Does Endothelin-1 Play a Role in the Pathogenesis of Cerebral Vasospasm?

Francesco Cosentino, MD; Zvonimir S. Katusic, MD, PhD

**Background**
Endothelin-1 is a very powerful endogenous vasoconstrictor substance produced by endothelial cells. Its long-lasting vasoconstrictor and hypertensive action has been well documented in several species, including humans.

**Summary of Review**
It is generally accepted that endothelin-1 may contribute to the pathogenesis of a number of cardiovascular diseases. In the cerebral vasculature, endothelin-1 has been proposed as a key mediator of cerebral vasospasm following subarachnoid hemorrhage. Availability of endothelin-1 antagonist provided a pharmacologic tool to test the role of endothelin in the development of vasospasm.

**Conclusions**
This brief review is focused on the controversial results reported by different groups concerning the possible role of endothelin-1 in narrowing of cerebral arteries exposed to autologous blood. (Stroke. 1994;25:904-908.)

**Key Words**
- cerebral vasospasm
- endothelin

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Endothelial cells play a key role in the local regulation of the vascular smooth muscle tone by producing and releasing relaxing and contracting factors. Although the physiological role of endothelium-dependent contractions in regulation of the cardiovascular system is unclear, existing evidence supports the concept that vasoconstrictor substances may become important regulators of vascular tone under pathological conditions. Endothelin-1 (ET-1), one of the most potent endogenous vasoconstrictor substances known, is produced by endothelial cells. Its long-lasting vasoconstrictor and hypertensive action has been well documented, and it is generally accepted that increased production of ET-1 may contribute to the pathogenesis of a number of cardiovascular diseases. In the cerebral vasculature, ET-1 has been proposed as a key mediator of cerebral vasospasm following subarachnoid hemorrhage (SAH).

Cerebral vasospasm remains one of the major causes of morbidity and mortality in patients with aneurysmal SAH. Angiographic vasospasm, defined as a focal or diffuse narrowing of the major cerebral arteries, appears most often 4 to 10 days after the onset of SAH. Approximately 60% of patients with SAH exhibit delayed vasconstriction; however, its pathogenesis is still not well understood. A strong correlation has been found between the presence of a thick clot in the subarachnoid space and the future development of symptomatic vasospasm. A very large number of putative spasmogens released from the intracisternal clot have been proposed. They may be directly vasoconstrictive or induce the release of vasoconstrictors and/or impair endothelium-dependent relaxations. This brief review focuses on the current knowledge concerning the potential pathophysiological role of ET-1 in cerebral vasospasm after SAH.

**Background**
There are three structurally and pharmacologically separate endothelin (ET) isopeptides in human and other mammalian species named ET-1, ET-2, and ET-3. The only one produced by endothelial cells is ET-1. The production of ET-1 is well characterized and involves the final transformation of big ET-1 catalyzed by a putative endothelin-converting enzyme (ECE). The relatively slow rate of production of ET-1 suggests that the peptide is more likely to participate in long-term regulation of vascular tone rather than in acute responses. Two different ET receptor subtypes have been identified: ET₁ and ET₂. The ET₁ receptor has a greater affinity for ET-1 and ET-2, whereas the B subtype has about equal affinity for all endothelins. Cerebral arterial endothelial cells may produce ET-1. The contracting effect of the peptide on vascular smooth muscle can be explained not only by a direct activation of ET₁ receptor but also by sensitizing blood vessels to other vasoconstrictor substances. Such responses may favor the occurrence of abnormal vasoconstrictions and therefore be important in cerebral arterial vasospasm after SAH.

**Endothelin-1 and Cerebral Vasospasm**
To imply a major role for ET-1 in the pathophysiology of cerebral vasospasm after SAH, one would have to determine (1) cerebral vascular reactivity to ET-1, (2) levels of ET-1 after SAH, and (3) whether or not ECE inhibitors or selective ET-1 antagonists may prevent development of cerebral vasospasm.

**Can ET-1 Induce Cerebral Vasospasm?**
Several studies have confirmed the vasoconstrictor effect of ET-1 in cerebral arteries in vitro and in vivo. In isolated arteries ET-1-induced contractions are concentration dependent, long lasting, and difficult to wash.
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Is ET-1 Production Increased During SAH?

One of the consequences of SAH is exposure of brain tissue to direct contact with blood. Production of ET-1 is stimulated by various vasoactive substances present in the blood, including arginine vasopressin, angiotensin II, and thrombin; excessive local production of these substances is reported after SAH. In addition, ET-1 is formed from big ET-1 via a putative ECE. The existence of blood-brain barrier prevents a vasoconstrictor effect of circulating ET-1. However, ET-1 produced in the endothelium of cerebral arteries may be secreted abuminally, having access to its receptor on the smooth muscle cells.

Considering the slow onset and long duration of the ET-1 vasoconstrictor effect, it has been proposed that this peptide may be the mediator of chronic decrease in arterial diameter after SAH. However, the onset of arteriographic vasospasm in humans is usually between 4 to 10 days after SAH. Thus, the arterial narrowing produced by intracisternal injection of ET-1 occurs in a time course that is not entirely consistent with that of cerebral vasospasm.

Do ECE Inhibitors or ET, Receptor Antagonists Prevent Cerebral Vasospasm?

ET-1 is formed from big ET-1 via a putative ECE. Because big ET-1 is two or three orders of magnitude less potent a vasoconstrictor than ET-1, inhibition of ECE should effectively block the biological effects of ET-1. Although no specific inhibitors are available, a metalloprotease inhibitor, phosphoramidon, has been shown to reduce the conversion of big ET-1 to ET-1 in vitro and its pressor activity in vivo (Figure).

In dogs intracisternal administration of big ET-1 caused a profound decrease in the diameter of the basilar artery that was inhibited by pretreatment with phosphoramidon. Furthermore, phosphoramidon prevented development of cerebral vasospasm in a "double-hemorrhage" canine model of the disease. In contrast, there are studies that did not detect any preventive effect of phosphoramidon.

In particular, the results of our study demonstrated that a daily intracisternal injection of a high dose of phosphoramidon did not significantly affect SAH-induced cerebral vasospasm.

Only a few studies have been reported concerning the effects of ET, receptor antagonists in experimental models of SAH so far. The ET-1 receptor antagonists 45 However, the existence of a correlation between plasma or cerebrospinal fluid (CSF) ET-1 concentrations of oxyhemoglobin may also inactivate endothelin-1. 45 Several studies have demonstrated elevation in immunoreactive ET-1 levels in the CSF and plasma of animals and patients with SAH. Conversely, an equal number of convincing studies failed to uncover augmented levels of the peptide. The problem of determining ET-1 in its very low concentrations is probably an important source of variability. These conflicting findings may be explained by methodological differences, in particular, cross-reactivity of certain antibodies used to measure ET-1 with ET isofoms and or its precursors as well as with other peptides. Studies reporting elevated levels of ET-1 in plasma and CSF were done in patients subjected to neurosurgical interventions, and ET levels were measured in the perioperative period. A nonsurgical therapeutic approach may lead to different results. It is impossible to rule out that the augmentation of ET-1 levels may be the consequence of surgically induced vascular disturbances.

The existing literature is inconclusive regarding the correlation between circulating levels of ET-1 and development of cerebral vasospasm after SAH. However, it should be stressed that circulating ET levels may not reflect the local modulatory role of the peptide. Indeed, ET-1 most likely acts in a paracrine fashion, regulating vascular smooth muscle cells nearby. Endothelial cells in culture release twice as much ET-1 toward vascular smooth muscle compared with luminal direction. Therefore, circulating levels of the peptide may well be within the normal range even in the presence of an increased concentration in the immediate vicinity of smooth muscle cells. This concept is supported by the findings of a recent study in which immunoreactive ET-1 measured in canine basilar artery wall after SAH was increased only on day 2 and not on day 7, when the presence of vasospasm was confirmed by angiography. The contrasting findings reported in the literature concerning the circulating levels of ET-1 and the availability of ECE inhibitors and ET, receptor antagonists prompted new studies to probe the potential link between ET-1 and this pathological condition.
Summary of the Reports Concerning the Effect of $\mathrm{ET}_{\alpha}$ Receptor Antagonists on Cerebral Vasospasm

<table>
<thead>
<tr>
<th>$\mathrm{ET}_{\alpha}$ Antagonist</th>
<th>Experimental Model</th>
<th>Administration Regimen</th>
<th>Placebo</th>
<th>Active Drug</th>
</tr>
</thead>
<tbody>
<tr>
<td>BQ123$^{*}$</td>
<td>Rat (one hemorrhage)</td>
<td>Single IC injection</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>FR13931$^{†}$</td>
<td>Dog (double hemorrhage)</td>
<td>IC injections on days 0, 2, 4</td>
<td>62</td>
<td>76$^{†}$</td>
</tr>
<tr>
<td>BQ123$^{*}$</td>
<td>Dog (double hemorrhage)</td>
<td>Daily IC injection</td>
<td>56</td>
<td>62</td>
</tr>
<tr>
<td>BO485$^{*}$</td>
<td>Dog (double hemorrhage)</td>
<td>Continuous systemic infusion</td>
<td>60</td>
<td>75$^{†}$</td>
</tr>
</tbody>
</table>

Values are expressed as percent diameter of basilar arteries in placebo and $\mathrm{ET}_{\alpha}$ antagonist-treated groups compared with 100% control diameter before injections of autologous blood.

$\mathrm{ET}_{\alpha}$ indicates endothelin-$\alpha$; IC, intracisternal.

*Significant prevention of subarachnoid hemorrhage-induced decrease in cerebral blood flow ($P<.01$).

$^{†}$Significantly different from placebo group ($P<.01$).

tested were synthetic peptides named BQ123, BQ485, and FR13931.$^{53,64-68}$ Recent reports on isolated arteries demonstrated selective inhibitory effect of BQ123 on ET-1–mediated contractions in systemic and pulmonary arterial vessels.$^{69-71}$ (Figure). In our study, BQ123 (10$^{-5}$ mol/L) selectively inhibited contractions caused by ET-1 in canine basilar artery. However, daily intracisternal administration of BQ123 in a concentration 10 times higher than the concentration that in vitro abolished the contractile effect of ET-1 did not prevent experimentally induced cerebral vasospasm.$^{52}$ These findings suggest that ET-1 may not be the major mediator responsible for the decrease in cerebral arterial diameter associated with SAH. By contrast, other reports showing that administration of $\mathrm{ET}_{\alpha}$ receptor antagonists reduces cerebral vasospasm support the hypothesis that ET-1 plays a key role in this condition.$^{64-68}$ Differences in the experimental design of these studies may explain these contrasting findings, such as use of different species and models of the disease (rat, rabbit, dog; one hemorrhage versus double hemorrhage), and different concentrations and means and regimens of drug administration (intracisternal versus systemic; single or daily injections versus continuous infusion). After intracisternal administration, diffusion of the active drug to the arterial wall may be prevented by the thick clot surrounding the vessel. Therefore, it is necessary to acknowledge that the local concentration of active compounds may not be high enough to prevent the effect of ET-1.$^{53}$ On the other hand, the reports of positive findings obtained in the groups treated with $\mathrm{ET}_{\alpha}$ receptor antagonist are not definite. Only a slight prevention of arterial narrowing after SAH was achieved (Table).

Historically, the research for single causal factors of cerebral vasospasm has been disappointing, implying a more likely multifactorial origin of this pathological condition. However, since the discovery of ET-1 and the characterization of its potent and long-term vasoconstrictor effect, an increasing number of studies have focused on the pathophysiological importance of ET-1 in cerebral vasospasm. With the recent development of ECE inhibitors and $\mathrm{ET}_{\alpha}$ receptor antagonists, research tools to better address this question have come to be available. The contradictory findings reported in the literature so far do not allow any definitive conclusion concerning the role of ET-1 in cerebral vasospasm. However, the attempt to implicate ET-1 as a putative mediator of cerebral vasoconstriction deserves further investigation. It is necessary to obtain more information on the pharmacokinetics of newly developed compounds to optimize the therapeutic regimen in terms of dose and timing. Moreover, additional studies with regard to characterization of expression of ET receptor subtypes in smooth muscle cells may help to determine if increased sensitivity to ET-1 contributes to chronic narrowing of cerebral arteries.$^{72}$ These studies will certainly resolve the existing controversy surrounding the role of ET-1 in the pathogenesis of cerebral vasospasm.

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