Tenascin-C–coated platinum coils for acceleration of organization of cavities and reduction of lumen size in a rat aneurysm model

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Objective. Detachable platinum coils are widely used in the endovascular treatment of intracranial aneurysms. The use of coil placement produces a higher incidence of aneurysm recurrence compared with surgical clipping. To reduce the incidence of recurrence by promoting clot organization, the authors designed a platinum coil coated with tenascin-C (TNC), an extracellular matrix glycoprotein, and then histologically examined tissue responses.

Methods. Platinum coils were prepared by successive coatings with cationic polyethyleneimine and anionic heparin and then TNC or basic fibroblast growth factor (bFGF) was immobilized by affinity binding to the heparin. Six unmodified, six heparin-coated, six bFGF-coated, or eight TNC-coated platinum coils were inserted into ligated common carotid arteries (CCAs) of adult male rats, and CCA segments were harvested after 14 or 28 days.

The percentages of organized areas occupying the luminal cavity in unmodified, heparin-coated, bFGF-coated, and TNC-coated groups were 4.8 ± 4.6, 1.6 ± 1.1, 17.9 ± 10.7, and 93.4 ± 6.9%, respectively. In addition, the mean lumen size in the TNC-coated group (0.35 ± 0.23 mm²) was reduced to less than half that of the unmodified group (0.72 ± 0.21 mm²). Immunohistochemical analysis revealed that α-smooth muscle actin–positive cells were a major cellular component of the organized tissue within the TNC-coated coils but not in the bFGF group. Collagen fibrils in the organized areas were also much thicker and denser with TNC-coated coils than with bFGF-coated coils.

Conclusions. Placement of TNC-coated coils can remarkably accelerate organization of luminal cavities and reduce their volume, providing improved efficacy of these coils for endovascular embolization.

Key Words • tenascin-C • basic fibroblast growth factor • endovascular treatment • intracranial aneurysm • rat

Endovascular treatment of intracranial aneurysms with detachable platinum coils, such as Guglielmi detachable coils, has been widely accepted as a less invasive alternative to standard surgical clipping. According to the results of the International Subarachnoid Aneurysm Trial, endovascular treatment of acutely ruptured aneurysms is both safe and effective compared with surgery and can improve the outcome of patients who have suffered subarachnoid hemorrhage. An endovascular approach, however, produces a higher incidence of recurrence compared with surgical clipping, especially in large and giant aneurysms. Histological analysis of aneurysms in human autopsy cases has demonstrated a lack of clot organization in the lumens, probably due to the relative biological inertness of platinum coils. Therefore, to enhance the repair processes, a variety of surface modifications of platinum coils for endovascular aneurysm treatment have been attempted in experimental studies. Tenascin-C, an extracellular matrix glycoprotein, is expressed in adult tissues undergoing tissue remodeling; for example, during neoplasia and in inflammation and wound healing. Tenascin-C is also closely related to neointimal formation after angioplasty and atherosclerosis and it promotes migration and growth factor–dependent proliferation of arterial SMCs. In addition, adventitial myofibroblasts expressing TNC are thought to be actively involved in neointimal hyperplasia and so this molecule is considered a key molecular target for prevention of arterial stenosis.

We hypothesized that TNC could promote organization of the aneurysm cavity in endovascular treatment when using platinum coils. To test this hypothesis, heparin-coated coils were used to deliver TNC molecules to the lumen of blind-ended sacs in a rat model because it is known that two sites that bind to heparin exist in the TNC subunit.

Materials and Methods

Coil Preparation

Straight unmodified platinum coils 300 μm in diameter and 12.5
mm in length (Maruho Hatsujo Kogyo, Kyoto, Japan) were prepared by successively coating them with cationic polyethyleneimine and anionic heparin. Details of the preparation of the heparin-coated coils have been described by Ohyama, et al. Heparin-coated coils were used to deliver two different heparin-binding proteins: bFGF and TNC. Just before coil implantation, surface modifications were made to the heparin-coated coils by immersion in 100 \( \mu \)g/ml of bFGF (Kaken, Tokyo, Japan) or 100 \( \mu \)g/ml of TNC, which had been purified from conditioned medium of human glioma cells (U251MG) in 10 mM phosphate-buffered saline at 4˚C for 1 hour. The coils were then washed with phosphate-buffered saline. Unmodified platinum (control group) and heparin-coated (heparin-coated group) coils were used as controls.

**Animal Selection**

The experiments were performed in accordance with the Guidelines for Animal Experiments of Mie University Faculty of Medicine. Adult male Sprague–Dawley rats, weighing 300 to 400 g each (SLC, Hamamatsu, Japan), were used. No anticoagulant or antiplatelet agents were administered before or after coil placement. Rats were divided randomly into four groups: six in the control, six in the heparin-coated, six in the bFGF-coated, and eight in the TNC-coated group.

**Coil Implantation**

All rats were anesthetized by an intraperitoneal injection of sodium pentobarbital (25 mg/kg of body weight). After a subcutaneous injection of lidocaine was administered, a paramedian skin incision was made from the angle of the mandible to the midclavicular line. The right CCA was exposed by dissection along the anterior border of the sternocleidomastoid muscle and a permanent ligature was then placed just proximal. A temporary clip was placed more than 15 mm proximal to the CCA bifurcation. A small arteriotomy was made 2 mm proximal to the distal ligature. A 12.5-mm-long coil segment was inserted into the CCA and new ligatures were placed just proximal to the arteriotomy to exclude it from the circulation and to fix the coil in the blood flow. The temporary clip was released to re establish blood flow in the carotid segment, and the wound was closed. The animals were returned to their cages to recover.

**Animal and Tissue Preparation**

Rats were killed 14 days after coil implantation with a lethal dose of sodium pentobarbital. The experiments were performed in accordance with the Guidelines for Animal Experiments of Mie University Faculty of Medicine. Adult male Sprague–Dawley rats, weighing 300 to 400 g each (SLC, Hamamatsu, Japan), were used. No anticoagulant or antiplatelet agents were administered before or after coil placement. Rats were divided randomly into four groups: six in the control, six in the heparin-coated, six in the bFGF-coated, and eight in the TNC-coated group.

**Fig. 1.** Photomicrographs showing organization of arterial cavities after coil implantation in a rat model. In control and heparin-coated cases blind-ended sac lumens are filled by a blood clot; organization was scarce 2 weeks after implantation. In the bFGF-coated example, the clot is partly organized with cellular components; in the TNC-coated case, the lumen is almost completely organized and reduced in size, the arterial media being wrinkled (arrowheads). Asterisks indicate tissue-free areas after removal of the coils during tissue preparation. Bar = 100 \( \mu \)m.
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![Diagram of HE, α-SMA, CD68, vWF staining](image)

**Fig. 2.** Photomicrographs showing characterization of organized tissues in arterial cavities after coil implantation. Hematoxylin and eosin staining (HE) shows organized areas containing spherical cytoplasm-rich cells in the bFGF case but spindle-shaped cells in the TNC example. Immunohistochemical analysis of α-SMA demonstrates dense accumulation of positive cells limited to the TNC case. Macrophages (CD68) are apparent in both groups, but accumulation of macrophages near the coil surface is shown in the TNC example. For the VWF-stained tissue, the interface region between the unorganized clot and organized area is positive with bFGF (left upper quadrant), whereas small vessels are positive with TNC. Arrows indicate arterial media. Asterisks indicate tissue-free areas after removal of the coils during tissue preparation. Bar = 100 μm.

Morphometric Analysis

Morphometric analyses were performed on cross-sections of each artery stained with H & E. Samples of the proximal region within 5 mm of the permanent ligature were photographed using a digital camera and processed with image analysis software (Scion Corp., Frederick, MD). The inside area of the arterial media encircled by the internal elastic lamina was determined as the vascular lumen, and cellular and fibrotic compartments in the lumen were defined as organized areas. The organized areas in a mean of five cross-sections of each individual artery were calculated, and the overall mean for individual groups was obtained. All data are expressed as the mean ± standard deviation. The unpaired two-tailed Student t-test was used to compare data between two groups, with a probability value of less than or equal to 0.05 being statistically significant.

Histochemical and Immunohistochemical Analyses

Immunohistochemical staining was performed to identify macrophages, SMCs/myofibroblasts, and endothelial cells. Sections were first treated with a monoclonal antibody against rat CD68 (Serotec, Oxford, United Kingdom) followed by peroxidase-conjugated goat anti–mouse immunoglobulin G Fab’ (MBL, Nagoya, Japan). Alpha-smooth muscle actin was stained using the EPOS system (Dako Japan, Kyoto, Japan), and endothelial cells were labeled with a polyclonal anti-VWF antibody (Dako Japan). Collagen fibers of Sirius red-stained sections were also detected under a polarized microscope.

**Results**

**Clot Organization After Coil Implantation in the Rat Aneurysm Model**

In groups in which uncoated and heparin-coated coils were implanted, luminal cavities of the blind-ended sac models were filled with blood clots, and organization began to be observable 2 weeks postimplantation (Fig. 1). In the bFGF-coated group, clots were partly organized with a cellular component consisting of spherical cells (Fig. 2). In the TNC-coated group, lumens were almost entirely organized and spaces between vascular walls and coils were filled with fibroblasts, macrophages, small vessels, and fibrotic components; that is, granulation tissue. The vascular walls appeared atrophic and the lumen was reduced in size.

Results of morphometric analyses of the organized areas and the sizes of the vascular lumens after coil implantation are given in Fig. 3. The percentage of organized areas occupying the luminal cavity in the bFGF-coated group (17.9 ± 10.7%) was significantly higher than in the control (4.8 ± 4.6%) and heparin-coated (1.6 ± 1.1%) groups (Fig. 3A). The effect of TNC coating was significantly stronger than that of bFGF and, as a result, almost the entire cavity (93.4 ± 6.9%) was occupied. In addition, TNC reduced the luminal area (0.35 ± 0.23 mm²) to one half that of the control.
trol group (0.72 ± 0.21 mm²), and bFGF showed a similar tendency (0.52 ± 0.27 mm²) (Fig. 3B).

Characterization of Organized Tissue

Next, we characterized the cellular components and the degree of fibrosis in organized tissues of cavities in the bFGF and TNC groups. Immunohistochemical analysis of αSMA demonstrated dense accumulation of positive cells in organized tissue of the TNC group but not in that of the bFGF group (Fig. 2). Macrophages labeled with CD68 were seen to a similar extent in both groups, although accumulation adjacent to the coil surfaces was prominent in the TNC group. Positive labeling with VWF was seen at interfaces between unorganized clots and organized areas in the bFGF group, and small vessels were positive in organized tissue of the TNC group. Formation of collagen fibers was analyzed by observing birefringence of collagen fibers stained with Sirius red (Fig. 4). In the bFGF group, collagen fibrils were scarcely detected in the organized tissue between the coils and vascular walls. In contrast, the TNC group exhibited dense accumulation of thick collagen fibrils in the organized tissue. As cell numbers gradually decreased, the collagen fibrils became denser and the surface of tissues around the coils finally became covered with mature collagen bundles at 28 days.

Discussion

The importance of endovascular treatment of intracranial aneurysms by using detachable platinum coils has now been established; however, aneurysm recurrence after coil placement remains a problem, especially in large or giant aneurysms and in those individuals presenting with signs of neural compression related to an aneurysm mass effect, which might be worsened by regrowth. In one series, additional treatments, such as repeated coil placement, surgery, and/or parent vessel occlusion, were required in 58% of 29 patients with very large or giant aneurysms treated with detachable coils at a median 50-month follow up. Authors of another study showed that 25% of large and giant aneurysms were incompletely occluded, and an additional one fourth were not occluded at all. Histopathological analysis of material obtained at autopsy or surgical removal of human cerebral aneurysms after coil placement also revealed that intraaneurysm organization is often incomplete, with blood spaces frequently encountered between coils and the aneurysm neck. Therefore, acceleration of the intraaneurysm organization is considered an important component in successful aneurysm obliteration when using platinum coils.
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To accelerate clot organization, a number of authors have modified coil surfaces with various materials, such as extracellular matrix proteins, growth factors, biostable or biodegradable polymers, and fibroblasts transfected with the bFGF gene.1 Murayama and colleagues20–22 demonstrated that platinum coils covered with bioabsorbable polymeric material accelerated fibrosis and neointima formation in a swine aneurysm model. A coil coated with collagen and vascular endothelial growth factor also promoted vascular wall thickening in a rat aneurysm model.2 In addition, a platinum coil with a bFGF-releasing polyvinyl alcohol core accelerated intraaneurysm organization.17 More recently, recombinant human vascular endothelial growth factor immobilized on heparin-coated coils, as in this study, promoted endothelialization at the orifices of rat external carotid artery sacs.24

In the present study in rats, TNC-coated coils dramatically accelerated clot organization and resulted in the complete occlusion of the luminal cavity with fibrotic tissues. Furthermore, this was accompanied by a marked reduction in the size of the vascular lumens. Organized tissues that formed around the TNC-coated coils were characterized by the accumulation of numerous αSMA-positive cells and dense collagen fibrils.

Tenascin-C is highly expressed in human atherosclerotic plaques and in neointima after percutaneous transluminal coronary angioplasty.10 Tenascin-C might be upregulated in the early stages of neointima formation in association with the accumulation of αSMA-positive cells in an arteri
graft stenosis rat model, as well as in human restenotic neointima after percutaneous transluminal coronary angioplasty, subsequently followed by deposition of collagen.11 In TNC-deficient mice, much less neointimal hyperplasia at the arterial anastomotic site of a simple aortotomy model was seen than in wild-type mice.12 These findings imply that TNC plays significant roles in neointimal formation. Recently, it was suggested that TNC might also contribute to adventitial remodeling, which involves migration of αSMA-positive myofibroblasts.5,20 Myofibroblasts share characteristics with fibroblasts and SMCs expressing αSMA, and when tissue injury occurs they migrate into the damaged lesion, form granulation tissue, exert strong contractions to minimize the wound size, synthesize and organize collagen fibrils, and finally cause shrinkage of the collagen matrix with corresponding wound closure.5,26 A close relationship between TNC expression and myofibroblasts has been reported in various tissues, and it is suggested that TNC might promote their recruitment.3,10

The present results indicate that myofibroblast recruitment occurs with TNC-coated coil implantation; the resultant migration of SMCs/myofibroblasts into the arterial cavity accelerates the processes involved in organization. Strong contraction forces generated by densely gathered SMCs/myofibroblasts together with contraction of collagen fibers during mature bundle formation could contribute to a reduction in lumen size. In addition, the organized tissues also showed infiltration of macrophages, which also secrete cytokines to induce migration and proliferation of SMCs.23 They might accelerate remodeling of the organized tissue by secreting proteases to degrade the extracellular matrix.

Because we used a simple blind-ended sac model of the right CCA, endothelialization across the aneurysm neck or effects on the parent artery of the aneurysm could not be assessed in this study. The TNC coating on coils may bring about a potential risk of parent artery stenosis or occlusion. Immunohistochemical analysis of TNC showed that labeling was limited in the tissue adjacent to the coils on Day 14, whereas labeling had disappeared by Day 28 (data not shown), suggesting that the effect of TNC could be limited to a small localized area and to an acute stage. To elucidate these issues, long-term observation of the tissue response to TNC-coated coils in experimental saccular aneurysms when using larger animals will be necessary.

Conclusions

Implantation of TNC-coated coils accelerated organization of cavities and reduced lumen size in a rat blind-ended sac model, indicating the possibility of preventing aneurysm recurrence after coil embolization. Further investigations of the potential future application of these coils in human aneurysms are therefore clearly warranted.

References

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