Prospective, randomized, double-blind trial of BQ-123 and bosentan for prevention of vasospasm following subarachnoid hemorrhage in monkeys

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Thirty-one monkeys were randomly divided into three groups to undergo baseline cerebral angiography followed by induction of subarachnoid hemorrhage by placement of autologous blood clot along the right-sided arteries of the anterior circle of Willis (Day 0). The monkeys were then given drug vehicle or one of two endothelin (ET) antagonists, BQ-123 (6 mg/kg/day) or bosentan (5 mg/kg/day) intracisternally. The BQ-123 was administered by continuous infusion from a subcutaneous pump and the bosentan was given by twice-daily injections into an Ommaya reservoir in the subcutaneous space with a catheter along the right middle cerebral artery (MCA). Seven days later (Day 7), angiography was repeated and the animals were killed. Comparison of arterial diameters shown on angiograms between Day 0 and Day 7 groups given placebo and bosentan showed significant reductions in the diameters of the right intradural internal carotid (28% ± 6% and 30% ± 6%, respectively, paired t-test, p < 0.05), anterior cerebral artery (29% ± 8% and 32% ± 6% respectively ± 6%, respectively) and MCA (34% ± 6% and 46% ± 4%, respectively). Animals injected with BQ-123 had significant narrowing of the right extradural internal carotid artery (7% ± 6%) and the basilar artery (11% ± 3%), but not of the right MCA. Comparison of arterial diameters between groups at Day 7 showed significant variance in the right extradural internal carotid, both intradural internal carotid, right middle cerebral, and left anterior cerebral arteries; the animals injected with BQ-123 developed significantly less arterial narrowing than those receiving bosentan and placebo. Bosentan was not detected in the cerebrospinal fluid aspirated from the cisterna magna on Day 7, whereas BQ-123 was detected in two animals. We can infer from these results that BQ-123 prevents vasospasm following subarachnoid hemorrhage in monkeys, that further investigations of ET antagonists are warranted, and that ET may be an important pathophysiological mediator of vasospasm. The lack of efficacy of bosentan may be related to inadequate cerebrospinal fluid levels obtained by administration twice-daily through an Ommaya reservoir.

KEY WORDS • bosentan • BQ-123 • endothelin • subarachnoid hemorrhage • vasospasm • cynomolgus monkey

THE endothelins (ETs) are 21–amino acid peptides that are potent vasoconstrictors.3,28 Three distinct genes (ET-1, 2, and 3) for ETs have been identified.4,25 Endothelin-1 is the most potent vasoconstrictor and has been implicated as a mediator of vasospasm following aneurysmal subarachnoid hemorrhage (SAH).7 Some evidence that supports an etiological role for ETs in vasospasm has been produced, although there are conflicting reports about whether ET levels are elevated after SAH and about the efficacy of using ET antagonists against vasospasm.1 The current experiment was based on the hypothesis that increased synthesis of ETs by endothelial cells contributes to smooth-muscle contraction and vasospasm after SAH and that antagonism of the action of ETs might diminish vasospasm. The primary endpoint was the degree of vasospasm assessed by comparison of arterial diameters on angiograms taken at baseline and 7 days after clot placement and drug administration. A monkey model was used because this model most reliably reflects the features of vasospasm as they occur in humans and because spasm in this model is resistant to treatments for vasospasm that have not substantially prevented vasospasm in humans. Two ET antagonists were tested: BQ-123 is a relatively specific antagonist of ETα receptors and bosentan is a competitive antagonist of both ETα and ETβ receptors.

Materials and Methods

Animal Preparation

Thirty-one cynomolgus monkeys were randomly divided into three groups. Over a period of 7 days the groups received intracisternal injections of placebo (11 animals), BQ-123 (6 mg/kg/day) (10
constant. Mannitol (0.6 gm/kg) was given intravenously, the PaCO₂
angiogram was obtained. Exposure factors and magnification were
was inserted. A single midarterial phase, anteroposterior cerebral
position and sterile technique, and a 20-gauge polyethylene catheter
right axillary artery of each monkey was exposed using magnifica-
pressure, and heart rate were monitored. Transcranial Doppler ultra-
BQ-123, is an antagonist of ETA receptors6 that remains stable for 7
dose and Administration of Placebo, BQ-123, and Bosentan
and Use Committee of the University of Chicago and met the stan-
other adverse effects caused by intracisternal injection of ET antag-
exsanguination under anesthesia. The brains were removed, speci-
placed along the right MCA. In the placebo group, five animals
Palo Alto, CA) or the Ommaya reservoir to implant, so that he was
geon was given an osmotic pump (Alzet, model 2ML1; Alza Corp.,
fluid (CSF) was aspirated from the cisterna magna and stored at
were under general anesthesia as described above. Cerebrospinal
were under Day 7, transcranial Doppler ultrasound and cerebral angiography were repeated while the animals
were under general anesthesia as described above. Cerebrospinal
fluid (CSF) was aspirated from the cisterna magna and stored at
30%–50% reduction), or severe (≥ 50% reduction). Comparisons
within groups and at different times were made by paired t-test,
and intergroup comparisons at Day 0 and Day 7 were determined
by one-way analysis of variance followed by a Bonferroni multi-
ple comparison test if significance variance was found. Transcra-
nal Doppler flow velocities were compared to angiographically
determined arterial diameters by linear regression. All data analysis
was performed by a statistician blinded to the identity of the groups,
and significance was taken at p < 0.05. Values are reported as the
mean ± the standard error of the mean. See text for description of statistical analysis. SAH = subarachnoid
hemorrhage.

### Physiological Variables of Monkeys Receiving Placebo, BQ-123, or Bosentan After SAH*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Placebo Group</th>
<th>BQ-123 Group</th>
<th>Bosentan Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>3.16 ± 0.21</td>
<td>3.26 ± 0.24</td>
<td>3.03 ± 0.22</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>123 ± 5</td>
<td>128 ± 6</td>
<td>122 ± 4</td>
</tr>
<tr>
<td>Mean blood pressure (mm Hg)</td>
<td>63 ± 5</td>
<td>63 ± 10</td>
<td>77 ± 9</td>
</tr>
<tr>
<td>PaCO₂ (mm Hg)</td>
<td>42 ± 3</td>
<td>42 ± 2</td>
<td>45 ± 3</td>
</tr>
</tbody>
</table>

* Values are expressed as the mean ± standard error of the mean. See text for description of statistical analysis. SAH = subarachnoid hemorrhage.

Dose and Administration of Placebo, BQ-123, and Bosentan

Two ET antagonists were chosen. The first, the synthetic peptide
BQ-123, is an antagonist of ET₁ receptors7 that remains stable for 7
days in physiological buffer at body temperature. In previous exper-
iments involving dogs, intrathecal administration of more than 0.6
gm/kg per day BQ-123 significantly prevented vasospasm induced
by two intrathecal injections of blood.37 Differences in brain and
CSF volumes between the species preclude a direct comparison, but
a high dose of BQ-123 (6 mg/kg/day) was chosen for use in our
study of monkeys to replicate the dose used in dogs. The BQ-123
was administered as a continuous intracisternal infusion from a sub-
cutaneous osmotic pump for 7 days. The second antagonist, bosen-
tan (Ro47-0203), is a competitive antagonist of ET₁, and ET₂,
receptors that is orally active and soluble in physiological buffer
containing 10% (v/v) dimethyl sulfoxide.43 Previous work indicat-
ed that 3 mg/kg bosentan administered intravenously augmented
cerebral blood flow after SAH in rats.6 A slightly higher dose (5
mg/kg/day), similar to the dose of BQ-123, was selected for use in
the present investigation. There has been no information about the
relative efficacy of bosentan administered intravenously or intracis-
ternally. An intracisternal route was chosen on the basis of the
favorable results obtained with BQ-123 in dogs. The low solubility
at high concentrations of bosentan in solution necessitated twice-
daily intrathecal injections through an Ommaya reservoir. Five an-
imals from the placebo group received an infusion of physiological
saline via an osmotic pump and five animals received twice-daily
injections of 10% dimethyl sulfoxide (v/v) in physiological saline
through an Ommaya reservoir. Two methods of placebo adminis-
tration were used to account for the two methods of drug adminis-
tration. No differences in the degree of vasospasm were detected
between the two methods of placebo administration and the results
for these animals are presented as a single group. The sterility of all
injected solutions was confirmed by microbiological culture.

Data Analysis

Arterial diameters from cerebral angiograms were measured
using an optical micrometer at predetermined points along the
extradural ICA, intradural ICA, ACA, MCA, and basilar artery
(BA). Measurements were conducted by an investigator without
knowledge of the monkey’s group. The extent of vasospasm was
assessed by comparing angiograms taken before and 7 days after
induction of SAH. Vasospasm was categorized as absent (0%–10%
reduction in diameter), mild (10%–25% reduction), moderate
(25%–50% reduction), or severe (≥ 50% reduction). Comparisons
within groups and at different times were made by paired t-test,
and intergroup comparisons at Day 0 and Day 7 were determined
by one-way analysis of variance followed by a Bonferroni multi-
ple comparison test if significance variance was found. Transcra-
nal Doppler flow velocities were compared to angiographically
determined arterial diameters by linear regression. All data analysis
was performed by a statistician blinded to the identity of the groups,
and significance was taken at p < 0.05. Values are reported as the
mean ± the standard error of the mean.

### Results

**Physiological Variables**

Comparisons between groups at Day 0 showed significant
variance in weight (Table 1). Initially, the mean weight of animals receiving bosentan was significantly
greater than the mean weight of animals receiving BQ-
123. At Day 7, however, there were no significant differ-
ences in physiological variables between groups. There
were no significant differences in physiological variables in the placebo and BQ-123 groups when comparisons were made within groups between Day 0 and Day 7. Within the bosentan group, the animals had significantly lower mean weight and significantly higher heart rate after 7 days (p < 0.005).

Angiographically Verified Vasospasm

Intergroup comparisons of arterial diameters on Day 0 did not reveal significant differences (Tables 2 and 3, Figs. 1–3). On Day 7, there was significant variance between groups with regard to diameters of the right extradural ICA and intradural ICA, right MCA, and left ACA. Multiple comparisons showed no pairwise differences for the right intradural ICA. For the right and left extradural ICAs, right MCA, and left ACA, the mean diameter in the bosentan group was significantly smaller than the mean diameter in the BQ-123 group. The mean diameter of the right MCA in the placebo group was also significantly smaller than that in the BQ-123 group.

Comparisons of arterial diameters within groups between Day 0 and Day 7 showed significant reductions in the right intradural ICA, MCA, and ACA in animals injected with placebo and with bosentan (Table 3, Figs. 1 and 2). Animals treated with bosentan also developed significant arterial narrowing of the right and left intradural ICAs and the extradural ICA. For animals given BQ-123, there were significant reductions in the diameters of right intradural ICA and BA and no significant narrowing of the right MCA.

**TABLE 2**

<table>
<thead>
<tr>
<th>Artery</th>
<th>Placebo Group (mm)</th>
<th>BQ-123 Group (mm)</th>
<th>Bosentan Group (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 0 (11 animals)</td>
<td>Day 7 (10 animals)</td>
<td>Day 0 (10 animals)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Day 7 (9 animals)</td>
</tr>
<tr>
<td>rt C3</td>
<td>2.34 ± 0.14</td>
<td>2.16 ± 0.13</td>
<td>2.70 ± 0.13</td>
</tr>
<tr>
<td>rt C4</td>
<td>2.00 ± 0.14</td>
<td>1.36 ± 0.11</td>
<td>2.08 ± 0.10</td>
</tr>
<tr>
<td>rt ACA</td>
<td>1.25 ± 0.09</td>
<td>0.89 ± 0.12</td>
<td>1.25 ± 0.11</td>
</tr>
<tr>
<td>rt MCA</td>
<td>1.61 ± 0.11</td>
<td>1.02 ± 0.10</td>
<td>1.71 ± 0.11</td>
</tr>
<tr>
<td>lt C3</td>
<td>2.41 ± 0.11</td>
<td>2.18 ± 0.09</td>
<td>2.47 ± 0.13</td>
</tr>
<tr>
<td>lt C4</td>
<td>1.68 ± 0.07</td>
<td>1.00 ± 0.09</td>
<td>1.49 ± 0.08</td>
</tr>
<tr>
<td>lt ACA</td>
<td>1.34 ± 0.12</td>
<td>1.18 ± 0.07</td>
<td>1.49 ± 0.12</td>
</tr>
<tr>
<td>lt MCA</td>
<td>1.56 ± 0.14</td>
<td>1.61 ± 0.15</td>
<td>1.62 ± 0.19</td>
</tr>
<tr>
<td>basilar</td>
<td>1.67 ± 0.08</td>
<td>1.69 ± 0.13</td>
<td>1.67 ± 0.13</td>
</tr>
</tbody>
</table>

* Values are expressed as the mean ± standard error of the mean. SAH = subarachnoid hemorrhage; C3 = extradural internal carotid artery; C4 = intradural internal carotid artery; ACA = anterior cerebral artery; MCA = middle cerebral artery. See text for description of statistical analysis.

**TABLE 3**

<table>
<thead>
<tr>
<th>Artery</th>
<th>Placebo Group</th>
<th>BQ-123 Group</th>
<th>Bosentan Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 0 (10 animals)</td>
<td>Day 7</td>
<td>Day 0 (10 animals)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Day 7 (9 animals)</td>
<td></td>
</tr>
<tr>
<td>rt C3</td>
<td>−7.5 ± 3.9</td>
<td>−6.7 ± 5.6†</td>
<td>−18.9 ± 6.1†</td>
</tr>
<tr>
<td>rt C4</td>
<td>−27.5 ± 6.4†</td>
<td>−5.2 ± 11.9</td>
<td>−29.7 ± 6.2†</td>
</tr>
<tr>
<td>rt ACA</td>
<td>−29.2 ± 8.2†</td>
<td>−6.3 ± 10.7</td>
<td>−32.1 ± 6.2‡</td>
</tr>
<tr>
<td>rt MCA</td>
<td>−33.5 ± 5.98</td>
<td>−6.8 ± 7.1</td>
<td>−45.8 ± 4.0‡</td>
</tr>
<tr>
<td>lt C3</td>
<td>−3.8 ± 4.6</td>
<td>−1.6 ± 7.5</td>
<td>−13.2 ± 6.9‡</td>
</tr>
<tr>
<td>lt C4</td>
<td>−6.7 ± 6.8</td>
<td>−8.2 ± 7.7</td>
<td>−18.8 ± 6.4‡</td>
</tr>
<tr>
<td>lt ACA</td>
<td>−4.4 ± 8.9</td>
<td>−2.5 ± 9.2</td>
<td>−15.0 ± 8.7</td>
</tr>
<tr>
<td>lt MCA</td>
<td>−6.4 ± 7.4</td>
<td>−0.2 ± 8.4</td>
<td>−14.7 ± 13.2</td>
</tr>
<tr>
<td>basilar</td>
<td>−1.0 ± 5.9</td>
<td>−10.7 ± 3.4†</td>
<td>−13.5 ± 8.8</td>
</tr>
</tbody>
</table>

* Values are expressed as mean ± standard error of the mean. SAH = subarachnoid hemorrhage; C3 = extradural internal carotid artery; ACA = anterior cerebral artery; MCA = middle cerebral artery.
† p<0.05; ‡ p<0.005; and § p<0.001.

**FIG. 1.** Bar graph showing percent reduction in diameter of right-sided arteries between Day 0 and Day 7 for each group. Bars represent mean ± standard error of the mean. Statistically significant reductions in diameters occurred in animals in the placebo and bosentan groups (paired t-test, p < 0.05). C4 = intradural internal carotid artery; ACA = anterior cerebral artery; and MCA = middle cerebral artery.
Because the monkeys were entered into the study over a 5-month period, we checked for a correlation between the degree of vasospasm and the time of entry into the study. There was no relationship, indicating that time could not account for the marked variation in degree of vasospasm between the groups (data not shown). Several animals became ill and died or underwent angiography before Day 7. The analysis provided above includes these animals but a repeat analysis that excluded them did not significantly alter the results (data not shown).

Transcranial Doppler Ultrasound

The groups did not differ at baseline in mean Doppler ultrasound velocities of the MCA. At Day 7, there was significant variance between groups in right MCA velocity but there were no pairwise differences (Table 4). Velocities were significantly increased by Day 7 in both left and right MCA in the placebo and bosentan groups and in the right MCA in the BQ-123 group. There was a significant linear correlation between angiographically determined diameter of the MCA and Doppler flow velocity \( (r = 0.64, p < 0.005) \) (Fig. 4). Inspection of the relationship, however, suggests that the relationship is not linear. Because flow velocity is directly related to blood flow and inversely related to the square of the radius of the artery, we tested different transformations of MCA diameter and flow velocity to determine which provided a linear relationship. We found that flow velocity was directly related to the inverse of the square of arterial diameter \( (r = 0.61, p < 0.05) \) (Fig. 4).

Clinical Observations, Pathology, and Drug Levels in CSF

Among the animals injected with BQ-123, one monkey died of surgical complications immediately after induction of SAH, and one developed seizures on Day 4, which were caused by a temporal lobe intracerebral hematoma found at autopsy. One animal in the placebo group died of a temporal lobe hematoma on Day 4. Four monkeys injected with bosentan became ill with seizures, hemi-
BQ-123, bosentan, and vasospasm

TABLE 4  
Mean transcranial Doppler flow velocities in middle cerebral arteries of monkeys receiving placebo, BQ-123, or bosentan after SAH*

<table>
<thead>
<tr>
<th>Group</th>
<th>Day 0 cm/sec</th>
<th>Day 7 cm/sec</th>
<th>Percent Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>placebo</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>right</td>
<td>44 ± 2 (11)</td>
<td>101 ± 6 (8)</td>
<td>129 ± 21 ‡</td>
</tr>
<tr>
<td>left</td>
<td>46 ± 2 (11)</td>
<td>59 ± 4 (8)</td>
<td>27 ± 8 †</td>
</tr>
<tr>
<td>BQ-123</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>right</td>
<td>39 ± 3 (10)</td>
<td>72 ± 13 (8)</td>
<td>66 ± 17 †</td>
</tr>
<tr>
<td>left</td>
<td>41 ± 3 (10)</td>
<td>51 ± 6 (8)</td>
<td>19 ± 10</td>
</tr>
<tr>
<td>bosentan</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>right</td>
<td>38 ± 2 (8)</td>
<td>67 ± 7 (6)</td>
<td>73 ± 19 †</td>
</tr>
<tr>
<td>left</td>
<td>40 ± 4 (8)</td>
<td>50 ± 5 (6)</td>
<td>23 ± 8 †</td>
</tr>
</tbody>
</table>

* Values are expressed as the mean ± standard error of the mean. Numerals in parentheses indicate numbers of animals. SAH = subarachnoid hemorrhage.
† p<0.05; and ‡ p<0.001.

Examinations of sections of brain tissue from monkeys in each group did not reveal inflammation or changes in the brain parenchyma in the drug-treated groups when compared to changes seen in animals in the placebo group.

Levels of bosentan were measured in fluid aspirated from the Ommaya reservoir prior to each injection and in CSF aspirated from the cisterna magna on Day 7 (Table 5). Despite a mean level of bosentan in the Ommaya reservoir measuring 2.4 ± 1.5 mg/ml (40 ± 26 mM), bosentan was not detected in the CSF aspirated from the cisterna magna. The concentrations of bosentan injected into the Ommaya reservoir were approximately 8 mg/ml. The concentration of BQ-123 in CSF aspirated from the cisterna magna on Day 7 was not detectable in six animals (< 0.05 μM) and measured 0.1 to 0.18 μM in two animals.

Discussion

Evidence that ET Mediates Vasospasm

Injection of ET-1 into the CSF of dogs produced vasoconstriction of the BA, respiratory arrest, hypertension, bradycardia, and death. Other investigators have found similar doses of ET-1 to cause severe, long-lasting contraction of dog and cat BAs in vivo. Intraluminal infusion of ET-1 had no effect on the diameter of the cat BA although intracisternal injection of this substance produced contraction lasting up to 12 hours. Therefore, ET-1 can contract cerebral arteries when administered into the subarachnoid space, but there is no information concerning whether this effect lasts for weeks or whether concentrations of ET that occur in CSF after SAH can produce such prolonged contractions. Effects of other ETs are not known. There are great differences in activity among ET peptides depending on the species of animal investigated, dose of ET, vascular bed, and type of vessel examined. Evidence is divided about whether ETs are elevated in CSF in association with vasospasm and data can be found both to support and to refute the correlation between increased ET-1 and vasospasm. Following SAH in dogs, increased immunoreactivity to ET-1 was found in the BA after 2 days but not after 7 days, although vasospasm was still present during the latter time. Endothelin also is elevated in response to other central nervous system insults such as head injury, suggesting that elevations during vasospasm could reflect a nonspecific response of the brain.
TABLE 5
Cerebrospinal fluid levels in monkeys receiving BQ-123 or bosentan after SAH*

<table>
<thead>
<tr>
<th>Day After SAH</th>
<th>Source of Fluid</th>
<th>BQ-123 (ng/ml)</th>
<th>Bosentan (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ommaya reservoir</td>
<td>0.14 ± 0.06 (2)</td>
<td>&lt;0.00002 (6)</td>
</tr>
<tr>
<td>2</td>
<td>Ommaya reservoir</td>
<td>2.1 ± 0.6 (3)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Ommaya reservoir</td>
<td>2.5 ± 1.1 (2)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Ommaya reservoir</td>
<td>2.6 ± 0.6 (3)</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Ommaya reservoir</td>
<td>1.8 (1)</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Ommaya reservoir</td>
<td>2.8 ± 2.1 (2)</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Cisterna magna</td>
<td>3.8 (1)</td>
<td></td>
</tr>
</tbody>
</table>

* Values are expressed as the mean ± standard error of the mean. Numerals in parentheses indicate numbers of animals. SAH = subarachnoid hemorrhage.

Other reasons to consider a role for ET-1 in vasospasm are the observations that hemoglobin, a potential mediator of vasospasm, increases secretion of ET-1 from cultured bovine pulmonary arterial and bovine aorta endothelial cells.13,25 Cerebral arteries of some species are sensitized by ET-1 after SAH to contract to other agonists,1,8 and the efficacy of ET antagonists in some studies of vasospasm.7

Previous Studies of ET Antagonists for the Treatment of Vasospasm

The synthetic peptide BQ-123 proved to be efficacious for prevention of vasospasm in rabbits and dogs and for increasing cerebral blood flow after SAH in rats.5,15,17 Synthetic peptides that antagonize the action of ET on ETA receptors, such as FR139317 and cyclo(D-ASP-L-Pro-D-Val-L-Leu-D-Trp), decreased vasospasm in dogs and rabbits.10,24 Bosentan administered systemically was effective for preventing vasospasm in rabbits and for increasing cerebral blood flow after SAH in rats.5 The ETs are peptides that become physiologically active after cleavage from larger proteins, termed big ETs, and the enzymes that perform this cleavage are metalloproteinases. Phosphoramidon, an inhibitor of metalloproteinase enzymes, prevents the conversion of inactive big ET into active ET. Although phosphoramidon was shown to prevent vasospasm following SAH in dogs,22,23 Cosentino and coworkers8 found that phosphoramidon and BQ-123 were not effective against vasospasm when administered to dogs in a similar fashion to that used by other investigators who reported them to be efficacious. Such differences in efficacy, noted Cosentino and coworkers,8 might be related to species and technical differences.

Findings of This Study and Their Explanation

A relatively specific antagonist of ETA receptor, BQ-123 prevented vasospasm in a monkey model of vasospasm. The effectiveness of BQ-123 far exceeds that of any other drug treatment for vasospasm in monkeys that we have evaluated, other than tissue plasminogen activator, which acted by evacuating the vasospasm-causing subarachnoid clot.9 The inhibition constant of BQ-123 is 22 nM for ETA receptors and 18 μM for ETB receptors. Our findings indicate that contraction mediated by ETA receptors is important in the pathogenesis of vasospasm in monkeys. There is no information on the location of these receptors in monkeys, although evidence from other species suggests that they are located on vascular smooth muscle where they mediate contraction.12 The effects of BQ-123 observed in this study could not be explained by bias of the investigators because the study was blinded, or by time of surgery during the study.

Bosentan, a competitive antagonist of both ET and ETA receptors with inhibition constants of 4.7 nM and 95 nM, respectively, was not efficacious against vasospasm.5 There are several potential explanations. Drug levels in the cisterna magna were not detectable; therefore, bosentan may not have been efficacious because adequate CSF levels were not obtained. In other models, bosentan was found to be effective against vasospasm when administered intravenously.26 The efficacy of BQ-123, despite low cisternal levels, however, would argue against this theory, although the pharmacokinetics of the drugs might differ. Bosentan may exert deleterious effects when administered intracisternally, although we found no histopathological evidence that bosentan caused any inflammation or brain damage. More animals injected with bosentan, however, became ill and developed seizures, which suggests but does not prove an adverse effect of intracisternal bosentan. It may be that successive intracisternal boluses exert deleterious effects unlike continuous infusions because bosentan may be a local irritant at high concentration (S Roux, personal communication, 1994). Because bosentan blocks the effects of ETs at both the ETA and ETB receptors, the beneficial effects of ETA receptor blockade might be counteracted by blockade of ETB receptors that mediate relaxation of smooth muscle.12 Endothelin-B receptors mediate contraction caused by some agonists, and current knowledge of ET receptors and the processes that they mediate is too rudimentary to provide mechanistic explanations at present.15 Finally, the drug vehicle itself could produce vasospasm not prevented by bosentan. Vasospasm in placebo animals injected with drug vehicle, however, was not different from vasospasm in placebo animals receiving infusions of saline through an osmotic pump.

Conclusions

Administration of BQ-123 significantly prevented vasospasm in monkeys in a randomized, blinded, placebo-controlled trial. From this we infer that ETs are important in the pathogenesis of vasospasm in this model and that further investigations of BQ-123 for use in the prevention of vasospasm are warranted. More work is required to understand the biochemistry of ETs and ET receptors in this model, along with a full explanation for the failure of bosentan to prevent vasospasm when administered in the manner used in this study.

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References


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