Temporal Relationship Between Endothelin-1 Concentrations and Cerebral Vasospasm in Patients With Aneurysmal Subarachnoid Hemorrhage

L. Mascia, MD; L. Fedorko, MD, PhD; D.J. Stewart, MD; F. Mohamed, BSc; K. terBrugge, MD; V.M. Ranieri, MD; M.C. Wallace, MD

Background and Purpose—Endothelin 1 (ET-1) is a potent vasoconstrictor that may play a role in cerebral vasospasm following subarachnoid hemorrhage (SAH). However, data regarding its pathogenic role in the development of vasospasm are controversial. We planned a prospective, observational clinical study to investigate the temporal relationship between increased ET-1 production and cerebral vasospasm or other neurological sequelae after SAH.

Methods—ET-1 levels in cerebrospinal fluid (CSF) were measured in 20 SAH patients from admission (within 24 hours from the bleeding) until day 7. Patients received a daily transcranial Doppler study and a neurological evaluation. On day 7, angiography was performed to verify the degree and extent of vasospasm. Patients were then classified as having (1) clinical vasospasm, (2) angiographic vasospasm, (3) no vasospasm, or (4) poor neurological condition without significant vasospasm (low Glasgow Coma Scale score [GCS]).

Results—On admission, ET-1 levels were increased in the low-GCS group compared with the other groups ($P=0.04$). On day 4 ET-1 levels were not significantly different among groups, whereas on day 7 ET-1 levels were significantly increased in both the clinical vasospasm and low-GCS groups compared with the angiographic vasospasm and no vasospasm groups ($P<0.005$). Moreover, when the low-GCS group was excluded, there was a significant relationship between vasospasm grade and CSF ET-1 levels ($R^2=0.73$).

Conclusions—CSF ET-1 levels were markedly elevated in patients with clinical manifestations of vasospasm (day 7) and with a poor neurological condition not related to vasospasm. However, ET-1 levels were low in clinical vasospasm patients before clinical symptoms were evident (day 4) and remained low in angiographic vasospasm patients throughout the study period. Thus, our data suggest that CSF ET-1 levels are increased in conditions of severe neuronal damage regardless whether this was due to vasospasm or to the primary hemorrhagic event. In addition, CSF ET-1 levels paralleled the neurological deterioration but were not predictive of vasospasm. (Stroke. 2001;32:1185-1190.)

Key Words: cerebral ischemia ■ cerebral vasospasm ■ endothelins ■ subarachnoid hemorrhage ■ ultrasonography, Doppler, transcranial

Delayed cerebral vasospasm is one of the most serious consequences following aneurysmal subarachnoid hemorrhage (SAH). The incidence of angiographic vasospasm is in excess of 50%, with symptomatic vasospasm occurring in 30% of patients. Progression to cerebral infarction occurs in 50% of symptomatic cases. Because of this high morbidity rate, considerable research efforts have been directed at identifying the mechanism of vasospasm and in identifying potential candidate factors. Endothelin (ET) is a family of 3 vasoconstrictor isopeptides with common structural features (ET-1, ET-2, ET-3) expressed by several cell types in the brain, including neurons, glial cells, and macrophages. Experimental studies have shown that ET exerts a potent and long-lasting vasoconstrictor effect associated with morphological changes mimicking the delayed, SAH-associated vasospasm. Several studies in humans have demonstrated there are increased ET-1 levels in cerebrospinal fluid (CSF) and in plasma after SAH and that ET-increased production is associated with vasospasm. In these studies, increased ET-1 production correlated with the presence of symptomatic vasospasm. However, there are some studies in humans that do not support this hypothesis. Increased ET levels have also been reported in ischemic stroke and after severe brain injury. All these pathological conditions share the feature of the presence of cerebral ischemia rather than the develop-
ment of vasospasm. Therefore, ET-1 may play a role in ischemic brain injury, irrespective of any effect on vascular tone. The aim of this study was to define the temporal relationship between increased ET-1 production and the development of cerebral artery vasospasm and neurological sequelae after SAH.

Subjects and Methods
Following approval of institutional review boards and after obtaining informed consent from the patient or next of kin, 20 patients with SAH confirmed by CT scan (13 women, 7 men; mean±SD age 55±16 years) were recruited from the intensive care unit of a large university teaching hospital. All the patients fulfilled the following inclusion criteria: (1) angiographic proof of aneurysm, (2) admission within 24 hours from the SAH, and (3) presence of an intraventricular catheter placed either after admission or at the time of the surgery. Patients were graded clinically according to the World Federation of Neurological Surgeons (WFNS) scale\(^{20}\) and were also classified according to the CT distribution of blood as described by Fisher et al.\(^{20}\)

All patients underwent neurosurgical intervention or endovascular procedure to secure the aneurysm within 2 days of admission. No patients exhibited signs of severe cardiac insufficiency, cardiac ischemia, concomitant infection, or acute or chronic renal failure. Furthermore, patients with signs of hemodynamic instability (defined by the presence of episodes of systemic hypotension for at least 2 hours: systolic blood pressure <85 mm Hg or reduction to >40 mm Hg from baseline, or need for inotropic agents to maintain systolic blood pressure >85 mm Hg) were excluded.\(^{21}\)

Neurological status was evaluated daily using the Glasgow Coma Scale (GCS).\(^{22}\) On day 7 after hemorrhage, a second angiogram was routinely performed, and patients were then classified into the following groups: (1) angiographic vasospasm (AV), if the angiogram showed ≤25% reduction from baseline diameter without any clinical deterioration; (2) clinical vasospasm (CV), if a reduction in the angiographic diameter of ≥50% was accompanied by contralateral weakness to the middle cerebral artery (MCA) studied or global neurological deterioration (2-point reduction in GCS) occurring after day 3 for anterior or diffuse vasospasm; (3) no vasospasm (NV), if there was no significant reduction in the angiographic diameter; and (4) low GCS score, if patients were in poor neurological condition from the time of admission or immediately after surgery (GCS <8) and did not show a reduction in angiographic diameter on day 7. The diameter of the MCA close to the bifurcation was measured and corrected for magnification. At baseline, a diameter of ≥2 mm was considered normal (ie, no vasospasm).\(^{23}\)

The severity and extent of vasospasm, the total vasospasm grade (TVG), was defined according to the score derived from the angiogram: grade 1 indicated no significant vasospasm (<25%); grade 2, mild vasospasm; grade 3, moderate vasospasm (25% to 50%); and grade 4, severe vasospasm (≥50%). The extent of vasospasm was reported in the following territories: internal carotid artery, MCA, anterior cerebral artery, and basilar artery. The TVG was calculated by adding the individual scores for all 7 vessels. Angiograms were reviewed in a blinded and unbiased manner by 2 experienced neuroradiologists. The accuracy of the angiographic report was evaluated considering the percentage diameter reduction as standard: sensitivity equal to 0.7 and specificity equal to 0.9 were calculated.

Flow velocities in the MCA were measured daily by means of transcranial Doppler ultrasonography (TCD).\(^{24}\)

Measurement of ET-1
In all patients, 2 mL CSF was drawn into tubes containing EDTA from the intraventricular catheter left in place during the patient’s stay in the ICU. Tubes were transported in ice water, centrifuged at 3000 rpm for 15 minutes at 4°C, and stored at –80°C until the assay. Frozen CSF samples were thawed on ice and filtered through Amicon 30 000-molecular-weight-cutoff membranes (Millipore) to remove any hemoglobin that might interfere with the assay. The filtrate was then assayed for ET-1 using the ELISA kit from Biomedica, which consists of a 96-well plate coated with a polyclonal rabbit anti-ET antibody. After adding the standards, controls, or samples, a monoclonal anti-ET antibody was added as the detection antibody to form a sandwich, and the plate was incubated overnight at room temperature. After several washes, the wells were incubated with anti-mouse IgG conjugated to horseradish peroxidase for 1 hour at 37°C. Color was developed using tetramethylbenzidine as substrate, after which STOP solution (1 mol/L sulfuric acid) was added and the plate was read in a plate reader at 450 nm. The standard curve was plotted using a 4PL algorithm and the samples read off the curve, since the amount of color developed is directly proportional to the amount of ET-1 immunoreactivity (IR) present in the sample. The ET antibody exhibited a cross-reactivity of 100% with ET-2, <5% with ET-3, and <1% with big ET (both 1–38 and 22–38). The detection limit was 0.05 fmol/mL. The intra-assay and interassay coefficients of variation were 4.5% and 7.25%, respectively.

Statistical Analysis
Values are presented as mean±SD. Data within each group were compared by ANOVA for repeated measurements, and if significant, a post hoc comparison by paired t test was performed between days 4 or 7 and day 1. Comparison of data between different groups was also performed at each time point (days 1, 4, and 7) by ANOVA. A regression model was applied to describe the relationship between ET-1 IR levels and the extent of vasospasm.

Results
Eleven patients had an aneurysm in the anterior circulation, whereas in 9 patients the aneurysm was in the posterior circulation. The aneurysm treatment was performed within 2 days from admission in all patients. The median value for the WFNS scale was 3 (range 1 to 5); 18 patients had a Fisher grade 3 on CT scan, while the remaining 2 patients had grades 2 and 4, respectively. The overall incidence of angiographic vasospasm was 45% (9 patients), with clinical vasospasm occurring in 4 (20%). The median onset of neurological deterioration related to vasospasm occurred on day 6.5 (range 6 to 8 days); 6 patients were in poor neurological condition (GCS <8) from time of admission or immediately after surgery. A mean TCD velocity >100 cm/s\(^{25}\) was considered an indicator of vasospasm and confirmed by angiography.

Comparison Among Groups
On admission (day 1), ET-1 IR levels were significantly increased only in the low-GCS group (P=0.04) compared with the other 3 groups. On day 4, ET-1 IR levels were not significantly different between groups. On day 7, ET-1 IR concentration was significantly increased in both the CV and low-GCS groups (P<0.005) compared with AV and NV groups. TCD mean velocity was normal in the 4 groups at admission. On days 4 and 7, TCD velocity in the CV and AV groups was significantly increased (P<0.0005) compared with that in the NV and low-GCS groups, which showed a level of blood velocity within the normal range. The neurological condition expressed by GCS was significantly deteriorated in the low-GCS group on days 1 and day 4 compared with the other 3 groups; on day 7, the neurological condition was impaired in the CV and low-GCS groups compared with AV and NV groups (Figure 1).
Analysis of Temporal Changes Within Groups
In patients with CV, CSF ET-1 IR levels were significantly increased on day 7 compared with days 1 and 4 (P<0.005; Figure 1). In patients with AV or NV, ET-1 IR levels remained low but above the detection threshold of the assay (0.05 fmol/mL) throughout the study period. In the low-GCS group, ET-1 IR levels, while increased at baseline, did not change significantly from day 1 to day 7. In the CV and AV groups, TCD mean velocity significantly increased on day 4 (P<0.001) and was further elevated on day 7 (P<0.0001) compared with day 1. In the NV and low-GCS groups, it remained within the normal range (33 to 90 cm/s) throughout the study period, although there was a significant increase in both groups at days 4 and 7 (P<0.005). In patients with CV, GCS dropped significantly on day 7 compared with days 1 and 4, whereas in patients with AV and NV it remained stable throughout the study period. Patients defined as having a low GCS demonstrated a severe neurological impairment from admission (GCS <8) until day 7 (Figure 1).

The relationship between ET-1 level and the degree and extent of vasospasm (quantified by the TVG) was described by an exponential regression model (R²=0.73, P<0.0001; Figure 2).

Discussion
Our data demonstrate that levels of ET-1 in CSF were markedly elevated in patients with SAH associated with clinical manifestations of vasospasm and in patients with SAH who did not develop vasospasm but were in poor neurological condition throughout the study period. Patients with significant narrowing of the arterial cerebral vessels not accompanied by clinical deterioration and patients without any detectable vasospasm had stable levels of ET-1 during the study period. Therefore, our data suggest that ET-1 concentration in CSF is increased in conditions of neuronal damage, as demonstrated by a deterioration of the neurological status, but cannot be used as a predictor of vasospasm.

Definition of Patient Population
In our patient population, the diagnosis of vasospasm was established by angiography on day 7. The demonstration that arterial narrowing was not always associated with delayed neurological deficit allowed us to distinguish between angiographic and clinically significant vasospasm. Furthermore, patients with NV on angiography, but with a persistent poor
neurological condition, clearly represented a different patient group. According to these integrated criteria used to define patient groups (neurological status, angiography, TCD evaluation), we were able to identify patients with cerebral ischemia due to vasospasm and patients with global hypoperfusion related to the primary hemorrhagic or other event, and to consider separately patients who developed angiographic vasospasm but did not show any clinical deterioration. The incidence of vasospasm was lower than generally reported, but we believe that inclusion and diagnostic criteria may explain this difference.

**Time Course of Vasospasm and ET Production**

In our study, the AV group subclinical vasospasm was clearly present according to TCD and angiographic results. Daily measurements of TCD were obtained to follow the temporal evolution of the arterial narrowing. Patients who had vasospasm on day 7 were identified on day 4 by TCD. ET-1 levels on day 4 were not predictive of vasospasm.

Data reported in literature regarding the pathogenic role of ET in the development of vasospasm are controversial. In 1989 the first report of elevated plasma ET in aneurysmal SAH patients was published. On admission, plasma ET-1 levels were higher than control in patients with and without vasospasm. At day 7 there was a further significant increase in the group with symptomatic vasospasm. Suzuki et al did not distinguish between patients with and without vasospasm and reported an increased CSF level on day 6 in the global SAH patient population. Subsequently, more reports established the association between symptomatic vasospasm and increased ET-1 levels. Of note, other reports were unable to confirm an increased production of ET-1 and big-ET in patients who developed vasospasm. However, these investigations had important limitations, including the reliance on plasma rather than CSF ET-1, the diagnosis of vasospasm by TCD not confirmed by angiography, and the grouping together, for analysis, of patients with both angiographic and clinical vasospasm. In our study we evaluated the presence of ET-1 in CSF not only at the peak interval for clinical vasospasm (day 7) but also at an earlier time preceding clinical manifestation of vasospasm (day 4) as confirmed by TCD studies. The absence of increased ET-1 levels in the CV group on day 4, and in patients with subclinical vasospasm at any time, suggests that ET-1 in CSF is a marker of severe neuronal damage and cannot be used to predict the occurrence of vasospasm. However, we cannot exclude a causal role of ET-1 in vasospasm secondary to SAH. Ventricular CSF levels may not reliably reflect vascular ET-1 production, expressed at lower levels. Indeed, there was a significant relationship between TVG and CSF levels, which was driven mainly by the CV group. It is possible that vascular production of ET-1 was also increased in the AV group, but this did not reach the threshold of detection in CSF. However, the high levels found in the low-GCS group support the view that CSF ET-1 is a marker of CNS ischemia rather than a mediator of vasospasm. Of note, Shaw et al have recently looked at the efficacy and safety of the ET antagonist TK-044 in the treatment of SAH: the primary end point of the study was to determine the effect of TK-044 on the incidence of delayed neurological deterioration caused by ischemia.

Increased ET production has also been reported in other diseases, such as acute ischemic stroke with large cortical or lacunar infarction, severe brain injury, and meningitis. All these pathological conditions are linked by the presence of hypoperfusion and hypoxia leading to impaired neurological function. In these situations, ET production may represent a nonspecific reaction in response to a general stress. ET-1 produced locally or from the systemic circulation can lead to further deleterious effects in the area of cerebral ischemia and compromise the recovery of the already-injured neurons. This process contributes to a vicious circle, with a further reduction in blood flow enhancing the damage and worsening the neurological outcome. Furthermore, it has been recently reported that in patients with mild to moderate ischemic stroke (GCS 15), plasma ET-1 levels were normal. All these data suggest that the extent of cerebral ischemia may be the key factor in ET production. In support of this hypothesis, there are studies regarding ET production after myocardial infarction. ET-1 plasma levels rise rapidly in patients after acute myocardial infarction, and in states of markedly depressed cardiac performance they might also reflect abnormalities of systemic perfusion. This confirms the view that the extent of tissue ischemia might be a crucial determinant of ET release.

The time course of ET production after vasospasm in SAH patients is quite different from that of other pathological conditions: astrocytes exposed to hypoxia need only 6 hours to produce ET; after experimental focal or global ischemia and after ischemic stroke in humans, cerebral tissue starts to produce ET within 24 hours; after complicated myocardial infarction, ET plasma production is increased within 6 hours. Differently from all these situations, in SAH patients, ET production is more likely increased after 7 days, both in plasma and in CSF. This discrepancy suggests that if ET is responsible for vasospasm, plasma and CSF levels are not adequate methods by which to monitor ET production.

We could not determine the source of ET-1 production, but we hypothesize that neuronal tissue and glial cells were both involved in increased ET-1 production. ET is a vasoconstrictor peptide expressed in the brain by neurons, glial cells, macrophages, and endothelial cells. ET-1 is thought to be a paracrine mediator rather than an endocrine hormone and its secretion by endothelial cells is largely abluminal toward the adjacent vascular smooth muscle cells, while ET-1 produced by glial cells or neurons in response to pathological conditions, such as ischemia, can be detected in CSF. Therefore, if ETs are present in CSF, this should be interpreted as a sign of neuronal injury rather than related to the pathogenesis of vasospasm.

Our data demonstrate that ET-1 is identified in the CSF in association with symptomatic vasospasm and after neuronal damage that occurs in SAH patients in the early phase. ET-1 present in CSF may be a reflection of neuronal tissue damage rather than a direct contributor to vasospasm. In any event, CSF ET-1 levels cannot be used to predict vasospasm. Further studies in larger patient cohorts are required to...
definitively rule in or out the role of ET-1 in the pathogenesis of this important complication of SAH.

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References


Endothelin-1 in Vasospasm After SAH

Despite years of research, the pathophysiology of delayed cerebral vasospasm after subarachnoid hemorrhage (SAH) remains obscure. Endothelins are close relatives of a snake venom family of sarafotoxins, and among them, endothelin-1 (ET-1) is a potent vasoconstricting peptide. ET-1 exerts its long-lasting vasoconstricting activity through stimulation of an ET₃ receptor on smooth muscle cells. Therefore, it was only natural that after the discovery of ET-1, researchers...
embraced the idea of ET-1 being responsible for the development of delayed cerebral vasospasm after SAH. The studies presented the ET-1 levels in plasma and/or cerebrospinal fluid (CSF) in the patients after SAH. Increased levels of ET-1 observed in plasma and sporadically in CSF seemed to confirm the hypothesis that ET-1 is responsible for vasospasm. Furthermore, since Ohlstein and Storer have shown that oxyhemoglobin (a putative agent responsible for the development of cerebral vasospasm) releases ET-1 from endothelial cells, this hypothesis has become even more attractive. Several methods of blocking of ET-1 production or its action have been proposed and used experimentally or clinically. However, ET-1 acts also as a stimulator of endothelial nitric oxide synthase via the ETB receptor and produces an increase in nitric oxide, a potent vasorelaxant. Therefore, it is not surprising that some experimental and clinical studies suggest the direct involvement of ET-1 in the development of vasospasm and a positive effect of blocking the action of ET-1. Others deny the existence of such a connection between ET-1 and vasospasm. Recently, it has even been shown that inhibition of the ET-1 pathway resulted in an increase in severity of vasospasm (A. Raabe, MD, et al, personal communication).

Because an increase of ET-1 in CSF has been described in patients with cerebral vasospasm and delayed ischemic neurological deficits after SAH, it is clear that the production of ET-1 may also be induced by ischemia. On the basis of experimental studies, it has been proposed that the increased ET-1 levels in CSF after SAH are not solely responsible for the development of vasospasm but result from ischemia occurring directly after SAH and/or as an effect of vasospasm.

It is neither glorious nor popular to present a negative study; therefore, I did not expect to see confirmation of experimental observations, especially considering how many different agents have been develop for clinical studies to block ET-1 activity (eg, AETN-40, BQ-123, FR139317, R047-0203, and phosphoramidon). Thus, it was a pleasure to find this valuable, well-planned, carefully executed, and, most importantly, convincing clinical prospective study by Mascia and colleagues. The authors elucidated the role of ET-1 in the development of vasospasm by defining “the temporal relationship between increased ET-1 production and the development of cerebral artery vasospasm and neurological sequelae following SAH.” The results clearly defined the lack of a causative relationship between ET-1 and delayed cerebral vasospasm after SAH. This study confirms the epiphenomenal rather than causative role of ET-1 in vasospasm after SAH and explains many clinical failures related to the use of ET-1 blockage as a treatment of delayed vasospasm.

Ryszard M. Pluta, MD, PhD, Guest Editor
Surgical Neurology Branch
National Institute of Neurological Disorders and Stroke
Bethesda, Maryland

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