Diltiazem Protects Against Functional Changes in Chronic Cerebrovasospasm in Monkeys

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Diltiazem given 48 hours before experimental subarachnoid hemorrhage protects the cerebral vasculature of monkeys against the widespread cerebrovascular spasm seen on angiography after 5–6 days and against associated neurologic defects. In vitro examination of the cerebral arteries from treated monkeys shows that compared with untreated animals, the functional changes in the vascular smooth muscle cells, the increase in arterial wall stiffness, and the decrease in contractility, all of which were prominent in untreated monkeys, were relatively small. Other changes such as abnormal spontaneous myogenic tone, decreased responsiveness to constrictor and dilator nerve activation, and other indexes of neuronal function were little influenced by the drug. We suggest that chronic cerebrovasospasm may be initiated by the combined action of exceptionally high concentrations of a number of putative spasmogens causing injury to the larger cerebral arteries. However, the later development of intractable spasm is related to the location of blood clot and to the involvement of the vascular wall in an inflammatory process. The combined insult results in pathologic changes in the artery wall resulting in increased thickness and stiffness. Diltiazem acts on cerebrovascular smooth muscle in lower concentrations than on smooth muscle in other vascular beds, interfering with calcium entry through receptor-operated and potential-sensitive channels, and may protect against calcium-induced cell death through these and additional actions. Protection against early events presumably prevents the genesis of the subsequent chronic state. (Stroke 1988;19:73–79)

We have previously described some of the structural and functional changes found in cerebral arteries of monkeys associated with chronic cerebrovasospasm 5–6 days after subarachnoid hemorrhage.‡ 1,2 Bleeding was induced by withdrawal of a needle previously placed in the intracranial portion of the internal carotid artery according to the method of Frazee.3 Subsequently, a predominantly unilateral widespread arterial narrowing was seen by angiography; although the exact distribution is not the same in all monkeys, there is invariably a neurologic deficit. In vitro studies of the larger arteries show that the arterial narrowing is associated with a marked increase in the stiffness of the arterial wall, abnormal spontaneous increases in tone, and a marked reduction in both constrictor and dilator nerve control. The arteries are modestly hypersensitive to agonists, particularly serotonin, and there is a very marked reduction in contractility, presumably associated with vascular smooth muscle cell damage.

A number of theories of chronic vasospasm have been advanced (see Bevan et al1 for brief discussion). Our findings favor the conclusion that, at least in the larger arteries, narrowing after 1 week is predominantly due to passive changes in the artery wall subsequent to injury and associated fibrotic change. Our experiments are not pertinent to the origins of vasospasm although our findings are consistent with the initial role of high concentrations of vasoconstrictor substances released from sympathetic nerves, blood, or blood clot and possibly from other adjacent tissues and their subsequent damage to the arterial vasculature.4–14

When these experiments were initiated, we hypothesized that putative spasmogens caused constriction of vascular smooth muscle by opening receptor-operated and/or potential-sensitive channels in cerebrovascular smooth muscle cells, allowing the entry of extracellular calcium.15 Development of cerebrovascular smooth muscle tone depends almost exclusively on the entry of calcium from the extracellular space through such channels.16 Furthermore, the calcium entry pathways in cerebrovascular smooth muscle are more sensitive to calcium channel blockers such as diltiazem and nimodipine than those in blood vessels from many other vascular regions.17–19 It seemed reasonable to us that chronic cerebrovasospasm originates from the action of these spasmogens; it might be prevented by a calcium channel antagonist. Accordingly, monkeys destined for experimental subarachnoid hemorrhage were pretreated with diltiazem, a calcium channel antagonist that shows cerebrovascular selectivity,19–22 48 hours prior to its induction. Subsequent angiographic and neurologic examination showed marked amelioration of the cerebral spasm and the absence of neurologic change.23 Angiographic data of the monkeys has been described.23 This drug regimen was adopted to test the hypothesis that calcium entry into cells was an essential causal step in the genesis of the chronic spastic state.

In this article we describe the in vitro study of functional changes of cerebral arteries in a series of mon-
keys pretreated with diltiazem and subjected to experimental subarachnoid hemorrhage. These findings are compared with those previously found in an untreated group. In keeping with the angiographic and clinical evidence, our analysis revealed considerable protection by diltiazem, particularly of the nonneuronal elements of the artery wall. We suggest that this drug interferes with the entry of calcium and possibly with its subsequent cellular toxicity, which lead to wall damage and rigidity.

Materials and Methods

The methodology employed was similar to that described previously. Diltiazem (25 mg/kg twice daily) was administered in apple juice to six monkeys commencing 24 hours before the baseline angiography, neurologic examination, and measurement of arterial pressure by arterial catheter. Subarachnoid hemorrhage was induced by puncture of the internal carotid pressure by arterial catheter. Subarachnoid neurologic examination, and measurement of arterial mencing 24 hours before the baseline angiography, was administered in apple juice to six monkeys compared with those previously found in an untreated article) and the untreated (see reference 1) were compared. Data are expressed, except where stated otherwise, as means = SEM. Significance was inferred when p < 0.05.

Results

Relation Between Passive Wall Force and Vessel Circumference: Wall Thickness

Although arterial segments from the injured side showed a tendency to develop greater passive wall force compared with arterial segments from the control side of the same monkey, a statistical comparison at a variety of lengths showed no significant difference between the two sides (Figure 1). The mean percent increase in passive wall force at lengths 2, 4, 6, and 8 mm beyond the maximum unstretched half-circumference (L0) for all pairs of segments was 155 ± 37% of control, compared with 449 ± 125% in the untreated series of monkeys.

In two instances (both middle cerebral artery segments) a satisfactory passive wall force–vessel circumference relation on the injured side could not be obtained experimentally. The tissues did not show normal stress relaxation after stretch. The experimental records, although irregular, showed sudden, abrupt, irreversible reductions in wall force after stretch as though the segment had suddenly and irreversibly elongated. A “normal”-looking relation was observed in control segments. These two cerebral artery segments corresponded with angiographic measurements of lumen diameters 105 and 82% of the control side.

There was no significant difference between wall thickness and vessel circumference of corresponding artery segments from the two sides; values from the injured compared with the control side for thickness and radius were 94 ± 6% and 93 ± 8%, respectively. Significant increases in wall thickness were found on the injured side in the untreated series.

![Figure 1. Passive wall force-length (1/2 vessel circumference) curves from anterior and middle cerebral arteries of monkeys 5-6 days after experimental subarachnoid hemorrhage. Monkeys were treated with diltiazem commencing 48 hours before hemorrhage and throughout experimental period. Corresponding segments were taken from the side of arterial puncture and the contralateral side. Particular segments are not identified on graph.](http://stroke.ahajournals.org/)
Spontaneous Tone Activity

Large spontaneous excursions of tone, usually lasting many minutes but often as long as an hour, were seen in most segments. Occasionally these excursions seemed to be generated by changing the bath solution, but this was not consistent even in the same segment. This activity was not seen in sham-operated monkeys. The extreme variability of this phenomenon makes a quantitative comparison between segments from different monkeys virtually impossible. It is our impression that the irregular activity was greater on the injured side and was usually greater in anterior compared with middle cerebral artery segments. It occurred in segments of arteries that on angiography showed little or no narrowing (97 ± 3.7%; n = 10, expressed as percent diameter of injured compared with control side).

Due to the irregular spontaneous activity, the following measurements could not be made in all segments and were not always determined under comparable conditions.

Contractility

The maximum capacity of anterior and middle cerebral artery segments from injured compared with control sides to develop force to 10⁻⁴ M NE plus 10⁻³ M serotonin was not significantly different (115 ± 27%; n = 10); this contrasts with a value of 32 ± 10% found between contractility and angiographic diameter (Fig. 2). Spontaneous Tone Activity

**Efferent Nerve Function**

Electrical field stimulation. Transmural nerve stimulation, when the artery had little or no active intrinsic tone, resulted in contraction. This was presumably the result of adrenergic nerve activation since in previous experiments field-induced tetrodotoxin-sensitive contraction was prevented by 5 x 10⁻⁶ M guanethidine. Mean values of injured:control anterior middle cerebral artery segments for the three tested frequencies (2, 4, and 8 Hz) were 38.63 ± 11.8% (n = 16; means for six grouped experiments); the corresponding value for the untreated series was 23.41 ± 6.0%. These means are not significantly different. Electrical field stimulation after 5 x 10⁻⁶ M guanethidine and after the artery was precontracted with prostaglandin F₂₀ (PGF₂₀) caused a frequency-dependent relaxation when expressed in the same way. The total mean ratio was 19.54 ± 5.55% (n = 26), which contrasts with a value of 54 ± 22.7% seen in the untreated series. Neither pairs of values for the two series are different from each other. There is no relation between the size of reduction of neurogenic vasoconstriction and neurogenic vasodilation, nor between the reduction in contractility and reduction in neurogenic response.
The increase in vascular smooth muscle tone with PGE$_2$ was reduced by 10$^{-2}$ M sodium nitrite or 10$^{-5}$ M papaverine but not by 10$^{-6}$ M acetylcholine.

**Choline Acetyltransferase**

The enzyme activity expressed as percent injured: control levels was significantly less reduced in the treated compared with the untreated series, 65.24 ± 6.1% compared with 31 ± 10.2%.

**Neuronal Uptake of Tritiated Norepinephrine**

In contrast to the untreated series, uptake of [3H]NE into the nerve terminal was not different in segments from the two sides and was 0.983 ± 0.526 and 0.96 ± 0.39 pM/g wet weight, respectively.

**Discussion**

The calcium channel antagonist diltiazem has been shown to protect the cerebral artery bed from the vasospastic consequences of experimental subarachnoid hemorrhage.$^{23}$ This conclusion is based on the prevention of a reduction in arterial lumen observed angiographically and of a neurologic deficit. This article describes the results of a functional in vitro examination of cerebral arteries from diltiazem-treated monkeys and includes a comparison with the findings of an in vitro study of arteries from an untreated series.$^1$ In summary, cerebral arteries of the treated monkeys did not show the increase in arterial wall rigidity and the decrease in vascular smooth muscle contractility as did the untreated group. Abnormal, presumably myogenic, activity of the larger cerebral arteries was present, but its irregularity precluded quantitative comparison. The relative increase in contractile response to serotonin compared with NE that occurred after hemorrhage was prevented by diltiazem. Except in the case of choline acetyltransferase activity, measurements of neuronal function (force change to stimulation of adrenergic and dilator nerves) and neuronal uptake of [3H]NE were not influenced. Preliminary structural studies$^2$ are consistent with these functional findings. The changes observed, particularly in the tunica media and tunica adventitia of untreated monkeys, were reduced in the diltiazem-pretreated monkeys. There was less evidence of infiltration by inflammatory cells and fibrosis. On the other hand, in the segments examined the adventitial nerves showed variable degrees of reduction in catecholamine fluorescence and evidence of nerve terminal and axonal degeneration in electron micrographs.

Artery function was tested in vitro in the absence of diltiazem, which, in view of its protective effect, we must presume has access to the cerebral artery wall in vivo. Thus, in vitro findings do not necessarily reflect the function of arteries in vivo. It seems quite likely that diltiazem present in the vessel wall would wash out in vitro by the time that these measurements were made. However, we think it unlikely that diltiazem would have an effect on passive wall force–vessel circumference relations in vitro or in vivo. The irregular spontaneous myogenic activity is calcium-dependent and manganese- and diltiazem-sensitive (J.A. Bevan, unpublished results). Since this activity occurred in segments from vessels that on angiography showed little or no narrowing, it seems likely that such activity was inhibited in vivo by diltiazem. Maximum contractility, measured at the end of the in vitro experimental protocol of about 7–8 hours duration and after many changes in the bath solution, is the maximum contraction recorded to a combination of agonists. It was measured when diltiazem presumably had been washed out of the tissue, and thus it seems unlikely that the measurement was compromised because the artery segment was taken from a diltiazem-treated monkey. Furthermore, unless diltiazem preferentially reduced the contractility of segments from the control compared with the injured side, the perceived protection of contractility by the drug would be valid.

Diltiazem is a calcium channel antagonist with some propensity for the vascular smooth muscle cells of the cerebral circulation. However, it might have actions on other calcium-dependent mechanisms. For example, diltiazem might alter the amount of hemorrhage that followed the arterial puncture and therefore the size and localization of the clot; it might alter mechanisms involved in clotting or fibrinolysis or the inflammatory process. Since clot size and blood staining were not quantified, there is no definitive answer to these questions. There is little evidence that diltiazem changes coagulation processes. Damage to efferent nerves was present irrespective of diltiazem treatment. If the protection of diltiazem were due to an alteration in clotting, it would be expected that nerve function as well as vascular function would be less compromised. Clearly this was not the case.

To understand the protective action of diltiazem in chronic cerebrovasospasm (Figure 3), it is important to appreciate that the time course of the spasm is complex and probably two-phased.$^{24–26}$ It seems likely that the initiating event that eventually leads to chronic arterial narrowing in subarachnoid hemorrhage is the presence around the arteries at the base of the brain of a variety of constrictor substances derived from nerves, blood clot, and other tissue and of circulating vasoactive...
substances associated with increased sympathetic activity (see Table 1 for a limited list of putative spasmogens and calcium channel antagonists reported to be active against them). These spasmogens presumably cause substantial increases in tone through the entry of calcium through plasmalemmal channels. This effect, together with the associated ischemia, would lead to a loss of endothelium, as occurs in myocardial infarction and which, because of the loss of endothelial dilator influences, would exacerbate the constriction. The local adherence of platelets to the intimal surface would tend to reinforce these vasoconstrictor effects. Cell damage may result from the intense long-lasting contraction. Both NE in high concentration and blood applied directly to cerebral arteries are known to cause myonecrosis.

Whatever the explicit cause of the cell damage (and it may result from free radical generation), it would result in inflammation, edema, and fibrosis of the artery wall. This would form the basis for the decreased wall distensibility, the increase in total wall thickness, and the reduced contractility. The concept that inflammation may be pivotal in the pathogenesis of vasospasm is supported by the findings that anti-inflammatory agents given before hemorrhage significantly lessen the resulting spastic change.

We suggest that diltiazem protection may be due to the prevention of calcium entry into the smooth muscle cells during the initial part of the sequence that leads to chronic narrowing, but possibly via other actions as well. Calcium in high concentrations is known to cause cell death. Cells appear to be protected by calcium

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<th>Putative spasmogen</th>
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<th>Calcium channel blocker</th>
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<td>Blood</td>
<td>Diltiazem</td>
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<td>Murata et al, 1982</td>
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<td>D600</td>
<td>Nimodipine</td>
<td>White and Robertson, 1983</td>
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<td>Nicardipine</td>
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<td>Yamamoto et al, 1983</td>
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<td>J.A. Bevan, unpublished</td>
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<td>Diltiazem</td>
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<td>Allen and Banghart, 1970</td>
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<td>Diltiazem</td>
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<td>Bevan, 1982</td>
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<td></td>
<td>Nifedipine</td>
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<td>Allen and Banghart, 1979</td>
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<td>Van Neuten and Vanhoutte, 1981</td>
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<td>Nicardipine</td>
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<td>Yamamoto et al, 1983</td>
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<td>Diltiazem</td>
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<td>Shimizu et al, 1980</td>
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channel antagonists in ways that are not understood. In addition, diltiazem probably interferes with the abnormal myogenic activity that occurs in these cerebral arteries.

Nosko et al. examined the efficacy of nimodipine in preventing chronic cerebral vasospasm and delayed ischemia after subarachnoid hemorrhage in monkeys. Spasm was induced by placing autologous hematoma against the major vessels at the base of the brain. Unlike our present study, the calcium channel antagonist was administered starting 14–20 hours after clot placement. They found no significant difference in the incidence of chronic cerebral vasospasm. Obvious possible reasons for this difference include the use of a different calcium channel blocker (diltiazem is of a different chemical class than nimodipine). Furthermore, the timing of commencement of treatment might be of some importance. Preliminary results of our own trial (J.G. Frazee, R.D. Bevan, and J.A. Bevan, unpublished observations) with diltiazem administered 24 hours after hemorrhage does indicate some protective effect of this agent given after the initiating event.

The concept that chronic cerebrovasospasm evolves from an initial potentially reversible state is consistent with other observations. Varsos et al. showed that intravenous aminophylline given on the first and third days after intracisternal blood reversed cerebral constriction in dogs. Neither aminophylline, papaverine, nor nifedipine were effective on the fifth day. The lack of action of drugs on established vasospasm is well documented. It must, however, be noted that Fleischer et al. reported clinical improvement associated with the use of aminophylline, a drug that might be expected to reverse myogenic activity. Clinical improvement has been noted with nimodipine. Norwood et al. claimed reversal of presumably established chronic cerebrovasospasm using salbutamol and aminophylline in monkeys and found that the diameter of the vessels after the drug was administered approached control levels. We can only comment that neither aminophylline nor salbutamol would be expected to change or reverse established rigidity of the arterial wall. Gioia et al. found in a canine model of chronic cerebral vasospasm that nimodipine reversed blood-induced narrowing. However, we know very little about the exact nature of the arterial narrowing that was studied in that instance.

In summary, diltiazem exerted a protective effect against experimental chronic cerebrovasospasm in monkeys. It appeared to markedly protect against vascular smooth muscle damage but not against nerve damage. We suggest that this protection inhibited the sequence of events that culminates in chronic spasm, as evidenced by the absence of change in passive wall properties, and in contractility and hypersensitivity. It seems likely that the abnormal myogenic activity may be inhibited in vivo in diltiazem-treated animals. This protection is most likely due to antagonism of excessive early vasoconstriction caused by release of vasoconstrictor agents from a number of sources close to the arteries at the base of the brain.

Acknowledgments

It is a pleasure to acknowledge assistance in the experimental execution of these studies by M. Owen, PhD, J. Brayden, PhD, I. Laher, PhD, J. Walsmsley, PhD, J. Hwa, J. Laher, and Henry Young, MD.

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Bevan et al Diltiazem and Cerebrovasospasm

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KEY WORDS • calcium channel blockers • diltiazem • monkeys • cerebral vasospasm • subarachnoid hemorrhage
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Stroke. 1988;19:73-79
doi: 10.1161/01.STR.19.1.73

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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