

# Elevated level of endothelin-1 in cerebrospinal fluid and lack of nitric oxide in basilar arterial plasma associated with cerebral vasospasm after subarachnoid haemorrhage in rabbits

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## Abstract

**Background** The role of endothelin-1 (ET-1) and nitric oxide (NO) as two important mediators in the development of cerebral vasospasm (CVS) after subarachnoid haemorrhage (SAH) is controversial. The objective of this study was to determine whether local levels of ET-1 and NO in cerebral arterial plasma and/or in cerebrospinal fluid (CSF) are associated with the occurrence of CVS after SAH.

**Methods** CVS was induced using the one-haemorrhage rabbit model and confirmed by digital subtraction angiography of the rabbits' basilar artery on day 5. Prior to sacrifice, local CSF and basilar arterial plasma samples were obtained by a transclival approach to the basilar artery. Systemic arterial plasma samples were obtained. ET-1 levels were determined by immunometric technique (pg/ml  $\pm$  SEM) and total nitrate/nitrite level spectrophotometrically ( $\mu$ mol/l  $\pm$  SEM).

**Findings** Angiographic CVS was documented after SAH induction (n=12, P<0.05). The ET-1 level in CSF was significantly elevated by 27.3% to 0.84 $\pm$ 0.08 pg/ml in SAH animals (n=7) in comparison to controls (0.66 $\pm$ 0.04 pg/ml,

n=7, P<0.05). There was no significant difference in ET-1 levels in systemic and basilar arterial plasma samples of SAH animals compared to controls. A significant lack of local NO metabolites was documented in basilar arterial plasma after SAH (36.8 $\pm$ 3.1  $\mu$ mol/l, n=6) compared to controls (61.8 $\pm$ 6.2  $\mu$ mol/l, n=6, P<0.01).

**Conclusion** This study demonstrates that an elevated ET-1 level in CSF and local lack of NO in the basilar arterial plasma samples are associated with CVS after experimental SAH.

**Keywords** Subarachnoid haemorrhage · Cerebral vasospasm · Endothelin-1 · Nitric oxide

## Abbreviations

ET-1	endothelin-1
NO	nitric oxide
CVS	cerebral vasospasm
SAH	subarachnoid haemorrhage
CSF	cerebrospinal fluid
SEM	standard error of the mean
DSA	digital subtraction angiogram
CI of diff	confidence intervals of difference
iNOS	induced nitric oxide synthase
nNOS	neuronal nitric oxide synthase
eNOS	endothelial nitric oxide synthase

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## Introduction

Cerebral vasospasm (CVS) develops in 30–70% of patients suffering from subarachnoid haemorrhage (SAH) [29] and still remains an unsolved complication leading to cerebral

ischaemia, permanent neurological dysfunction and death [21]. Despite substantial progress in the understanding of the development of CVS after SAH, its pathogenesis remains unclear with regard to its complexity. In addition to haemolytic metabolites recently discovered to be pathogenetic factors, such as oxyhaemoglobin and bilirubin oxidation products [4], the probably most important factors contributing to the narrowing of cerebral arteries after SAH are dysfunctions observed in the endothelin-1 (ET-1) and nitric oxide (NO) system as a result of haemolytic processes in the subarachnoid space [21].

ET-1 is the most potent vasoconstrictive endogenous peptide produced by various cells, including endothelial cells, smooth muscle cells, astrocytes and neurons [13]. In both experimental and clinical studies, elevated levels of ET-1 in either cerebrospinal fluid (CSF) and/or peripheral plasma have been observed to be associated with the occurrence of CVS [9, 12, 35]; however, none of the studies has focused on the local cerebral plasma levels as the bridge between CSF and the periphery. The therapeutic approach in affecting the system by selective endothelin A receptor antagonism seems to be promising according to preliminary clinical results [3, 31]. However, ET-1's source and cause of release remain unclear. Some researchers propose that ET-1 is induced by cerebral ischaemia and released from astrocytes, leading to elevated levels in CSF [12, 22]. Others propose that elevated ET-1 levels observed in peripheral plasma samples are directly associated with CVS as a result of endothelial dysfunction [9, 10].

Nitric oxide synthase mediates oxygen-dependent conversion from L-arginine to radical NO, leading to vasodilation through the cyclic guanylate monophosphate pathway [2]. Decreased bioavailability of endogenous NO has been widely accepted as an important part of the pathogenesis of delayed CVS after SAH, more likely caused by decreased production than by increased scavenging by haemolytic metabolites as was initially assumed [21]. Controversies about the origin of decreased availability remain because of conflicting data published about NO metabolite levels in the CSF and peripheral plasma [8, 21, 25].

On the basis of a novel sampling approach, our study seeks to include local cerebral plasma levels as a further variable to focus on for bridging a gap between conflicting data published about CSF and peripheral plasma levels of ET-1 and NO. With the use of an experimental SAH model, it compares local basilar arterial plasma levels of ET-1 and NO metabolites with those in CSF as well as compares them to systemic arterial plasma levels. This may possibly contribute to the clarification of conflicting data published on the source of release of ET-1 and reduced bioavailability of NO.

## Methods and materials

### In vivo

The protocol of this study was reviewed and approved by the Swiss Institutional Animal Care and Use Committee and meets the Swiss guidelines for laboratory animal use (Canton Bern, Switzerland, approval no. 15/06).

### Experimental groups

A total of 24 adult New Zealand white rabbits of either sex weighing 2.6 to 3.6 kg were randomly assigned to two groups (12 animals each). One group underwent experimental SAH on day 0, and one served sham-operated as control.

### Angiography

After induction of general anaesthesia by intramuscular administration of ketamine (30 mg/kg) and xylazine (6 mg/kg), digital subtraction angiograms (DSA) were performed on day 0 prior to SAH and on day 5 post SAH. The rabbits' left (day 0) or right (day 5) subclavian artery was microsurgically cannulated using a 0.5-mm plastic tube. The catheter was fixed distal to the vertebral branch. Subsequently, DSA was performed by intraarterial bolus injection of non-ionic iopamidol (0.6 ml/kg) as contrast agent. Meanwhile, the arterial blood gas status was analysed to monitor ventilation parameters from a collected arterial blood sample. Images of the vertebrobasilar system were obtained using a rapid sequential angiographic technique with the small focal spot at 66 kV 125 mA (DFP 2000A, Toshiba Corporation®, Japan). The average diameter of the basilar artery was digitally calculated in  $\mu\text{m}$  using the automatic measurement tool of ImagePro Discovery® analysis software (MediaCybernetics, Bethesda, MD).

### Cerebral vasospasm model

Following baseline DSA on day 0, the rabbits' atlantooccipital membrane was exposed by a microsurgical posterior approach in prone position, and a 25-gauge needle was inserted into the cerebromedullar cistern while maintaining general anaesthesia. After relief of 1.0 ml CSF, an equal amount of unheparinised autologous arterial blood in the SAH group was injected into the cistern under microscopic view to imitate SAH in order to induce CVS. An equal amount of isotonic saline solution instead was injected in controls. Postsurgical pain relief was managed by subcutaneous administration of buprenorphine (0.1–0.2 mg/kg).

### Collection of samples

Following post-treatment DSA on day 5, the rabbits underwent coniotomy to keep up general anaesthesia by inhalation of an isoflurane/oxygen/air mixture (2.5/5/92.5 vol%). Systemic arterial plasma samples were collected via the central arterial catheter. Basilar arterial plasma and CSF samples were collected by a transclival approach. In brief, the rabbits' head was reclined and fixed at a 20° angle, the trachea and oesophagus were cautiously laterally retracted and the musculature underneath bluntly microsurgically dissected. The paravertebral venous plexus exposed in the depth was coagulated using a pair of bipolar forceps. The exposed dorsocaudal edge of the clivus was followed frontocranially by cautious dissection until the clivus' surface on the base of the skull could fully be displayed. A compression of the carotid arteries and the descending vagus nerves was avoided throughout the procedure.

An ellipsoid osteotomy (2-mm diameter) with the use of a high-speed microdrill (Fine Science Tools, North Vancouver, BC, Canada) exposed the dura and the basilar artery underneath. The CSF sample was collected by transdural insertion of a 26-gauge bent needle into the subarachnoid space. The part of the dura exposed was fully excised with microscissors in order to allow a direct approach to the distal segment of the basilar artery. The basilar arterial blood sample was collected by intraluminal insertion of a 29-gauge bent needle into the basilar artery. The collected blood samples were centrifuged for 15 min at 1,500 rpm, and the plasma remnants and CSF samples were stored at -80° C. The animals were killed by intraarterial bolus injection of sodium thiopental (40 mg/kg). All surgical procedures were performed under sterile conditions.

### In vitro

#### Detection of ET-1

In seven randomly chosen animals of each group, ET-1 systemic and basilar plasma levels as well as CSF levels were quantitatively determined by the extraction-free human ET-1 chemiluminescent sandwich immunoassay technique (QuantiGlo QET00B, R&D Systems, Minneapolis, MN) as previously validated [18]. The amino acid sequence of human ET-1 matches rabbit ET-1 exactly. The quantitative range of the assay is 0.32 to 1,000 pg/ml. According to the manufacturer, cross-reactions of antibodies used are observed for human ET-1 (100%), human big ET-2 (0.01%), human big ET-38 (0.019%), human ET-3 (7.8%) and human ET-2 (27.4%).

### Measurement of NO metabolites

In six random animals of each group, nitrite/nitrate levels were quantitatively determined as a relative measure for basilar, systemic plasma and CSF levels of NO production, as is common practice. Following reduction of nitrate to nitrite with a modified Griess method, the total nitrite level was spectrophotometrically detected at 540 nm (QuantiChrom Nitric Oxide Assay Kit, Bioassay Systems, Hayward, CA) as previously validated [24].

### Drugs

The following drugs were used: buprenorphinum (Temgesic® 0.3 mg/ml, Essex Chemie, Lucerne, Switzerland), iopamidol (Iopamiro® 300 mg/ml, Bracco, Milan, Italy), isoflurane (Forene® 99%, Abbott Laboratories, Abbott Park, IL), ketamine HCl (Ketalar®50 mg/ml, Pfizer AG, Zurich, Switzerland), sodium thiopentalum (Pentothal® 25 mg/ml, Abbott Laboratories, Abbott Park, IL) and xylazine HCl (Xylapan® 20 mg/ml, Vetoquinol AG, Bern, Switzerland).

### Statistical methods

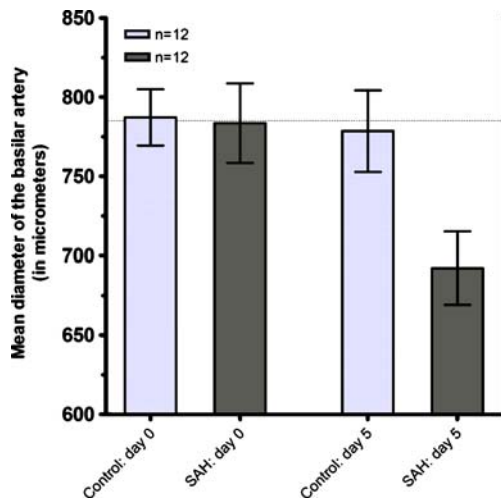
Values are expressed as mean  $\pm$  SEM (n=number of animals). Statistical difference between two means and multiple means was determined by Student's unpaired t-test and one-way ANOVA followed by Bonferroni's post-testing, respectively. The statistical significance was considered if the P-value was less than 0.05 ( $p < 0.05$ ) in context of the 95% confidence intervals of the difference examined (95% CI of diff).

## Results

### Arteriographic vasospasm

On day 5 after SAH, the diameter of the basilar artery was significantly narrowed by 11.7% to  $692.2 \pm 23.0 \mu\text{m}$  (n=12) compared to the animals' baseline angiogram on day 0 ( $783.5 \pm 25.1 \mu\text{m}$ , n=12,  $P < 0.01$ , 95% CI of diff: 15.4 to 167.2  $\mu\text{m}$ ); on the same day, the basilar artery diameter remained unchanged ( $778.5 \pm 25.8 \mu\text{m}$ , n=12) in the control group compared to the baseline on day 0 ( $787.2 \pm 17.8 \mu\text{m}$ , n=12,  $P > 0.05$ , 95% CI of diff: -84.6 to 67.2  $\mu\text{m}$ ) (Fig. 1).

The animals' initial blood pressure at baseline angiography (SAH group:  $71.1 \pm 2.8$  mmHg in mean arterial pressure, n=12; controls:  $68.8 \pm 2.0$  mmHg, n=12) was not different from the values monitored on day 5 in both the SAH group ( $70.6 \pm 2.2$  mmHg, n=12,  $P > 0.05$ ) and the controls ( $70.8 \pm 2.1$  mmHg, n=12,  $P > 0.05$ ). Correspond-

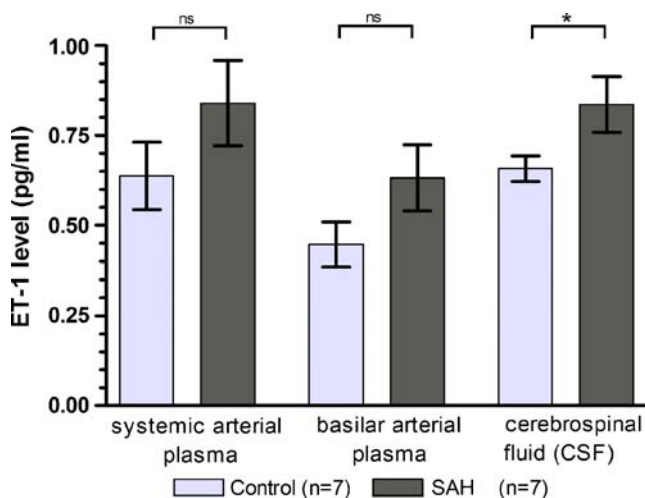


**Fig. 1** Box plot with the absolute change of the basilar artery in diameter: Baseline angiography in SAH and control group on day 0 vs. on day 5 (in  $\mu\text{m} \pm \text{SEM}$ ). (\* $P < 0.05$ ; ns=non-significant)

ingly, the initial blood gas parameters such as  $\text{pO}_2$  (SAH group:  $57.9 \pm 3.6$  mmHg; controls:  $56.8 \pm 2.1$  mmHg),  $\text{pCO}_2$  (SAH group:  $50.6 \pm 1.1$  mmHg; controls:  $50.1 \pm 1.3$  mmHg) and pH (SAH:  $7.39 \pm 0.01$ ; controls:  $7.38 \pm 0.01$ ) did not change compared to the values monitored during follow-up angiography on day 5 in both the SAH ( $P > 0.05$ ) and control group ( $P > 0.05$ ) and did not statistically differ between the two groups ( $P > 0.05$ ).

#### Local ET-1 levels

The ET-1 level in CSF samples was significantly elevated by 27.3% to  $0.84 \pm 0.08$  pg/ml in SAH animals ( $n=7$ ) in comparison to controls ( $0.66 \pm 0.04$  pg/ml,  $n=7$ ,  $P < 0.05$ ,

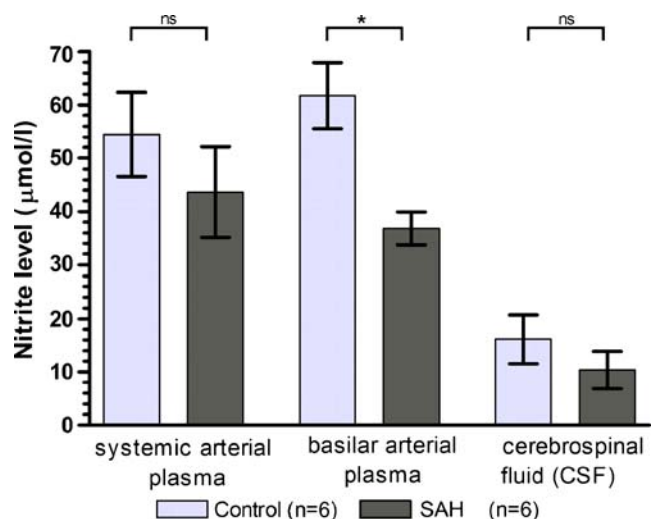


**Fig. 2** Box plot with SAH vs. control: ET-1 levels in CSF, basilar and systemic arterial plasma on day 5 after SAH (in pg/ml statistically differ SEM). (\* $P < 0.05$ ; ns=non-significant)

95% CI of diff: 0.01 to .35 pg/ml). There was a slight increasing tendency observed in plasma samples as well, but no significant difference of ET-1 levels was remarked in systemic or basilar arterial plasma samples of SAH animals compared to controls. Local levels of ET-1 in the basilar arterial plasma samples did not statistically differ from those in the systemic plasma in SAH ( $n=7$ ,  $P \geq 0.05$ , 95% CI of diff: -0.12 to 0.53 pg/ml) or control groups ( $n=7$ ,  $P \geq 0.05$ , 95% CI of diff: -0.05 to 0.44 pg/ml), nor did they statistically differ from those in CSF of the SAH ( $P \geq 0.05$ , 95% CI of diff: -0.51 to 0.10 pg/ml) or control groups ( $P \geq 0.05$ , 95% CI of diff: -0.51 to 0.09 pg/ml) (Fig. 2).

#### Local levels of NO metabolites

The level of total nitrate/nitrite in the basilar arterial plasma samples was significantly reduced, by 40.4% to  $36.8 \pm 3.1$   $\mu\text{mol/l}$  in SAH animals ( $n=6$ ) compared to controls ( $61.8 \pm 6.2$   $\mu\text{mol/L}$ ,  $n=6$ ,  $P < 0.01$ , 95% CI of diff: 9.5 to 40.3  $\mu\text{mol/l}$ ). A slightly decreasing tendency of the local NO metabolite level was also observed in CSF ( $n=6$ ) and systemic arterial plasma ( $n=6$ ) samples in comparison to the controls ( $n=6$ ), but remained insignificant ( $P > 0.05$ ). The local level of total nitrate/nitrite in basilar arterial plasma samples did not differ statistically from the level in systemic samples for both the SAH group ( $n=6$ ,  $P > 0.05$ ) and the control group ( $n=6$ ,  $P > 0.05$ ). It was significantly higher than the level detected in CSF samples for both the SAH group ( $n=6$ ,  $P < 0.001$ , 95% CI of diff: 15.9 to 37.1  $\mu\text{mol/l}$ ) and the control group ( $n=6$ ,  $P < 0.001$ , 95% CI of diff: 28.5 to 62.7  $\mu\text{mol/l}$ ) (Fig. 3).



**Fig. 3** Box plot with SAH vs. control: total nitrite levels in CSF, basilar and systemic arterial plasma on day 5 after SAH (in  $\mu\text{mol/l} \pm \text{SEM}$ ). (\* $P < 0.01$ )

## Discussion

### Origin of increased bioavailability of ET-1

ET-1 is produced by various cells, including neurons, glial cells, smooth muscle cells and endothelial cells, within minutes after being stimulated by hypoxia, ischaemia and shear stress [13]. Its release from vascular endothelial and smooth muscle cells is more likely to follow a paracrine than an endocrine pattern, as ET-1 abluminally binds to ETA receptors in the smooth muscle cell layer to mediate long-lasting vasoconstriction [13, 32]. Despite broad acceptance of ET-1 as a contributing mediator of CVS, conflicting data have been reported about the correlation of ET-1's plasma level and CVS. On one hand, ET-1's peripheral plasma level has been found to correlate with the severity of vascular diseases [13], as shown in clinical studies of Juvela et al. [9, 10] and Suzuki et al. [30] in regard to the occurrence of delayed cerebral ischaemia and vasospasm after SAH. On the contrary, other authors were not able to correlate ET-1 plasma levels to CVS [11, 12, 27].

In our experimental study neither the local ET-1 level in the basilar arterial nor the one in systemic plasma samples after SAH differed significantly from those in controls. These findings support the clinical observations reported elsewhere [11, 12, 27]. In addition, our data did not show significant differences between the local ET-1 level obtained from basilar arterial plasma samples compared to the systemic samples within either the SAH or the control group. Nevertheless, our results demonstrated a trend towards lower levels of this peptide in the basilar arterial samples than in the peripheral plasma in both groups. This observation might suggest that the elevated peripheral plasma levels of ET-1 observed in clinical studies are rather unlikely to be caused by increased release of ET-1 into the cerebral circulation [9, 10, 30]. One reason might be that the systemic plasma ET-1 concentration could be significantly influenced by physiological and pathological conditions in structures other than the brain and cerebral vessels, as shown, for example, in systemic hypertension and heart failure [13, 35].

Instead, our data demonstrated a significantly elevated ET-1 level in CSF on day 5 after experimental SAH. This observation supports the hypothesis of mediated CVS partly due to elevated ET-1 levels in CSF. In experimental studies on felines and canines, intracisternal bolus injections of ET-1 have previously demonstrated ET-1's potency in mediating vasoconstriction from the abluminal site [16]. Pluta et al.'s experimental study in primates found the ET-1 level in CSF to be dependent on astrocyte release [22]. Our finding of an elevated level of ET-1 in CSF confirms the clinical results reported earlier [11, 12, 14, 27, 28]. Among

these reports the correlation of the CSF to the plasma levels of ET-1 in SAH-induced CVS is still controversial. With respect to the apparently very limited specificity and sensitivity of ET-1 in systemic plasma to correlate with CVS, some authors showed ET-1's peripheral plasma concentration during CVS to act differently from the one in CSF [12, 27], while Kastner et al. demonstrated them to be relatively similar in concentration averaged over time [11]. Keeping in mind that ET-1 levels of local cerebral plasma and CSF had not been previously examined, we documented the basilar arterial plasma levels of ET-1 to be statistically indifferent from the ones in CSF. These findings might possibly be explained epiphenomenally due to the ET-1 conversion rate in the endothelial basilar arterial layer or due to ET-1 release from the CSF into the cerebral circulation. This hypothesis might also be supported by Menon et al. who found high arterio-jugularvenous ET-1 gradients in patients suffering from SAH-induced CVS [15].

Though our study results tend to agree with these hypotheses, one needs to keep in mind that the SAH model used in this study is certainly limited in mimicking the complex clinical conditions of an aneurysmal SAH in humans. Due to single time point assessment during the peak time of CVS, the study is also limited in providing data about possible metabolite level changes during the development of CVS.

### Origin of reduced NO bioavailability

Endogenous NO is a radical product of the oxidation of l-arginine to citrulline by nitric oxide synthase, which causes vasodilation through activation of the soluble guanylate cyclase pathway. Three isoenzyme forms are known to play a role in vasculature-mediating NO release: induced nitric oxide synthase (iNOS), periadventitial neuronal nitric oxide synthase (nNOS) and endothelial nitric oxide synthase (eNOS). While iNOS possibly plays a pathophysiological role in the acute and early states after SAH, there is debate over whether the activity of nNOS and eNOS has a role in the delayed development of CVS after SAH [5, 21]. After a short half-life, radical NO is metabolised by being oxidised to stable nitrite and nitrate. This serves as an acceptable relative measure of NO level in both experimental and clinical research. Decreased bioavailability of NO has been shown to be associated with the development of delayed CVS after SAH, more likely caused by deprived production rather than just increased scavenging by haemolytic metabolites such as oxyhaemoglobin as initially assumed [5, 21].

This study demonstrated the novel finding that locally reduced NO availability in basilar arterial plasma is

associated with CVS after SAH. It hypothesises that there is decreased production of NO in the endothelial layer of local cerebral vasculature. This local lack of NO in the basilar plasma might hypothetically be explained by dysfunctional endothelium [7] or by inhibited enzyme activity of eNOS [8]. Whether these results are caused by downregulation of eNOS expression itself, however, remains uncertain because of the conflicting data reported: Hino et al. [6] found decreased eNOS mRNA in cerebral arteries of primates and Park et al. [20] in the microvasculature of rats after induced SAH. On the contrary, Vatter et al. could recently not reproduce those findings by histochemical staining of eNOS in the rats' basilar artery after SAH [33]. In support of our hypothesis, Jung et al. found that the level of asymmetric dimethylarginine, an endogenous competitive inhibitor of nitric oxide synthase and a metabolism product of bilirubin, was elevated in CSF during delayed CVS in an experimental primate model [8]. It is suggested to contribute to the endogenous inhibition of eNOS [8] and, thus, possibly to the lack of NO in the basilar arterial plasma found in our study.

Uncertainty remains about conflicting data reported for NO metabolites after SAH concerning the CSF level. While some authors reported in clinical studies with small sample sizes that NO metabolite levels in CSF were increased after SAH [17, 25, 29, 34]; others observed CVS to be associated with reduced CSF levels of nitrite [26], suggesting possible affection of nNOS [8, 23]. However, our data do not confirm any significant change in nitrite level in CSF in either direction. Possible explanations for the conflicting data published are a lack of large clinical sample sizes, different time courses, and different spots of vessel catheterisation and sample collection.

Our data also do not demonstrate a significant difference between nitrite levels in systemic plasma after SAH compared to controls, in agreement with clinical data provided by Suzuki et al. [29]. It suggests that systemic plasma may be too unspecific to detect a locally limited pathophysiological process in the cerebrovascular NO system.

#### Interaction of ET-1 and NO

Experimental data have been published suggesting that there may be a direct or indirect interaction between the ET-1 and NO systems on the basis of a yet unclear mechanism. Ohkita et al. provided evidence for inhibition of ET-1 production by NO, assuming a modulatory role of NO [19]. Impairment of this possible modulatory role of NO on ET-1 during CVS after SAH has been found by Alabadi et al. [1] and supports our observation that an elevated ET-1 level in CSF accompanies a lack of local NO.

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## Comments

The authors used a well established animal model (rabbit single hemorrhage) of cerebral vasospasm (CVS) with the aim to investigate the source of ET-1 and NO in the development of CVS. They determined levels of ET-1 and nitrite in the basilar artery (BA), the systemic plasma and the CSF. They observed an increased level of ET-1 in the CSF after experimental SAH but no changes in the BA and the systemic circulation. For nitrate they found decreased levels in the BA after SAH but no changes in the systemic circulation and the CSF.

The investigation is well performed, the data is presented in an adequate and clear form. It supports the pathophysiological concept, that for the development of CVS relevant changes of the ET metabolism are localized inside the cerebrospinal space but not in the systemic circulation. Furthermore, the present data support a decreased function of the NO metabolism mainly inside the cerebrovasculature after SAH, which represents another factor for the development of CVS.

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It has been more than 50 years since delayed cerebral vasospasm in patients after aneurysmal subarachnoid hemorrhage (aSAH) has been linked to a poor outcome.[1] Most unfortunate is that this rather uncommon but most devastating form of stroke affects those patients who have already been treated by surgical or endovascular securing of the aneurysm. In extensive worldwide research, thousands of neurosurgeons and scientists have examined innumerable chemicals and drugs using simple, sophisticated and ingenious *in vivo* and *in vitro* models testing numerous hypotheses hoping to cure or prevent delayed cerebral vasospasm. Still, it is an effort in vain with regard to finding the magic bullet but because of this effort, we have significantly advanced our knowledge of cerebral vessels physiology and developed a better understanding of the pathophysiological changes in the brain and the cerebral vessels evoked by aSAH. In spite of these advances, there appears to be an increasing uneasiness that the failure to find treatment may be due to a “barking up the wrong tree”.[2]

The discoveries that nitric oxide (NO), a toxic gas from fumes of gasoline engines is Furchgott’s endothelium-derived relaxing factor

(EDRF),[3] that endothelin-1 (ET-1), a venom from the sarafotoxin family, is produced in our bodies by endothelial cells as the most potent vasoconstrictor[4] and that both regulate cerebral blood have given new hope to develop a cure for vasospasm.

In the 1990s, numerous studies suggested that NO or ET-1 was involved in the development of delayed cerebral vasospasm,[5, 6] while other reports denied such a relationship.[7, 8] The controversy is still ongoing although preclinical and clinical trials using intracarotid infusion of NO gas solution,[9] intracarotid or intrathecal administration of NO donors[10-12] and ET-1 receptor inhibitors have claimed clinical success.[13, 14]

However, better understanding of aSAH pathophysiology, discovery of new aSAH-related events like cortical spreading ischemia[15] and, probably most importantly, the failure of Clazosentan, a selective ET-1 A receptor inhibitor, in Phase II clinical study[2] to improve the outcome despite prevention of vasospasm all lead to a paradigm shift with the focus on the possibility that a stroke after aSAH may be evoked by another nonvasospasm-related mechanism(s). Growing body of evidence suggests that these pathomechanisms are a result of the presence of an arterial blood clot in the subarachnoid space, increased ET-1 in CSF, and decreased availability of NO in cerebral arteries.[2, 15]

In their clever, well-planned and executed, but most confirmatory *in vivo* experimental study published in this issue of *Acta Neurochirurgica*, V. Neuschmelting and colleagues simultaneously examined the role of both NO and ET-1 in the development of vasospasm in a rabbit model of aSAH. To assure the relevance of their findings, the authors used an innovative surgical approach. They collected blood samples directly from the basilar artery to compare to the peripheral arterial blood and CSF samples. The authors convincingly showed that the presence of ET-1 in the CSF and endothelial NOS dysfunction were associated with vasospasm development. These findings, while not altogether new,[6, 10, 11, 13, 14] are a novel approach to sample collection and shed new light on the pathophysiology of vasospasm after SAH by separating the extra- and intravascular events. Doing this further opens the door to a Kuhnian revolution in our understanding the role of vasospasm in stroke after aSAH and hopefully leads to successful treatment of this dreadful complication of a ruptured intracranial aneurysm.

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