BQ-123, A PEPTIDIC ENDOTHELIN ET\textsubscript{A} RECEPTOR ANTAGONIST, PREVENTS THE EARLY CEREBRAL VASOSPASM FOLLOWING SUBARACHNOID HEMORRHAGE AFTER INTRACISTERNAL BUT NOT INTRAVENOUS INJECTION

Martine Clozel and Hiroshi Watanabe
Pharma Division, Preclinical Research
F.HOFFMANN-LA ROCHE LTD
Grenzacherstrasse 124
CH-4002 Basel, Switzerland

(Received in final form December 16, 1992)

Summary
The aim of this study was to evaluate the role of endothelin and endothelin ET\textsubscript{A} receptor in the early cerebral vasoconstriction following subarachnoid hemorrhage (SAH) in the rat. SAH induced by injection of autologous blood in the cisterna magna reduced by 22 to 38% cerebral blood flow (CBF) measured with radioactive microspheres at 30, 60 and 120 min after SAH. The cyclic pentapeptide BQ-123, a selective antagonist of the ET\textsubscript{A} receptor, injected intravenously (3 mg/kg) had no effect on this decrease in CBF. However, intracisternal BQ-123 (10 nmol) completely prevented the decrease in CBF at 60 and 120 min after SAH. These results suggest that BQ-123 does not cross the blood-brain barrier, but demonstrate that endothelin acting on ET\textsubscript{A} receptor plays a role in the pathogenesis of cerebral vasoconstriction in this rat model of SAH.

One of the major complications of subarachnoid hemorrhage (SAH) after rupture of intracranial aneurysms is a delayed cerebral ischemia due to abnormal vasoconstriction and usually encountered at 6-10 days after SAH. The incidence of clinical symptoms of delayed cerebral ischemia in patients with SAH varies between 20 and 30% (1), while the incidence of death or of permanent cerebral dysfunction varies between 5% (2,3) and 14% (1). However, angiographic vasospasm seems to be much more frequent: 40 to 70% of patients have reduction in the caliber of one or several cerebral arteries by 7 days after SAH (1,4,5,6). By transcranial Doppler, even if an early operation on the SAH is performed, 95% of the patients show some degree of increase in flow velocity in the middle cerebral artery (2). By inference, Seiler et al (2) estimated that, if a blood flow velocity higher than 140 cm/sec was indicative of an arterial narrowing visible by angiography, 52% of the patients with SAH would have shown angiographic vasospasm.
The mechanism of this delayed vasoconstriction is not well understood. There is a relationship between vasospasm and the amount of blood in the subarachnoid space (7). A very large number of putative spasmogens released from the intracisternal blood (or clot) have been proposed (for review, see 1 and 8). These substances released by the clot could be directly spasmogenic or could induce the release of vasoconstrictors by endothelial cells (9) or hypothalamus (10) or impair endothelium-dependent vasodilation (11,12,13). Among these substances, oxyhemoglobin is a likely candidate (13,14).

Recently, endothelin was shown to be one of the most potent vasoconstrictors known (15). Endothelin is present in the brain, especially in hypothalamus (16,17), and its release can be stimulated by various blood elements, including thrombin (18), platelets (19) and oxyhemoglobin (20). Endothelin is an extremely potent and long-lasting cerebral vasoconstrictor (21,22). A single intrathecal injection of endothelin-1 in dogs leads to a constriction of the basilar artery which is still present after 3 days (23).

The first endothelin receptor antagonists have been recently described (24,25,26,27). The cyclic pentapeptide BQ-123 in one of the most potent ones (25). It is an antagonist of the ETA receptor, which is the main or the only endothelin receptor on vascular arterial smooth muscle cells and is the main receptor responsible for the vasoconstrictor effect of endothelin-1. Intravenous BQ-123 can block the pressor effect of intravenous endothelin in rats (25) but not the pressor effect of other vasoconstrictors.

We therefore used BQ-123 to determine the role of endothelin in the early cerebral vasoconstriction in a rat model of SAH. Since intravenous BQ-123 was found to be inactive, we examined also the effect of intracisternal BQ-123. Cerebral blood flow was measured with the radioactive microspheres technique.

Materials and Methods

Wistar rats (380-420 g) were anesthetized with Inactin (125 mg/kg i.p.) and paralyzed with alloferin (1.0 mg/kg, then 0.5 mg/kg/hr i.v.). The rats were tracheotomized and mechanically ventilated with a mixture of air and 28-30% oxygen using a rodent ventilator. Both femoral arteries were cannulated. The right femoral arterial catheter was used for continuous monitoring of arterial blood pressure and heart rate (HR). The left femoral catheter was advanced into the aorta and used for withdrawal of the reference blood sample. The left ventricle was catheterized via the right common carotid artery for injection of radioactive microspheres, and the left femoral vein for drug injection and constant infusion of alloferin. The atlanto-occipital membrane was exposed and a 25-gauge needle was inserted into the cisterna magna for the subsequent injection of arterial blood. The proper placement of the needle was assessed by the possibility to withdraw cerebrospinal fluid (CSF) through the needle. Body temperature was monitored and maintained near 37°C. Arterial blood gases were determined before the first microsphere injection and at the end of the study using a blood gas analyzer (ABL 300, Radiometer, Copenhagen, Denmark). Throughout the experiments, end-expiratory O₂ and CO₂ concentrations were monitored (Normocap, Datex, AVL Medical Instruments, Schaffhausen, Switzerland).
SAH was induced by a single injection of autologous blood in the cisterna magna, following a technique slightly modified from Solomon (28). In this model, cerebral blood flow (CBF) decreases by about 40% at 30 to 120 min after SAH (28). Briefly, 0.35 ml of nonheparinized arterial blood was withdrawn in a syringe from the femoral artery and replaced by an equal volume of normal saline. 0.3 ml of this autologous arterial blood was injected over 1 min into the cisterna magna via the catheter fixed previously while the rat was held in a head-drop position. Immediately after SAH and every 30 min thereafter, heparin (LIQUEMIN® F.Hoffmann-La Roche LTD, Basel, Switzerland) was given intravenously at a dose of 150 U/kg. We had shown in preliminary experiments that this dosage of heparin did not modify the decrease in CBF induced by SAH but improved the stability of arterial blood pressure and CBF in sham-operated rats.

CBF was measured 15 min before and 30, 60 and 120 min after SAH by the radioactive microspheres technique (29,30). Microspheres (15 μm diameter) labeled with either Indium-114, Scandium-46, Strontium-85 or Tin-113 were used. Microspheres were suspended in 0.9% saline solution with 0.005% Tween 80. To prevent aggregation of microspheres, the vial containing the microsphere solution was placed in an ultrasonic bath for 5 min and then shaken for 1 min with a vortex mixer prior to injection. For each measurement, a 0.2 ml suspension containing approximately 100,000 microspheres was injected into the left ventricle over a 10-s period and flushed with 0.3 ml saline. A reference blood sample was withdrawn simultaneously from the left femoral artery into a heparinized syringe using a Harvard pump at a rate of 0.6 ml/min for 1 min beginning 10 s before the microsphere injection. After the completion of the experiments, rats were sacrificed and the brain (hemispheres, brainstem and cerebellum) and both kidneys were removed, weighed and placed in scintillation vials. Radioactivity in each tissue sample was counted for 2 min in a multichannel gamma scintillation counter equipped with a germanium crystal (Enertec, Strasbourg, France). Organ blood flow was computed from the radioactivity in each tissue sample and in the reference blood sample after appropriate correction for the background activity as follows: Regional blood flow (ml/min/g) = reference blood sample withdrawal rate x radioactivity per gram of tissue / radioactivity in reference blood sample. Blood flow in both kidneys was determined to check the evenness of the distribution of microspheres. Kidney blood flow was found to vary between both kidneys by less than 10%.

In a first study, BQ-123 (synthesized de novo at F.Hoffmann-La Roche LTD, Basel, Switzerland) or its vehicle (0.15% sodium carbonate in saline) was injected intravenously at a dose of 3 mg/kg, 10 min before SAH. Six rats were used in each group. In a second study, BQ-123 or its vehicle was injected intracisternally 10 min before SAH at a dose of 10 nmol (6.1 μg) in a volume of 0.1 ml. This dose was chosen to allow a concentration of the drug in CSF of about 10 μM, assuming a volume of distribution of the drug in CSF of 1 ml. Six rats were used in each group.

All results are given as mean ± SEM. Statistical analysis was performed by analysis of variance for repeated measures and compared the effects of BQ-123 to
the effects of its vehicle at each time point. A p value less than 0.05 was considered significant.

Results

Effects of intravenous BQ-123

Intravenous BQ-123 (3 mg/kg) had no acute effect and no effect at 30, 60 or 120 min after SAH on mean arterial blood pressure (MABP), HR or blood gases (Table 1). These parameters remained stable throughout the experiments, inasmuch as SAH had no significant effect on any of them (beside a short-lasting increase in MABP immediately after subarachnoid blood injection).

<table>
<thead>
<tr>
<th>Study</th>
<th>Group</th>
<th>Time after SAH (min)</th>
<th>Mean arterial blood pressure (mmHg)</th>
<th>Mean Heart rate (beats/min)</th>
<th>Mean pH</th>
<th>Mean PCO2 (mmHg)</th>
<th>Mean PO2 (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intravenous</td>
<td>Control</td>
<td>baseline</td>
<td>129±2</td>
<td>430±12</td>
<td>7.45</td>
<td>39±1</td>
<td>134±5</td>
</tr>
<tr>
<td>BQ-123</td>
<td></td>
<td>30</td>
<td>126±2</td>
<td>446±13</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>60</td>
<td>127±1</td>
<td>430±13</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>120</td>
<td>125±2</td>
<td>427±14</td>
<td>7.44</td>
<td>37±1</td>
<td>138±3</td>
</tr>
<tr>
<td>BQ-123 baseline</td>
<td></td>
<td>127±3</td>
<td>413±11</td>
<td>7.46</td>
<td>38±0</td>
<td>141±3</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>30</td>
<td>123±3</td>
<td>428±9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>60</td>
<td>124±3</td>
<td>420±12</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>120</td>
<td>127±2</td>
<td>415±11</td>
<td>7.43</td>
<td>37±0</td>
<td>122±4</td>
</tr>
<tr>
<td>Intracisternal</td>
<td>Control</td>
<td>baseline</td>
<td>126±3</td>
<td>418±9</td>
<td>7.45</td>
<td>39±1</td>
<td>128±7</td>
</tr>
<tr>
<td>BQ-123</td>
<td></td>
<td>30</td>
<td>126±2</td>
<td>423±11</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>60</td>
<td>123±2</td>
<td>415±13</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>120</td>
<td>125±2</td>
<td>422±9</td>
<td>7.46</td>
<td>38±1</td>
<td>130±5</td>
</tr>
<tr>
<td>BQ-123 baseline</td>
<td></td>
<td>129±2</td>
<td>427±5</td>
<td>7.46</td>
<td>38±1</td>
<td>120±8</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>30</td>
<td>126±2</td>
<td>443±6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>60</td>
<td>126±1</td>
<td>437±8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>120</td>
<td>127±1</td>
<td>447±14</td>
<td>7.43</td>
<td>38±1</td>
<td>116±5</td>
</tr>
</tbody>
</table>

In contrast, CBF was markedly affected by SAH, with a decrease of 22 to 38% at 30 min after SAH and a slight recovery at 60 and 120 min. This decrease in CBF was maximal in the cerebellum (0.69±0.06 at 30 min vs 1.10±0.04 ml/min/g at baseline) (Table 2 and Figure 1). Intravenous BQ-123 had no significant effect on this decrease in CBF, except in one area (left hemisphere) where it induced a moderate but significant increase in CBF at 60 and 120 min (Table 2 and Figure 1).
TABLE 2

Changes in cerebral blood flow (CBF) in different brain area before (baseline) and 30, 60 and 120 min after subarachnoid hemorrhage (SAH) in control rats and in rats treated with intravenous or intracisternal BQ-123

<table>
<thead>
<tr>
<th>Study</th>
<th>Group</th>
<th>Time after SAH (min)</th>
<th>Cerebral blood flow (ml/min/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>left hemisphere</td>
</tr>
<tr>
<td>Intravenous</td>
<td>Control baseline</td>
<td>30</td>
<td>0.66±0.05</td>
</tr>
<tr>
<td>BQ-123</td>
<td>Control baseline</td>
<td>60</td>
<td>0.49±0.03</td>
</tr>
<tr>
<td></td>
<td>BQ-123 baseline</td>
<td>30</td>
<td>0.52±0.05</td>
</tr>
<tr>
<td></td>
<td>BQ-123 baseline</td>
<td>60</td>
<td>0.66±0.07*</td>
</tr>
<tr>
<td></td>
<td>BQ-123 baseline</td>
<td>120</td>
<td>0.67±0.05*</td>
</tr>
<tr>
<td>Intracisternal</td>
<td>Control baseline</td>
<td>30</td>
<td>0.73±0.03</td>
</tr>
<tr>
<td>BQ-123</td>
<td>Control baseline</td>
<td>60</td>
<td>0.48±0.06</td>
</tr>
<tr>
<td></td>
<td>BQ-123 baseline</td>
<td>30</td>
<td>0.49±0.04</td>
</tr>
<tr>
<td></td>
<td>BQ-123 baseline</td>
<td>60</td>
<td>0.53±0.06</td>
</tr>
<tr>
<td></td>
<td>BQ-123 baseline</td>
<td>120</td>
<td>0.75±0.07</td>
</tr>
<tr>
<td></td>
<td>BQ-123 baseline</td>
<td>60</td>
<td>0.73±0.06**</td>
</tr>
<tr>
<td></td>
<td>BQ-123 baseline</td>
<td>120</td>
<td>0.64±0.02</td>
</tr>
</tbody>
</table>

* p < 0.05, ** p < 0.01 compared to control

Effects of intracisternal BQ-123

The control group receiving intracisternally the vehicle of BQ-123 was similar to the control group of the intravenous study, with no change in MABP or HR (Table 1) and a 34 to 60% decrease in CBF at 30 min with a slight recovery at 60 and 120 min (Table 2 and Figure 2).

In marked contrast to intravenous BQ-123, intracisternal BQ-123 had a highly significant effect on the changes in CBF induced by SAH (Table 2 and Figure 2). Intracisternal BQ-123 prevented almost completely the decrease in CBF at 60 and 120 min after SAH. In the right hemisphere, this effect was not statistically significant, probably because of the dependence of this hemisphere on other brain areas for vascularization (the right common carotid artery had been occluded for left ventricle catheterization) increased CBF variability. In brainstem and cerebellum, CBF was back to baseline level at 60 and 120 min. In contrast, at 30 min after SAH, there was no significant effect of BQ-123 (Table 2 and Figure 2).
Percent changes in cerebral blood flow (CBF) in rats subjected to subarachnoid hemorrhage (SAH) treated with intravenous BQ-123 (3 mg/kg) or its vehicle. n = 6 in each group. Statistics were made on absolute values and are shown in Table 2.

Percent changes in cerebral blood flow (CBF) in rats subjected to subarachnoid hemorrhage (SAH) treated with intracisternal BQ-123 (10 nmol) or its vehicle. n = 6 in each group. Statistics were made on absolute values and are shown in Table 2.
Discussion

Our results show that blockade of the endothelin $\text{ET}_A$ receptor is a valid concept for preventing early cerebral vasospasm in this rat model of SAH, but that BQ-123 is only effective after intracisternal, not intravenous injection.

The rat model of SAH was adapted from Solomon (28) and the changes in CBF we observed were very similar to those described (28). We had shown in previous experiments that in sham-operated rats receiving intracisternal artificial CSF instead of blood, there was no decrease in CBF and that indeed CBF changes could be attributed to SAH (not shown). This model might not be a perfect reflection of the clinical situation of delayed cerebral vasospasm, since in rats the decrease in CBF is relatively acute and does not seem to last over 24 hrs (31). However, vascular reactivity is also modified very early after SAH in rabbits (32) and man (33).

Among the spasmogens which are released from the blood clot or whose release is stimulated by a product of the blood clot (8), endothelin is a likely candidate. A single intracisternal injection of endothelin in dogs was able to reduce basilar artery diameter for more than 3 days, a pattern similar to the effects of a single injection of blood (23). Endothelin infused intracisternally for 7 days in dogs reproduced both the angiographic and histological features of a double-hemorrhage model of SAH (34). The role of endothelin in cerebral vasoconstriction after SAH was also suggested by the observation that phosphoramidon, which inhibits the conversion of big endothelin-1 to endothelin-1, and actinomycin D, a ribonucleic acid synthesis inhibitor which also suppresses endothelin synthesis, were both able to inhibit almost completely the development of delayed vasospasm after SAH in dogs (35,36). Endothelin probably plays a role in human SAH as well, as shown by the increased levels of endothelin in plasma and CSF of patients with SAH (37,38,39).

Our finding that blockade of endothelin receptors can prevent the early decrease of CBF in a rat model strongly reinforces the evidence for a role of endothelin in experimental SAH. The fact that BQ-123, which is a selective antagonist of the $\text{ET}_A$ receptor (25), could prevent the decrease in CBF after SAH shows that the $\text{ET}_A$ receptor is involved in this vasoconstriction. The role of $\text{ET}_A$ receptor in the cerebral vasoconstrictor effect of endothelin was already suggested by the difference in potency between endothelin-1 and endothelin-3 for contracting isolated canine cerebral arteries (40). However, we found no significant effect of BQ-123, even intracisternally, at 30 min after SAH, suggesting that the very early vasoconstriction may not be due to endothelin or to the $\text{ET}_A$ receptor. Experimental studies in larger animals are needed to evaluate whether the chronic vasospasm, the one which occurs several days after SAH, can be prevented by endothelin receptor antagonists. Our study does not allow us either to determine whether BQ-123 was active for dilating large vessels such as the basilar artery or small resistance vessels. Such a question should be answered on larger animal models by comparing angiographic and CBF data.
The lack of efficacy of intravenous BQ-123 in opposition to intracisternal BQ-123 was probably due to a lack of penetration through the blood-brain barrier. The dose of intravenous BQ-123 (3 mg/kg) was not insufficient, since this dose was able to inhibit the pressor effect of endothelin-1 (25 and personal observations). Despite the alteration of the hemato-encephalic barrier in SAH, BQ-123 was virtually ineffective after systemic injection. It was interesting to notice that neither intravenous nor intracisternal BQ-123 modified arterial blood pressure, suggesting that BQ-123 does not have any systemic vasodilator effect.

At the present time, the calcium antagonist nimodipine is the only drug on the market registered for SAH. It seems to improve the clinical outcome of patients with delayed cerebral ischemia, however without increasing CBF (41). In addition, its systemic vasodilator effects may be detrimental in SAH patients with poor cerebral autoregulation. If the present results are also found in situations of chronic vasospasm, endothelin antagonists might represent a new therapeutic approach for preventing cerebral ischemia after SAH in man.

Acknowledgements

We wish to thank Arnold Trzeciak for the synthesis of BQ-123, Patrick Hess for his technical help and Doris Brütsch for typing the manuscript.

References

15. M. YANAGISAWA, H. KURIHARA, S. KIMURA, Y. TONOBE, M.
KOBAYASHI, Y. MITSUI, Y. YAZAKI, K. GOTO, T. MASAKI, Nature 332

16. A. GIAID, S.J. GIBSON, M.T. HERRERO, S. GENTLEMAN, S. LEGON, M.
YANAGISAWA, T. MASAKI, N.B.N. IBRAHIM, G.W. ROBERTS, M.L.

17. M.E. LEE, S.M. DE LA MONTE, S.C. NG, K.D. BLOCH, T. QUERTEMOURS,

18. M. KOHNO, K. YASUNARI, K. YOKOKAWA, K.I. MURAKAWA, T.
HORIO, Y. KANAYAMA, M. FUZISAWA, T. INOUE, T. TAKEDA,

19. E.H. OHLSTEIN, B.L. STORER, J.A. BUTCHER, C. DEBOUCK, G.


25. M. IHARA, K. NOGUCHI, T. SAEKI, T. FUKURODA, S. TSUCHIDA, S.

26. S. MIYATA, M. HASHIMOTO, Y. MASUI, M. EZAKI, S. TAKASE, M.

27. S. MIYATA, M. HASHIMOTO, K. FUJIE, M. NISHIKAWA, S. KIYOTO, M.

28. R.A. SOLOMON, J.L. ANTUNES, R.Y.Z. CHEN, L. BLAND, S. CHIEN,


30. S.F. FLAIM, S.H. NELLIS, E.J. TOGGART, H. DREXLER, K. KANDA, E.D.


33. K. HATAKE, I. WAKABAYASHI, E. KAKISHITA, S. HISHIDA, Stroke 23

34. H. KOBAYASHI, M. HAYASHI, S. KOBAYASHI, M. KABUTO, Y.

35. Y. MATSUMURA, R. IKEGAWA, Y. SUZUKI, M. TAKAOKA, T.
UCHIDA, H. KIDO, H. SHINYAMA, K. HAYASHI, M. WATANABE, S.

36. T. SHIGENO, T. MIMA, M. YANAGISAWA, A. SAITO, K. GOTO, K.
YAMASHITA, T. TAKENOUCHI, N. MATSUURA, Y. YAMASAKI, K.