Collagen deficiency and ruptured cerebral aneurysms

A clinical and biochemical study

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Skin and temporal arterial biopsies were obtained from 17 patients undergoing surgery for ruptured cerebral aneurysm, and specimens were taken from six age- and sex-matched control surgical patients. Radioactively labeled and control tissue collagen patterns were studied by interrupted polyacrylamide gel electrophoresis (PAGE), using the trisborate buffer system or by carboxymethyl cellulose (CMC) chromatography. Type III/I collagen ratios were then measured from autoradiographs of the radioactively labeled samples using the Joyce Loebl gel scanner adapted for flat bed gels. In the case of the CMC labeled material, the ratios were measured by the ratios of the summed radioactively labeled $\alpha_1(III)$, $\alpha_2(II)$, and $\alpha_2(I)$ peaks. Eleven of the 17 patients were Type III collagen-deficient while all of the six control patients had normal collagen ratios. The implications of these findings are discussed.

KEY WORDS: cerebral aneurysm, collagen, Type III collagen deficiency, fibroblast, cultures, subarachnoid hemorrhage

In the United Kingdom, 4000 deaths yearly are directly caused by bleeding into the subarachnoid space, mainly from ruptured cerebral aneurysms. There are blister-like arterial outpouchings which affect between 1% and 2% of the population, and are most commonly situated on the anterior half of the circle of Willis. The abnormalities originate at arterial bifurcations where the internal elastic lamina becomes interrupted and the medial layer thins. As the elastic interruptions or gaps occur in normal individuals as well as in patients with aneurysms, they cannot be the sole cause of aneurysms. However, if combined with a separate abnormality of the arterial wall, the elastic gaps would be the obvious place for aneurysm formation to occur. Because of our previous experience with various inherited abnormalities of collagen Type III, in which aneurysms of arteries other than the cerebral circulation commonly cause premature death, we have studied Type III collagen patterns in cerebral aneurysm patients. Our preliminary data have shown that some cerebral aneurysm patients are indeed deficient in Type III collagen. In this more detailed communication, we report our further studies on 17 patients with cerebral aneurysm.

Clinical Material and Methods

Skin and temporal arterial biopsies were obtained from 17 patients undergoing surgery for ruptured cerebral aneurysm in the South East Thames Regional Neurosurgical Centre at the Brook General Hospital, London. Specimens were also taken from six age- and sex-matched control surgical patients, three of whom had gliomas and three meningiomas. A skin sample approximately 1 mm wide and 5 mm long was taken from the edge of the craniotomy incision. A piece of superficial temporal artery, about 1 cm long, was removed from its division by the incision. Some skin and arterial samples were frozen unfixed for biochemical examination of their collagens, and others were placed in Dulbecco's medium for primary fibroblast culture. Collagens synthesized by these cells were labeled by adding 1 $\mu$C/mC of uranium-carbon-14 glycine and proline in the presence of ascorbate and $\beta$-amino proprionitrile.

Radioactively labeled and control tissue collagens were then liberated by a limited pepsin digestion at 15°C and collagen patterns were studied by interrupted polyacrylamide gel electrophoresis (PAGE), using the trisborate buffer system, or by carboxymethyl cellulose chromatography. Type III/I collagen ratios were then measured from autoradiographs of the radioactively labeled samples using the Joyce Loebl gel scanner adapted for flat bed gels. In the case of the CMC labeled material, the ratios were measured by the ratios of the summed radioactively labeled $\alpha_1(III)$, $\alpha_2(II)$, and $\alpha_2(I)$ peaks. Eleven of the 17 patients were Type III collagen-deficient while all of the six control patients had normal collagen ratios. The implications of these findings are discussed.

KEY WORDS: cerebral aneurysm, collagen, Type III collagen deficiency, fibroblast, cultures, subarachnoid hemorrhage
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TABLE 1
Aneurysm patients with normal collagen ratios *

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age (yrs), Sex</th>
<th>Presenting Symptoms</th>
<th>Site of Aneurysm</th>
<th>Collagen Type III:I Ratio</th>
<th>Aneurysm Surgery</th>
<th>Outcome at 3 Mos</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>22, F</td>
<td>headache, vertigo</td>
<td>lt ICA, rt PCA</td>
<td>13.2 normal</td>
<td>clipped</td>
<td>good</td>
</tr>
<tr>
<td>2</td>
<td>27, M</td>
<td>headache, coma</td>
<td>ACA</td>
<td>17.8</td>
<td>clipped</td>
<td>good</td>
</tr>
<tr>
<td>3</td>
<td>46, F</td>
<td>coma</td>
<td>rt MCA</td>
<td>11.0</td>
<td>clipped</td>
<td>good</td>
</tr>
<tr>
<td>4</td>
<td>52, M</td>
<td>headache, coma</td>
<td>lt MCA</td>
<td>variable Normal</td>
<td>clipped</td>
<td>fair</td>
</tr>
<tr>
<td>5</td>
<td>56, M</td>
<td>coma</td>
<td>lt PCA</td>
<td>10.5 Normal</td>
<td>clipped</td>
<td>good</td>
</tr>
<tr>
<td>6</td>
<td>58, M</td>
<td>headache, vomiting</td>
<td>ACA</td>
<td>normal</td>
<td>clipped</td>
<td>good</td>
</tr>
</tbody>
</table>

* CMC = carboxymethyl cellulose chromatography; ICA = internal carotid artery; PCA = posterior cerebral artery; MCA = middle cerebral artery; ACA = anterior cerebral artery; -- = test not performed.

TABLE 2
Aneurysm patients with Type III collagen deficiency *

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age (yrs), Sex</th>
<th>Presenting Symptoms</th>
<th>Site of Aneurysm</th>
<th>Collagen Type III:I Ratio</th>
<th>Aneurysm Surgery</th>
<th>Outcome at 3 Mos</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>29, M</td>
<td>coma</td>
<td>ACA</td>
<td>8.2</td>
<td>clipped</td>
<td>good</td>
</tr>
<tr>
<td>8</td>
<td>30, F</td>
<td>headache</td>
<td>lt PCA</td>
<td>7.6 low</td>
<td>clipped</td>
<td>good</td>
</tr>
<tr>
<td>9</td>
<td>33, F</td>
<td>headache, double vision</td>
<td>lt PCA</td>
<td>-- low</td>
<td>clipped</td>
<td>good</td>
</tr>
<tr>
<td>10</td>
<td>36, F</td>
<td>headache, coma</td>
<td>rt PCA</td>
<td>-- low</td>
<td>clipped</td>
<td>fair</td>
</tr>
<tr>
<td>11</td>
<td>36, F</td>
<td>headache, double vision</td>
<td>rt PCA</td>
<td>7.1 --</td>
<td>clipped</td>
<td>died</td>
</tr>
<tr>
<td>12</td>
<td>46, F</td>
<td>coma</td>
<td>lt MCA</td>
<td>6.6 variable</td>
<td>wrapped</td>
<td>died</td>
</tr>
<tr>
<td>13</td>
<td>47, F</td>
<td>coma</td>
<td>lt ICA, lt MCA</td>
<td>6.9</td>
<td>clipped</td>
<td>good</td>
</tr>
<tr>
<td>14</td>
<td>50, M</td>
<td>headache, dysphasia</td>
<td>lt MCA</td>
<td>6.9 low</td>
<td>clipped</td>
<td>poor</td>
</tr>
<tr>
<td>15</td>
<td>53, F</td>
<td>coma</td>
<td>ACA</td>
<td>-- low</td>
<td>clipped</td>
<td>fair</td>
</tr>
<tr>
<td>16</td>
<td>55, F</td>
<td>headache, dysphasia</td>
<td>lt PCA</td>
<td>7.0 --</td>
<td>clipped</td>
<td>good</td>
</tr>
<tr>
<td>17</td>
<td>62, M</td>
<td>headache, confused</td>
<td>rt PCA</td>
<td>-- low</td>
<td>clipped</td>
<td>good</td>
</tr>
</tbody>
</table>

* CMC = carboxymethyl cellulose chromatography; ICA = internal carotid artery; PCA = posterior cerebral artery; MCA = middle cerebral artery; ACA = anterior cerebral artery; -- = test not performed.

Results

Clinical Data

Eleven of the 17 patients with ruptured cerebral aneurysms were demonstrated to have Type III collagen deficiency. Tables 1 and 2 contain the essential clinical data of the 17 patients, including age, sex, mode of clinical presentation, past medical history, and the eventual clinical outcome at 3 months after rupture. The age range and mode of presentation are very similar in patients with normal collagen ratios (Table 1) and those with Type III collagen deficiency (Table 2); however, there were more women than men (a ratio of 8:11 compared with 2:6) in the latter group. There were no obvious differences in the aneurysm sites in either group, and two of the Type III deficient patients were hypertensive.

Surgical Treatment

The aneurysms of all patients in this study were treated conventionally by either clipping or wrapping. There is some suggestion that the clinical recovery at 3 months was rather poorer in the Type III collagen-deficient group; however, the numbers are small and not statistically significant (chi-square = 1.41, p > 0.1).

Biochemical Results

Typical biochemical data are illustrated in Figs. 1 and 2, and tabulated along with the clinical data in Tables 1 and 2.

Chromatographic Data. Typical CMC chromatograms are shown in Fig. 1, which separates the \( \alpha_1(III) \) collagen peak from the \( \alpha_1(I) \) and \( \alpha_2(I) \) chains of Type I collagen. Here the amounts of radioactively labeled collagen are expressed as a percentage of Type III, compared with the \( \alpha_1(I) \) and \( \alpha_2(I) \) chains. It can easily be seen both visually and numerically that some aneurysm patients produce significantly less Type III collagen than normal control patients (7.5% compared with 10.5%).

 Autoradiographic Data. Because column chromatography of labeled collagen is time-consuming and
labor intensive, wherever possible we have tried to measure Type III collagen with a faster technique which requires smaller numbers of cultured skin fibroblasts and less radioactive label. Using slab gel electrophoresis and interrupted reduction of disulfide bands from Type I collagen it is possible to process up to 20 samples on two gels very quickly. A typical series of gels are shown in Fig. 2. Tracks 2, 7, 10, 12, 14, 18, 19, and 20 show diminished quantities of Type III collagen compared with the others. Tracks 1, 8, 9, 11, and 17 are from control patients. This rapid method is ideal for screening large numbers of samples, although it is somewhat less reliable than column chromatography when the levels of Type III collagen are in the borderline range. This method is, however, capable of identifying obvious Type III deficiency.

Discussion

Any theory which accounts for congenital aneurysms must also explain their common origin from arterial bifurcations and why branches elsewhere in the arterial circulation so rarely form aneurysms. No doubt there are a number of factors that influence arterial aneurysm formation. These include intrinsic congenital or inherited abnormalities of the vessel wall, the diastolic arterial pressure, and perhaps other more complex influences, like viral infections and arterial vasoconstriction.

Systemic aneurysms sparing the cerebral circulation have been described in association with a variety of inherited connective-tissue defects in which collagen abnormalities are already recognized or strongly suspected. These include Ehlers-Danlos syndrome (EDS) I, EDS III (FM Pope, J Levi, unpublished data, 1980), EDS IV, EDS VI, Marfan syndrome, Menkes' kinky-hair syndrome, the mottled brindled mouse defect, and copper deficiency in swine and sheep.

The biochemical abnormalities include complete collagen Type III deficiency, collagen Type I α2 chain heterogeneity, lysyl hydroxylase deficiency, and inherited or acquired lysyl oxidase deficiency causing abnormalities of collagen cross-link formation. In pseudo-xanthoma elasticum, elastin protein or cross linking abnormalities are likely. Abnormalities of mucopoly-saccharides have been described in some forms of the Marfan syndrome, but no defects of arterial muscle proteins have ever been described in any of this disease group.

In view of these facts, we measured Type III collagen levels in skin, and cultured skin fibroblasts from a variety of patients with cerebral aneurysms. Three clear facts emerge: there is a group of aneurysm patients 1) who are clearly Type III collagen-deficient; 2) who are clearly not Type III collagen-deficient; and 3) in whom Type III collagen deficiency is possible but less certain. In contrast to our previous study, we did not find measurement of pepsin-released tissue collagen easy to interpret, and have abandoned this as a method of assessment. Radioactive labeling of collagen ratios, estimated by slab gel autoradiography and scanning densitometry or chromatographic separation of radioactive collagen produced consistently reliable results.

We are not aware of any previous study that demonstrates Type III collagen deficiency in patients with primary subarachnoid hemorrhage from a ruptured aneurysm. This protein lack is not an “all or none” phenomenon but, rather like EDS Type IV, there is a variation in the severity of Type III collagen deficiency and possibly also in the fine molecular structure Type III produced. It is likely that other factors are involved in the production of intracranial aneurysms. These might include age, sex, and blood pressure.

The incidence of rupture of intracranial aneurysms increases with age. The majority of ruptures occur in patients between 40 and 70 years of age, although familial aneurysms have a peak incidence at between 30 and 40 years. The age range of our patients with Type III collagen deficiency was 29 to 62 years.
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The combination of this with the uniquely unsupported nature of the major cerebral vessels, local stresses at the bifurcation, and Type III collagen abnormality allows a self-perpetuating process to start, leading to aneurysm formation. Further important questions that will now require study include the frequency of Type III collagen deficiency in the general population, and the inheritance of Type III collagen levels in the relatives of aneurysm patients. Type III collagen levels may be a useful indicator of a predisposition to aneurysm formation, and it will be very important to discover whether the presence of the deficiency inevitably implies future aneurysm formation. If this information can be combined with a safe, simple, reproducible method of measuring arterial distensibility, then the management and prognosis of intracranial aneurysm patients may make considerable progress.

Fig. 2. Separation of 14C radioactively labeled collagen by slab gel electrophoresis. Type III is separated from Type I collagen by interrupted reduction of disulfide bands. The radioactively labeled bands are then visualized by autoradiography. Tracks 1, 8, 9, 11, and 17 are from normal control patients.

though the period of life from 40 to 70 years is a time at which acquired diseases are common, the age of presentation of a genetic disorder may be very variable. For example, thalassemia occurs early in life, while Huntington’s chorea is delayed to middle age. So the fact that aneurysm patients commonly present in middle age or later does not preclude a significant genetic predisposition.

Gautier-Smith has described a two-thirds increased frequency of ruptured intracranial aneurysms in women, and they fare less well than men. Although our series is small, there were more women particularly in the group of patients with Type III collagen deficiency.

Another factor that may influence aneurysm formation is hypertension, which may act as an aggravating or accelerating factor in aneurysm formation. Andrews has suggested that genetic and environmental factors (connective tissue disease and hypertension) may predispose to aneurysm formation in cases of familial intracranial aneurysms. Interestingly, both hypertensive patients in our small series were Type III collagen-deficient.

We conclude that Type III collagen deficiency is associated with ruptured intracranial aneurysms, but we do not know how the deficiency allows aneurysm formation. One possibility may be that the branching points at which aneurysms occur are inherently weak.

References


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