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Vascular

Endothelin-1 levels in plasma and cerebrospinal fluid of patients with cerebral vasospasm after aneurysmal subarachnoid hemorrhage

Iruena Moraes Kessler, MD, MSc[a], Yolanda Galindo Pacheco, PhD[b], Silene Paulino Lozzi, PhD[c], Antônio Santos de Araújo Jr., MD[d], Franz Jooji Onishi, MD[d], Paulo Andrade de Mello, MD, PhD[a,*]

[a]*Neurosurgical Service, Departments of* [b]*Anatomy and* [c]*Biochemistry, and* [d]*Neurosurgical Laboratory, Medical School, University of Brasilia, 71680-070 Brasília-DF, Brazil*

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Abstract

Background: Plasma and cerebrospinal fluid (CSF) concentrations of endothelin-1 (ET-1) were measured in patients with subarachnoid hemorrhage (SAH) after aneurysmal rupture and compared with levels of ET-1 in volunteers. We analyze the relationship between levels of ET-1 in both CSF and plasma with the risk of developing cerebral vasospasm (CVS).

Methods: Cerebrospinal fluid and blood samples were collected from 30 selected patients after SAH and from 10 healthy volunteers who were used as control. All samples were stored at −70°C and the levels of ET-1 in CSF and blood were measured by using enzyme-linked immunosorbent assay and Western blot. All patients were submitted to angiography to confirm vasospasm.

Results: From the 30 patients admitted at different days of SAH, 18 (60%) developed clinical CVS and 10 (33%) presented angiographic CVS. The levels of ET-1 in the CSF were significantly higher ($P = .0001$) in patients (1.618 ± 1.05 fmol/mL) than in controls (0.365 ± 0.328 fmol/mL). There was statistical difference ($P < .05$) in CSF levels of ET-1 between each group of the Hunt-Hess scale and controls. The mean plasma concentration of ET-1 was similar ($P > .05$) in the control group (1.531 ± 0.753 fmol/mL) and in patients with SAH (1.920 ± 1.15 fmol/mL).

Conclusions: These findings indicate that a significant rise in ET-1 levels in the CSF, but not in the plasma, occurs in patients who develop CVS after SAH. Our observation suggests that ET-1 might be involved in the pathogenesis of SAH-associated CVS.

Keywords: Cerebral aneurysm; Cerebral vasospasm; Endothelin-1; Subarachnoid hemorrhage

1. Introduction

The development of cerebral vasospasm (CVS) is responsible for high morbidity and mortality in subarachnoid hemorrhage (SAH) due to cerebral aneurysm rupture [1,8]. Despite many clinical and experimental trials, the etiology and pathophysiology of CVS remain unknown. Nevertheless, some previous trials have demonstrated high concentrations of vasoactive substances in cerebrospinal fluid (CSF) and plasma of patients with CVS and SAH [2,11]. A novel class of peptides originally isolated from porcine endothelial cells, named endothelins (ETs), have a strong vasoconstrictor effect and were evident in those patients [21,22]. These peptides were found to be present in other types of cells including neurons, glial cells, choroid plexus, and macrophages under pathological conditions [10]. When ETs are injected in the adventitia of blood vessels, they produce a powerful and lasting vasoconstrictor effect [14].

In more recent studies, Clozel and Watanabe [3] studied the action of BQ123. This drug is a potent antagonist to the endothelin A receptor, and when used in a rat model of SAH, it prevents CVS. Matsumura et al [13] showed that phosphoramidon, another antagonist to endothelin A, improved CVS in 2 SAH canine models. Those findings

* Corresponding author. Tel.: +55 61 366 1018.
E-mail address: paulomello@terra.com.br (P.A. de Mello).

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have raised the possibility that ETs may play an important role in the pathophysiology of CVS [15,18]. Both endothelin-1 (ET-1) and endothelin-3 are found in human CSF and plasma [6,9].

There are few trials involving human models of CVS for technical and ethical reasons. The objectives of our research were to evaluate the correlation between CSF and plasma levels of ET-1 and CVS in patients with SAH and to discuss a possible role of ET-1 in the pathogenesis of CVS.

2. Patients and methods

Fifty patients with aneurysm SAH were admitted to the Neurosurgical unit of Hospital de Base in Brasilia from June 1999 to May 2000. Only 30 patients admitted within 23 days of aneurysm rupture were included in the study. The other 20 patients exceeded 23 days or had cardiac failure, coronariopathies, and renal failure and were excluded because such conditions can raise ET levels.

Ten volunteers, who did not have any neurologic disease, were recruited at the same hospital as a control group.

The protocol was submitted to the Ethics Committee of the Medical School, University of Brasilia, and is filed under number 25000130535/2002-13.

All patients were subjected to a daily neurologic examination including the Glasgow Coma Scale [19] and the Hunt-Hess (HH) scale [7]. The computed tomographic scan was analyzed in terms of the amount of blood according to the Fisher scale [4]. The next step consisted of an angiography that was used together with the neurologic examination to diagnose and classify the CVS according to Fisher [5].

Five milimeters of blood was collected from a brachial vein with a BD23 needle avoiding the use of a garrot. The blood sample was centrifuged at 1200 rpm for 5 minutes to isolate plasma. Cerebrospinal fluid was collected using a peridural BD23G needle. Both plasma and CSF samples were maintained at −70°C in liquid nitrogen.

The ET-1 concentration was determined by using an enzyme-linked immunosorbent assay (ELISA) (Catalog BI 20052, Biomedical Group, Viena, Austria).

All patients were treated with nimodipine (Nimotop, Bayer, Inc) administered orally at 120 mg/d divided into 4 doses.

We performed the statistical analysis with analysis of variance (ANOVA), followed by the post hoc Tukey test, to compare 3 or more parametric groups [20]. For 2 parametric groups, we used the *t* test, and for 2 nonparametric groups, we applied the Mann-Whitney *U* test. We considered the following variables: plasma levels of ET-1 (ELISA-PLASMA), CSF levels of ET-1 (ELISA-CSF), and Fisher and HH scales. The criterion for significance was $P < .05$.

Of the 30 participating subjects, 50% were women and 50% were men. The ages of the patients ranged from 20 to 68 years with an average of 45.37 years, whereas the ages of the control group ranged from 18 to 37 with an average of 24.9 years.

The most frequent clinical presentation was HH II in 15 (50%) patients, followed by HH III in 10 (33%) patients and HH I in 5 (16.7%) patients.

Computed tomographic scan findings, according to the Fisher score [4], were as follows: 8 (26.7%) patients with Fisher 1; 8 (26.7%) patients with Fisher 2; 10 (33%) patients with Fisher 3; and 4 (13.3%) patients with Fisher 4.

Clinical diagnosis of CVS was given to 18 (60%) patients and was confirmed with angiography in 10 (33%). The grade of CVS according to angiographic Fisher [5] was null in 20 (66.7%) patients, grade 1 in 3 (10%),

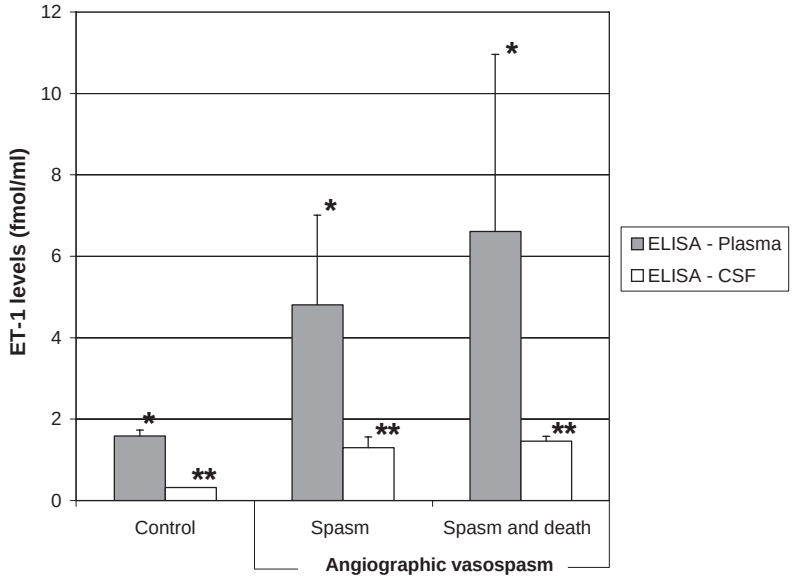

Fig. 1. CSF and plasma ET-1 levels in controls and patients, according to angiographic vasospasm and deaths. The angiographic vasospasm group was compared to the control band with no angiographic vasospasm. One asterisk means $P < .5$. Two asterisks means $P < .001$ (Tukey test, ANOVA).

grade 2 in 2 (6.7%), grade 3 in 2 (6.7%), and grade 4 in 3 (10%) patients.

3. Results

There was statistical difference in CSF levels of ET-1 between each HH scale group and the controls ($P < .05$, Tukey test, ANOVA). Cerebrospinal fluid level of ET-1 in the patients ranged from 0.568 to 2.668 fmol/mL, with an average ± SD of 1.618 ± 1.05 fmol/mL. Cerebrospinal fluid levels of ET-1 in controls ranged from 0.037 to 0.694 fmol/mL, with an average ± SD of 0.365 ± 0.328 fmol/mL. Therefore, CSF concentration of ET-1 was significantly higher in the patients than in the control group ($P = .0001$, t test).

Plasma levels of ET-1 in the patients greatly varied, ranging from a minimum value of 0.772 fmol/mL to a maximum of 3.068 fmol/mL with an average value ± SD of 1.92 ± 1.15 fmol/mL. Plasma levels of ET-1 in controls ranged from 0.778 to 2.284 fmol/mL, with an average ± SD of 1.531 ± 0.753 fmol/mL. Therefore, there was no statistical difference between patients and controls ($P = .426$, Mann-Whitney U test).

We analyzed samples of CSF and plasma continually, between day 0 to 4 days after ictus and day 4 to 7 days and after day 7 after ictus, and we did not find significant differences across groups ($P > .05$, ANOVA).

There were 2 deaths, one due to rebleeding and another due to severe vasospasm. Both patients were recorded as HH III at admission to the hospital.

We organized the data based on the occurrence of vasospasm. The results showed that the highest levels of plasma ET-1 were found in the patients with vasospasm and death. Such ratios were significantly higher than controls ($P = .041$, Tukey test, ANOVA) (Fig. 1).

4. Discussion

The participation of several vasoactive peptides, including ETs, has been related to the pathophysiology of CVS, but the exact mechanisms of CVS remain obscure [12,16].

The present study is in line with the contents of literature reviews. We found an expressive difference in CSF ET-1 levels between patients compared with the control group. Suzuki et al [17] found similar results, where CSF ET-1 levels were measured until the 18th day after ictus. Starting from a basal CSF level of 0.4 ± 0.2 fmol/mL, there was an increase until the sixth day (n = 11), with a progressive decrease afterward.

As for plasma ET-1 levels, our study did not find significant differences between patients and controls. Perhaps this result would have been more expressive if the plasma samples had been collected serially, as suggested by Masaoka et al [11], who found elevated plasma ET-1 levels starting on the third symptomatic CVS day (9.1 ± 3.2 pg/mL) and the highest levels on the seventh day (12.0 ± 4.3 pg/mL). They attributed those findings to endogenous ET-1 production.

When patients with angiographic CVS are considered, elevated levels of CSF ET were detected when compared with controls. In the plasma, there was a statistical difference between ET-1 levels of patients who evolved with CVS and death compared with the patients without vasospasm.

Considering the powerful constrictive effect of ET-1 and the undoubted fact that it is found in the nervous system mainly under the pathological condition of CVS after SAH, the blockage of its production or action could prevent the harmful effects of CVS.

5. Conclusions

In the present study, we have found significant increase in CSF ET-1 concentrations in patients with CVS after SAH. Plasma ET-1 concentration increased mainly in association with angiographic findings of CVS.

Our results suggest an involvement of ETs as an important mediator in the pathogenesis of CVS. They also suggest that ET levels could be used as a predictor of the outcome of this pathology. Determining the exact level of ET that is associated with the triggering of the vasospasm chain would be very useful as a next step in the investigation of this potential marker.

There are many trials using antagonists to ETs such as BQ123, an ET receptor antagonist. As mentioned by Clozel and Watanabe [3], this compound prevented CVS after intracisternal but not intravenous injection. On the other hand, Matsumura et al [13] used phosphoramidon, an antagonist of ETs that prevented the occurrence of CVS after SAH in dogs.

The next step would be to study the molecular mechanisms of ET regulation, including the signaling pathways involved in the regulation of ET expression.

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Commentary

This study compared the ET-1 levels in CSF and plasma of 30 patients with aneurysmal SAH with those of 10 controls. Twenty aneurysmal SAH patients were excluded from the analysis because they presented more than 23 days after SAH or had cardiac or renal failure or coronary artery disease. The latter 3 conditions are reported to increase ET-1 levels. The epidemiologic features of the study population are appropriately described. Although 60% of patients had a delayed deterioration, only 33% were confirmed to have vasospasm by angiography. It was found that CSF levels, but not plasma levels, of ET-1 were increased in patients with aneurysmal SAH ($P = .0001$). The patient group was broken down by days after SAH at which the sample was collected (0-4, 4-7, and >7 days) but no difference was found in ET-1 levels across groups. When the patients with suspected vasospasm were further analyzed by grouping them according to those that had no angiographic vasospasm and those with angiographic vasospasm, ET-1 levels were significantly higher in the angiographic vasospasm group.

This is a well-designed study with interesting results. The report of increased ET-1 levels in the CSF of patients after aneurysmal SAH, however, is not new. This was first reported in 1990 by H Suzuki and in 2000 by K Suzuki in *Surgical Neurology* and in 2001 by L Mascia in *Stroke*, to mention a few papers. The authors quote some of these studies. I feel that this report is an important confirmatory contribution. Its main strength is the correlation between ET-1 levels and symptomatic vasospasm.

Rafael J. Tamargo, MD, FACS
Department of Neurosurgery
Johns Hopkins Hospital
Baltimore, MD 21287, USA