Aneurysm Organization Effects of Gellan Sulfate Core Platinum Coil with Tenascin-C in a Simulated Clinical Setting and the Possible Mechanism

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Background: This study aimed to deliver gellan sulfate core platinum coil with tenascin-C (GSCC-TNC) into rabbit side-wall aneurysms endovascularly and to evaluate the organization effects in a simulated clinical setting. *Methods:* Elastase-induced rabbit side-wall aneurysms were randomly coiled via a transfemoral route like clinical settings with platinum coils (PCs), gellan sulfate core platinum coils (GSCCs), or GSCC-TNCs ($n = 5$, respectively). Aneurysm-occlusion status was evaluated angiographically and histologically at 2 weeks post coiling. As each rabbit coiled aneurysm provided only 2-3 tissue slices due to technical limitations and prevented immunohistochemical evaluations, a PC, GSCC, or GSCC-TNC was randomly implanted in a rat blind-ended model ($n = 3$, respectively) and the organization effects were immunohistochemically evaluated for expressions of tenascin-C (TNC), transforming growth factor-beta (TGF-β), and matrix metalloproteinase-9 (MMP-9) 2 weeks later. *Results:* Coil handling was similar among the 3 kinds of coils. GSCCs showed a significantly higher ratio of organized area to the aneurysmal cavity than PCs, but GSCC-TNCs had the greatest organization-promoting effects on aneurysms (the ratio of organized area/aneurysmal luminal area: PC, 17.9 ± 7.1%; GSCC, 54.2 ± 18.3%; GSCC-TNC, 82.5 ± 5.8%). GSCC-TNCs had intense immunoreactivities for TNC, TGF-β, and MMP-9 in the organized thrombosis and tunica media. GSCCs also showed intense immunoreactivities for TNC, TGF-β, and MMP-9, although the extent was less than GSCC-TNCs. The immunoreactivities were hardly found in unorganized thrombus and the tunica media of aneurysm wall in the PC group. *Conclusions:* This study first showed that GSCC-TNCs promote intra-aneurysmal clot organization in simulated clinical settings using rabbits

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possibly through the TGF-β and MMP-9 upregulation. **Key Words:** Tenascin-C—gellan sulfate—transforming growth factor-beta—elastase-induced rabbit aneurysms—coil embolization.

Introduction

The endovascular treatment of cerebral aneurysms has been widely accepted as an alternative treatment to surgical clipping.[1] However, problems of recanalization and coil compaction still remain in aneurysms treated with platinum coils (PCs). Although previous studies demonstrated that PCs induce thrombus formation within the aneurysm sac shortly after embolization,[2,3] fresh thrombus is unstable and subject to thrombolysis. The subsequent process, that is, organization of thrombus, is thought to be necessary for aneurysm healing to be completed. However, in many cases, thrombus organization occurs very late and remains incomplete in the long term, causing the recanalization after coil embolization of cerebral aneurysms.[2]

For decreasing the recanalization after coil embolization, we developed a new coil using a matricellular protein tenascin-C (TNC), gellan sulfate core platinum coil with tenascin-C (GSCC-TNC). TNC has fibrosis-promoting effects.[4,5] Gellan is an extracellular polysaccharide that pseudomonas elodea produces, and is applied to food as gellan gum, guaranteeing its safety for human.[6,7] Gellan sulfate (GS), which was developed by sulfating gellan, is a heparin-like molecule and therefore binds to TNC in a concentration-dependent fashion.[4,8] Gellan sulfate core platinum coil (GSCC) was developed by the insertion of a thread of GS into the central hollow of PC, and was then immersed in TNC solution to produce GSCC-TNC.[6] Thus, locally applied TNC may not affect thrombogenicity, but promotes intra-aneurysmal clot organization possibly by recruiting macrophages, which secrete cytokines to induce migration and proliferation of smooth muscle cells (SMCs).[4] The authors previously reported preliminary data that a straight-shaped GSCC-TNC promoted clot organization in a carotid artery-stump model of rats.[6] As the next step before clinical application of this new device, it is necessary to evaluate the safety and efficacy in bigger animal models that adequately mimic human pathophysiology, and to analyze the underlying mechanisms of the efficacy.

The aims of the present study were thus for the first time to obliterate a rabbit side-wall aneurysm with GSCC-TNC endovascularly like a clinical setting, and to evaluate the organization effects and the possible mechanisms.

Materials and Methods

Study Protocol

All procedures were approved by the Animal Ethics Review Committee of Mie University.

First, experiment 1 was conducted to examine the organized effects of GSCC-TNC in a simulated clinical setting. In 15 rabbits, elastase-induced, saccular aneurysms were produced and randomly coiled with PCs, GSCCs, or GSCC-TNCs ($n = 5$ each) via the femoral artery. After 2 weeks, the aneurysm-occlusion status was evaluated angiographically and histologically.

Second, experiment 2 was conducted to examine the possible mechanisms for GSCC and GSCC-TNC to promote aneurysm organization because GSCC also had intra-aneurysmal organization-promoting effects. In rabbit coiled aneurysms used in the present study, it was impossible to remove metabolic coil fragments within the aneurysm and to prepare the section for immunohistochemical evaluations while intra-aneurysmal thrombus and organized tissues remained intact due to technical limitations. Therefore, we used a rat blind-ended model, in which straight coils were used and easily removed while tissues around the coil were preserved. Nine rats randomly underwent implantation of a straight PC, GSCC, or GSCC-TNC (n = 3 each) in the blind end of the right common carotid artery (CCA), which was evaluated using hematoxylin and eosin (HE) staining and immunohistochemistry for TNC, transforming growth factor-beta (TGF-β) and matrix metalloproteinase-9 (MMP-9) at 2 weeks after coil implantation.

Animal Models and Coil Embolization

In experiment 1, elastase-induced, saccular aneurysms were produced in 15 New Zealand white rabbits (body weight, 3-4 kg; SLC, Hamamatsu, Japan) using previously published methods.[8] Anesthesia was induced by an intramuscular injection of ketamine and xylazine (60/6 mg/kg body weight) and was maintained by inhalation anesthesia of isoflurane (.5-1.5% end-tidal concentration). The distal portion of the right CCA was ligated. A small arteriotomy was performed at approximately 3 cm cephalad to the origin of the right CCA, and a 3-French balloon catheter (Fogarty; Baxter Healthcare, Irvine, CA) was advanced and inflated to occlude the origin of the right CCA under fluoroscopic guidance. Elastase (1.0 mg protein/mL) (Sigma-Aldrich, St. Louis, MO) was incubated within the lumen of the proximal right CCA for 20 minutes, after which the balloon was deflated, the catheter system was removed, and the right CCA was ligated just proximal to the arteriotomy. Thus, aneurysms formed from the stump of the right CCA. At least 28 days later, under general anesthesia and sterile conditions, the right common femoral artery was exposed, a 4-French sheath