Nitric oxide metabolites in cisternal CSF correlate with cerebral vasospasm in patients with a subarachnoid haemorrhage

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Summary

Background. The pathogenesis of cerebral vasospasm is likely to be multifactorial. Exposure of the adventitia of large cerebral arteries to blood breakdown products initiates a cascade of changes in both morphology and vasomotor regulation of the exposed vessels. The role of nitric oxide (NO) in development of cerebral vasospasm process is controversial. Basal cerebral vascular tone requires the continuous release of NO, nevertheless NO is involved in free radical mediated injury of endothelial cell membrane. Concentrations of nitrate/nitrite (stable endproducts of NO metabolism) were studied in cisternal cerebrospinal fluid (cCSF) in patients suﬀering from aneurysmal subarachnoid haemorrhage (SAH).

Method. 21 patients suﬀering from aneurysmal SAH were investigated. Treatment included aneurysm clipping, cisternal drainage of CSF and intravenous nimodipine in all patients as well as triple H therapy when indicated. TCDS was performed on a daily basis. A mean flow velocity of more than 150 cm/sec and the development a delayed neurological deficit was defined as vasospasm. CSF samples were collected on the day of surgery and for the 7 days following. NO-M (nitrite and nitrate) were measured using a commercially available test kit.

Findings. 5 of 21 patients developed clinically symptomatic vasospasm. There was a significant diﬀerence in NO levels between the groups. Patients with cerebral vasospasm showed signiﬁcantly higher levels of NO-M in CSF than patients with a uncomplicated follow-up between day 2 and 8.

Interpretation. Our preliminary results indicate that SAH leads to an increase in NO-M in CSF. This increase of NO-M signiﬁcantly correlates with the flow velocities in TCDS measurement suggesting that NO plays an important role in the pathogenesis of cerebral vasospasm.

Keywords: Subarachnoid haemorrhage; nitric oxide; cerebral vasospasm; free radicals; nitric oxide synthetase; cerebrospinal fluid.

Introduction

Aneurysmal subarachnoid haemorrhage (SAH) has a high morbidity and mortality, mainly as a result of the effects of the initial bleeding, surgical complications, and a delayed ischemic neurological deﬁcit in part due to cerebral vasospasm [6, 31, 32, 54]. The pathogenesis of delayed cerebral vasospasm is likely to be multifactorial. The exposure of the adventitia of large cerebral arteries to blood after SAH leads to a cascade of changes in both the morphology and vasomotor regulation of the exposed vessel. The duration as well as extent of exposure have been correlated with the subsequent development of vasospasm [10, 17]. Haemoglobin and its break-down-products bind vasodilative substances, stimulate the production of mediators of vasoconstriction, induce the production of inflammatory agents like free radicals and cytokines, and thereby cause changes of the endothelial and smooth muscle cell structure [22, 43, 56, 64, 66, 74].

Nitric oxide (NO) has been postulated to be involved in the pathogenesis of cerebral vasospasm [21, 35, 72]. NO causes vasodilation in cerebral arteries and mediates the relaxant effect of many other vasodilators [71]. The basal cerebral vascular tone requires the continuous release of NO by endothel cells [8, 9, 52, 53, 62, 70]. Under physiological conditions NO is produced by constitutive NO synthase (cNOS). This isoform functions transiently and depends on intracellular calcium levels. After SAH, haemoglobin and oxyhaemoglobin bind NO and lead to a loss of NO. However, the immunological response after SAH, especially the exposure of the vessel to oxyhaemoglobin, causes a reactive increase in expression of the inducible form of NOS (iNOS) in macrophages and activated...
microglia. The result is a long lasting overproduction of NO. A high NO concentration may lead to peroxidative injury of cell membranes, resulting in a pathological alteration of the endothelial and the smooth muscle cell layers of the arterial wall. Changes of the cell wall structure may disturb the diffusion of NO from endothelial cells to smooth muscle cells. The result is a disruption of the balanced regulation of the cerebral vascular tone, thus causing vasospasm [44]. If this mechanism plays a major role in the development of vasospasm after SAH, a NO metabolite levels may differ in patients with and without cerebral vasospasm.

The aim of this study was to investigate if there is a difference in the CSF concentration of NO between SAH-patients who develop cerebral vasospasm and patients with an uncomplicated clinical course. Because NO is extremely instable, the stable endproducts of the NO metabolism, nitrate and nitrite (NO-M) were measured in cisternal CSF (cCSF).

Patients and methods

Patients

21 patients suffering from a spontaneous SAH were included in the study. For clinical assessment of severity of SAH the Hunt and Hess (H&H) [24] grading system was used. The amount of blood observed on the initial cerebral computed tomography (CCT) was classified according to the Fisher scale [10]. The aneurysm was diagnosed and located by four vessel digital subtraction angiography.

Operation

All patients were treated by early surgery within 72 hours after ictus. In case of an acute hydrocephalus a ventricular catheter was placed prior to angiographic examination or during aneurysm surgery. Aneurysm surgery was performed under continuous somatosensory evoked potential electrophysiological monitoring. Aneurysm neck clipping was possible in all cases. At the end of surgery a catheter (Codman) was placed in the basal cisterns under microsurgical control in all patients regardless if a ventricular catheter has been inserted or not.

Intensiv care unit

After the operation, all patients were treated in the neurosurgical intensive care unit. Treatment included intravenous application of nimodipine and triple H therapy as necessary. Cisternal CSF was sampled from the first to the eighth postoperative day.

Transcranial Doppler Sonography (TCDS)

Cerebral blood flow velocity was measured transcranially with (DWL X4-RC Multi-Dop X2). For quantification, mean blood flow velocity from the middle cerebral artery (MCA) was measured. The measurements were performed one day after the operation and for the following 10 days.

Definition of cerebral vasospasm

Cerebral vasospasm was defined as an elevation of cerebral blood flow velocity in MCA \( > = 150 \text{ cm/sec} \) for at least three days and the development of a delayed neurological deficit.

Nitrate/nitrite assay kit

The final and stable endproducts of NO in vivo are nitrite and nitrate. The best index of total NO production is the sum of nitrite and nitrate. Therefore these NO-metabolites were determined by means of a commercially available assay kit (Alexis corporation, Nitrate/ Nitrite Colorimetric Assay Kit). The first step of the analyzing procedure is the conversion of nitrate to nitrite utilizing nitrate reductase. After conversion spectrophotometric measurement of nitrite is accomplished using the Griess reaction. The amount of nitrite is then determined as a colored azo-dye product of the Griess reaction that absorbs visible light at 540 nm by spectrophotometry.

Statistics

Statistical analysis was performed using the SPSS software.

Results

The clinical presentation of all patients is summarized in Table 1. 5 of 21 (23.8%) patients developed an increase in cerebral blood flow velocity (mean flow MCA \( > = 150 \text{ cm/sec} \), detected by TCDS) for at least three days as well as a delayed ischemic neurological deficit. These five patients formed the “vasospasm group”. 16 (76.2%) patients never had long lasting elevated cerebral blood flow velocities and none of them developed a delayed ischemic neurological deficit.

Mean age in the vasospasm group was 43.2 \( \pm 5.0 \) years, in the other group 51.6 \( \pm 9.8 \) years. This difference is statistically not significant. Two out of the five patients with vasospasm (40%) and three out of the 16 patients with an uncomplicated clinical course (18.5%) were classified as Hunt & Hess Grade IV. Acute hydrocephalus was present in 4 patients of the vasospasm group (60%) and in 8 patients (50%) of the other group, respectively.

The extent of the haemorrhage determined according to the Fisher scale was III in 4 patients with vasospasm group (60%) and in 8 patients (50%) of the other group, respectively.

Mean NO-M concentration and cerebral vasospasm

Patients with cerebral vasospasm showed higher levels of NO-M in cCSF than patients without vasospasm (Fig. 2). Mean NO-M concentrations were sig-