ABSTRACT

BACKGROUND AND PURPOSE: While WEB devices have been shown to be safe and effective for aneurysm treatment, WEB-shape modification compression has been associated with incomplete aneurysm occlusion. We explored the relationship between occlusion rates and WEB-shape modification in different WEB device types in an experimental aneurysm model.

MATERIALS AND METHODS: Elastase-induced aneurysms were created in rabbits and treated with dual-layer (n = 12), single-layer (n = 12), or single-layer sphere (n = 12) WEB devices. Aneurysms were followed up either at 3 or 12 months. Angiographic occlusion was graded using the WEB Occlusion Scale: grade I, complete; grade II, complete but recess filling; grade III, residual neck; or grade IV, residual aneurysm. WEB-shape modification and histologic features were also analyzed.

RESULTS: Grade I or II occlusion was seen in 16 (44%) aneurysms, and grade I, II, or III (“adequate”) occlusion was observed in 22 (61.1%) aneurysms at follow-up. WEB-shape modification was observed in 22 (61.1%) aneurysms. WEB-shape modification was higher in single-layer (9/12) and dual-layer (10/12) devices compared with single-layer sphere devices (3/12). Aneurysms with WEB-shape modification had a higher level of thrombus organization in the dome compared with those without WEB-shape modification (68% [15/22] versus 50% [7/14]). WEB-shape modification was not correlated with angiographic or histologic outcomes but was significantly correlated with levels of fibrosis and smooth muscle cells in the aneurysm.

CONCLUSIONS: WEB-shape modification is not associated with incomplete aneurysm occlusion of WEB devices in the rabbit model but may be related to connective tissue formation and the healing response to WEB device implantation.

ABBREVIATIONS: FD = flow diverter; SMA = smooth muscle actin; WSM = WEB-shape modification; DL = dual-layer; SL = single-layer; SLS = single-layer sphere
potentially be associated with the approximately 15% rate of incomplete/inadequate aneurysm occlusion observed in the published WEB Intrascalar Therapy (WEB-IT) trial. However, this phenomenon has not been well-understood until now because other researchers have reported that this shape change did not impact the anatomic outcome after WEB deployment. In this study, we compared the occlusion rates of DL, SL, and single-layer sphere (SLS) WEBs in rabbit elastase (Worthington Biochemical)-induced aneurysm models and evaluated the relationship between WSM using histologic results and angiographic occlusion rates.

MATERIALS AND METHODS

Aneurysm Creation
Elastase-induced aneurysms were created in 36 New Zealand white rabbits. Animal procedures were approved by the Institutional Animal Care and Use Committee at Mayo Clinic. Aneurysm-creation procedures were performed using an elastase-induction model as previously described. Endovascular treatment of each aneurysm was undertaken at least 3 weeks after aneurysm creation.

Devices
WEB devices are classified according to shape and the number of mesh layers each one contains. DL devices consist of 2 layers of braided, nitinol wire mesh. SL and SLS devices consist of a single layer of braided, nitinol/platinum wire mesh; however, SLS implants have a more rounded 3D shape than the “barrel-like” SL and DL devices. The specific qualities of the WEB devices have been previously described.

Device Deployment
The WEB device deployment procedure has been reported previously. The right femoral artery was briefly exposed. A 5F sheath (Envoy; Cordis) was inserted, 500 U of heparin was injected, and a 5F catheter was then advanced into the brachiocephalic trunk from the aortic arch. DSA was performed through the guide catheter. A 0.027-inch ID (interior diameter) microcatheter (VIA-27; Sequent Medical) was advanced into the aneurysm lumen over a microguidewire (Transend-14; Stryker). Appropriately sized devices were placed in the aneurysm cavity for each device (n = 12 [DL], n = 12 [SL], n = 12 [SLS]). DSA was performed immediately following device placement. No animals received anti-platelet therapy during the course of the study.

Follow-Up and Euthanasia
Angiographic evaluation was completed immediately after device implantation and again at follow-up. The cohort was divided into 2 subgroups preselected for euthanasia at 3 months (n = 6 [DL], n = 6 [SL], n = 6 [SLS]) and 12 months (n = 6 [DL], n = 6 [SL], n = 6 [SLS]), respectively, by intravenous injection with a lethal dose of pentobarbital through the ear vein. Following euthanasia, aneurysm tissue was harvested and placed in a 10% formalin solution.

Aneurysm Grading and WSM Assessment
The degree of angiographic aneurysm occlusion at follow-up was graded as follows: grade I, complete; grade II, complete but with recess filling; grade III, residual neck; or grade IV, residual aneurysm. Two reviewers independently evaluated the angiographic occlusion, and disagreements were resolved by a third reviewer. Angiographic occlusion outcome was dichotomized into either complete occlusion (grade I or II) or incomplete occlusion (grade III or IV). Grades I, II, and III were considered adequate. The distance between proximal and distal device markers was measured on unsubtracted angiographic images. WSM was defined as a change in distance (≤−10% to ≥10%) between markers at follow-up compared with immediate postdevice deployment.

Histopathologic Processing and Analysis
A histopathologist who was blinded to the angiographic results did the processing and analysis for healing evaluation. Aneurysm samples were processed at 1000-μm intervals in a coronal orientation, permitting long-axis sectioning of the aneurysm neck, with use of an Isomet Low Speed Saw (Buehler). After the device segments were removed under a dissecting microscope, the samples were then re-embedded in paraffin, sectioned at 4 μm, and stained with hematoxylin-eosin.

Masson trichrome staining was performed to evaluate collagen deposition within the aneurysm; collagen deposition within the aneurysm dome was segmented and quantified using the previously described method. The fibrosis ratio (total area of fibrosis within the aneurysmal cavity divided by the total area of the aneurysmal cavity) was calculated for each aneurysm.

Statistical Analysis
Continuous variables were described as mean [SD] and compared using a Student t test. Categoric variables were presented as number (percentage) and compared using the Fisher exact test. The correlation between WSM and aneurysm occlusion was assessed by the Spearman rank correlation. The correlation between WSM and aneurysm geometries, histologic healing, fibrosis, and smooth muscle actin levels was evaluated by simple linear regression. A P value < .05 was considered statistically significant.

RESULTS

Angiographic Findings
All aneurysms were implanted with appropriately sized devices. No morbidity or mortality was observed throughout the study.

In the group of rabbits selected for euthanasia at 3 months (n = 18), grade I or II occlusion was achieved in 9 (50%)
aneurysms at follow-up. Within this group, 6 rabbits were treated with the DL device: 1 (16.7%) showed grade I occlusion, 3 (50.0%) showed grade II occlusion, 1 (16.7%) showed grade III occlusion, and 1 (16.7%) showed grade IV occlusion. For the 6 rabbits treated with the SL device, 1 (16.7%) showed grade I occlusion, 3 (50.0%) showed grade II occlusion, 1 (16.7%) showed grade III occlusion, and 3 (50.0%) showed grade IV occlusion. In the final subgroup (n = 6) treated with the SLS device, 3 (50.0%) rabbits showed grade I occlusion, 1 (16.7%) showed grade II occlusion, 1 (16.7%) showed grade III occlusion, and 2 (33.3%) showed grade IV occlusion.

In the 12-month group at follow-up (n = 18), 7 (38.9%) aneurysms had grade I or 2 occlusion. Of the 6 rabbits treated with the DL device, 3 (50.0%) showed grade I occlusion, 1 (16.7%) showed grade II occlusion, and 2 (33.3%) showed grade IV occlusion. For the rabbits treated with the SLS device, 1 (16.7%) showed grade I occlusion, 2 (33.3%) showed grade III occlusion, and 3 (50.0%) showed grade IV occlusion. For the rabbits treated with the SLS device, 1 (16.7%) showed grade I occlusion, 1 (16.7%) showed grade II occlusion, 1 (16.7%) showed grade III occlusion, and the remaining 3 (50.0%) showed grade IV occlusion (Table 1).

In a pooled angiographic analysis, 16 (44%) aneurysms (9 at 3 months and 7 at 12 months) demonstrated complete occlusion and 22 (61.1%) aneurysms (12 at 3 months, and 10 at 12 months) showed adequate occlusion. No significance was found in complete or adequate occlusion rates between the 3- and 12-month groups (P = .74). DL (75%, 9/12) devices demonstrated a higher percentage of adequate aneurysm occlusion compared with SLS (58.3%, 7/12; P = .68) and SL (50%, 6/12; P = .04) devices.

**WEB-Shape Modification**

WSM was observed in 22 (61%) aneurysms (Fig 1), of which half (5 with complete occlusion, 6 with incomplete occlusion) were in the 3-month group and the remaining half (4 with complete occlusion, 7 with incomplete occlusion) were in the 12-month group (Table 2). WSM was not statistically associated with aneurysm neck width or height (Fig 2). There were no significant differences in occlusion grades in aneurysms with or without WSM (9 with complete occlusion, 13 with incomplete occlusion versus 7 with complete occlusion and 7 with incomplete occlusion; P = .73) (Fig 1). Furthermore, no correlation was found between the percentage of WSM and the occlusion grade. Most interesting, SLS had significantly less WSM (25%, 3/12) compared with the DL (83%, 10/12; P = .012) and SL (75%, 9/12; P = .039) devices.

**Histologic Findings**

The mean histologic healing score was not significantly different between the 3- and 12-month groups (5.6 [SD, 2.6] versus 4.2 [SD, 2.5]; P = .11), but it was significantly higher in aneurysms with complete occlusion compared with those with incomplete occlusion (6.39 [SD, 2.07] versus 2.80 [SD, 1.75]). Histologic evaluation of aneurysm sacs showed a combination of loose connective tissue and thrombus (unorganized, organized, and poorly organized).

### Table 1: Aneurysm occlusion grading summary

| Group | DL No. (%) | SL No. (%) | SLS No. (%) | Total No. |
|-------|------------|------------|-------------|-----------|
| 3-Month group | | | | |
| Grade I | 3 (50.0) | 1 (16.7) | 1 (16.7) | 5 (27.8) |
| Grade II | 3 (50.0) | 3 (50.0) | 0 (0) | 6 (33.3) |
| Grade III | 1 (16.7) | 2 (33.3) | 0 (0) | 3 (16.7) |
| Grade IV | 1 (16.7) | 3 (50.0) | 2 (33.3) | 6 (33.3) |
| Total | 6 | 6 | 6 | 18 |
| 12-Month group | | | | |
| Grade I | 3 (50.0) | 1 (16.7) | 1 (16.7) | 5 (27.8) |
| Grade II | 1 (16.7) | 0 (0) | 1 (16.7) | 2 (11.1) |
| Grade III | 0 (0) | 2 (33.3) | 1 (16.7) | 3 (16.7) |
| Grade IV | 2 (33.3) | 3 (50.0) | 3 (50.0) | 8 (44.4) |
| Total | 6 | 6 | 6 | 18 |

* Grade I represents complete occlusion; grade II, complete occlusion with recess filling; grade III, residual neck; grade IV, residual aneurysm.

### Table 2: WSM and aneurysm occlusion by device type

| Follow-Up, Occlusion Group | DL (n = 12) | SL (n = 12) | SLS (n = 12) | Total (n = 36) |
|---------------------------|------------|------------|-------------|---------------|
|                           | With WSM, No. (%) | Without WSM, No. (%) | With WSM, No. (%) | Without WSM, No. (%) | With WSM, No. (%) | Without WSM, No. (%) | With WSM, No. (%) | Without WSM, No. (%) |
| 3 Months                  |             |            |             |               |
| Grade I or II             | 4 (33.3)    | 0          | 0           | 1 (8.3)       | 1 (8.3)         | 3 (25.0)          | 5 (13.9)         | 4 (11.1)         |
| Grade III or IV           | 2 (16.7)    | 0          | 3 (25.0)    | 2 (16.7)      | 1 (8.3)         | 1 (8.3)          | 6 (16.7)         | 3 (8.3)          |
| 12 Months                 |             |            |             |               |
| Grade I or II             | 3 (25.0)    | 1 (8.3)    | 1 (8.3)     | 0             | 0              | 2 (16.7)         | 4 (11.1)         | 3 (8.3)          |
| Grade III or IV           | 1 (8.3)     | 1 (8.3)    | 5 (41.7)    | 0             | 1 (8.3)         | 3 (25.0)         | 7 (19.4)         | 4 (11.1)         |
| Total                     | 10 (83.3)   | 2 (16.7)   | 9 (75.0)    | 3 (25.0)      | 3 (25.0)        | 9 (33.3)         | 22 (61.1)        | 14 (38.9)        |

* Grade I represents complete occlusion; grade II, complete occlusion with recess filling; grade III, residual neck; grade IV, residual aneurysm.
Aneurysms that demonstrated complete occlusion had more organized thrombus (76%, 16/21), while aneurysms that exhibited incomplete occlusion had unorganized thrombus (60%, 9/15) in the aneurysm dome. Most aneurysms showed open areas with no tissue filling and a neck remnant with a concave surface toward the dome (75% [12/16] in the complete occlusion group versus 90% [18/20] in the incomplete occlusion group). Inflammation within the aneurysm lumen was absent or mild.

Aneurysms with the WSM showed a high level of organized thrombus compared with those without WSM (68% [15/22] versus 50% [7/14], \( P = .09 \)) (Fig 3). The mean histologic healing score, inflammation score, fibrosis percentage, and SMA percentage was 5.0 [SD, 2.3], 1.5 [SD, 0.6], 15.9 [SD, 14.8], and 12.6 [SD, 9.6], respectively, in aneurysms with WSM, and 4.7 [SD, 3.2], 1.2 [SD, 0.8], 9.6 [SD, 8.0], and 9.5 [SD, 8.2], respectively, in aneurysms without WSM. There were no statistically significant differences in histologic healing, inflammation, fibrosis, and SMA between aneurysms with and without WSM. However, the percentage of WSM was moderately correlated with both fibrosis \( (r = 0.37, P = .02) \) and SMA levels \( (r = 0.36, P = .032) \) (Fig 4). WSM was not significantly correlated with either total histologic healing or inflammation.

**DISCUSSION**

Our study, which aimed to delineate the underlying mechanisms of WSM in WEB devices, demonstrates that WSM does not correlate with angiographic aneurysm occlusion or total histologic healing outcomes in the rabbit aneurysm model. However, WSM is positively associated with collagen and SMA levels, supporting the hypothesis that WSM is likely more related to aneurysm healing than external (eg, hemodynamic) compression alone.

The underlying mechanisms of WSM or compression could be multifaceted (eg, device size and construction, exact anatomic...
configuration of aneurysm geometry and surrounding vessels, parent artery, aneurysm neck). Computational fluid dynamics simulations have shown that WEB device compression was positively correlated with computational fluid dynamics–derived inflow into the aneurysm. In endoluminal FDs, pore density has been positively associated with aneurysm occlusion. Pore density at the proximal WEB device marker is relatively high compared with that in endoluminal devices, and it is highly unlikely that device compression would lead to reduced pore density at the neck. Rouchaud et al demonstrated, in coiled aneurysms, that a higher level of collagen in the aneurysm dome could trigger coil retraction from the neck orifice into the aneurysm cavity, resulting in aneurysm recurrence. Our findings suggest that WEB devices could behave like coils in the vascular microenvironment and result in device compression, and the contractile properties of connective tissue components in WEB-implanted aneurysms could lead to WSM. Proper endothelial cell growth across the pores of FDs in the aneurysm neck is also considered critical for aneurysm occlusion, in addition to the change in blood hemodynamics, for the mechanistic action of FDs. Endoluminal FDs placed in the healthy parent artery act as scaffolds for endothelial cell migration and neo(intima) formation. In contrast, intrasaccular FDs are implanted in the aneurysm cavity, which represent a nonfunctional endothelium and a smooth-muscle layer and could explain the lack of neointimal coverage at the neck. The observed WSM rate of 62% in the rabbit model is similar to that reported in clinical studies for the WEB device. Although DL WEB devices had higher adequate occlusion rates compared with SL devices in this study, the DL devices also demonstrated higher WSM. Given the small sample sizes and the difference of a single result providing statistical significance, taken together, these results suggest that WSM is not well-correlated with either device type.

Our study has several limitations. First, the variation in the angiographic working projection angle resulted in inaccurate measurements of the distance between device markers. Second, we arbitrarily defined 10% of the device as a threshold for WSM. A high stringent limit may provide different results. Third, we did not evaluate numerous other factors, including mechanical and hemodynamic factors, which could influence the device shape changes.

CONCLUSIONS

WSM is not associated with incomplete aneurysm occlusion in the rabbit model but may be related to connective tissue formation and collagen deposition after WEB implantation.

FIG 4. Correlation of the degree of device shape change with histologic features. Linear regression shows a significant positive correlation between the percentage of device length change and fibrosis (A) and SMA (B). Straight lines indicate regression; bowed lines indicate 95% CI.

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