Histological Changes in the Rat Common Carotid Artery Following Simultaneous Topical Application of Cotton Sheet and Cyanoacrylate Glue

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Abstract
Histological changes in and around the arterial walls of rats were investigated following simultaneous topical application of cotton sheet and cyanoacrylate glue. The bilateral common carotid arteries were exposed using sterile techniques, and the test materials were applied to the right artery. The left artery served as a control. Changes in arterial histology were evaluated at 2 weeks, 1 month, 2 months, and 3 months after surgery. Extensive inflammation consisting primarily of histiocytes and multinuclear giant cells was observed around the materials, but tended to decrease by 3 months. Necrosis in the media and fibrosis in the adventitia initially appeared around 2 weeks, and became advanced by 2-3 months. At 2-3 months, disruption of elastic fibers and marked fibrosis in the media were seen, and endothelial proliferation in the intima appeared. Intimal proliferation was observed at both the experimental and other sites of the vessels. The present results suggest that simultaneous use of the test materials can cause the arterial occlusive lesions observed following aneurysmal surgery.

Key words: cotton, cyanoacrylate glue, histology, rat

Introduction
Neck clipping is the most widely accepted treatment for intracranial aneurysms, but this is not always possible, particularly for fusiform and atherosclerotic aneurysms. Reinforcement is often applied in such cases. Various wrapping and coating materials are used to reinforce aneurysms, including cyanoacrylate glues and cotton. However, arterial occlusive lesions and subsequent stroke may occur following reinforcement of unruptured aneurysms using cotton sheet (Bemsheet®) or cyanoacrylate glue (Biobond®). The present study investigated the histological changes following topical application of cotton sheet and cyanoacrylate glue in and around the arterial walls of rats.

Materials and Methods
Non-fabric sheet made of 100%-cellulose cotton (Bemsheet®; Kawamoto Co., Ltd., Osaka) and cyanoacrylate glue (Biobond®; Yoshitomi Pharmaceuticals Co., Ltd., Osaka) were tested.

Twenty male Sprague-Dawley rats (Charles River Japan, Inc., Atsugi, Kanagawa), weighing 200–300 g, were anesthetized with pentobarbital sodium (50 mg/kg, i.p.), and surgery was performed under sterile conditions. Under the operating microscope, 5 mm of the outer half of the bilateral common 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 not to directly touch the CCA wall. The right CCA was wrapped with 3 × 5 mm² of cotton sheet, and then coated with one drop of cyanoacrylate glue from a 22-gauge needle. The left CCA of each rat was used as the sham operation control.

Five animals 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.), followed by transcardiac perfusion fixation with 10% formalin solution. Both CCAs were then removed with the surrounding tissue. Sections (3 μm thick) were cut.
and stained with HE and elastica Masson stains. Histological changes in the granulation tissue and the vascular walls induced by the test materials were assessed. The chronology of the histological changes were evaluated semi-quantitatively as mild, moderate, and marked by one of the authors (Y.Y.).

Results

No microscopic evidence of abnormality or infection was observed in any of the control vessels (not shown). 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.

Cotton sheet appeared as rod shaped fibers (10–15 × 50–60 μm), and cyanoacrylate glue appeared as an irregularly shaped material containing small particles (0.5–2 μm in diameter). The extravascular granulation tissue showed severe inflammatory reaction at 2 weeks, when histiocytes and multinuclear giant cells were most prominent. The test materials were not directly attached to the arterial wall. Fibroblasts were seen in the adventitia, and necrosis in the media was observed in two of five rats (Fig. 1).

The inflammatory response at 1 month was similar to that at 2 weeks, but the number of eosinophiles in the granulation tissue had decreased (not shown). Fibrosis in the adventitia and necrosis in the media were seen in all rats.

The inflammatory response in the granulation tissue at 2 months was similar to that at 2 weeks, whereas no eosinophiles were present. The test materials were located very close to the CCA wall. Disruption of elastic fibers had appeared in the media, and infiltration of collagen fibers (or fibrosis) was observed in the superficial layers. Necrosis in the media was more advanced compared with that at 1 month. Intimal proliferation was observed both on the experimental side and in other regions of the CCA (Fig. 2).

At 3 months, the test materials were attached to the CCA. The number of inflammatory cells had decreased in the granulation tissue. Lymphocytes were still present in all rats. Endothelial proliferation could still be observed (Fig. 3 upper). The changes in the arterial wall resembled those seen at 2 months. Histiocytes were present in the adventitia. The media was very thin in places, and was substituted by collagen fibers believed to derive from the adventitia (Fig. 3 lower). Cotton sheet fibers showed no changes, but the amount of cyanoacrylate glue had decreased gradually, although it was still present at 3 months.

Table 1 Extravascular cell chronology in the granulation tissues

<table>
<thead>
<tr>
<th></th>
<th>Time after surgery</th>
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<tbody>
<tr>
<td></td>
<td>2 wks</td>
</tr>
<tr>
<td>Histiocytes</td>
<td>#</td>
</tr>
<tr>
<td>Multinuclear giant cells</td>
<td>#</td>
</tr>
<tr>
<td>Fibroblasts</td>
<td>#</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>+</td>
</tr>
<tr>
<td>Eosinophiles</td>
<td>+</td>
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</tbody>
</table>

Animal number: five in each group. +: mild, #: moderate, #: marked.

Table 2 Intravascular histological changes

<table>
<thead>
<tr>
<th></th>
<th>Time after surgery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 wks</td>
</tr>
<tr>
<td>Intimal proliferation</td>
<td>no</td>
</tr>
<tr>
<td>Media necrosis</td>
<td>+</td>
</tr>
<tr>
<td>fibrosis</td>
<td>no</td>
</tr>
<tr>
<td>elastic fiber disruption</td>
<td>no</td>
</tr>
<tr>
<td>Adventitia fibrosis</td>
<td>+</td>
</tr>
<tr>
<td>histiocyte</td>
<td>no</td>
</tr>
</tbody>
</table>

Animal number: five in each group. +: mild, #: moderate.

Fig. 1 Photomicrograph showing histological changes 2 weeks after topical application of both cotton sheet and cyanoacrylate glue. Necrosis in the media is evident (arrows). CCA: lumen of the common carotid artery. HE stain, ×100.
Fig. 2 Photomicrographs showing histological changes 2 months after topical application of both cotton sheet and cyanoacrylate glue. *upper:* Necrosis in the media and endothelial proliferation in the intima are visible. CCA: lumen of the common carotid artery. HE stain, ×100. *lower:* Disruption of elastic fibers in the media (arrow), and increased number of collagen fibers (fibrosis) in both the adventitia and media are evident. Elastica Masson stain, ×100.

**Discussion**

In the present study, extracranial vessels rather than intracranial vessels were used. The major difference is that the intracranial vessels have thinner adventitia, and thus histological changes due to application of vasculotoxic material should be more marked in the intracranial vessels. We previously studied the histological changes in rat CCAs in the presence of either cyanoacrylate glue or cotton sheet, and found necrosis in the media and fibrosis in the adventitia. In this study, the simultaneous use of these materials was investigated. Compared with the previous study, the extravascular cell reaction particularly by multinuclear giant cells, as well as necrosis in the media and fibrosis in the adventitia, was more pronounced in this study. Furthermore, we found intimal changes, which were not observed in the previous study.

The present results clearly demonstrate the occurrence of endothelial proliferation in the intima, as well as necrosis in the media and fibrosis in the adventitia, following simultaneous wrapping with cotton sheet and coating with cyanoacrylate glue. Intimal proliferation was observed not only at the site where the test materials had been applied, but also at other sites. This suggests that these materials can induce vascular wall abnormality, resulting in luminal stenosis or occlusion. Our clinical observations further indicate that proliferative pathology may progress centripetally to induce stenosis. We performed a second craniotomy on a patient who had received aneurysm reinforcement following...
clipping 2 years before and subsequently suffered asymptomatic stenosis in the parent arteries. Intraoperative observations following the second craniotomy showed that the outer surface of the arteries at the location of the angiographical stenoses appeared normal. Based on the present results, as well as the previous clinical and experimental studies, the toxic effect of the cyanoacrylate glue on the vessels was probably enhanced by simultaneous use with cotton cloth reinforcement, because the cyanoacrylate glue was in contact with the vessels for a longer period of time, thus allowing the glue to exert its toxic effects on the surrounding tissue.

In the present study, we also observed medial necrosis, elastic fiber disruption, and subsequent thinning of the media, suggesting aneurysm formation may be a risk. Ethyl 2-cyanoacrylate (Aron Alpha) may induce de novo aneurysm. Furthermore, experimental studies have shown that methyl 2-cyanoacrylate induces fusiform dilations in relatively large arteries (3–5 mm in diameter) during the first week following surgery. Therefore, cyanoacrylate glues appear to be a risk factor for aneurysm development, although the present study did not detect any aneurysmal changes.

Biodegradation of cyanoacrylate polymers can produce free radicals, which react with polyunsaturated lipids, leading to activation of the synthesis of prostaglandins and thromboxanes, which can induce inflammation and tissue toxicity. Cyanoacrylate glue alone can induce major vessel occlusion and subsequent stroke. However, the mechanisms involved in intimal proliferation remain unclear. Intimal change, as well as fibrosis in the adventitia, may indicate a defense mechanism against the toxic material applied outside the vessels.

Cotton fibers have no direct toxicity against any tissue. However, cotton alone used as an aneurysm reinforcement can result in occlusive lesions following granuloma formation. The cotton acts as a reinforcement material following progressive fibrosis, and this may lead to granuloma formation. Although the present study did not observe occlusive lesions due to granuloma, cotton may be a possible hazard for induction of vascular occlusive lesions.

The combination of cotton sheet and cyanoacrylate glue applied to the arterial walls of rats induced fibrosis in the adventitia, fibrosis, necrosis, and elastic fiber disruption in the media, and endothelial proliferation in the intima. These results suggest that simultaneous use of these materials may induce the occlusive vascular lesions observed previously in the clinical setting.

Acknowledgments
The technical assistance of Mr. Yozo Ito and Mr. Mitsuru Shirasawa, and the secretarial assistance of Miss Toshiko Aburaya and Mr. Yoshitaka Tozawa are gratefully acknowledged.

References


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Commentary

The authors should be commended for a well-designed and executed study of the reaction of the rat common carotid artery (CCA) on wrapping with cotton sheet and cyanoacrylate glue. The inflammatory reaction to a foreign body on the outer surface of the CCA is well documented in the second week already, and then further after one month and after two months, as well as the changes due to the inflammation during the third postoperative month. The contralateral CCA, which was only exposed, was the best control group. The authors, on the basis of their previous studies, claimed that the reaction to the same foreign material(s) in intracranial arteries is even more pronounced, since the wall of those arteries is thinner. Is it clear that the reaction of the CCA to the foreign materials occurs when these materials are applied on the surface of the CCA either in combination or each alone? The authors could not give the reader an exact answer as to why this reaction occurs and why the reaction passes through the artery wall from the adventitia through the muscular layer and also involves the intima. There is the possibility that the degrading products of the material(s) themselves, or in biochemical reaction with other substances, may provoke the inflammatory reaction. However, having some experience with "muslin tissue" wrapping of the intracranial arteries with the aim of reinforcing the walls, in cases of fusiform aneurysms or not completely clipped aneurysms (MCA or others), we found that some of the daughter arteries were occluded during the control angiography. We always thought that this could be caused either by granuloma formation around the artery and progressive narrowing of the lumen of the artery(ies), or might be the result of too tight wrapping reinforced with additional tightening of this wrapping material with additional clip(s).

This study, as well as previous studies and other neurosurgeons' experiences, supports the statement that the placing of wrapping materials and glue may induce inflammatory reaction and that the later changes on the foreign body may — and in many cases do — result in the stenosis, if not in the occlusion of the daughter arteries. The message is clear: In clinical work, do not perform the wrapping of any of the intracranial arteries unless it is of vital importance. However, the answer is not yet at hand: it could be either shunting or performing of a bypass. Also, these two answers are not only experience-dependant, but both also do require additional biological and neurophysiological studies.

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This is an important and useful report for aneurysmal surgery. Although occlusive complications by wrapping materials following aneurysmal surgery had been recognized clinically, basic experimental studies have rarely been reported. This experimental study revealed important evidence, that is, simultaneous topical application of cotton sheet and cyanoacrylate glue can cause arterial occlusive lesions on rats. Cotton fibers have no direct toxicity against any tissue. However, this may lead to granuloma formation. The combination of cotton sheet and cyanoacrylate glue applied to the arterial walls of rats induced fibrosis in the adventitia, fibrosis, necrosis, and elastic fiber disruption in the media and endothelial proliferation in the intima. In aneurysmal surgery, some neurosurgeons prefer to use those materials for reinforcement of the residual aneurysmal wall. This research suggests the risk of such a wrapping procedure.

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The authors report the risk of occlusive vascular lesions after simultaneous use of cotton sheet and cyanoacrylate glue as coating materials for cerebral aneurysms. The model was the extracranial carotid artery, which has different characteristics from the intracranial artery other than histology. The latter is surrounded by CSF, and its isolation from CSF by coating materials may be involved in the development of vascular lesions. Many neurosurgeons already know that wrapping by cotton sheet alone can also cause arterial stenosis by foreign body reaction. On the other hand, chemical or foreign body reactions against the coating materials may cause fibrosis of the aneurysmal wall which could prevent rupture. To prevent adverse effects of coating, I think we should not use both materials simultaneously, and should be careful not to use excessive amounts of glue. It is also desirable not to surround the parent artery totally by coating materials, especially by cotton sheet.

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