Effects of blood coagulation Factor XIII on the development of experimental cerebral aneurysms in rats

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Pathological and experimental studies have shown that cerebral aneurysms develop in part as a result of injury to the blood vessel wall. One of the peculiar aspects of aneurysm development is a defective proliferative or healing response to such injury. To examine this phenomenon, blood coagulation Factor XIII, which is known to enhance the healing process of wounds in general, was given to rats to induce experimental cerebral aneurysms. The rats were subjected to ligation of one common carotid artery and induction of hypertension, and were fed beta-aminoproprionitrile. Two weeks thereafter, Factor XIII was injected intravenously daily for 5 days (10 U/100 gm body weight/day). Twelve days after the start of Factor XIII injections, the rats were sacrificed and examined under light and electron microscopy. In seven of 12 bifurcations which developed small aneurysms, prominent intimal thickening was observed in the aneurysm lumen. In the most advanced cases, the aneurysm lumen was completely filled with proliferated smooth-muscle cells and collagen. In five of nine bifurcations that showed no aneurysm development, apparent intimal thickening was found at the site where aneurysms might be expected to grow. In the group of rats studied for induction of cerebral aneurysms but not given Factor XIII, none of 11 bifurcations with or without aneurysms showed such intimal thickening. The results indicated that the proliferative response at the sites of aneurysm development was modified by exogenous Factor XIII.

KEY WORDS: aneurysm, intimal proliferation, Factor XIII, endothelial injury, smooth-muscle cell

In order to clarify the etiology of saccular cerebral aneurysms, it is necessary to study the early stages of these lesions. Stehbens classified three types of changes at the cerebral arterial bifurcation that occur early in aneurysm formation: areas of thinning, funnel-shaped dilatations, and microscopic evaginations. We have developed a method of inducing cerebral aneurysms in rats and monkeys by ligation of one common carotid artery and rendering the animals hypertensive, with or without feeding beta-aminoproprionitrile. Beta-aminoproprionitrile is a lathyrogen that is known to inhibit crosslinking of collagen and elastin and to cause fragility of connective tissue. In this aneurysm model, early aneurysmal changes resemble the areas of thinning and microscopic evaginations described by Stehbens. Associated with these early aneurysmal changes are degeneration of the endothelial cells, fragmentation and disappearance of the internal elastic lamina, and thinning of the medial muscle layer. In this animal model, hemodynamic stress exacerbated by experimental manipulation causes injury to the intima near the apex of the bifurcation, followed by further degenerative changes of the vessel wall. In general, injury of the arterial wall intima causes a proliferative response, as in cases of wound healing of other tissues; however, at least at the early stages of aneurysm formation in this animal model, we have never seen proliferative changes in experimental aneurysms or at the sites where aneurysms may be expected to grow. Although intimal thickening is often observed at the entrance of small evagination sites, this is the intimal pad or cushion and is later involved in the wall of small evaginations and disappears in the very early stage of aneurysm development. These findings indicate that cerebral aneurysms develop partly because of decreased proliferative response to injury at cerebral arterial bifurcations.

Blood coagulation Factor XIII (also known as "fibrin-stabilizing factor") has an important function not only in hemostasis but also in wound healing.
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Factor XIII stabilizes fibrin and stimulates proliferation of fibroblasts, their adhesion to fibrin threads, and migration in the fibrin clot. In animal studies, tensile strength of skin wounds has been reported to be significantly raised by Factor XIII. An effect of Factor XIII on bone fractures has also been demonstrated in animals. In the present study, exogenous Factor XIII was given to rats with experimental cerebral aneurysms to identify whether similar proliferative reaction occurs at the site of injury: that is, at the site of early aneurysm development.

**Materials and Methods**

**Animal Preparation**

Thirty-two male Sprague-Dawley rats, ranging in age from 7 to 9 weeks, were subjected to cerebral aneurysm induction. The procedure for inducing cerebral aneurysms in rats was as follows. In each animal, the left common carotid artery and the posterior branches of both renal arteries were ligated under intraperitoneal sodium pentobarbital anesthesia (40 mg/kg). One week after the operation, 1% saline was substituted for the animals' drinking water. One week later, beta-aminopropionitrile fumarate was added in a 0.12% concentration to the standard laboratory chow fed to the animals.

**Experimental Groups**

Four weeks after the operation, the rats were separated into two groups. Twenty-one rats were injected with Factor XIII (Fibrogammin, 10 U/100 gm body weight/day) via the tail vein for 5 consecutive days and 11 rats were maintained without Factor XIII injection to serve as an untreated control group. Blood pressure was measured by the tail-cuff autopickup plethysmographic method in rats in an unanesthesized state just before surgery and just before sacrifice.

**Postmortem Examination**

Both groups of rats were sacrificed 12 days after the start of Factor XIII administration or at an equivalent time. At the end of the experiment, they were perfused through the abdominal aorta with 0.15 M heparinized phosphate buffer (pH 7.4) followed by 2.5% glutaraldehyde in 0.15 M phosphate buffer (pH 7.4). After perfusion fixation, the major arteries at the base of the brain were carefully dissected free from the brain under a dissecting microscope and immersed in the same fixative for 24 hours.

In each animal, the junctions between the anterior cerebral artery (ACA) and the olfactory artery (OA) on both sides were excised for histological examination. The specimens were washed three times in 0.15 M phosphate buffer (pH 7.4) and postfixed in 2% osmium tetroxide for 1 hour. After dehydration in a graded concentration of alcohol, they were embedded in epoxy resin. Serial semithin sections at 1 μm along the longitudinal axis of the branches were stained with 0.5% toluidine blue for light microscopic examination. Additional thin sections were cut and stained with uranyl acetate followed by lead hydroxide for examination by transmission electron microscopy.

**Results**

In the Factor XIII-treated group, maximum blood pressure was 120 ± 13 mm Hg at surgery and 190 ± 29 mm Hg at sacrifice. In the untreated control group, maximum blood pressure was 126 ± 8 mm Hg at surgery and 185 ± 23 mm Hg at sacrifice. There was no significant difference in maximum blood pressure between the two groups (p < 0.1, Student's t-test).

**Arterial Junctions on Nonligated Side**

**Untreated Group.** In the 11 untreated rats, various stages of aneurysms formation were observed at the bifurcation apex in seven (Table 1). A small aneurysmal bulge was found at one bifurcation (Fig. 1). Light mi-

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**TABLE 1**

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of Bifurcations</th>
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<tr>
<td></td>
<td>With Aneurysm</td>
</tr>
<tr>
<td>untreated group</td>
<td>7 (0)</td>
</tr>
<tr>
<td>treated group</td>
<td>12 (7)</td>
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*Anterior cerebral/olfactory artery (ACA/OA) junctions contralateral to the side of carotid ligation. The number of bifurcations with intimal proliferation is shown in parentheses.

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* Beta-aminopropionitrile supplied by Tokyo Kasai Co., Tokyo, Japan.
† Fibrogammin (Factor XIII concentrates, placental fibrin-stabilizing factor) obtained from Hoechst Japan Ltd., Tokyo, Japan.
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FIG. 2. Junction between the anterior cerebral artery (ACA) and the olfactory artery on the nonligated side in a rat not treated with Factor XIII. The internal elastic lamina distal to the intimal pad (arrow) on the side of the ACA is thinned and partly fragmented (arrowheads). Here, the luminal surface shows mild depression. Toluidine blue, × 270.

FIG. 3. Junction between the anterior cerebral artery and the olfactory artery on the nonligated side in two Factor XIII-treated rats. Toluidine blue, × 240. Upper: The original aneurysmal lumen is traced by the fragmented elastic lamina (arrowheads). The lumen of the aneurysm is completely filled with intimal proliferation. Lower: The aneurysmal lumen is completely obliterated by proliferated tissue.

Microscopy revealed that the wall of the bulge was composed of connective tissue. Normal arterial components such as smooth-muscle cells and internal elastic lamina were not identified. In another bifurcation, a small evagination of the lumen into the arterial wall was found just distal to the apex on the side of the ACA. The intimal pad was located just proximal to this evagination and was partly involved in the wall of the evagination. In five bifurcations, shallow but visible depressions on the luminal aspect of the arterial wall were found just distal to the intimal pad on the side of the ACA (Fig. 2). At the proximal margin of the intimal pad, the internal elastic lamina had split into several layers, then fragmented and disappeared under the pad. In the depression near the apex, fragmented and sparse, weakly stained elastic tissue was visualized.

Four bifurcations showed neither evagination nor depression at the site distal to the intimal pad on the side of the ACA. At this site however, the internal elastic lamina was thinned and partly fragmented.

Factor XIII-Treated Group. Among the 21 Factor XIII-treated rats, frank aneurysms were found in three bifurcations, small evaginations were found in three, and depressions were found in six. Nine bifurcations showed no aneurysm bulges or depressions. In this group with or without aneurysmal change, various degrees of intimal thickening were also observed. The intimal thickening extended distally to cover the bifurcation apex area where aneurysms might be expected to originate (Fig. 4). In some cases the proliferation was continuous with the apical intimal pad, and in others the apical intimal pad was totally involved with tissue proliferation. Endothelial cells with large, round, or oval nuclei were frequently observed lying over proliferated intima. Such proliferative changes were observed nowhere in the arterial wall other than at the luminal surface at sites of aneurysmal change or at the apical region of the bifurcation (Fig. 4 right).

Transmission electron microscopy showed that these proliferating cells contained a large number of subcellular organelles with dense plaque at their margins. They were surrounded by basement membrane and had many processes. The intercellular space was filled with collagen fibers and basement membrane-like lamellar structures (Fig. 5). These findings indicated that the
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![Image of aneurysm junctions](image)

**FIG. 4.** Junction between the anterior cerebral artery (ACA) and the olfactory artery (OA) on the nonligated side in Factor XIII-treated rats. *Left:* As seen in Fig. 2, the internal elastic lamina is thinned and fragmented. However, in contrast to the specimen in Fig. 2, intimal proliferation is significant at the site where an aneurysm might be expected to originate (arrows). Toluidine blue, × 170. *Right:* Lower-magnification view of another specimen showing marked intimal thickening at the apical region. This thickening is not observed in other parts of the lumen. Toluidine blue, × 14.

proliferating cells were so-called "synthetic phenotypes" of smooth-muscle cells.

**Arterial Junctions on Ligated Side**

In the untreated group, no apparent aneurysmal or proliferative intimal changes were observed at the apical area of the junctions. The group treated with Factor XIII similarly showed neither aneurysmal nor proliferative changes at their bifurcations.

**Discussion**

The present study clearly demonstrates that administration of exogenous Factor XIII causes tissue proliferation in the intima of the aneurysm sacs, on the surface of early aneurysm formations, and at the site where aneurysms might be expected to originate in rats treated to induce cerebral aneurysms. In the most advanced cases, the lumen of fully developed aneurysms was completely filled with such proliferated tissue.

**Effect of Factor XIII on Induced Cerebral Aneurysms**

In rats that had received ligation of one common carotid artery, induction of renal hypertension, and beta-aminopropionitrile feeding but were not treated with Factor XIII, various stages of aneurysm formation were observed at the ACA/OA junction contralateral to the side of carotid ligation. This junction was chosen for the present study because the angle of bifurcation varies very little, the number of branches is constant, and this is one of the most frequent sites of aneurysm development. At this bifurcation, shallow depressions or grooves were often observed on the luminal surface just distal to the intimal pad. Here, the arterial wall showed thinning of the media. The elastic lamina both beneath the intimal pad and in the area of the depression showed mild degenerative changes or partial disappearance; these were revealed to be early changes of aneurysm formation by our previous studies. In the advanced cases, the wall of the bulge had lost smooth-muscle cells and elastic lamina and was mainly composed of connective tissue. Histopathological details of these various stages of aneurysm formation in this animal model have been reported previously.

In animals treated with exogenous Factor XIII, various stages of aneurysm formation were also found. The major difference between this and the nontreated group was that many of the bifurcations in the treated animals showed significant proliferation of cells and accumulation of matrix in the intima near the apex of bifurcations. The intimal proliferation covered the luminal surface just distal to the intimal pad on the side of the ACA where aneurysms frequently develop. In the most advanced cases, the aneurysms were completely obliterated by proliferated tissue. In cases without aneurysm...
indicated that proliferative tissue was composed mainly with Factor XIII. The electron microscopic findings indicated that proliferative tissue was composed mainly of smooth-muscle cells of synthetic phenotype and collagen. Smooth-muscle cells in the proliferated intima were different from the cells of contractile phenotype in the medial layer. Such intimal proliferation was not found in the group without Factor XIII treatment.

We have previously conducted histopathological studies of several hundreds of bifurcations in experimental animals, including animals without beta-aminopropionitrile feeding, but we have never seen a proliferative response of the intima at the site of aneurysm development other than those in the Factor XIII-treated rats in the present study.

In common vascular pathology, injury or denudation of endothelial cells usually results in intimal thickening with cell proliferation and accumulation of connective tissue mainly in the intimal layer. Such proliferative changes are an essential part of the vascular pathology of atherosclerosis. In our animal model, the endothelial cells just distal to the apical intimal pad showed degenerative and regenerative changes even at the stage before aneurysm formation. At bifurcations with early aneurysmal alterations, degenerated endothelial cells were dominant and, at bifurcations with an apparent aneurysm, endothelial cells were lost almost completely. In contrast to the formation of atherosclerosis, neither apparent proliferation of cells nor deposition of connective tissue in the intimal layer was observed at the site of aneurysm formation. Although the mechanism of the different responses to endothelial injury elicited by aneurysm and atherosclerosis development is not clear, it can be said that aneurysm formation is partly caused by a decreased or defective proliferative response to vascular injury.

With these observations, the present study was undertaken to find a substance or treatment to modify the process of progressive degenerative changes at the site of aneurysm development.

Effect of Factor XIII on Proliferation of Smooth-Muscle Cells

Blood coagulation Factor XIII is reported to accelerate wound healing both clinically and experimentally. Factor XIII covalently crosslinks fibrin monomers to form a highly organized, stable fibrin clot. It stimulates migration of fibroblasts into a stabilized fibrin clot and promotes their proliferation. In vitro experiments show that the growth of fibroblasts cultured in Factor XIII-deficient plasma is defective both quantitatively and qualitatively. Normal fibroblast proliferation can be restored by adding Factor XIII. It may act as a growth promoter which triggers and facilitates the cell growth steps into deoxyribonucleic acid (DNA) synthesis and stimulates the growth of fibroblasts.

Although it has not been reported that Factor XIII promotes the proliferation of smooth-muscle cells and it is not known whether Factor XIII is defective at the site of aneurysm development, the present study showed that exogenous Factor XIII has a proliferative effect at the site of aneurysm development. In animals treated with Factor XIII, only the site where aneurysms were located or might be expected to develop showed intimal proliferation; other portions proximal or distal to the apical area did not show any proliferative changes. Such specific localization of cell proliferation is very similar to the response to injury of other tissues. This may, in turn, indicate that cerebral aneurysm formation is at least partly the result of injury to the arterial wall and of a defective or decreased healing process there. Further elucidation of the mechanism of smooth-muscle cell proliferation at the site of aneurysm development in animals treated with Factor XIII may add some information about the control mechanism of smooth-muscle cell proliferation at the site of vascular injury.

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References

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