New developments in imaging and treatment of intracranial aneurysms

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Chapter 2

Transforming growth factor ß-coated platinum coils for endovascular treatment of aneurysms: an animal study

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ABSTRACT

OBJECTIVE
To test the hypothesis that coating platinum coils with transforming growth factor β (TGFβ) would improve the cellular proliferation within experimental aneurysms relative to uncoated coils.

MATERIALS AND METHODS
Elastase-induced saccular aneurysms were created in 12 New Zealand White rabbits. These aneurysms were embolized with platinum coils, either “control” (unmodified) coils or “test” (coated with TGFβ) coils. Subjects were killed either 2 weeks (n= 3, control; n= 3, test) or 6 weeks (n= 3, control; n= 3, test) after embolization. Aneurysm tissue was embedded in plastic, sectioned, and stained with hematoxylin and eosin. The thickness of tissue covering the coils at the coil-lumen interface was measured by use of a digital microscope, and was compared between groups by use of the Student’s t test.

RESULTS
Two-week implantation samples demonstrated mean thickness of tissue overlying TGFβ -coated coils of 36 ± 15 μm and mean thickness of overlying control coils of 3 ± 5 μm, indicating significantly thicker tissue growth covering test versus control coils (P = 0.02). Six-week implantation samples demonstrated mean thickness of tissue overlying TGFβ -coated coils of 86 ± 74 μm versus mean thickness overlying control coils of 37 ± 6 μm this difference did not reach statistical significance (P = 0.30). Thickness of tissue covering TGFβ -coated coils did not change significantly from 2 to 6 weeks (P = 0.31). Tissue thickness over control coils increased significantly between 2 and 6 weeks (P = 0.002).

CONCLUSION
TGFβ -coated platinum coils undergo earlier cellular coverage than standard platinum coils, but differences in coverage between coated and control coils are no longer present at later time points. These data suggest that improvements in intra-aneurysmal cellular proliferation resulting from coil modifications, although significant in the early postembolization phase, may dissipate over time.

KEY WORDS
Aneurysm, Animal model, Embolization, Growth factors
INTRODUCTION

Guglielmi detachable coils (GDCs) (Target Therapeutics, Fremont, CA) represent an important advance in minimally invasive therapy of cerebral aneurysms. Numerous reports have demonstrated the clinical efficacy of GDCs in diminishing rates of rehemorrhage in ruptured aneurysms \(^{9,11,16}\). The relative efficacy of GDCs is influenced by aneurysm size; small aneurysms demonstrate excellent long-term occlusion rates, but large and giant aneurysms demonstrate disappointing rates of stable, aneurysm occlusion \(^{5,6,10,11,16,21}\).

Because of relatively disappointing results in the treatment of large and giant aneurysms with GDCs, several investigators have proposed modifications to the coil to improve occlusion rates in these aneurysms \(^{1,3,6,7,12,14,15,17,19,22,23}\). These coil modifications are aimed primarily at increasing the “biological activity” of the GDC, because standard GDCs are relatively biologically inert and fail to induce scar formation in large aneurysms \(^{8,10,11,16}\). Three classes of biological modification have been proposed previously. First, the addition of a collagen filament to the core of the GDC has been tested in a swine model \(^{6,7,23}\). Second, tissue allografts, including fibroblasts and smooth muscle cells, have been added to embolic agents to improve cell growth in experimental aneurysms \(^{15,19,22}\). And last, a number of coil coatings have been proposed to increase biological activity. These coatings include basement membrane and extracellular matrix proteins, proteins involved in the fibrinolytic pathway, and peptide growth factors \(^{1,3,12,14,17}\). Several of these coatings have demonstrated promise in a swine model of saccular aneurysms, but a recent report in a rabbit model with modulators of the fibrinolytic system demonstrated no improved efficacy in increasing intra-aneurysmal fibrosis as compared with standard coils \(^{9}\).

Transforming growth factor β (TGFβ) is an ubiquitous growth factor that has been demonstrated to increase collagen synthesis, and endothelialization in vivo. These biological functions would be considered beneficial in the setting of coil embolization of aneurysms. TGFβ has been demonstrated previously to adhere to the surface of platinum coils in vitro \(^{13}\). In the current study, we explore the histological differences, by use of a rabbit aneurysm model, between standard platinum coils and platinum coils coated with TGFβ, specifically regarding the rate and degree of cell growth over intra-aneurysmal coils.

MATERIALS AND METHODS

ANEURYSM CREATION

All animal experimentation was approved by the animal research committee at our institution. Details of aneurysm creation are available in published reports \(^{2,4}\). Twelve New Zealand White Rabbits (3–4 kg) were used for this study. Each animal was anesthetized with 60 mg/kg of intramuscularly administered ketamine (Ketaved; Vedco, St. Joseph, MO) and 6 mg/kg xylazine (Traquived; Vedco). Heparin, 100 U/kg, was administered intravenously. By use of sterile technique, a 3-cm longitudinal incision was made in the midline of the neck.
The right common carotid artery (CCA) was exposed for approximately 1.5 cm. Three 3-0 silk sutures were passed around the artery. The distal aspect of the artery was ligated with one of the silk sutures. An arteriotomy was performed and a 5-French vascular sheath was passed retrograde into the CCA, with its tip resting approximately 3 cm cephalad to the origin of the CCA. Under fluoroscopic guidance, a 3-French Fogarty balloon catheter (Baxter Healthcare Corp., Deerfield, IL) and a Tracker 10 microcatheter (Target Therapeutics) were passed through the diaphragm of the sheath. The balloon was inflated at the bifurcation of the right CCA and the right subclavian artery. Porcine elastase, 200 U, (Sigma Chemical Co., St. Louis, MO, and Worthington Biochemical, Lakewood, NJ) was mixed with Omnipaque 300 iodinated contrast (Nycomed, Princeton, NJ) and saline. This elastase-contrast-saline mixture was injected through the microcatheter and incubated within the CCA lumen for 20 minutes. The Fogarty balloon was deflated and removed. After the procedure, the animals were allowed to recover for 2 weeks before embolization.

**COATING OF PLATINUM COILS WITH TGFβ**

Previous studies have demonstrated prolonged adherence of TGFβ to the surface of platinum coils by simple co-incubation of the coils with the growth factor (13, 20). TGFβ (Sigma) reconstituted in bovine serum albumin to a concentration 1 μg/ml was prepared. Coil samples were incubated in this solution for 2 hours, followed by immediate implantation into aneurysms.

**ANEURYSM EMBOLIZATION PROCEDURE**

With the elastase-induced aneurysm technique as used in New Zealand White Rabbits, it is impossible to precisely predict the size and shape of resultant aneurysm. For the current study, we specifically chose aneurysms with relatively narrow necks (dome-to-neck ratio ≥ 1.5:1) to facilitate coil embolizations. Aneurysm sizes were approximately 4 mm diameter for all cases. Before embolization, the animals each were premedicated as indicated previously. Heparin (100 U/kg) was given intravenously to prevent thromboembolic complications. A 4-French vascular sheath was placed in the right common femoral artery. A 4-French catheter was advanced to the origin of the brachiocephalic artery. An external sizing device was placed on the chest to aid in choice of appropriate coils. Digital subtraction angiography was performed. By use of coaxial technique, a Tracker 10 microcatheter was advanced into the aneurysm cavity. GDC embolization of the aneurysms was performed by use of either control (untreated) GDCs (n=6) or TGFβ-coated T10 coils (n=6), sized appropriately to the diameter of the aneurysm cavity. A single T10 coil, 4 mm x 10 cm, was used for embolization in each case. Because we were highly focused on the interface between the coil and flowing blood within an aneurysm, we chose to use a single coil for embolization to ensure some ongoing intra-aneurysmal flow after coil insertion. After embolization, the microcatheters and guiding catheters were removed, the femoral sheath was removed, and the femoral artery was ligated. The skin over the femoral artery was closed with running suture.
HISTOLOGICAL EVALUATION
Animals survived for either 2 weeks (n=3, control animals; n=3, TGFβ-coated coil animals) or 6 weeks (n=3, control animals; n=3, TGFβ-coated coil animals). The animals were killed with a lethal dose of pentobarbital. The aortic arc and the brachiocephalic vessels were removed en bloc and placed in formalin for at least 24 hours. The samples were embedded in methylmethacrylate, sectioned at 30 μm increments, and stained with hematoxylin and eosin. Qualitative histological evaluation was performed by two experienced observers. These observers characterized the cellular infiltration within the aneurysm lumen globally, with particular attention to the type of cellular infiltration (nucleated cells, red blood cells, or thrombus). Detailed characterization of the cells immediately adjacent to the coils also was performed.

QUANTITATIVE HISTOPATHOLOGY
Stained tissue sections were viewed by two experienced observers, blinded to the treatment group, by use of an Olympus BH2 microscope (Olympus Optical Co., Tokyo, Japan) connected to an MTI (Irvine, CA) digital camera. A calibrated slide with 10-m minor units and 100-m major units was used to set the calibration scale. The thickness of tissue overlying two coil loops was measured for each sample. The thickness of tissue was compared between control versus test coils at 2 weeks and at 6 weeks, between control coils at 2 weeks versus control coils at 6 weeks, and between test coils at 2 weeks versus 6 weeks by use of the Student’s t test.
RESULTS

QUALITATIVE HISTOPATHOLOGY
Samples of the aneurysms with standard GDCs collected 2 weeks after embolization demonstrated unorganized thrombus involving the majority of the aneurysm cavity. Red blood cells were noted within the central lumina of the coil winds. There was no evidence of cellular proliferation on the implanted coils, within the central lumen of the coils, or across the necks of the aneurysms (Fig. 1).

Conversely, TGFβ-coated coil samples at 2 weeks demonstrated multiple nucleated cells proliferating over the surface of the coils and within the central lumen of the coils. Measurable tissue was present over the surface of the coils at their point of contact with the aneurysm neck (Fig. 2).
Fig 2. TGF-ß-coated coil, 2 weeks after implantation. Coil winds have been displaced slightly from their original position, leaving serrated clear spaces that indicate the initial position of the coil. There is a measurable thickness of cell growth over the coil loop (arrows). Nucleated cells also are present within the central lumen of the coil (hematoxylin and eosin; original magnification, x 100).

Fig 3. Control coil, 6 weeks after implantation. There is a measurable thickness of cell growth over the coil loop (arrows). Nucleated cells also are present within the central lumen of the coil (hematoxylin and eosin; original magnification, x 100).
Among the samples of the aneurysms with standard GDCs collected 6 weeks after embolization, coil winds were found primarily along the periphery of the aneurysms. As such, it was straight forward to compare similar portions of the aneurysm in different cases.

Although most of the aneurysm lumens were filled with involuting thrombus, measurable areas of cellular proliferation were present over the surface of the coils in these 6-week control samples (Fig. 3). Qualitative histopathology findings for the 6-week TGFβ-coated GDCs were nearly identical to those observed in the 2-week TGFβ-coated samples.

Fig 4. TGFβ-coated coil, 6 weeks after implantation. Cell growth is observed over the surface of the coil (arrows) (hematoxylin and eosin: original magnification, x 100).
Specifically, there were multiple nucleated cells proliferating over the surface of the coils and within the central lumen of the coils. Measurable tissue was present over the surface of the coils at their point of contact with the aneurysm neck (Fig. 4).

QUANTITATIVE HISTOPATHOLOGY
We noted a statistically significant difference at 2 weeks in the thickness of the cellular layer covering the TGFβ-coated coils (mean thickness, 36 ± 15 μm) versus that of covering control coils (mean thickness, 3 ± 5 μm) (P = 0.02). Cellular coverage was nearly completely absent over all coil loops in the control samples at 2 weeks, and a measurable thickness of tissue was noted over all TGFβ-coated coils at 2 weeks.

At 6 weeks after implantation, the control coils demonstrated a significant increase in thickness covering the coils as compared with control coils at 2 weeks (37 ± 6 μm at 6 wk versus 3 ± 4 μm for control coils at 6 and 2 wk, respectively; p = 0.002), TGFβ-coated coils demonstrated a trend toward increased thickness from
2 to 6 weeks, but this difference did not reach statistical significance. Notably, there was no statistically significant difference at 6 weeks between the thickness covering control coils versus that covering TGFβ-coated coils (37 ± 6 μm versus 86 ± 74 μm for control and TGFβ-coated coils, respectively; P=0.30).

**DISCUSSION**

In this study, we compared the "biological activity," defined as the degree of cellular proliferation around and within coils, of standard platinum coils with that of platinum coils coated with TGFβ in a rabbit model of secular aneurysms. TGFβ was chosen for this study because it is a growth factor that plays a prominent role in wound healing and because it binds nonselectively to various inorganic surfaces, including platinum coils (13,20). We demonstrated that coating with TGFβ improves cellular coverage early after coil implantation as compared with standard coils. However, the difference in degree of cellular proliferation over coils dissipated over time; in our model, no difference was observed between TGFβ-coated coils and control coils at 6 weeks.

Numerous authors have proposed methods for increasing the "biological activity" of platinum coils to improve occlusion rates in cerebral aneurysms. Collagen and other basement membrane coatings, collagen filaments, growth factors and other proteins, polymers, and cells have been added to platinum coils to improve fibrotic reaction to implanted coils (6,7,17,23). Although some of these techniques demonstrated promise in preclinical testing, none has reached clinical application. Most of these coil modification techniques were tested in a swine model of aneurysms; this model evidently undergoes spontaneous thrombosis and fibrosis even without treatment. To our knowledge, none of the preclinical testing in swine of modified platinum coils has included an untreated control arm, rendering it impossible to interpret histological findings in treated aneurysms. A recent report used a surgically created aneurysm in rabbits to test the efficacy of plasminogen activation inhibitor in improvement of intra-aneurysm healing (3). These latter authors failed to demonstrate improvement in cellular proliferation over the coils by use of plasminogen activation inhibitor coatings. We hypothesize that thin coatings of bioactive substances on platinum coils are unable to effect metabolic changes at any significant distance from the coil surface. Because only a small minority of the volume of a coil-embolized aneurysm contains coil, and the majority of the volume represents blood, it is reasonable to assume that improvements in healing must reach long distances relative to coating thickness. Our TGFβ coating was able to improve cellular coverage at early time points after embolization, but these differences did not persist. We hypothesize that more durable improvements may occur with the use of bioactive substances that either permeate further into the thrombus surrounding the coil loops or are produced during a prolonged period of time. Although our study failed to demonstrate prolonged "bioactivity" within experimental aneurysms by use of a TGFβ coating, we remain optimistic that TGFβ, when delivered in larger amounts or in a more prolonged fashion than in...
this study, may improve intra-aneurismal fibrosis. As stated above, we chose to focus on TGF\(\beta\) among many available growth factors, not only because it plays a prominent role in wound healing but also because it has been demonstrated to bind nonselectively to various inorganic surfaces (18,20). TGF\(\beta\) is released from platelet a-granules and influences many of the cells involved in wound healing. TGF\(\beta\) enhances epithelial cell coverage (12) and stimulates fibroblasts to produce Type I collagen and fibronectin (17). All of these biological actions are considered by us to be desirable within coil-embolized aneurysms. Prolonged delivery of growth factors may be achieved either by binding the proteins to various nonmetallic carriers or by implantation of tissue allografts that have been biologically modified to produce and secrete the protein (12, 14).

Our study had several important limitations. As observed with many protocols that use metallic implants, detailed immunohistochemical processing is difficult or impossible. Because of these severe limitations in tissue processing, we have not attempted to identify the cell types observed proliferating adjacent to implanted coils. We have simply identified nucleated versus no nucleated cells. The former probably represent fibroblasts, smooth muscle cells, or inflammatory cells, and the latter almost certainly represent red blood cells in various stages of breakdown. Even within this limitation, we consider it useful to identify any type of nucleated cell adjacent to implanted coils, if the control coils fail to demonstrate such findings. Another limitation of this study was the small sample size. Even with the small sample size, however, we were able to demonstrate statistically significant differences between test and control coils at an early time point. Furthermore, our study was performed by use of a rabbit model of aneurysms that may not perfectly simulate human intracranial aneurysms. Among the many animal models available, however, the model used herein has numerous advantages. It shares the morphological features observed in humans, with parent arteries of approximately 3 to 4 mm and aneurysm cavities of approximately 6 mm, and it arises at a prominent vessel curve. The wall is arterial, rather than venous as observed in other animal models of aneurysms, and there is no local surgery around the aneurysm cavity. Last, we did not include angiographic follow-up as a part of this study. Our primary interest was to analyze the histological reaction to the coated coil rather than coil morphology.

A further limitation of our study was that we could not match packing density exactly in each aneurysm, and the degree of packing density probably is an important determinant in the biological response to implanted coils. We remain highly focused on the interaction between the coil surface and the intra-aneurysmal tissue, and our model of a single coil allows clear identification of this interaction. Numerous coil winds were readily assessed regarding thickness of overlying tissue in each sample. Another reason to purposely “under pack” the aneurysm cavities is that biological modifications probably will have most impact in large and giant aneurysms, in which dense packing is impossible in most cases. Because our animal model usually results in small aneurysms, we used the “underpacked” small aneurysm as a surrogate for moderately packed large and giant aneurysms.
REFERENCES


