The effect of timing of clot removal on chronic vasospasm in a primate model

Yuji Handa, M.D., Bryce K. A. Weir, M.D., Michael Nosko, M.D., Ph.D., Russ Mosewich, Tsutomo Tsuji, M.D., and Michael Grace, Ph.D.

Department of Surgery, Walter Mackenzie Health Sciences Centre, University of Alberta, Edmonton, Alberta, Canada

The effect of complete clot removal at times from 48 to 96 hours after subarachnoid hemorrhage (SAH) on the development of chronic cerebral vasospasm was evaluated to determine whether there is a critical point after which clot removal is ineffective in preventing vasospasm. Thirty cynomolgus monkeys were randomized to one of five groups: sham-operated group, clot removal at 48 hours after SAH (48-hour group), clot removal at 72 hours after SAH (72-hour group), clot removal at 96 hours after SAH (96-hour group), and clot placement only (clot group). Standard microsurgical techniques were used to dissect bilaterally the major cerebral arteries free of arachnoid. An autologous blood clot averaging 4.2 gm was placed around the vessels in the subarachnoid space of the monkeys in the 48-hour, 72-hour, 96-hour, and clot groups. Physiological saline was instilled into the subarachnoid space of the sham-operated animals. Animals in the clot-removal groups underwent surgical clot removal at the determined times for each group. Two animals in each of the sham-operated and clot groups were subjected to reoperation at each of 48, 72, and 96 hours after SAH. The incisions were reopened and then simply reclosed. Neurological status, angiographic cerebral vessel caliber, and physiological status were evaluated before and 7 days after SAH induction. There were no significant neurological deficits in the sham-operated, 48-hour, or 72-hour groups. Two animals in each of the 96-hour and clot groups showed deterioration in level of consciousness developing on Day 4 or 5 after SAH induction. All the major cerebral arteries of the animals in the clot and 96-hour groups showed significant vasospasm (p < 0.01) on Day 7. Animals in the 72-hour group had significant vasospasm (p < 0.05) of the internal carotid and middle cerebral arteries but not the anterior cerebral arteries. There was no significant vasospasm (p > 0.05) in any of the cerebral arteries in the 48-hour group. Severity of vasospasm paralleled the duration of contact between the blood clot and the cerebral vessels. Evacuation of the subarachnoid hematoma later than 48 hours after SAH resulted in no significant reduction in the degree of chronic cerebral vasospasm. It is suggested that clot removal at early operation is likely to be useful only if it is performed within 48 hours of SAH.

Key Words • subarachnoid hemorrhage • vasospasm • clot removal timing • cynomolgus monkey

Although the pathophysiology of chronic cerebral vasospasm following subarachnoid hemorrhage (SAH) is still unclear, it is certain that blood clot in the subarachnoid cisterns is related to the development of chronic cerebral vasospasm, and its amount is predictive of the severity of cerebral vasospasm. Mechanical removal of clot in the subarachnoid space, especially during early surgery, has been performed in an attempt to decrease the degree of vasospasm and the incidence or severity of delayed ischemic deficit caused by it. The efficacy of early operation and clot removal is still uncertain.

The primate model of SAH developed in our laboratory reliably produces chronic cerebral vasospasm, thereby affording the opportunity to evaluate mechanical clot removal. A previous study by Nosko, et al., demonstrated that clot removal 24 hours after induction of SAH in the primate model completely prevented the development of chronic cerebral vasospasm. The present study evaluated the effect of clot removal at various times from 48 to 96 hours after SAH on the development of chronic cerebral vasospasm in order to determine whether there is a critical time after which clot removal is ineffective.

Materials and Methods

Protocol

Thirty female cynomolgus monkeys (Macaca fascicularis), weighing an average of 3.3 kg (range 2.6 to
Timing of clot removal in vasospasm

4.1 kg), were separated by a restricted randomization method into five groups of six animals. After subarachnoid placement of clot, the groups underwent: sham operation (sham-operated group), clot removal 48 hours after induction of SAH (48-hour group), clot removal 72 hours after SAH (72-hour group), clot removal 96 hours after SAH (96-hour group), or no clot removal (clot group).

Before induction of the SAH, cerebral angiography was performed on all animals to evaluate the baseline cerebral artery calibers, mean arterial blood pressure (MABP), and heart rate. All animals then underwent bilateral craniectomy and dissection of the arachnoid membrane from around the cerebral arteries on Day 0. Forty-eight hours after the craniectomy the animals in the 48-hour group underwent repeat craniotomy for clot removal. At 72 hours after induction of SAH the animals in the 72-hour group underwent repeat craniotomy for clot removal. At 96 hours the 96-hour group underwent repeat craniotomy for clot removal. The sham-operated group and the clot group were each randomly divided into three subgroups of two, each of which underwent repeat craniotomy at either 48 hours, 72 hours, or 96 hours after induction of SAH; the incision and the dura were merely opened and closed in these animals in order to provide controls for the second craniotomy procedure of the clot-removal groups.

Neurological status of all animals was assessed at least twice daily after the first craniectomy. A five-grade system was used in which Grade 1 indicated a normal status and Grade 5 represented a moribund animal. Individual assessment was made for each limb with respect to motor power and muscle control. On Day 7, all animals underwent repeat evaluation of baseline parameters and were then sacrificed by exsanguination under general anesthesia. This protocol was evaluated and approved by the Animal Ethics Review Committee at the University of Alberta. The care and surgery of the animals were performed according to the standards of the Canadian Council on Animal Care.

Operative Procedure

Anesthesia was induced with intramuscular ketamine hydrochloride (6 to 10 mg/kg) for the angiography, or intravenous sodium pentobarbital (26 mg/kg) for the craniotomy. Animals were intubated and ventilated with a 2:1 mixture of N\textsubscript{2}O:O\textsubscript{2} administered with a variable-phase respirator. Blood gas analysis was performed to adjust and maintain pCO\textsubscript{2} between 38 and 39 mm Hg during angiography and between 30 and 35 mm Hg during craniotomy. Animals were paralyzed with gallamine, 2 mg/kg, injected intravenously every 45 minutes. Procaine penicillin, 100,000 IU/kg, was given intramuscularly 30 to 60 minutes before the operation. Body temperature was maintained at 37°C by a heating pad and monitored by a rectal thermometer.†

Using a sterile technique, the femoral artery on either side was catheterized with a No. 5 French radiopaque polyethylene catheter with a sigmoid tip. The catheter was advanced under fluoroscopic control and placed in the innominate artery for cerebral angiography. The catheter was connected via a three-way stopcock to a pressure transducer for monitoring arterial blood pressure and to a Cordis injector for administering iothalamate meglumine for angiography.‡ One arterial-phase anteroposterior angiogram was obtained on each occasion by injecting 10 ml of iothalamate meglumine at 300 psi.

After each procedure, paralysis was reversed with intravenous injections of prostigmine, 0.07 mg/kg, and atropine sulfate, 0.02 mg/kg. The animals were extubated after the return of the gag reflex.

Induction of Subarachnoid Hemorrhage

A right frontotemporal craniectomy was performed. Under the microscope, the dura was opened and the sylvian fissure was split with sharp dissection. The arachnoid over the middle cerebral, internal carotid, anterior cerebral, and posterior communicating arteries was opened, the temporal lobe was then retracted, and the remaining arachnoid over the posterior communicating artery was opened. Liliequist's membrane was incised and the arachnoid over the tip of the basilar artery and the posterior cerebral artery was opened. An autologous blood clot was then carefully placed around the exposed arteries. The dura was closed with 6-0 silk sutures, and the scalp incision was closed in layers with 2-0 silk and 3-0 monofilament polyethylene sutures.§ The same procedure was then repeated immediately on the left side. The total amount of blood clot placed in the bilateral subarachnoid space was recorded. In the case of sham-operated animals, 5 ml of normal saline was instilled into the dissected subarachnoid space in place of the blood clot.

Removal of Clot

Forty-eight hours after the SAH induction, the 48-hour group animals were anesthetized and the incisions were reopened. The dura was opened and the previously placed blood clot was removed carefully under the microscope. All fragments of removed hematoma were saved for weighing. The subarachnoid spaces were flushed with copious amounts of saline until all traces of blood were removed. The incisions were closed as in the first operation. The same procedure was carried out at 72 hours after SAH induction in the 72-hour group animals.

‡ Tele-thermometer manufactured by Yellow Springs Instrument Co., Yellow Springs, Ohio.
§ Polyethylene sutures (Dermalon) manufactured by Davis and Geck, New York, New York.
and at 96 hours after SAH induction in the 96-hour group.

In each subgroup of two animals in the sham-operated and the clot groups, the craniotomy was merely opened and closed at either 48, 72, or 96 hours after induction of the SAH with no manipulation of the hematoma or cerebral arteries. The animals were maintained under anesthesia for an average of 3 hours to duplicate the experience of the clot-removal groups.

**Radiological Assessment**

One arterial-phase anteroposterior angiogram was obtained before SAH induction and another on Day 7 just before sacrifice. Exposure factors were maintained constant, and a radiopaque control standard was used for correction to constant magnification. Calibers of cerebral vessels were measured six times in a blinded fashion with a calibrated optical micrometer, and mean values were determined.

Ten arterial measurements were obtained as follows: bilateral external carotid arteries; bilateral internal carotid arteries between the posterior communicating artery and the ophthalmic artery; bilateral anterior cerebral artery; bilateral middle cerebral artery; and the proximal and distal pericallosal artery. Serial measurements were recorded as percentage change from baseline values in individual animals and by group.

**Data Analysis**

All data were coded, entered into a computer, and edited. Data for hemodynamic variables were compared by Student’s t-test for paired variables among the five groups and between Days 0 and 7. The percent decrease of vessel caliber on Day 7 compared to baseline was analyzed for each vessel among the five groups. The level of significance for all tests of comparison was p < 0.05, unless otherwise stated.

**Results**

Comparison of data within each group and across the five groups revealed no significant differences in baseline values, in Day 7 values, or between baseline and Day 7 values in body weight or measured physiological indices (PaCO₂, MABP, and heart rate) (Table 1). Comparison between the clot group and the clot-removal groups revealed no statistically significant differences in the weight of clot placed (p > 0.05). The differences in the weight of clot placed and weight of clot removed in each of the clot-removal groups were statistically significant (p < 0.05). Mean percentages of the original weights of clot, removed at the second operation, were 26% for the 48-hour clot-removal group, 24% for the 72-hour clot-removal group, and 17% for the 96-hour clot-removal group. There were no significant differences in the weight of clot removed across the clot-removal groups.

**Neurological Status**

No animals developed hemiparesis due to delayed ischemic deficit. No animals in the sham-operated, 48-hour, and 72-hour groups showed neurological signs or significant disturbances of consciousness during the course of the experiments. Two animals in the 96-hour group became somnolent on Day 4. Two animals in the clot group also developed a decreased level of consciousness with a loss of aggressive behavior on Day 5.

### TABLE 1

**Measurements of physiological parameters and vessel diameters by group**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>Sham-Operated Group</th>
<th>Clot Removal Groups</th>
<th>Clot Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>48 Hrs</td>
<td>72 Hrs</td>
<td>96 Hrs</td>
<td>48 Hrs</td>
</tr>
<tr>
<td>no. of monkeys</td>
<td>30</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>body weight (kg)</td>
<td>3.29 ± 0.3</td>
<td>3.34 ± 0.2</td>
<td>3.02 ± 0.3</td>
<td>3.13 ± 0.4</td>
</tr>
<tr>
<td>MABP (mm Hg)</td>
<td>111 ± 10</td>
<td>104 ± 11</td>
<td>111 ± 9</td>
<td>113 ± 13</td>
</tr>
<tr>
<td>heart rate (min⁻¹)</td>
<td>149 ± 9</td>
<td>152 ± 5</td>
<td>154 ± 14</td>
<td>158 ± 5</td>
</tr>
<tr>
<td>PaCO₂ (mm Hg)</td>
<td>38.5 ± 0.4</td>
<td>38.7 ± 0.3</td>
<td>38.6 ± 0.5</td>
<td>38.4 ± 0.5</td>
</tr>
<tr>
<td>vessel caliber (mm)</td>
<td>rt extradural ICA</td>
<td>1.57 ± 0.09</td>
<td>1.51 ± 0.11</td>
<td>1.41 ± 0.16</td>
</tr>
<tr>
<td></td>
<td>lt extradural ICA</td>
<td>1.51 ± 0.09</td>
<td>1.43 ± 0.13</td>
<td>1.39 ± 0.17</td>
</tr>
<tr>
<td></td>
<td>rt supraclinoid ICA</td>
<td>0.97 ± 0.09</td>
<td>1.01 ± 0.11</td>
<td>0.88 ± 0.10</td>
</tr>
<tr>
<td></td>
<td>lt supraclinoid ICA</td>
<td>0.96 ± 0.09</td>
<td>0.97 ± 0.11</td>
<td>0.84 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>rt ACA</td>
<td>0.67 ± 0.09</td>
<td>0.65 ± 0.06</td>
<td>0.54 ± 0.11</td>
</tr>
<tr>
<td></td>
<td>lt ACA</td>
<td>0.73 ± 0.09</td>
<td>0.69 ± 0.05</td>
<td>0.65 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>rt sphenoidal MCA</td>
<td>0.80 ± 0.09</td>
<td>0.74 ± 0.04</td>
<td>0.70 ± 0.11</td>
</tr>
<tr>
<td></td>
<td>lt sphenoidal MCA</td>
<td>0.79 ± 0.09</td>
<td>0.76 ± 0.04</td>
<td>0.65 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>proximal pericallosal artery</td>
<td>0.82 ± 0.09</td>
<td>0.88 ± 0.10</td>
<td>0.77 ± 0.12</td>
</tr>
<tr>
<td></td>
<td>distal pericallosal artery</td>
<td>0.81 ± 0.10</td>
<td>0.84 ± 0.11</td>
<td>0.80 ± 0.09</td>
</tr>
<tr>
<td>weight of clot placed (gm)</td>
<td>4.3 ± 0.8</td>
<td>4.2 ± 0.5</td>
<td>4.1 ± 0.9</td>
<td>4.1 ± 1.0</td>
</tr>
<tr>
<td>weight of clot removed (gm)</td>
<td>1.1 ± 0.5</td>
<td>1.0 ± 0.3</td>
<td>0.7 ± 0.1</td>
<td>6</td>
</tr>
</tbody>
</table>

* Values are means ± standard error of the means. MABP = mean arterial blood pressure; ICA = internal carotid artery; ACA = anterior cerebral artery; MCA = middle cerebral artery.
Timing of clot removal in vasospasm

Fig. 1. Baseline angiogram on Day 0 (upper left), and Day 7 angiograms of a monkey in the sham-operated group (center left), the 48-hour group (lower left), the 72-hour group (lower right), the 96-hour group (center right), and the clot group (upper right). There is no evidence of vasospasm in the sham-operated monkey. The angiogram of the monkey in the 48-hour group shows mild vasospasm and that of the monkey in the 72-hour group shows moderate vasospasm of the intracranial vessels. Angiograms from the monkeys in the 96-hour group and the clot group show severe vasospasm of the intracranial cerebral arteries and also of the extracranial internal carotid artery. The severity of vasospasm parallels the duration of clot contact with the vessels.

Degree of Cerebral Vasospasm

Average reduction in vessel caliber compared to the baseline value was calculated individually for each vessel as well as for the combined values of the internal carotid, middle cerebral, and anterior cerebral arteries in each group. Severity of vasospasm was graded according to reduction in vessel caliber as follows: no vasospasm (< 10% reduction), mild vasospasm (10% to 30% reduction), moderate vasospasm (31% to 50% reduction), and severe vasospasm (> 50% reduction).

Typical vasospasm seen in each group is illustrated in Fig. 1, and the results are summarized in Fig. 2. One monkey in the sham-operated group exhibited no vasospasm in any of the vessels on Day 7, and the other five monkeys showed mild vasospasm of some vessels. The average reduction (−3.7%) in caliber of the intracranial vessels demonstrated no significant vasospasm.
Five monkeys in the 48-hour group developed mild vasospasm and another monkey developed moderate vasospasm. The average reduction in vessel caliber (−11.12%) in this group indicated mild vasospasm. Four of six monkeys in the 72-hour group developed mild vasospasm and the other two developed moderate vasospasm. In this group the average reduction in vessel caliber (−15.1%) represented mild vasospasm. In the 96-hour group, one monkey developed severe vasospasm, three developed moderate vasospasm, and one developed mild vasospasm. The average reduction in vessel caliber (−20.6%) in this group indicated moderate vasospasm.

In the clot group, one of six monkeys developed severe vasospasm and the other five exhibited moderate vasospasm. Average reduction in vessel caliber (−24.3%) in this group constituted moderate vasospasm.

An intergroup comparison of percentage decrease in vessel caliber on Day 7 compared to baseline was evaluated for each vessel (Table 2). There were no significant differences between the sham-operated and the 48-hour groups with any of the vessels (p > 0.05). In the 72-hour group, the reduction in vessel caliber of all cerebral arteries except the anterior cerebral artery showed statistically significant differences (p < 0.05) compared to the sham-operated group. The reduction in vessel caliber of all the vessels in the 96-hour group showed statistically significant differences (p < 0.01) between those in the sham-operated group and no significant differences (p > 0.05) between those in the clot group.

**Discussion**

There are many hypotheses regarding the pathogenesis of chronic vasospasm after SAH. The spasmogenic factors are suspected to be derived from the blood clot or the blood breakdown products in the cerebrospinal fluid.12,21 It is believed that there is some delay between the time of first blood contact with the vessels before there is effective constriction of the cerebral vessels. Removal of the subarachnoid clot at early operation has been suggested in order to remove the blood clot from around the vessels prior to the appearance of a sufficient concentration of spasmogenic agents by clot lysis to cause severe vasospasm.14

A possible role for the mechanical removal of subarachnoid blood clot in preventing vasospasm was suggested as early as 1958.6 Recently, with the development of computerized tomography, there has been no doubt that blood clot in the subarachnoid space is related to the development of vasospasm and that there is a close correlation between the development of vasospasm and the amount and location of the blood clot.4,13,17,19 These important discoveries have encouraged some neurosurgeons to remove the clot from the subarachnoid space in the acute stage following the SAH. Clinical studies performed by neurosurgeons in Japan infer that clot...
removal within a few days after the ictus is an effective means of preventing the development of vasospasm and of preventing or decreasing the incidence of delayed ischemic deficits. 3,11,12,18-20 These authors have suggested that clot removal should be performed as early as possible. Some neurosurgeons have reported that clot removal later than 72 hours after the SAH is ineffective in preventing the development of vasospasm. 14,18,20

The methods described for removing the subarachnoid clot vary with each report. Extensive and aggressive removal of clot has been performed and is recommended by some. 3,20 The importance of continuous cisternal drainage following mechanical clot removal is suggested by others. 14,19 Although the extent of mechanical clot removal is variable among studies and these studies were not performed with randomized controls, the critical time for clot removal suggested in these reports is in agreement with the results of the present study. Some authors do not advocate early operation and clot removal for the prevention of vasospasm and ischemic deficit. 3,8,11 Ljunggren et al. 8 reported that early operation with removal of subarachnoid clot did not eliminate the risk of delayed ischemic dysfunction. Ohta, et al., 11 wrote that extensive clot removal within 48 hours did not prevent the development of vasospasm but did decrease its severity. Excessive dissection to evacuate the clot may increase the degree of brain swelling and in some cases might precipitate intracerebral bleeding. In this study, some animals in both the clot and the clot-removal groups showed various degrees of cerebral edema at reopening. This was sometimes significant enough to present difficulty in retraction of the brain. The swelling and softening were thought to be effects of subarachnoid clot and the resultant ischemia.

In the present study, the severity of vasospasm correlated with the timing of clot removal (that is, with the duration that the clot was in contact with the vessels). In this model of experimental vasospasm, all of the clot fragments seen microscopically were removed from the subarachnoid cisterns. This mechanical clot removal appeared to be effective in decreasing the severity of vasospasm. The following may be possible mechanisms: removing or decreasing the concentration of spasmogenic agents derived from the blood clot; removing the blood clot prior to the generation of spasmogenic substance(s) derived from clot after some finite time; or improving the normal cerebrospinal fluid circulation around the blood vessels in the basal cisterns and thereby enhancing the washout or dilution of spasmogenic agents. The identification of specific factors or spasmogenic agents was not investigated. If some single spasmogenic agent exists, it is suspected from the present study that its effectiveness or the amount of it increases with time so that constriction of the vessels continues after removal of the clot.

The infiltration of blood elements into the arterial wall in experimental SAH has been reported as an important feature of vasospasm. 1 In the present study, all microscopic clot was removed and cisterns were washed out with copious amounts of physiological saline, but moderate vasospasm still developed in the late clot-removal groups. Alexander, et al., 1 reported that cisternal lavage 24 hours after hemorrhage in experimental animals has no effect on the development of vasospasm in spite of evidence of clot removal as seen at sacrifice. The results of our experiment suggest that direct removal of clot is more effective than simple cisternal lavage.

In a study by Nosko, et al., 10 25% of animals in the clot group developed severe ischemic deficits. In the present study, two of six animals in the clot group developed a deterioration in their levels of consciousness without hemiparesis or severe focal neurological deficit. There are differences in the severity of vasospasm that developed between the two studies. These differences may result from technical variations between operators and the variations in the amount of autologous blood clot placed.

Clot removal later than 48 hours does not significantly affect the development of vasospasm. This suggests that early surgery with aggressive removal of subarachnoid clot may significantly reduce the incidence and severity of chronic vasospasm provided that it is performed within 48 hours after the ictus. Extensive removal of clot from patients in the acute stage of SAH is more difficult than from the monkeys utilized in this experimental model. Removal of a sufficient amount of clot from the subarachnoid cisterns to prevent the development of vasospasm may not be possible without the risk of worsening the neurological state by trauma from small-vessel or retractor injury. Recently, cisternal lavage with artificial cerebrospinal fluid, with or without clot lytic agents, has been attempted in order to prevent vasospasm. 1,2,16 These therapies may help to prevent vasospasm with lesser risk of iatrogenic brain injury than mechanical clot removal or they may be useful adjuncts to clot removal.

References

6. Johnson R, Potter JF, Reid RG: Arterial spasm in sub-
Y. Handa, et al.


Manuscript received January 30, 1987.
This work was supported in part by a grant to Dr. Weir from the Medical Research Council of Canada.
Address reprint requests to: Bryce K. A. Weir, M.D., Department of Surgery, 2D1.02 Walter Mackenzie Health Sciences Centre, University of Alberta, Edmonton, Alberta T6G 2B7, Canada.