Histological Changes in the Rat Common Carotid Artery Induced by Aneurysmal Wrapping and Coating Materials

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Abstract

Histological changes in and around the arterial walls of rats were investigated following topical application of aneurysmal wrapping and coating materials, including a fibrin glue, a cyanoacrylate glue (Biobond), and cotton fibers (Bemsheet). Bilateral common carotid arteries were exposed using sterile techniques, and one of the test materials was applied to the right artery. The left artery was used as the control. Changes in arterial histology were evaluated at 2 weeks, 1 month, 2 months, and 3 months after surgery. The fibrin glue was surrounded by intense inflammation at 2 weeks after surgery. Both the fibrin glue and inflammation had disappeared at 2 months, but the glue had induced mild inflammation in the adventitia. Biobond caused chronic inflammation, necrosis of the media, and thickening of the arterial wall due to fibrosis in both the media and adventitia. Bemsheet produced chronic inflammation, progressive fibrosis, and granuloma. Connective tissue increased in the adventitia, but no major changes were observed in the media. The Bemsheet fibers remained unchanged, and adhered to the arterial wall. Although arterial stenoses were not observed in the present study, the results suggest that cyanoacrylate glue can cause the arterial occlusive lesions observed following aneurysm surgery.

Key words: cotton, cyanoacrylate glue, fibrin glue, histology, rat

Introduction

The ideal surgical procedure for the treatment of intracranial aneurysms is complete neck clipping. However, wrapping and coating techniques for residual aneurysms are occasionally required, such as when the complete neck closure induces stenosis of parent arteries or occlusion of adjacent perforating arteries.

The development of wrapping and coating materials that prevent bleeding from the aneurysms, but do not adversely affect surrounding tissue, has resulted in the commercial availability of many wrapping and coating materials. However, no commercially available material provides ideal reinforcement without secondary side effects. Arterial occlusive lesions and subsequent cerebral ischemia have occurred following aneurysm surgery using wrapping and coating materials such as cotton fibers and cyanoacrylate glue. The present study investigated the histological changes in and around the arterial walls of rats following topical application of three wrapping and coating materials (fibrin glue, Biobond, and Bemsheet).

Materials and Methods

Three materials were tested: a fibrin glue (Bolheal; Fujisawa Pharmaceutical Co., Ltd., Osaka), consisting of human fibrinogen, human antihemophilic factor-XIII fraction, human thrombin, bovine aprotinin, and calcium chloride; a cyanoacrylate glue (Biobond; Yoshitomi Pharmaceuticals Co., Ltd., Osaka), consisting of a cyanoacrylate monomer, a nitrile rubber, and a polyisocyanate; and a non-fabric cloth of 100%-cellulose cotton (Bemsheet; Kawamoto Co., Ltd., Osaka).

Sixty male Sprague-Dawley rats (Charles River Japan, Inc., Atsugi, Kanagawa), weighing 250–300 g had free access to food and water. The animals were anesthetized with pentobarbital sodium (50 mg/kg, i.p.), and surgery was performed under sterile conditions. Under the operating microscope, approximately 5 mm of the outer half of the bilateral com-
monic carotid arteries (CCAs) was exposed via a ventral midline skin incision in the neck. The inner half of the CCAs and the surrounding nerves were not manipulated, and care was taken to not directly touch the CCA wall. The test material was applied to the right CCA. Each material was applied in 20 animals. Both fibrin glue and Biobond were applied as one drop from a 22-gauge polyethylene tube, and Bemsheet was applied as a 3 × 5 mm². The left CCA of each rat was used as the sham operation control.

Five animals from each group were sacrificed at intervals of 2 weeks, 1 month, 2 months, and 3 months following surgery. The animals were first anesthetized with pentobarbital sodium (100 mg/kg, i.p.), and following transcardiac perfusion-fixation with a 10% formalin solution, both CCAs were removed with the surrounding tissue. Sections (1 μm thick) were stained with HE and Elastica Masson stains. Histological changes induced by the test materials in the granulation tissue and the vascular walls were assessed using light microscopy.

Results

No microscopic evidence of abnormality or infection was observed in any of the control vessels (data not shown). No changes in the intima and no stenoses were evident in any of the control or experimental vessels examined. The chronology of extravascular reactive cell infiltration in the granuloma and the histological changes in the vascular walls of the right CCAs are summarized in Tables 1 and 2, respectively.

### I. Fibrin glue

Fibrin glue was identified by HE staining as an amorphous, acellular, and eosinophilic substance, surrounded by intensive inflammation of mainly histiocytes and fibrocytes at 2 weeks (Fig. 1). A slight increase in fibroblasts was observed in the adventitia. The glue had been almost totally absorbed at 1 month (not shown). Granulation tissues containing primarily macrophages remained around the CCA, where the glue had been applied previously. The inflammation had decreased in severity from that seen at 2 weeks. The glue and the inflammation had disappeared at 2 and 3 months. No histological changes were observed in the CCA walls at 3 months.

### II. Biobond

Biobond appeared as an irregularly shaped material, containing small particles (0.5–2 μm in diameter). Biobond was surrounded by inflammatory cells, consisting of mainly histiocytes, fibroblasts, and macrophage-derived multinuclear giant cells at 2 weeks. The connective tissue had increased inside and outside the adventitia, and surrounded the granulation tissue. Necrosis was evident in the media. The granulation tissue surrounding the Biobond had enlarged due to the presence of inflammatory cells and reactive blood vessels at 2 and 3 months (Fig. 2). The adventitia had thickened due to an increase in fibrous connective tissue. In the outer layer of the media, at the side of Biobond application, the nucleus of the muscle cells had disappeared, the circular smooth muscle layers had decreased in number (Fig. 3), and the elastic bands in the media were frequently disrupted (Fig. 4). Collagen fibers had increased in the media, and replaced the exter-

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±: slight, +: mild.
nal layers of the smooth muscles. Consequently, the vessel wall had thickened due to inflammatory processes. No distinct changes in the tunica intima were observed.

III. Bemsheet

Bemsheet appeared as rod-shaped fibers (10-15 × 50-60 µm²). Bemsheet had induced an inflammatory granuloma, consisting of cell infiltration by histiocytes, giant cells, and fibroblasts at 2 weeks. Thereafter, the granulation tissue showed progressive fibrosis (Fig. 5). The amount of connective tissue (fibroblasts and collagen fibers) in the adventitia had increased, making the CCA look stiff (Fig. 6). The tunica media showed degeneration at 2 months, by which time the smooth muscle cells had disappeared and been replaced with fibrous tissue. The number of collagen fibers dispersed among the Bemsheet fibers increased with time. The Bemsheet fibers and the surrounding giant cells were unchanged at 3 months, and were in direct contact with the CCA.

Discussion

I. Methodology

The present study used the major extracranial vessels of rats instead of the intracranial vessels of humans to examine possible mechanisms of arterial occlusive lesion observed in clinical settings following the use of aneurysmal wrapping and coating materials. Therefore, there are species differences and differences in the resulting inflammation between intracranial muscular-type and extracranial elastic-type vessels between the present and previously published studies of wrapping and coating materials.

Fig. 1 Photomicrograph showing histological changes 2 weeks after topical application of fibrin glue (arrow). CCA: lumen of the common carotid artery. HE stain, ×10.

Fig. 2 Photomicrograph showing histological changes 3 months after topical application of Biobond including irregular-shaped cavities containing Biobond. CCA: lumen of the common carotid artery. HE stain, ×16.

Fig. 3 Photomicrograph showing histological changes 3 months after topical application of Biobond (star) with necrosis of the media (arrowheads). CCA: lumen of the common carotid artery. HE stain, ×100.

Fig. 4 Photomicrograph showing histological changes 3 months after topical application of Biobond (star) including disruption of the elastic bands of the media (arrows). CCA: lumen of the common carotid artery. Masson stain, ×100.
II. Fibrin glue

Fibrin glue is used in some neurosurgical procedures, particularly to seal the dura mater, and also to prevent vasospasm in patients with aneurysmal subarachnoid hemorrhage (SAH). An experimental study has indicated the possibility of using fibrin glue to diminish epidural scar formation following spinal surgery. Fibrin glue is a material of human origin, so this material is expected to reduce degeneration of the vascular wall, caused by inflammation, to a minimum. Therefore, some neurosurgeons have used the glue for aneurysm reinforcement.

In the present study, fibrin glue induced an intense inflammation 2 weeks following application. This inflammation was most likely an immunological reaction against the xenogenic protein. Therefore, the glue was removed completely by the inflammatory reaction. We observed almost no inflammation, and complete disappearance of the glue at 2 and 3 months. These results are similar to those reported previously. Another experimental study found that fibrin glue, used as a coating material for aneurysms, disappeared from around the aneurysms at 3 months following application. Fibrin glue is primarily an allogenic protein compound, so may be identified as a foreign body, resulting in inflammation and ingestion by phagocytic cells. The results of the present and previous studies indicate that fibrin glue is absorbed completely within 2 months after application. Therefore, fibrin glue cannot provide adequate aneurysmal reinforcement and should not be used as the sole reinforcing material for aneurysms. Clinical vasospasm usually occurs 2 weeks following SAH, so we cannot conclude whether fibrin glue is safe for SAH patients. Moreover, fibrin glue contains a bovine protein, aprotinin, which can induce anaphylaxis in humans.

III. Biobond and Bemsheet

Biobond induced chronic and progressive inflammation. Inflammatory cells were observed in the adventitia at 3 months. Necrosis of smooth muscle cells and disruption of the elastic fibers in the media were also observed. Subsequently, the inflammatory processes caused an increase in the width of both the media and the adventitia. These findings are similar to those observed in the cerebral blood vessels of SAH patients. Changes in the vessels of patients who died within 17 days after SAH were principally localized in the adventitia and media, whereas the intima showed slight changes, primarily swelling. In patients who died 3 weeks after SAH, the adventitia had thickened due to edema with an exudate of lymphocytes and macrophages. The smooth muscle cells of the media showed necrosis. Changes in the intima were most prominent and the intima always showed concentric subendothelial thickening by fibrosis. We did not observe such intimal changes, but these might have...
occurred had the vessel been in contact with the Biobond for longer. The present results suggest that arterial stenoses may be the result of Biobond-induced chronic inflammatory processes in the arterial wall, such as modifications of the normal vessel architecture, replacement of the adventitia with collagen fibers, and necrosis and fibrosis in the media.

Both Bemsheet and Biobond have been used during aneurysm neurosurgery to obtain stronger reinforcement. In the present study, Biobond caused chronic inflammation in both the adventitia and media of the CCA. In contrast, Bemsheet invariably adhered to the vessel wall. If both materials are used at the same time, arterial lesions due to Biobond may progress more markedly than in the present study because the Biobond is in contact with the vessel for longer. This possibility may explain the clinical manifestations of parent artery stenosis within a few months following the application of both Biobond and Bemsheet during aneurysm surgery. The present results suggest that Biobond-induced changes, particularly in the media, may induce arterial occlusive lesions in the clinical setting.

Bemsheet induced inflammatory granuloma and eventual replacement of the adventitial wall with collagen fibers, but caused no marked changes in the media. Bemsheet adhered firmly to the vessel wall, making the wall appear rigid, suggesting that the vessel might have lost pulsatility. Although Bemsheet did not induce marked changes in the media, cotton alone is known to induce arterial occlusive lesions. Marked fibrosis or granuloma formation may cause stenosis, and investigation of granulation tissue contraction following inflammation has demonstrated that myofibroblasts but not collagen fibers are responsible for contraction. Although vessel stenosis due to granulomas was not observed in the present study, simple compression due to the surrounding granuloma can induce arterial steno-occlusion. Therefore, Bemsheet may cause arterial occlusive lesions, although arterial stenosis was not observed in the present study.

The present results suggest that the cyanoacrylate glue can cause the arterial occlusive lesions observed following aneurysm surgery, although arterial stenoses were not observed in the present study. Further study is needed to determine whether histological changes become more marked than in the present study when both cyanoacrylate glue and cotton fibers are used at the same time.

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Commentary

Herrera and colleagues have completed an interesting and practical study in which they documented the progressive histological changes in a rat common carotid artery induced by various materials that have been used clinically for wrapping and coating of unclippable aneurysms. As the authors point out, the findings in this study may not be directly applicable to the clinical situation because of differences between intracranial and extracranial arteries as well as interspecies differences. The results, however, assist in the understanding of the long-term effects of these agents and their potential for inducing changes that will result in a decreased incidence of subsequent subarachnoid hemorrhage.

Base upon their findings, and those from other studies, it is apparent that fibrin glue provides no lasting benefit when used for the purpose of reducing the risk of subsequent hemorrhage from an unclippable aneurysm. Cyanoacrylate glue produced chronic inflammation in both the adventitia and media whereas cellulose cotton produced granulation tissue and progressive fibrosis with an eventual replacement of the adventitial wall with collagen fibers. The cotton induced no changes in the media.

Wrapping or coating of intracranial aneurysms is clearly an inferior treatment option and should only be used in rare cases. As the authors point out, both cyanoacrylate glue and cellulose cotton may induce arterial stenosis. We and others have seen cases of granuloma formation that has produced progressive optic nerve compression and dysfunction. No wrapping material completely eliminates the risk of future of subarachnoid hemorrhage as does clip ligation. Importantly, many aneurysms are determined to be “unclippable” by inexperienced surgeons who then use wrapping or coating materials. Reoperation on ruptured enlarging aneurysms that have been previously wrapped or coated is significantly more difficult. Before ever using any wrapping or coating material, the operating neurosurgeon should question whether that particular “unclippable” aneurysm will be clippable in the hands of an experienced neurovascular surgeon.

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The ideal material for wrapping aneurysm is still controversial. The authors investigated the histological changes in the rat carotid artery induced by aneurysmal wrapping and coating materials, including fibrin glue, cyanoacrylate glue (Biobond), and cotton fibers (Bemsheet). It is well known that ethyl 2-cyanoacrylate adhesive (Aron Alpha) causes a histotoxic reaction. Aron Alpha, for example, causes pial granulomatous inflammation, angionecrosis, thrombosis or hemorrhage in the cat middle cerebral artery. The authors revealed similar results by using Biobond glue and suggest that cyanoacrylate glue can cause the arterial occlusive lesions observed following aneurysm surgery. Bemsheet also causes chronic inflammation, progressive fibrosis and granuloma. We have also experienced a patient with ischemic occlusive carotid lesion by using Bemsheet after aneurysm surgery. These data give us useful and important information. Inflammation may have an alternative effect to reinforce the aneurysmal wall or occlude the parent artery.

Reference


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Department of Neurosurgery
Neurol Med Chir (Tokyo) 39, February, 1999
The authors have performed an interesting study in which histological changes in and around the rat common carotid artery were investigated following topical application of three materials for wrapping and coating of cerebral aneurysms (fibrin glue, cyanoacrylate glue, and cotton fibers). Although the clinical occurrence rate of arterial narrowing using these materials is unknown, it is supposed to be very low. However, it is important to know the histological reaction to these materials because the residual neck of the aneurysm will be reinforced with them until alternative materials appear.

The wrapping materials could cause inflammation in and around the common carotid arteries of the rat, but not arterial stenoses in the present study. The precise mechanism of induction of arterial stenoses by topical use of those materials could not be elucidated. If necrosis occurs in the media and the elastic bands are disrupted, the artery will dilate and not be narrowed. In general, the artery can be stenosed or obliterated, following intimal thickening and organizing thrombus subsequent to severe endothelial injury. If a further study is designed to introduce the arterial stenosis, cyanoacrylate glue and cotton fibers must be used simultaneously or the authors should observe the inflammation for more than 3 months.

Hideaki Nukui, M.D.

This is an interesting, clinically useful article concerning the benefit and risk of three different aneurysmal wrapping and coating materials which are commonly used in neurosurgical practice. The authors studied the biochemical reaction following topical application of these materials in the arterial walls of the rat common carotid artery. We can understand the histological changes in the normal arterial walls from their well designed study. The main purpose of aneurysmal wrapping and/or coating is however to protect bleeding from the aneurysmal dome or bleb. The histological structure of the aneurysmal dome is quite different from those of the aneurysmal neck and parent artery. Therefore, the biochemical effect of those materials on the aneurysmal dome is still unknown from these results. Further study will be necessary to clarify this actual problem to determine the benefit of the materials. But this article gives us an important information on the availability, selection and direction for use of these materials not only in aneurysm treatment but also for other neurosurgical operations.

Minoru Shigemori, M.D.

Hideaki Nukui, M.D.