Clentiazem Protects Against Chronic Cerebral Vasospasm in Rabbit Basilar Artery

Peter Vorkapic, MD; John A. Bevan, MD; and Rosemary D. Bevan, MD

Background and Purpose: Experiments were carried out in rabbits to determine whether clentiazem (8-chlorodiltiazem), a cerebrovascular-selective calcium channel blocker, administered 24 hours before subarachnoid hemorrhage influenced the subsequent cerebral vasospasm.

Methods: Subarachnoid hemorrhage was induced by multiple injections of blood into the prepontine cisterns of 35 male New Zealand White rabbits, and clentiazem (5 mg/kg) was administered 4 times daily until sacrifice. Cerebral artery diameter was assessed in vivo by angiography. Functional features of basilar arteries were measured using conventional in vitro methodology.

Results: Clentiazem reduced the angiographic narrowing seen on days 2 and 5 from 35% and 34%, respectively (sham control, 1.42±0.31 mm [n=22]), to 8% and 11%, respectively, and prevented the narrowing (32%) that occurred on day 9. Narrowing in the untreated rabbits was only partly reversed by papaverine; all narrowing in clentiazem-treated animals was papaverine sensitive. Clentiazem prevented or reduced many of the changes in the basilar artery caused by the subarachnoid hemorrhage. Of particular relevance to arterial narrowing were the increased wall stiffness, the transient spontaneous changes in wall force, and the reduction in relaxation to acetylcholine. Reduction of the changes in wall force induced by agonists and by stimulation of intramural sympathetic nerves was observed.

Conclusions: The vascular damage associated with chronic cerebral vasospasm is related to calcium entry into the smooth muscle and endothelial cells, and possibly sympathetic nerve terminals, through calcium channels sensitive to clentiazem, which suggests that clentiazem may be of value in the management of chronic cerebral vasospasm. (Stroke 1991;22:1409-1413)

Increased vessel wall rigidity and decreased capacity to develop active tone are the dominant features of cerebral arteries in experimental chronic cerebral vasospasm.1-4 In a 9-day in vivo study of subarachnoid hemorrhage (SAH) in rabbits, angiographic narrowing exhibited immediate papaverine-sensitive and subsequent papaverine-insensitive components. This latter component increased between days 3 and 9.5 The increased wall stiffness and loss of capacity to develop wall force correlated with the papaverine-insensitive component of narrowing seen on angiography.

The mechanism responsible for chronic cerebral vasospasm remains controversial. One possibility is that high concentrations of vasoconstrictor substances released from perivascular sympathetic nerves, blood, and blood clot, and possibly from adjacent brain tissue,6,7 may cause abnormal vascular smooth muscle contraction8 that results in excessive calcium entry9 and subsequent cellular damage.10-14 If chronic cerebral vasospasm follows from excessive calcium entry from the extracellular space, it might be expected that it would be ameliorated by agents that block its entry.

Cerebrovascular-selective calcium-channel blockers have been studied experimentally15,16 and used clinically for the management of cerebral vasospasm.17,18 In a monkey model, pretreatment with diltiazem prevented neurologic deficit and angiographic narrowing and greatly reduced functional changes in the artery wall.7,16

In this article, we describe the influence of pretreatment with clentiazem, a derivative of diltiazem that exhibits enhanced cerebrovascular selectivity,19-22 on the in vivo and in vitro changes over 9 days in the basilar artery of rabbits after experimental SAH.23

Materials and Methods
The methodology has been previously described.5,23 Thirty-five male New Zealand White rab-
bits weighing 1.85–2.75 kg were used in this study. Baseline angiography was performed 7 days before SAH. To induce SAH under general anesthesia, the tip of a silastic tube was inserted into the prepontine cistern and unheparinized autologous blood was injected in aliquots over 4 hours, to a total of 3 ml/kg. Clentiazem (5 mg/kg, 4 times daily) was administered 24 hours before the SAH was induced and continued until sacrifice via a feeding gastric tube implanted 48 hours before. Serial angiography was performed at 2, 5, and 9 days after SAH, on each occasion before and after an intra-arterial bolus injection of papaverine. Angiographic diameters were calculated as percent of baseline pre-SAH diameter in each animal.

In vitro studies of 4 mm basilar artery segment wall properties were also undertaken at 2, 5, and 9 days after SAH. Animals were stunned and bled by severing both common carotid arteries. The brain was removed and the basilar artery dissected free. We then assessed the passive wall force/vessel circumference relationship, the level of spontaneous myogenic tone activity, reactivity to both constrictors and dilators (histamine, serotonin, norepinephrine, potassium, acetylcholine), and constrictor nerve function.

Data from clentiazem treated animals were compared with untreated and sham-operated animals using analysis of variance. Significance was inferred when $p<0.05$.

Results

The average basilar artery diameter of all rabbits determined by preprocedural angiography in this series was 1.42±0.31 mm (mean±SE) ($n=22$). Mean diameters expressed as percentage of pre-SAH diameter for clentiazem-treated animals were significantly less than those from the untreated animals at days 2, 5, and 9 (Figure 1). Diameters of the untreated group were significantly less. In the untreated rabbits on day 2, but not on days 5 and 9, and at days 2 and 5 of the treated animals, narrowing was completely reversed by intra-arterial papaverine. In the latter group, no significant angiographic narrowing was observed on day 9.

The wall force change upon stretching a standard segment of the basilar artery 0.4 mm beyond the wire separation, when a just-discernible increase in force was detected, was used as a measure of wall stiffness. This was compared with measurements in sham-operated animals. At day 2, no differences were detected (Figure 2). In the untreated group on day 5, the passive wall force was 213±22% of control, and this increased to 274±30% on day 9, which was indicative of increased vessel wall stiffness. These values in treated animals were not significantly different from sham measurements.

Large, transient, variable, spontaneous increases in tone approaching 45% of maximum tissue contractility were recorded during the first 2 days after SAH in the untreated, but not the treated, animals. The variability of this phenomenon precluded quantitative assessment.

The maximum capacity of the sham animal artery segments to develop active force on exposure to histamine ($10^{-4}$ M), 2.53±0.16 grams, was preserved throughout the observation period in the treated group. In the untreated animals, the maximum contractility decreased significantly to a mean of 75.2±6.9% ($n=5$) and 46.0±6.7% ($n=5$) on days 5 and 9, respectively.

At all three time points the serotonin ED$_{50}$ were unchanged for both the treated and untreated groups and were not different from sham values (0.241±0.16 $\mu$M [$n=6$]; Figure 3). In the untreated group, the
maximum contraction to serotonin was significantly enhanced from sham control values of 1.44±0.19 grams to a mean of 172.7±23.3% (day 2) and 171.7±34.9% (day 5), and decreased to 57.2±8.9% (day 9). Maximum contraction did not change in the treated group. In the untreated group at days 5 and 9, the mean percentages of vessels not responsive to either serotonin or norepinephrine were 47.8% and 26%, respectively. In the treated series, all vessels responded to both agonists. The contraction to raised potassium (89 mM), sham value 1.25±0.14 grams, was unchanged in the treated series, but was significantly reduced to similar amounts at all three time points in the untreated group; the pooled mean was 41.3±3.59%. In the presence of an approximate histamine ED$_{50}$, acetylcholine dose-dependently relaxed all vessels of sham-operated and treated animals to baseline without change in ID$_{50}$, sham value 0.61±0.20 μM. In the untreated group, vasorelaxation to acetylcholine ($10^{-4}$ M) was significantly reduced at all time points (Figure 3).

The averaged equilibrium response to electrical field stimulation of intramural sympathetic nerves at 16 Hz was significantly more depressed in the untreated than in the treated series. Values for the treated groups, expressed as percentage control responses, were significantly greater than those from the untreated at all three time periods (pooled mean 70.0±3.9% [n=15] versus 17.8±5.6% [n=15]).

**Discussion**

The calcium channel antagonist diltiazem protects cerebral arteries of the monkey from vasospasm when this is assessed by both in vivo and in vitro techniques. Clentiazem, a derivative of diltiazem, has greater cerebrovascular selectivity. When tested against diltiazem on the contraction of a series of dog regional arteries to calcium and also potassium, it exhibited enhanced selectivity for cerebral arteries. This finding is reflected in studies of rabbit vessels. Clentiazem also has cerebrovascular protective properties. In the study reported here, clentiazem prevented the late papaverine-insensitive angiographic narrowing, the increased artery wall rigidity, the decreased contractility to agonists, the abnormal spontaneous tone excursions, the change in reactivity and responsiveness to constrictor agonists, and the reduction in endothelial-dependent, acetylcholine-induced vasodilation found in the untreated rabbits. There was some protection of neurogenic vasoconstriction.

In experimental SAH, the protective effect of a calcium channel antagonist depends on the timing of treatment and on the species. Chronic cerebral vasospasm in the monkey was more effectively prevented when diltiazem treatment was started 48 hours before than when started 24 hours after SAH. In a two-hemorrhage dog model, nifedipine resolved angiographic narrowing at 2 days but not at 5 days. Artery segments of the dog and monkey are found to be resistant to nimodipine in vitro, and nimodipine...
did not counteract angiographic spasm and neurological deficit in monkeys or dogs.27-29

The crucial event that leads to chronic arterial narrowing after SAH seems to be the release in close approximation to the artery of constrictor substances from adventitial nerves, blood clot, and brain, and possibly of circulating vasoactive substances (for summary see References 6 and 7). The final common pathway for the constrictor action of these putative spasmogens is probably calcium entry channels in the vascular smooth muscle and endothelial cells. Because of the early breakdown of the blood–brain barrier, circulating spasmogens and corpuscular blood components have access to the artery wall.13 Impaired endothelium-based relaxation mechanisms2-4,22 and platelet adherence to damaged endothelium33 probably enhance vasoconstrictor effects.31 Intense smooth-muscle contraction can cause cell damage and necrosis that is followed by a nonspecific inflammatory process and fibrosis. An increase in mural collagen5,36 might account for increased vessel wall rigidity.2,4,22 Since contractility and wall compliance were preserved in the clentiazem-treated group, we assume that it interferes with the initial steps in the self-perpetuating cascade of damage and the subsequent vascular wall response. The protection afforded by clentiazem is probably due to the prevention of excessive calcium entry that prevents calcium overload.4 A critical period, perhaps only a few hours in length, has been proposed, during which a calcium-channel blocker may be effective in preventing the subsequent vasospasm.2,25

Increased vessel wall rigidity has been correlated directly with angiographic narrowing, and this is presumably due to altered physical properties of the artery wall.23 Since loss of contractility can also be correlated with irreversible narrowing, excessive active muscle tone does not make a sizeable contribution to narrowing. The arterial narrowing observed in the clentiazem-treated group was reversed by papaverine. This indicated the presence of a small, reversible component presumably resulting from peripheral vasoactive substances or due to spontaneous, presumably myogenic, increases in tone. It is more pronounced in pial resistance vessels than in large conducting vessels at the base of the brain.2,24

References


**KEY WORDS** • basilar artery • calcium channel blockers • cerebral vasospasm • rabbits
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