Plasma endothelin and big endothelin concentrations and serum endothelin-converting enzyme activity following aneurysmal subarachnoid hemorrhage

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Object. Pathogenesis of delayed ischemia after aneurysmal subarachnoid hemorrhage (SAH) seems to be complex. An important mediator of chronic vasospasm may be endothelin (ET)-1 with its powerful and long-lasting vasoconstricting activity. In this prospective study the author investigated the correlations between serial plasma concentrations of ET-1 and big ET-1 as well as the ET-1/big ET-1 molar concentration ratio and serum endothelin-converting enzyme (ECE)-1 activity, and ischemic complications after SAH.

Methods. To measure plasma ET-1 (51 patients), big ET-1 immunoreactivity (22 patients), and serum ECE-1 activity (13 patients), blood samples were obtained on admission, in the morning after aneurysm surgery, and during the 2nd week after hemorrhage in 51 consecutive patients (28 men and 23 women, with a mean age of 50.8 years) with aneurysmal SAH. Mean plasma concentrations of ET-1 in patients with SAH (mean ± standard deviation: on admission, 4.2 ± 2 pg/ml; after surgery, 4.3 ± 2.2 pg/ml; and during the 2nd week after SAH, 3.7 ± 1.9 pg/ml) differed from those in healthy volunteers (2.9 ± 1.2 pg/ml; p < 0.01). Plasma concentrations of ET-1 and big ET-1 as well as the ET-1/big ET-1 ratio did not change significantly with elapsed time following SAH; however, serum ECE-1 activity during the 2nd week after SAH was higher in patients with SAH than that in controls (121 ± 56 pg/ml, respectively; p = 0.028). Plasma ET-1 concentrations (p < 0.05) and the ET-1/big ET-1 ratios (p = 0.063) were higher but plasma big ET-1 concentrations were lower (p < 0.05) in patients who experienced symptomatic delayed cerebral ischemia, compared with other patients with SAH. In addition, in cases in which follow-up computerized tomography scans or magnetic resonance images demonstrated permanent ischemic lesions attributable to vasospasm, patients had higher ET-1 concentrations than did other patients with SAH.

Conclusions. The plasma ET-1 concentration correlates with delayed cerebral ischemia after SAH, suggesting that an increased ET conversion rate in the endothelium predicts ischemic symptoms. Increased serum ECE-1 activity during the 2nd week may reflect the severity of endothelial injury to cerebral arteries.

Key Words • delayed cerebral ischemia • endothelin • subarachnoid hemorrhage • vasospasm

NEURYSMAL SAH, despite recent improvements in surgical and medical treatment, is a serious disease associated with high rates of mortality and morbidity.8,13 Delayed cerebral ischemia, commonly attributed to vasospasm in the large cerebral arteries, is a notable cause of death and disability after primary hemorrhage.11,21,27,29 The pathogenesis of cerebral vasospasm and delayed ischemia after SAH is unclear and seems to be complex and multifactorial; available treatment is therefore unsatisfactory.

Endothelin-1, a 21-amino acid peptide, has potent and long-lasting vasoconstricting activity.12,18–20,30 Intracisternal injections of ET-1 mimic the angiographically demonstrated pattern and morphological changes observed during cerebral vasospasm better than other vasoconstrictors.16,30 Nonetheless, there are conflicting reports on ET concentrations in CSF and in plasma after aneurysmal SAH as well as on the effect of ET receptor antagonists on post-SAH cerebral vasospasm.12,21,24,27,30 This conflict may result from the different methods used in various studies and is also likely attributable to a lack of statistical power to control the effects of confounding factors, especially considering the small sample sizes in most studies.

In this study, the association of plasma ET-1 and big ET-1 (the ET-1 precursor) immunoreactivity, the ET-1/big ET-1 molar ratio (a marker of the ET-1 conversion rate), and serum ECE-1 activity were investigated in relation to time after SAH, time to surgery, ischemic symptoms, radiological findings, clinical variables, baseline characteristics, and patient outcome.

Clinical Material and Methods

Patient Population

This prospective study included 51 consecutive patients (28 men and 23 women) ranging in age from 23.3 to 75.7 years (mean 50.8 years), who were admitted to the hospital...
TABLE 1
Severity of bleeding and outcomes in 51 patients with aneurysmal SAH*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No. of Patients (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WFNS grade on admission</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>27 (53)</td>
</tr>
<tr>
<td>II or III</td>
<td>12 (24)</td>
</tr>
<tr>
<td>IV or V</td>
<td>12 (24)</td>
</tr>
<tr>
<td>Fisher grade on admission CT scan</td>
<td></td>
</tr>
<tr>
<td>thin layer or diffuse deposition</td>
<td>16 (31)</td>
</tr>
<tr>
<td>thick layer or localized clots</td>
<td>35 (69)</td>
</tr>
<tr>
<td>delayed cerebral ischemia (50 patients)</td>
<td></td>
</tr>
<tr>
<td>none</td>
<td>37 (74)</td>
</tr>
<tr>
<td>RIND</td>
<td>6 (12)</td>
</tr>
<tr>
<td>FIND</td>
<td>7 (14)</td>
</tr>
<tr>
<td>outcome at 3 mos†</td>
<td></td>
</tr>
<tr>
<td>good recovery or minimal disability</td>
<td>27 (53)</td>
</tr>
<tr>
<td>moderate disability</td>
<td>10 (20)</td>
</tr>
<tr>
<td>severe disability</td>
<td>5 (10)</td>
</tr>
<tr>
<td>vegetative state</td>
<td>2 (4)</td>
</tr>
<tr>
<td>death</td>
<td>7 (14)</td>
</tr>
</tbody>
</table>

* FIND = fixed ischemic neurological deficit; RIND = reversible ischemic neurological deficit.
† Based on the Glasgow Outcome Scale. Causes of death: one patient died of primary bleed, three of an early rebleed, one of pneumonia, and two of surgical complications.

within 48 hours after aneurysmal SAH between February 1998 and March 1999 and whose aneurysms were subjected to surgery or coil placement. Of the 20 patients (39%) with a history of hypertension (pre-SAH blood pressure readings > 140/90 or the use of antihypertension medication), four (8%) had blood pressure readings higher than 160/95 and 11 (22%) had used antihypertension medication. One patient (2%) had diabetes mellitus, three patients (6%) had coronary heart disease, 25 (49%) were current cigarette smokers, seven (14%) were previous smokers, seven (14%) were frequent consumers of alcohol, 11 (22%) had used nonsteroidal antiinflammatory drugs before SAH, and six (12%) had used aspirin.

Clinical Monitoring, Treatment, and Outcome

A patient’s clinical condition on admission, before surgery, and during the three blood samplings was scored according to the WFNS Grading Scale.2 The amount of subarachnoid blood was categorized according to the scale created by Fisher, et al.4 (Table 1). Five patients (10%) had an intracerebral hematoma larger than 10 mm in diameter. In addition, nine (18%) had moderate to severe and 16 (31%) had slight intraventricular extension of bleeding. Computerized tomography scans were routinely obtained on the 1st postoperative day, on discharge, and during the follow-up examination at 3 months; scans were repeated if clinical deterioration occurred.

Of the 51 patients, 45 (88%) underwent surgical clipping of the aneurysm, three (6%) endovascular coil placement in the ruptured saccular aneurysm, and three (6%) proximal clipping or trapping of the ruptured fusiform aneurysm. Ruptured aneurysms were excluded within a mean of 30 hours (median 24 hours, range 6–84 hours) after onset of SAH. Temporary clipping of the parent artery of the aneurysm was performed in 13 operations (median time 7 minutes, range 1–37 minutes; in three patients, > 11 minutes).

Intravenous nimodipine treatment was started within a mean of 15 hours (median 12 hours, range 2–50 hours) after onset of SAH and was continued until 10 to 12 days after bleeding had begun. Thereafter, nimodipine was administered orally for up to 21 days after SAH. No hypertension, hypervolemia, or endovascular vasospasm therapy was used routinely, but it was used if ischemic symptoms occurred. After admission, neurological examinations were performed daily. Delayed cerebral ischemia was defined as the gradual development of focal neurological signs and/or deterioration in the level of consciousness not due to any known reason (intracerebral hematoma, rebleeding, hydrocephalus, and so forth).12 Causes of poor clinical condition and outcome were determined with the aid of repeated CT scanning, routine postoperative angiography (performed < 4 days after SAH, median 2 days), autopsy studies, or laboratory investigations. Seven patients (14%) had hydrocephalus that required the insertion of a shunt.

Outcome was assessed at 3 months post-SAH according to the Glasgow Outcome Scale.10 Follow-up CT scans (43 scans) and MR images (40 images) were also obtained at this time to identify permanent lesions consistent with a cerebral infarct. In three patients who died within 3 months after SAH, the follow-up CT scans were considered to be the last image that had been obtained longer than 3 weeks after SAH. Permanent lesions had not been demonstrated on any CT scan obtained on admission.

Hypodense lesions could be visualized on CT scans obtained in 31 patients (67%); none was exhibited on scans obtained in 15 (33%). In nine patients, the lesion was caused by symptomatic vasospasm. Causes of hypodense areas on CT scans and lesions on MR images were grouped as follows: Group 1, a lesion in the same area as a previous intracerebral hematoma (four patients), a lesion caused by damage to a penetrating artery during surgery (six patients), or for other reasons (spatula pressure during surgery, proximal clipping or trapping of a fusiform aneurysm, and/or cardiovascular embolus; 12 patients); or Group 2, a lesion caused by delayed cerebral ischemia (nine patients). Any patient whose scan had separate hypodense areas due to both delayed ischemia and previous intracerebral hemorrhage (three patients) was included in Group 2.

Magnetic resonance imaging studies were performed with a Philips Gyroscan (1 tesla). Standard axial T1-weighted (TR 560 msec, TE 14 msec, matrix 205 × 256), T2-weighted (TR 2870 msec, TE 120 msec, matrix 238 × 256), and fluid-attenuated inversion recovery (TR 4460 msec, TE 105 msec, matrix 248 × 256) sequences as well as a coronal T1-weighted (TR 30 msec, TE 8.9 msec, matrix 256 × 256) sequence were obtained.

Laboratory Procedures

Blood samples were collected from patients one to three times. The first sample (51 patients) was drawn on admission, soon after the diagnosis of SAH (mean 22 ± 14 hours after onset of symptoms, median 19 hours). The second and third samples were each collected in the morning, while patients were fasting. The second sample (49 patients) was obtained in the morning after surgery (mean 1.86 ± 0.64 days after bleeding, median 1.7 days), and the third sample (45 patients) was acquired before the end of intravenous administration of nimodipine during the 2nd week after onset of SAH (mean 10.22 ± 1.28 days, median 10 days).
Endothelin activity after aneurysmal subarachnoid hemorrhage

Blood samples (10 ml) for measurements of ET-1 in 51 patients and big ET-1 in the first 22 consecutive patients were collected with minimal stasis from an antecubital vein and were placed into tubes containing potassium EDTA (final concentration 2 mg/ml) and aprotinin (final concentration 500 IU/ml). Plasma was prepared by centrifugation of blood at 4˚C for 15 minutes at 1000 G, and samples were stored at −70˚C until assayed.

For the method of studying plasma ET-1 and big ET-1 immunoreactivity, see information on the RIA method, described in detail previously. All samples were analyzed at the Minerva Institute for Medical Research, Helsinki, which is a laboratory with personnel experienced in ET research. The ET antiserum showed a 100% cross-reaction with ET-2 and ET-3 (human) and a lower than 0.1% cross-reaction with big ET-1. The big ET-1 antiserum displayed a 100% cross-reaction with big ET-1 (human 1–38), a 50% cross-reaction with the 22–38 fragment of big ET-1, and a lower than 0.1% cross-reaction with ET-1.

Plasma samples were extracted on C18-OH analytical columns. Standard or sample (0.1 ml) was incubated with either 0.1 ml of ET-1 antiserum (final dilution 1:24,000) or 0.1 ml of big ET-1 antiserum (final dilution 1:45,000) for 48 hours at 4˚C. 0.1 ml of [125I]-labeled ET-1 or big ET-1 (7000 cpm) was then added and the mixture was incubated for an additional 24 hours at 4˚C. The bound ligands were separated using a double antibody method with normal rabbit serum and goat anti-rabbit immunoglobulin G serum. The measurement range of the RIA was 0.8 to 100 pg/tube. Intraassay and interassay coefficients of variation were 8 and 9%, respectively. Recovery of added synthetic ET-1 and big ET-1 were 80 and 85%, respectively. Control plasma samples obtained in patients with SAH and those in control volunteers during the first 2 weeks after onset of SAH (Fig. 1). Plasma ET-1 immunoreactivity differed between samples obtained from 51 consecutive patients, were diluted to a concentration of 1:50 with a buffer containing 30 mM of HEPES, 3500 mM of NaCl, 50 μM of ZnCl2 (pH 7) and were preincubated on ice with 10 μM of thiorphan for 30 minutes. The reaction was started by adding 300 mM of big ET-1 and continued at 37˚C for 60 minutes. The reaction was terminated by cooling on ice and by the addition of Na2EDTA to a final concentration of 1 mM. Internal control samples of pooled serum were also included in the assay. The amount of ET-1 generated was quantified by performing RIA of ET-1, as mentioned earlier. Assay specificity was demonstrated by a broad panel of enzyme inhibitors. The ECE-1 activity in 83 healthy volunteers was 121 ± 56 pg/ml of ET-1.

Statistical Analysis

Data were analyzed with commercially available software. Categorical variables were compared using the twotailed Fisher exact test or the chi-square test. Continuous variables were compared between groups by performing the Mann–Whitney U-test, the Student t-test, or ANOVA with corrected multiple pairwise comparisons by use of the Dunnett method. The effect of elapsed time on laboratory values was tested using the paired t-test or the Wilcoxon signed-rank test. Univariate association of continuous variables was tested by calculating the Pearson correlation coefficient (r).

In patients with no missing samples, the effect of elapsed time after SAH and different grouping variables on ET variables (expressed as the means ± standard deviation) were compared using repeated measures ANOVA and analysis of covariance. For analyses of variance and covariance, values of ET variables were analyzed after logarithmic transformation if necessary to obtain equality of variances between different groups. A two-tailed probability value less than 0.05 was considered to indicate statistical significance.

Sources of Supplies and Equipment

The aprotinin (Trasylool) used in tubes during blood sampling was obtained from Miles Laboratories (Elkhart, IN). The endothelin antiserum (human), the big ET-1 antiserum (human), and the goat anti–rabbit immunoglobulin G serum were acquired from Peninsula (London, UK). The synthetic ET-1 and the big ET-1 were purchased from the Peptide Institute (London, UK), and big ET-1 for ECE-1 analyses from the Peptide Institute (Barnett, UK). The thiorphan was acquired from Sigma Chemical Corp. (St. Louis, MO). The Statistical Product and Service Solutions statistical package (SPSS for Windows, version 9.01) was purchased from SPSS, Inc. (Chicago, IL). The C18-OH analytical columns (Bond Elut) were obtained from Analytichem International (Harbor City, CA).

Results

Severity of Bleeding and Outcome

For baseline variables and patient outcomes, see Table 1. Delayed ischemia was correlated (p < 0.01) with the presence of a hypodense area on the follow-up CT scan. Of the 12 patients who had symptomatic vasospasm and had undergone follow-up CT scanning, seven (58%) had a hypodense lesion attributable to vasospasm. Correspondingly, four (67%) of the six patients suffering from delayed ischemia with fixed ischemic neurological deficit but only two (6%) of 34 patients without cerebral ischemia had such a lesion.

Plasma ET Variables in Patients and Control Volunteers

Plasma ET-1 immunoreactivity differed between samples obtained in patients with SAH and those in control volunteers during the first 2 weeks after onset of SAH (Fig. 1). Early surgery did not significantly affect values of ET variables. During the 2nd week after surgery for SAH (Phase III sample), serum ECE-1 activity was elevated significantly (p = 0.029) compared with values in controls.

Postoperative Phase II ET-1 values correlated highly (p < 0.01) with both preoperative and postoperative Phase III ET-1 values (r range 0.636–0.642). Similarly, Phases I and II big ET-1 values correlated highly with each other (r = 0.595). Preoperative and postoperative values of ECE-1 activity did not follow this pattern, however. Phase III ET-1 values correlated inversely (p < 0.05) with Phases I and II values of big ET-1 (r range −0.446 to −0.527). The plasma ET-1 values at different phases correlated more or less significantly but inversely with serum ECE-1 activity values (r range −0.580−0.072).
Values of plasma ET-1, big ET-1, and serum ECE-1 activity seemed not to be significantly associated with time elapsed after hemorrhage (Fig. 1). The level of ECE-1 activity was increased between Phases II and III samples \((p = 0.036)\), and ET-1 levels decreased almost significantly \((p = 0.067)\) during the same period.

**Relationship Between Plasma ET Variables, and Delayed Ischemia and Time After SAH**

The relationship between values of ET variables and the occurrence of delayed cerebral ischemia and time elapsed after SAH are depicted in Fig. 2. Patients with delayed ischemia had significantly \((p = 0.025)\) elevated ET-1 values during the first 2 weeks after SAH, and these levels did not decrease significantly during this time. There was no significant interaction with ET-1 level between delayed ischemia and time after SAH. Plasma ET-1 levels were also significantly \((p < 0.05)\) higher in patients with delayed ischemia after adjustment for history of hypertension or amount of subarachnoid blood.

**Plasma ET Variables and Lesions Visualized on CT Scans and MR Images**

Values for ET variables according to cause of the hypodense lesion on the follow-up CT scan are depicted in Fig. 3. On the whole, patients with hypodense lesions demonstrated on follow-up CT scans tended to have nonsignificant higher ET-1 values than did those without a hypodense lesion. This same tendency appeared when comparisons were made between those who had a lesion (27 patients; 10 lesions due to delayed ischemia) and those with no lesion (13 patients) on MR images obtained at the 3-month follow-up.

Analysis of repeated measures ANOVA indicated that the presence of ischemic lesions on either a CT scan or an MR image was associated with elevated levels of plasma ET-1, because those who harbored lesions due to delayed ischemia had higher plasma concentrations (Fig. 3). On the other hand, no significant interaction appeared between the presence of ischemic lesions and elapsed time from SAH and ET-1 values.

No significant associations existed between plasma ET-1, big ET-1, or ECE-1 values and patient sex and age, amount of subarachnoid blood, occurrence of intraventricular or intracerebral bleeding, clinical condition on admission or during blood sampling, history of hypertension or cigarette smoking, or temporary arterial clipping during surgery, except for the fact that big ET-1 levels during the second week after SAH correlated with Fisher grades on admission \((p = 0.011)\) and tended to correlate with duration of temporary clipping \((p < 0.1; \text{data not shown})\).

**Discussion**

Based on these findings, plasma ET-1 concentrations after aneurysmal SAH correlate with both delayed symptomatic cerebral ischemia and ischemic lesions demonstrated on CT scans or MR images, suggesting that an increased ET conversion rate in the endothelium is predictive of the ischemic symptoms. Increased serum ECE-1 activity during the 2nd week after surgery for SAH may reflect the severity of endothelial injury to the cerebral arteries.
Endothelin-1, Big ET-1, and ECE-1

There exist three structurally and pharmacologically separate endothelin isopeptides (ET-1, ET-2, and ET-3), of which only one—ET-1—is produced by endothelial cells. Endothelin-1 is also produced by neurons and astrocytes in the central nervous system as well as by mononuclear leukocytes of bloody CSF. From big ET-1, ET-1 is produced through proteolytic cleavage by ECE-1, a membrane-bound zinc metalloproteinase, which preferentially cleaves big ET-1 rather than big ET-2 or big ET-3. The ECE is key in the biosynthesis of endothelin, because the biological activities of big ET are negligible. Because a disproportionate increase or decrease in plasma big ET-1 relative to ET-1 occurs in different vascular diseases, it has been suggested that the ECE, being responsible for the conversion of big ET-1 to ET-1, may play a role in ET-1 production and the pathogenesis of these diseases. For example, during pregnancy, plasma ET-1 and big ET-1 levels decrease with an increased molar ratio of big ET-1 to ET-1, and disruption of this regulatory system appears to accelerate the development of preeclampsia. On the other hand, levels of plasma big ET-1 increase more markedly than levels of plasma ET-1 in patients with disseminated intravascular coagulation and those with non-insulin-dependent diabetes mellitus with microangiopathy, suggesting that either the enzyme activity of ECE in injured vascular endothelial cells may be limited or the increased release of big ET-1 may occur in injured endothelial cells. In these diseases, a lower ET-1 conversion rate (that is, ET-1/big ET-1 molar ratio) may also be the result rather than the cause of vascular injury.

Endothelin-1 and Post-SAH Vasospasm

According to data in this study, ET-1 concentrations and perhaps the ET conversion rate seem to rise soon after SAH, particularly in those with a later symptomatic vasospasm. Thereafter, during the second week post-SAH, ET-1 concentrations decrease somewhat, but serum ECE-1 activity increases, possibly because of damage to the endothelium. Endothelin-1 may cause or at least contribute to post-SAH cerebral vasospasm or ischemia by multiple mechanisms and intracellular signaling transduction pathways. After SAH, ET-1 production by endothelial cells can be stimulated by thrombin, epinephrine, transforming growth factor–β, angiotensin II, vasopressin, oxyhemoglobin, cytokines, platelets, or shear stress. Both plasma and CSF thrombin-antithrombin complex (marker of thrombin generation) and catecholamine levels as well as CSF oxyhemoglobin concentrations and expression of transforming growth factor–β in an artery wall are highly elevated soon after SAH. Because endothelium-derived relaxing factor (or nitric oxide) activity in the endothelial cells is diminished after SAH, there may be an ET-1/endothelium-derived relaxing factor imbalance locally in the cerebral arteries. This functional imbalance may favor ET-induced vasoconstriction even without any marked ET production. Endothelin binds to specific membrane receptors: ETA and ETB2 in smooth muscle cells, leading to vasoconstriction; and ETB1 in endothelial cells, leading to relaxation of muscle cells and stimulation of its own synthesis. This binding further leads to intracellular biochemical signals in-
volving the activation of phospholipase C, which hydrolyzes phosphatidylinositol-4,5-biphosphate to diacylglycerol (an activator of protein kinase C), and inositol 1,4,5-triphosphate, which releases calcium from intracellular calcium stores.18,21,27,29,30 In addition, it has been recently demonstrated in a non-SAH experiment that the contractile effect of ET-1 may also be mediated by signaling pathways other than the protein kinase C pathway. These pathways include the protein tyrosine kinase–Src tyrosine kinase–Janus tyrosine kinase–mitogen-activated protein kinase pathway and the phosphatidylinositol-3 kinase pathway.31

**Endothelin-1, Big ET-1, and ECE-1 Measurements After SAH**

There are contradictory reports on ET levels in plasma and CSF during delayed ischemic complications and vasospasm after aneurysmal SAH. For results of several previous studies, see recent review articles.12,25 In studies with the largest patient populations (20–70 patients),12,25,28 ET-1 or big ET-1 concentrations in plasma or CSF have been correlated with vasospasm or have been found to be elevated after SAH. Plasma ET-1 levels are highest soon after SAH, with a gradual decline through time post-SAH; the greatest values appear in patients with symptomatic delayed cerebral ischemia, angiographically demonstrated severe or diffuse vasospasm, and/or cerebral infarction caused by vasospasm.12,28 This was also supported by the results of several experimental studies in which ET concentrations were increased in the endothelium or vessel wall of a vasospastic artery at 2 to 3 days after SAH but not on the 7th day.12,30

Although plasma ET-1 levels are highest during the first few days after SAH, levels of ET-1 or big ET-1 in CSF increase simultaneously with ischemia later after SAH,25,28 suggesting that these may be released either from ischemic astrocytes or neurons22,24,28 or from mononuclear leukocytes of bloody CSF.2 In contrast to ET-1 levels in CSF, ET-1 levels in plasma are not increased secondarily after cerebral ischemia.3

In 20 patients who suffered from SAH and who had a mean date of surgery on Day 3 (range Days 1–4) after aneurysm rupture, Seifert, et al.,25 demonstrated an increased big ET-1/ET-1 (or reduced ET-1/big ET-1) molar ratio on admission before surgery compared with the ratios for controls. Thereafter, the ET-1, big ET-1, or big ET-1/ET-1 ratio in plasma gradually fell to normal levels with time but was not associated with delayed ischemia. From this fact one may infer that the ET-1 conversion rate (reduced ET-1/big ET-1 ratio) in the endothelium may have been lower preoperatively; however, the actual situation is likely the opposite, because plasma ET-1 concentrations were higher in patients with SAH than in controls.14

A new observation in the current study was that the increases in serum ECE-1 activity did not occur until during the 2nd week, or the vasospasm phase, after aneurysmal SAH. This increase may be an indication of damage to the endothelium in cerebral arteries during that phase, leading to a release of ECE-1 into the blood circulation; the elevated serum ECE-1 activity may reflect the extent of this injury. This may also be one explanation for the decrease in plasma ET-1 levels and ET-1 conversion rate during the 2nd week after SAH despite the increased occurrence of vasospasm.

**Treatment of Vasospasm by Using ET-1 Antagonists**

Data from most experimental studies have demonstrated that vasospasm may be reduced or prevented by inhibitors of ECE or by intracisternal, topical, oral, or intravenous administration of ETA, ETB, ETB1, or ETA/B receptor antagonists; however, after excluding data from studies in which medication was started before experimental SAH, the beneficial effect based on starting time for the inhibitors/antagonists decreases (quite quickly) within 24 hours after SAH.12,30

Fig. 3. Bar graphs demonstrating the relationship between plasma ET variables and lesions on follow-up CT scans (A–D) and MR images (E–H). Group 1, lesion due to a known cause; Group 2, lesion due to delayed cerebral ischemia. *p = 0.061 for differences in ET-1 values between CT scan groups in Phases I to III in repeated measures of ANOVA; p = 0.051 for difference between Group 2 and no hypodense area with pairwise comparison with the Dunnett method (A). **p = 0.029 for differences in ET-1 values between MR imaging groups in Phases I to III in repeated measures of ANOVA; p = 0.019 for difference between Group 2 and no lesion (panel E). Abbreviation: HA = hypodense area.
Endothelin activity after aneurysmal subarachnoid hemorrhage

Based on data from a recent multicenter randomized, double-blind, placebo-controlled phase II trial, investigators found that the ETA/B receptor antagonist TAK-044 tended to reduce delayed ischemic events in a patient population that had also received nimodipine. In the future, data from phase III trials may indicate whether ET receptor antagonists have a beneficial effect on post-SAH symptomatic vasospasm in addition to the use of nimodipine or nicardipine and hypertension and/or hypervolemia therapy.

Conclusions

Plasma ET-1 concentration after aneurysmal SAH correlates both with delayed symptomatic cerebral ischemia and with ischemic lesions visualized on CT scans or MR images, suggesting that an increased ET conversion rate in the endothelium is predictive of ischemic symptoms. Increased serum ECE-1 activity during the 2nd week may reflect the severity of endothelial injury to the cerebral arteries.

References