 1; n=5 for each time) after collagen sponge embolization (alone or coated with fibrinogen) to document the appearance of endothelial or α-actin–positive mesenchymal cells at the neck of aneurysms and the events related to the formation of a neointima or to the recanalization process.

Angiographic and pathological results found at 3 weeks in aneurysms deprived of endothelium were then compared with contralateral intact aneurysms, both treated by collagen sponge embolization, in 12 animals (group 2).

To study the effects of endothelialization of the embolic agent, the sponge was covered before intraoperative embolization by a vein graft, with the endothelium facing the lumen on one side, compared with the endothelium facing the sponge on the other side (the surface of the embolic agent is then made of adventitia), in 4 animals. Pathology and angiography were then performed as in group 2.

Two alternative experiments designed to study the role of the endothelial lining in aneurysmal persistence and recurrences were then performed: we compared in 4 animals (group 4) a normal venous pouch aneurysm embolized with an endothelialized sponge and a reversed venous pouch aneurysm (with the adventitial side facing the lumen) embolized with a sponge covered with adventitia. Group 5 animals had bilateral inverted venous pouch aneurysms; one aneurysm was embolized with an endothelialized sponge and compared with a control sponge on the other side.

To complete this study, we included 3 types of control aneurysms (Figure 1a); normal (n=4), de-endothelialized (n=3), and inverted venous pouch aneurysms (n=3) that were observed for 3 weeks.

Surgical Construction of Aneurysms and Intraoperative Embolization

Protocols for animal experimentation were approved by the institutional animal committee in accordance with guidelines of the Canadian Council on Animal Care. Beagles (n=45) weighing 10 to 15 kg were sedated with an intramuscular injection of 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 surgical anesthesia with 2% isoflurane. Postoperative analgesia was provided for 3 days by a 50-µg fentanyl skin patch. Lateral wall aneurysms were constructed on each common carotid artery by a technique previously described. Two segments of the same external jugular vein were harvested for construction of the venous pouches. After temporary occlusion of the common carotid artery, an oval 5-mm arteriotomy was performed, and the venous pouch was sutured to the arterial wall with 7-0 prolene. In the “normal” model (group 1), one 8×8-mm absorbable gelatin (Gelfoam) sponge was inserted from the fundus into the aneurysm to completely occlude it. To prepare fibrinogen-coated sponges, the Gelfoam fragment was placed in a 12-well plate with 1 mL of Dulbecco’s modified Eagle’s medium containing 2 mg/mL fibrinogen and 10% fetal bovine serum. After 2 hours, sponges covered by the fibrinogen gel were transferred in excess Dulbecco’s modified Eagle’s medium, as described previously. To remove the endothelial layer of the venous pouch (group 2), the vein graft was inverted, mechanically scraped with a scalpel, and then reverted to normal before anastomosis. Sections of normal or de-endothelialized vein segments were immediately fixed for pathological confirmation that the endothelium was

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**Figure 1.** Aneurysm construction, sponge embolization, and description of experimental groups. a, We used 3 types of venous pouch aneurysms. The “normal” model was constructed by anastomosing end to side a segment of the external jugular vein to the site of carotid arteriectomy. The aneurysmal lumen was lined by an endothelial layer; a de-endothelialized aneurysm was constructed in a similar fashion with a venous segment, inverted to mechanically scrape the endothelium, and reverted to normal before anastomosis. An inverted venous pouch model was similarly constructed, but the venous segment was inverted before anastomosis; the aneurysmal lumen was then lined with adventitia. b, Four types of sponges were used for embolization: bare or “normal” Gelfoam sponges, fibrinogen-coated sponges, and sponges covered with a normal or inverted venous wall (with the endothelium or the adventitia facing the lumen). c, In addition to control aneurysms shown in panel a, 5 groups of animals were treated to compare the effects of changing the nature of the lining of the aneurysms or the surface of the sponges on angiographic and pathological findings at 3 weeks.
effectively intact or removed. To graft a segment of venous wall along with the collagen sponge (groups 3 to 5), the sponge was wrapped with a normal or inverted segment of the same external jugular vein before introduction into the aneurysm. For “inverted venous pouch aneurysms” (groups 4 to 5), the vein segment was inverted before anastomosis. Transfemoral angiography was undertaken immediately after surgery in all animals. During recovery, the dogs were fed a normal diet, and their activities were not restricted. Carotid angiography was repeated in anesthetized animals before euthanasia, by barbiturate overdose, to document the degree of aneurysmal obliteration. The common carotid artery was excised after the animals were killed. The wall of the artery was longitudinally opened to expose the luminal surface of the neck of the aneurysm. After fixation, the neck of aneurysms was photographed with an operating microscope. The aneurysms were then sectioned in the axial plane and photographed again before paraffin embedding.

### Experimental Groups and Angiographic Results

<table>
<thead>
<tr>
<th>Group</th>
<th>Aneurysm</th>
<th>Embolic Agent</th>
<th>n</th>
<th>Immediate</th>
<th>Follow-Up</th>
</tr>
</thead>
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<td>With endothelium</td>
<td>Sponge</td>
<td>12</td>
<td>2.0</td>
<td>3.0</td>
</tr>
<tr>
<td>3</td>
<td>Without endothelium</td>
<td>Sponge</td>
<td>12</td>
<td>1.5*</td>
<td>2.0†</td>
</tr>
<tr>
<td>4</td>
<td>With endothelium</td>
<td>Sponge + adventitia</td>
<td>4</td>
<td>3.0</td>
<td>3.0</td>
</tr>
<tr>
<td>5</td>
<td>Without adventitia</td>
<td>Sponge + endothelium</td>
<td>3</td>
<td>3.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Control</td>
<td>With endothelium</td>
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<td>4</td>
<td>3.0</td>
<td>3.0</td>
</tr>
<tr>
<td>6</td>
<td>Without endothelium</td>
<td>None</td>
<td>3</td>
<td>3.0</td>
<td>1.0</td>
</tr>
<tr>
<td>7</td>
<td>With adventitia</td>
<td>Sponge + endothelium</td>
<td>3</td>
<td>3.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

*P=0.71, †P=0.02, ‡P=0.012, §P=0.003 compared with group 2 with endothelium (Wilcoxon’s test).

**Angiography**

Surgical embolization of aneurysms and healing at the neck of the aneurysms were assessed in vivo by angiography immediately after embolization and before the animals were killed (at 3 weeks except for group 1). Results were scored according to a previously described classification. A score of 0 indicated complete obliteration; 1, “dog ears”; 2, residual or recurrent neck; and 3, residual or recurrent aneurysm; a score of 4 indicated large saccular recurrences. All angiographic scores of aneurysms were compared by Wilcoxon’s test. A value of P<0.05 was considered significant.

**Pathology**

Healing phenomena at the surface of sponges and the presence of clefts between the embolic agent and the aneurysmal wall were studied after formalin fixation, axial sectioning, and staining with hematoxylin-phloxine-saffron and Movat’s pentachrome stain. Immunohistochemistry served to characterize neointimal cells and cells inside the sponge at different time intervals after embolization, with the use of antibodies to smooth muscle α-actin and factor VIII. Sections of normal or de-endothelialized vein segments harvested at the time of aneurysm construction were immunostained for factor VIII for confirmation that the endothelium was effectively intact or removed.

### Results

**Angiographic Results**

Angiographic results are summarized in the Table and illustrated in Figure 2. The angiographic results and evolution of control aneurysms observed without embolization depended on the nature of the venous pouch lining: de-endothelialized and inverted venous pouch aneurysms thrombosed spontaneously, while normal (endothelialized) venous pouch aneurysms remained patent for at least 3 weeks. De-endothelialization before collagen sponge embolization (group 2) led to similar initial angiographic results (P=0.7) but significantly improved angiographic scores (less recurrence) at 3 weeks (P=0.02) (Figure 2a to 2d). The addition of an adventitial or an adventitial layer to the sponge used for embolization of normal venous pouch aneurysms did not significantly change angiographic scores immediately or at 3 weeks (group 3). Best angiographic results initially and at 3 weeks were found in inverted venous pouch aneurysms embolized with sponges covered with adventitia (group 4). There was no difference in the angiographic results of group 5 (inverted venous pouch) aneurysms: they were all occluded at 3 weeks.

**Pathological Studies**

Pathological findings with the different modifications of the model are illustrated in Figures 3 and 4. Macrosopic examination of specimens revealed that aneurysms treated with collagen sponges (with or without fibrinogen) consistently showed a residual or recurrent neck limited by a thin, dark membrane at 3 weeks (Figure 3c and 3d). Histological findings, after collagen sponge embolization with or without fibrinogen, of lateral wall canine aneurysms have been reported previously.4-6

The surface of the sponge is covered by a thin neointima, composed of endothelial cells and a few layers of α-actin–positive cells in a collagenous matrix. This neointima is continuous, with crescentic endothelialized spaces that separate the embolic agent from the aneurysmal walls at the corners of the recurrent neck (Figure 4a). To better understand the pathological sequence responsible for this phenomenon, we studied earlier specimens, at 1 and 2 weeks. Immunohistochemical staining demonstrates that the clot at the surface of the sponge is covered at 1 week by a continuous
layer of endothelial cells before there is significant infiltration by mesenchymal cells (Figure 5). The embolic agent is already separated from the aneurysmal wall by deep, fully endothelialized clefts that extend from the residual neck.

Scraping was effective in removing the factor VIII–positive layer, visible in control endothelialized venous segments used for construction of the contralateral normal aneurysms in group 2 animals. De-endothelialized aneurysms showed a thicker neointimal membrane sealing the neck of aneurysms at 3 weeks (Figure 3e and 3f). A large amount of connective tissue was found at 3 weeks between the sponge and the neck of aneurysms (Figure 4b). Some residual clot could sometimes be found between the thick neointima and the sponge, suggesting that the connective tissue had replaced a thick thrombus. Previously described clefts between the embolic agent and the wall of the aneurysm (Figure 4a) were decreased or absent (Figure 4b).

The addition of an endothelial layer to sponges before embolization led to deep recurrent endothelialized spaces...
Figure 4. Histological findings 3 weeks after surgical construction and embolization. Comparative illustrations of histological findings of normal (a, c, d), de-endothelialized (b), or inverted (e) venous pouch aneurysm 3 weeks after embolization with bare sponges (a, b), sponge covered with endothelium (c), or sponge covered with adventitia (d, e) are shown. Note the very thin neo-intima and the endothelialized clefts in the normal aneurysm embolized with bare sponge (arrows in a). The neo-intima is thick and recanalization is absent when the venous pouch is de-endothelialized or inverted before sponge embolization (arrowheads in b and e). The neo-intima is very thin and the endothelialized clefts are exaggerated when the sponge is covered with endothelium (arrows in c). The neo-intima is thick but recanalizing clefts persist (arrow in d) when a sponge covered with the thrombogenic adventitia is used to embolize a normal (endothelialized) aneurysm. Excellent healing followed embolization of an inverted venous sponge aneurysm embolized with a sponge covered with adventitia (e). Axial sections were stained with Movat’s pentachrome stain. Magnification ×20. G indicates sponge.

Inverting the venous pouch eliminated the endothelialized clefts and led to complete sealing of the neck by a thick neointima (Figure 4e) with or without sponge embolization and irrespective of the nature of the sponges used.

Discussion

Residual Necks, Recanalization, and Recurrences in Experimental Aneurysms

Porcine aneurysms have a strong tendency to heal after embolization. Residual necks do not remain patent and do not progress to recurrences, as seen in many human aneurysms. This phenomenon seems to be due to a propensity, in pigs, for extensive thrombosis and neointima formation. The cells involved in healing the neck of porcine aneurysms, harvested in vitro by explantation techniques, are growth factor-sensitive, α-actin–positive mesenchymal (or “neointimal”) cells. Thus, we first focused our research efforts on smooth muscle cells and neointima formation. When similar experimental models are used in dogs, the angiographic evolution of residual necks is entirely different. We have extensively studied a canine aneurysm model that has a strong propensity to recur. Pathological studies performed at 3 weeks reveal a thin and incomplete neointima at the surface of the sponges used for embolization. An inverse relationship between the thickness of the neointima and the development of recurrences is an oversimplification, however. Pathological specimens recovered 1 week after collagen sponge embolization demonstrate early recanalization and endothelialization of the clot formed at the surface of the embolic agent, before significant collagen deposition by invading mesenchymal cells. This phenomenon creates slit-like endothelialized spaces between the wall of the aneurysm and the embolic agent, which remain connected to the parent artery (Figure 5). These recanalizing spaces can also be found in canine bifurcation aneurysm treated with platinum coils. We believe that these clefts are the first sign of recanalization of aneurysms. The present study explored surgical variations of our model designed to determine the role played by endothelial cells in the persistence of residual lesions and the development of recurrences after embolization of experimental aneurysms.

Surgical Modifications of the Model and Recurrences

The endothelium is essential to the long-term patency of surgically constructed aneurysms and of residual lesions after embolization. When the venous pouch is inverted before aneurysm construction or when the venous segment is deprived of endothelial cells, aneurysms thrombose, are not recanalized, and eventually heal, even without sponge embolization. When the venous pouch is endothelialized, residual necks remain patent after sponge embolization and progress to recurrences. When an endothelial lining is added to the sponges used for embolization of endothelialized venous pouch aneurysms (groups 3 and 4), the formation of clefts between the sponge and the aneurysmal wall is exaggerated, and recurrences at 3 weeks are constant. When both surfaces are covered with adventitia, that is, when the sponge is covered with adventitia and the venous pouch is inverted...
(group 4), immediate morphological results are improved, clefts are absent, and there is no recurrence at 3 weeks (Figures 2 to 4). These modifications of the model support the role played by the endothelial lining of the aneurysm in the persistence of residual lesions and in the development of recurrences after embolization. Endothelialized aneurysms treated with sponges covered with the thrombogenic adventitia showed an increased amount of organized thrombus at the surface of the graft, but aneurysms were also recanalized by clefts and recurred at 3 weeks. This experiment demonstrates the limits of strategies designed to increase thrombosis or neointima formation at the surface of the embolic agent if they do not somehow inhibit the recanalization process. The method we used to control de-endothelialization at the time of aneurysm construction, by studying a test section of the venous segment, does not prove that removal of the endothelial layer was complete and homogeneous over the entire venous pouch. Scraping of the endothelial layer, however, caused significant changes in the evolution of aneurysms.

When the endothelial covering of the venous pouch was mechanically removed before aneurysm construction and sponge embolization, there was extensive fibrous tissue replacement of the space not occluded by the sponge, at the neck. The aspect of these lesions was reminiscent of results obtained in porcine aneurysms, lesions with a strong propensity for spontaneous healing.\(^4,5\) The formation of clefts at the interface between the sponge and the aneurysmal wall was strikingly diminished. The angiographic score was significantly improved at 3 weeks, with fewer recurrences compared to contralateral lesions also treated with collagen sponges but without prior de-endothelialization.

**Potential Clinical Applications**

Residual lesions and recurrences are linked to the presence of endothelialized clefts between the embolic agent and the aneurysmal wall. Treatments designed to increase “endothelialization” at the surface of coils, as proposed by some investigators,\(^9,10\) may in fact promote the persistence of residual lesions and the development of recurrences.

Other strategies that would increase thrombogenicity of the embolic agent without affecting the formation of these clefts, as seen in experiments using fibrinogen\(^6\) or adventitia covering the sponge, do not affect the recanalization process and are unlikely to modify the evolution toward recurrences. Removal of the endothelial layer covering the aneurysmal wall not only increases thrombus formation but also prevents the formation of endothelialized clefts and improves the angiographic evolution after embolization. Immediate and long-term results of endovascular treatment of human aneurysms could gain from de-endothelialization of the aneurysmal wall. This strategy could be performed through the endovascular route with the use of physical, chemical, or biomolecular interventions. Alternatively, the embolic agent could be designed to somehow inhibit or delay the invasion of endothelial cells in favor of mesenchymal cells.\(^13\)

**Conclusion**

The endothelial lining is essential to the persistence of residual lesions after embolization. It is also involved in recanalization of the thrombus formed on the embolic agent. Future strategies designed to remove the endothelial layer or to prevent endothelial recanalization of thrombus could improve long-term results of endovascular treatment of aneurysms.

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