Absence of Elevation of Big Endothelin in Subarachnoid Hemorrhage

G. Hamann, MD; E. Isenberg, MD; M. Strittmatter, MD; and K. Schimrigk, MD

Background and Purpose: Endothelin peptides are potent vasoconstrictors and thus are seen as potential cause of cerebral vasospasm after subarachnoid hemorrhage (SAH). Earlier reports showed elevated or normal endothelin levels in plasma and cerebrospinal fluid in patients suffering from SAH. The present study was designed to determine whether endothelin is a causal factor in SAH.

Methods: We studied 11 patients with acute SAH. Seven of these 11 patients had a proven aneurysm and six had experienced vasospasm. Big endothelin levels were determined by a radioimmunoassay recognizing the C-terminal peptide (normal range, 1–11 fmol/ml).

Results: There were no elevations of big endothelin in the 59 plasma samples and the 17 simultaneously estimated cerebrospinal fluid samples. Differences between plasma and cerebrospinal fluid did not reach significant levels. Big endothelin values between patients with and without vasospasm showed no significant differences.

Conclusions: Our findings suggest that plasma elevation of the endothelins is not reproducible in SAH and that big endothelin is unlikely to be a causal plasma factor in the complex multifactorial development of vasospasm after SAH. (Stroke 1993;24:383–386)

Key Words • subarachnoid hemorrhage • cerebral vasospasm • endothelin

Since the first studies of endothelins in 1988,1 the number of reports concerning these peptides increased rapidly. This so-called “endothelin explosion”2 focused on the pathophysiologic importance of the endothelins in subarachnoid hemorrhage (SAH). Endothelin-1 and big endothelin are especially potent vasoconstrictors and are able to produce long-term effects on blood vessels.2–3 Thus, these peptides are seen as important causal factors of vasospasm after SAH. An increased plasma endothelin-1 level has been recently demonstrated to be associated with a poor prognosis in acute ischemic stroke.4 First reports showed especially elevated levels of endothelin-1 in the cerebrospinal fluid (CSF) and plasma of postoperative patients with SAH.2–3 However, other reports do not support this hypothesis of plasma endothelin activation in patients after SAH.5–9 Because big endothelin is the active precursor of endothelin-1, it probably gives the best information about activation of the endothelin system.2–3,10 Therefore, we measured big endothelin in plasma and in some cases in CSF during the first 3 weeks after spontaneous SAH.

Subjects and Methods

Patients

Eleven consecutive patients suffering from acute SAH were included in the study. Mean age was 53 years with a range of 37–75 years. There were seven women and four men. SAH was verified by means of cranial computed tomography and/or lumbar puncture in all cases. Time between acute bleeding and admission to the hospital ranged between 1 and 8 days. All patients underwent panangiography with oblique projections. In seven cases, at least one aneurysm was revealed as cause of the bleeding.

Flow velocities in the basal arteries of the circle of Willis were measured by means of transcranial Doppler sonography (Eden Medizin Electronic), and vasospasms were classified according to the criteria of Harders and Gilsbach.

No patient showed any sign of cardiac insufficiency, ischemia, or concomitant infections. Blood was drawn from arterial catheters in all patients and immediately transferred to chilled tubes with EDTA. Tubes were precooled at 4°C, and blood samples were transported in ice water, centrifuged at 3,000 rpm for 15 minutes at 4°C, and immediately assayed or stored at −70°C until the assay.

Radioimmunoassay

Big endothelin was determined using a radioimmunoassay recognizing the C-terminal peptide.10 This is a part of big endothelin, which is cleaved into C-peptide and endothelin-1 by a protease.1,10 The radioimmunoassay provides information about both peptides and is highly sensitive in determining activation of the endothelin system.1,10 The assay was performed with a big endothelin kit (Biomedica GmbH, A-1210 Wien, Divischasse 4). The antibody used in the present radioimmunoassay reacted 82% with big endothelin fragment 22–38 and cross-reacted less than 1% with endothelin-1, endothelin-2, and endothelin-3. The minimum detectable dose was 0.2 fmol/ml, and the range of the
TABLE 1. Endothelin Levels in Patients With Subarachnoid Hemorrhage

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>Location of aneurysm</th>
<th>Neurological status</th>
<th>Vasospasm by TCD</th>
<th>H+H</th>
<th>Outcome (GOS)</th>
<th>Big endothelin (fmol/ml)</th>
<th>Day</th>
<th>Serum</th>
<th>CSF</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>69</td>
<td>F</td>
<td>ACOA</td>
<td>Severe coma, died on day 4</td>
<td>–</td>
<td>5</td>
<td>5</td>
<td>1</td>
<td>3.65</td>
<td>...</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>37</td>
<td>M</td>
<td>ACOA</td>
<td>Severe coma</td>
<td>–</td>
<td>5</td>
<td>5</td>
<td>1</td>
<td>2.00</td>
<td>...</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>75</td>
<td>F</td>
<td>L ICA</td>
<td>Disorientation, intracranial pressure rise on day 4</td>
<td>Slight diffuse vasospasm</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>7.62</td>
<td>...</td>
<td></td>
</tr>
</tbody>
</table>

| 4           | 53       | M   | R MCA                | Disorientation, confusion, focal deficit, R hemiparesis | Severe vasospasm of L MCA | 3   | 1            | 8                    | 1.50 | 3.90  |     |

| 5           | 37       | F   | R MCA                | No disorientation, focal deficit, R hemiparesis | Severe vasospasm of L MCA (days 5–8) and R MCA (days 9 and 10) | 3   | 1            | 6                    | 1.60 | ...   |     |

| 6           | 55       | F   | Neg                  | Inconspicuous | –                |                 |                            |                     | 2.20 | ...   |     |

TCD, transcranial Doppler sonography; H+H, clinical grading according to Hunt-Hess scale; GOS, Glasgow coma scale; CSF, cerebrospinal fluid; F, female; M, male; ACOA, anterior communicating artery; –, no vasospasm by TCD; L, left; R, right; ICA, internal carotid artery; MCA, middle cerebral artery; Neg, negative panangiography; BA, basilar artery.

Slight vasospasm indicates TCD of 120–160 cm/sec mean flow velocity. Severe vasospasm indicates TCD of >160 cm/sec mean flow velocity. Subcritical vasospasm (80–120 cm/sec mean flow velocity) was not detected. Diffuse vasospasm indicates involvement of more than two arteries.

test was 3–243 fmol/ml (1 fmol=4.28 pg). Both interassay and intra-assay variabilities were less than 10%. Simultaneous radioimmunoassay of plasma and CSF from one patient in the same test procedure was performed. The normal range of big endothelin was between 1 and 11 fmol/ml (32 nonvascular and nonneurological patients served as a control group). CSF samples of four patients without SAH were in this range too.

Statistical Analysis

The significance of differences between CSF and plasma samples was evaluated by using the Wilcoxon test. The Mann-Whitney U test was used to test differences between samples of patients with and without vasospasm. Big endothelin levels are expressed as median±SD.

Results

In Table 1, patient data and single values of big endothelin are shown. The mean plasma big endothelin level was 2.56±1.26 fmol/ml. This mean value and every single value were in the normal range of this radioimmunoassay (1–11 fmol/ml). The highest single big endothelin level was 7.5 fmol/ml in CSF and 7.62 fmol/ml in plasma.

In four patients, a simultaneous determination of big endothelin in CSF and plasma was possible. The mean big endothelin level was 5.17±1.24 fmol/ml in plasma and 4.15±1.43 fmol/ml in CSF. Both mean levels were in the normal range. No significant difference between plasma and CSF could be established by the Wilcoxon test.

Three patients with severe vasospasm showed no elevation in big endothelin level. The mean big endo-
TABLE 1. Continued

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>Location of aneurysm</th>
<th>Neurological status</th>
<th>Vasospasm by TCD</th>
<th>H+H</th>
<th>Outcome (GOS)</th>
<th>Big endothelin (fmol/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>60</td>
<td>F</td>
<td>BA</td>
<td>Intracranial pressure rise</td>
<td>Slight diffuse vasospasm</td>
<td>4</td>
<td>3</td>
<td>7 3.30 5.80</td>
</tr>
<tr>
<td>8</td>
<td>50</td>
<td>F</td>
<td>R MCA, ACOA</td>
<td>Slight disorientation</td>
<td>Slight vasospasm</td>
<td>3</td>
<td>2</td>
<td>4 4.50 4.70</td>
</tr>
<tr>
<td>9</td>
<td>44</td>
<td>F</td>
<td>Neg</td>
<td>Inconspicuous</td>
<td>-</td>
<td>2</td>
<td>1</td>
<td>3 1.40 ...</td>
</tr>
<tr>
<td>10</td>
<td>55</td>
<td>M</td>
<td>Neg</td>
<td>Inconspicuous</td>
<td>-</td>
<td>2</td>
<td>1</td>
<td>1 2.40 ...</td>
</tr>
<tr>
<td>11</td>
<td>53</td>
<td>M</td>
<td>Neg</td>
<td>Disorientation, focal deficit, R hemiparesis</td>
<td>Severe vasospasm (days 6–14)</td>
<td>4</td>
<td>1</td>
<td>7 3.00 2.00</td>
</tr>
</tbody>
</table>

The endothelin level for these patients was 2.10±1.09 fmol/ml. There was no significant difference by U test between patients with severe vasospasms and other patients.

Four of the 11 patients had a negative panangiogram and therefore were thought to suffer from spontaneous SAH without aneurysm. The mean big endothelin level in this group was 2.12±0.80 fmol/ml, and the difference between this group and the total group was not significant by U test.

Discussion

The results of the present study do not support the hypothesis that plasma endothelin is elevated in patients after SAH.²⁻⁷ Former reports of such an elevation described patients with neurosurgical intervention, and endothelins were measured in the perioperative time course.²⁻⁷ Two other recently published studies failed to demonstrate this elevation.²⁻⁷ Thus, our results are in agreement with these two studies. These conflicting findings may be explained as follows: 1) The test procedures were not comparable. The problem of determining endothelin in its very low concentrations is probably a source of variability,¹¹ but our intra-assay and interassay coefficients of variation show reliable assays. 2) The patient number was not sufficient to confirm or negate former findings. The present study involved a substantial number of patients. 3) The nonsurgical therapeutic approach may yield different results; endothelin rise after aneurysm clipping may be the effect of surgically induced vascular disturbances. 4) In two studies, the elevation was only seen in CSF.²⁻⁷ This view supports more local and reduced systemic effects of the endothelins.⁶ However, our own CSF values do not support this theory. The four patients with collected CSF samples showing the course of big endothelin are a small group, and a larger cohort is needed to clarify this conflict. 5) The effects of endothelin are paracrine and not directly visible in CSF or plasma. This theory may explain the differences between the present study and previous experiments.⁷ The results of studies showing increased levels of endothelin-1 and big endothelin in plasma after myocardial infarction do not support this explanation¹² but support a systemic reaction or effect of endothelins in different vascular disturbances. 6)
Recently, Ziv et al. reported an elevation of endothelins in ischemic cerebral lesions. The accompanying ischemic damage is probably caused by endothelin activation in SAH, and these elevations have been mistakenly linked with vasospasm.

Plasma endothelin elevations are not reproducible in SAH and are thus not thought to be a basic parameter in vasospasm pathophysiology. The research into singular vasospasm-causing factors is disappointing, and its history reflects a changing focus on new substances, such as serotonin and prostaglandins. The complex vasospasm after SAH is seen today as a multifactorial development. We support this concept and advise against regarding endothelin as a single main causal factor.

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