Prevention of Experimental Subarachnoid Hemorrhage-Induced Intracranial Arterial Vasonecrosis with Phosphodiesterase Inhibitor Phthalazinol (EG-626)

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SUMMARY
Experiments in monkeys have demonstrated that the chronic vasospasm and arterial injury reaction produced by subarachnoid blood injection can be prevented by treatment with the phosphodiesterase inhibitor phthalazinol. This protective effect, which is present even when the drug treatment is initiated after the subarachnoid blood injection, is presumed to occur because of the platelet-inhibiting effect of phthalazinol.

THE INTRACRANIAL arterial narrowing which occurs after subarachnoid hemorrhage remains poorly understood in spite of extensive investigation. Most clinicians, nevertheless, believe that it may be responsible for significant morbidity in patients after rupture of an intracranial aneurysm. The clinical deterioration is presumably secondary to ischemia and the process is generally called vasospasm.

We have reported previously that the arteries from experimental animals with vascular narrowing induced by subarachnoid blood injection show evidence of arterial injury characterized by endothelial cell loss, smooth muscle death, and intimal proliferation. This observation has been confirmed by others. We have proposed, therefore, that the term vasospasm is a misnomer and that the term vasonecrosis would be more appropriate.

The mechanisms responsible for this vascular injury have not been elucidated but because of its similarity to the platelet-mediated arterial injury reaction seen in systemic arteries following a variety of insults, we suspect that blood platelets play a role in its progression. On this basis we have experimented with platelet-blocking agents in an attempt to prevent intracranial arterial narrowing and vasonecrosis. Our experience with the phosphodiesterase inhibitor phthalazinol is the subject of this report.

Methods
Sixteen adult Macaca arctoides (stump tail) monkeys weighing 6 to 11 kg were used in this study. All experiments were performed with the animal under sodium pentobarbital general anesthesia.

Using sterile precautions and microsurgical technique a C2 laminectomy was performed on each animal. After the dura was opened, the arachnoid was incised immediately beneath the C2 nerve root on the left side and a 4 cm silastic catheter, one mm in diameter, was passed ventral to the spinal cord cephalad into the preponine cistern. The final position of these radiopaque tipped catheters was determined by x-ray. The operative wound was closed in layers with the ligated end of the catheter left in the subcutaneous tissue for subsequent injection.

After 5 days the neck was opened superficially and the catheter connected to a 3-way stop cock for simultaneous injection and pressure recording. A femoral artery cut down was performed in one-half the animals to evaluate by retrograde catheter angiography the intracranial arteries before blood injection. All animals were injected with 4 cc of nonheparinized autologous arterial blood into the prepontine cistern. The 8 monkeys in the experimental group received phthalazinol 50 mgm/kg daily beginning 2 hours after the subarachnoid blood injection. The drug was administered orally, suspended in Tang (artificial orange juice). The control monkeys received a similar amount of Tang but without phthalazinol. All monkeys had individual cages and ate their usual diet.

The animals were sacrificed 10 days after the blood injection by rapid excision of the heart followed by total removal of the previously exposed brain. The same animals that had angiography at the time of blood injection had repeat angiography prior to sacrifice. The brain was immersed in 4% phosphate-buffered glutaraldehyde within one minute of opening the chest.

The tissue for electron microscopy was post fixed in 2% phosphate buffered osmium tetroxide, embedded in Epon, sectioned with an LKB microtome and examined with a Zeiss EM 9A electron microscope. Sections were stained with uranyl acetate and lead citrate.

Results
Angiograms performed on 4 of the phthalazinol-treated monkeys before and after blood injection revealed no vascular narrowing (fig. 1) whereas 3 of the 4 untreated monkeys evaluated angiographically had significant reduction of arterial calibre (fig. 2). Careful light and electron microscopic analysis of the basilar arteries removed from the 8 monkeys treated with phthalazinol revealed no evidence of...
FIGURE 1. Vertebal angiograms performed before (A) and 3 days after (B) subarachnoid blood injection into the prepontine cistern of a monkey. Note the normal calibre of the vertebral and basilar arteries in A. In B, careful inspection will reveal contrast within the vertebral artery at the base of the skull (V) but only faint contrast within the intracranial vertebral and basilar arteries.

Vasonecrosis in 4, a focal area of vasonecrosis in 1 and diffuse but mild vasoedema in 3. This is compared with the universal appearance of marked vasonecrosis in the 8 control monkeys which received the same amount of intracisternal blood but no phthalazinol.

Examples of normal, untreated, and treated basilar arteries are shown (figs. 3, 4, 5).

Because vasonecrosis occurs in 100% of the controls and because the electronmicrographs provide a much more graphic and unequivocal picture of the process,

FIGURE 2. Vertebal angiograms performed before (A) and 3 days after (B) subarachnoid blood injection into the prepontine cistern of a monkey treated with phthalazinol beginning 2 hours after the blood injection. Note the calibre of the vessels.
we will emphasize the pathology or lack thereof, not the angiography.

The basilar arteries of the untreated monkeys show a consistent pattern of damage. The endothelium is necrotic, edematous or stripped from the elastica. The smooth muscle of the media is contracted and the undulations of the attached elastica are sharp. Beneath these corrugations there are deposits of cell debris and organelles. The basement membrane is swollen and 2 or 3 layers of proliferating smooth muscle cells are surrounded by edematous ground substance. The endoplasmic reticulum is grossly expanded and the mitochondria in the proliferative cells and the media are degenerate. Smooth muscle necrosis in the media is widespread and accompanied by an increase in the number of collagenous fibers. The described damage involves 30-40% of the circumference of the vessel.

Of the 8 treated monkeys in this study none showed evidence of the severe vasonecrosis seen in the controls. The basilar arteries from 4 animals showed no evidence of injury and could not be distinguished from normal. The basilar arteries from 3 monkeys had mild diffuse edema but without loss of the endothelium or necrosis of the media. Careful examination of the basilar artery from one animal revealed one focal area of intimal proliferation. Except for this single incidence of focal abnormality the arteries from the treated animals appeared to be protected from significant vasonecrosis. No proliferating cells are seen in the subendothelial space. The endothelial cytoplasm appears normal and the intercellular junctions of the endothelial cells are tightly interdigitated. The basement membranes are not fragmented. No necrosis is apparent in the media.

**Discussion**

These experiments show that the arterial injury reaction which occurs after experimental subarachnoid hemorrhage in monkeys is prevented by the phosphodiesterase inhibitor, phthalazinol. Angio-
Because of the known profound effect of phthalazinol on blood platelets we postulate that its protective effect is due to inhibition of platelet aggregation. An alternative hypothesis would be that the smooth muscle relaxing effect of phthalazinol could prevent vascular constriction and secondary arterial injury. We subscribe to the platelet related hypothesis because experiments by others on systemic arteries have revealed that platelets are essential for animals to develop intimal proliferation.

It is known that aggregating platelets liberate the prostaglandin metabolite thromboxane A₂, which, in addition to being a potent vasoconstrictor, causes arterial injury and stimulates intimal proliferation. The intimal proliferation is thought to be secondary to duplication and migration of smooth muscle cells. Further investigation will be required, however, to prove that platelets are essential for the injury reaction seen in brain arteries and to establish the mechanism of action of phthalazinol in preventing the damage.

It is noteworthy that phthalazinol can be administered after the subarachnoid blood injection and still prevent the damage from occurring. Other compounds which have been reported to prevent vasospasm, such as phyribenzamine, require pretreatment of the animal to be effective. We have not determined the time interval for the initiation of treatment beyond which this protection does not exist.

Before phthalazinol is considered for a clinical trial in subarachnoid hemorrhage patients its effect on the bleeding and clotting time needs to be determined. It is conceivable that the antiplatelet function of this potent phosphodiesterase inhibitor would increase the risk of rebleeding in a patient with an intracranial aneurysm. No data on this subject are available.
Figure 5. Electronmicrograph of luminal surface of basilar artery from untreated monkey removed 10 days after subarachnoid blood injection. The endothelium (e) is vacuolated and separated from the elastica (el). The elastica is tightly folded and necrotic smooth muscle cell debris is present beneath the convolutions. The deeper smooth muscle cells (s) are contracted. ×7,000.
Figure 6 (top) & 7 (bottom). Electronmicrograph of luminal surface of basilar artery from monkey treated with phthalazinol for 10 days after subarachnoid blood injection. The endothelium (e), elastica (el), and smooth muscle cells appear normal. ×7,000.
References


Attenuation of Ischemic Brain Edema by Pentobarbital after Carotid Ligation in the Gerbil

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SUMMARY The efficacy of pentobarbital in the treatment of ischemic cerebral edema was evaluated in 160 gerbils. Animals underwent carotid ligature under ether or pentobarbital (50 mg/kg) anesthesia. The pentobarbital anesthetized group received an additional dose of 30 mg/kg 4 h after ligation. Animals were evaluated for neurologic deficit at 4 and 8 h after ligation, then sacrificed. Water content of each hemisphere and swelling percentage were calculated from the wet and dry weights of the hemispheres. Swelling percentage in animals anesthetized with ether was 6.37 ± 0.89 SE, whereas gerbils who underwent sham carotid ligature showed a negligible (0.491 ± 0.15) swelling percentage (p < 0.01). Pentobarbital animals had a swelling percentage of 3.359 ± 0.68. This represents a significant edema reduction compared to ether-anesthetized animals (p < 0.01). Neurologic deficit was decreased by 56.7% (17/60 vs 30/60) in pentobarbital animals compared with ether animals (p < 0.025). Mortality at 8 hours was reduced by 75% (2/60 vs 8/60) in pentobarbital animals (p < 0.05).

MANY INVESTIGATORS have shown that barbiturates provide a "protective" effect for the brain in regional, as well as global ischemia. Recently, barbiturates have also been shown to inhibit the formation of cerebral edema produced by cryogenic injury. The development of edema in the ischemic brain plays a significant role in the severity and final outcome of an ischemic process.

Unilateral carotid artery ligation in the Mongolian gerbil results in fatal cerebral infarction in 40 to 60% of animals. The affected animals develop neurologic deficit, including seizures, and usually die shortly thereafter with evidence of complete ipsilateral hemispheric infarction.

This study was designed to evaluate the effect of pentobarbital on the development of ischemic brain edema 8 h after unilateral carotid artery ligation in the gerbil.

Materials and Methods

The experimental series consisted of 160 adult Mongolian gerbils divided into 3 groups. Forty animals underwent a sham operation, 20 of them receiving ether anesthesia and 20 pentobarbital 50 mg/kg i.p. Sixty untreated-ligated were anesthetized with ether, and 60 barbiturate-treated animals were anesthetized with pentobarbital 50 mg/kg i.p. Prior to surgery all animals received 1% trypan blue 1 cc. i.p. and either pentobarbital or an equivalent volume of normal saline.

The right common carotid artery was exposed through a 2 cm midline cervical incision, carefully isolated from adjacent structures, coagulated with bipolar current and divided. In the sham-operated animals the right carotid artery was exposed and isolated, but neither coagulated nor divided.
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