Effects of Anticoagulants in an Animal Model of Septic Cerebral Embolization

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SUMMARY The effect of anticoagulation on lesions caused by cerebral emboli of different types was studied in 57 dogs. The resultant arterial and parenchymal lesions were assessed by pathologic and angiographic studies. Embolization with emboli that caused little or no inflammatory response in the artery (12 dogs) was not associated with hemorrhagic infarcts or with subdural or subarachnoid hemorrhage; furthermore, treatment with anticoagulants (9 dogs) did not change the character of the lesions. Embolization with emboli that caused arteritis, that is, bacterial contamination or presence of lead chromate in the embolus (21 dogs), was associated with hemorrhagic infarcts, focal subarachnoid hemorrhage, and increased incidence of acute subdural hemorrhage. Treatment with anticoagulants (16 dogs) was associated with a further increase in the incidence of subdural hemorrhage.

USE OF ANTICOAGULANTS in the presence of septic cerebral embolism is controversial. Various authors have considered bacterial endocarditis to be an absolute,1 relative,2 or no contraindication3 to the use of anticoagulants. Those who advise against their use claim an increased incidence of intracranial hemorrhage as a major complication of this therapy. While intracranial hemorrhage is a recognized complication of subacute bacterial endocarditis,4 information linking this complication to the use of anticoagulants is largely based on case reports of few patients.5-7

In order to study the effect of anticoagulants during septic cerebral embolization, we administered anticoagulants to dogs while embolization was carried out, using a technique originally developed by Molinari.8

Methods

In 57 adult mongrel dogs weighing 10.4 to 17 kg, emboli were introduced into the cervical internal carotid artery, where they lodged most often in the proximal portion of the middle cerebral artery. Details of the surgical technique and preparation of the emboli have been described.9

Silastic emboli were prepared from medical-grade silicone rubber molding compound (Silastic, Dow Corning) and were injected either sterile or contaminated with β-lactamase-positive Staphylococcus aureus (MIC for penicillin G > 1.0 μg/ml). Contaminated emboli contained transversely oriented canals. Microfil emboli were prepared from a commercially available silicone rubber compound containing chrome yellow pigment (Microfil MV-122, Canton Biomedical Products, Boulder, CO) which produced an inflammatory reaction microscopically identical to that caused by the contaminated Silastic emboli. Dogs were placed into 6 groups on the basis of the type of embolus injected and the treatment with anticoagulants: group 1 (12 dogs), sterile Silastic emboli, not anticoagulated; group 2 (10 dogs), contaminated Silastic emboli, not anticoagulated; group 3 (9 dogs), sterile Silastic emboli, anticoagulated; group 4 (8 dogs), contaminated Silastic emboli, anticoagulated; group 5 (11 dogs), Microfil emboli, not anticoagulated; and group 6 (8 dogs), Microfil emboli, anticoagulated.
Anticoagulation

Anticoagulation was established in group 6 by the daily oral administration of dicoumarin in doses of 6.25 to 25 mg daily. For groups 3 and 4, sodium warfarin was administered intravenously on a daily basis, with the therapeutic dosage ranging from 0.09 to 0.19 mg/kg per day. Prothrombin times were measured 5 times weekly on blood collected in citrate tubes, using rabbit brain thromboplastin. The normal prothrombin time of the dog using this method was 8.8 ± 1.4 seconds (mean ± SD). The therapeutic range was arbitrarily chosen as 12.5 to 21.5 seconds. Prothrombin times of each dog were within this range on the day of operation.

Clinical Examination

The neurologic status of the dogs was examined on the morning of the day after operation and subsequently if deterioration occurred.

Clinical severity of infarct was graded as follows: grade 0, no deficit; grade 1, minor nonlocalizing neurologic deficits; grade 2, definitely localizing neurologic deficits such as hemiplegia or hemianopia; grade 3, same as grade 2 with the addition of any defect in the level of consciousness (failure to respond to normal environmental stimuli, apathy, lethargy); and grade 4, coma, death.

Sacrificing of Dogs

Some of the dogs died and others were killed when they were moribund or at 6 to 8 days after operation.

Fixed brains were sectioned coronally into 6-mm or 3-mm slices. Microscopic sections of a representative area of infarct were stained with hematoxylin and eosin. Van Gieson-elastica and hematoxylin and eosin stains were used on sections through the affected artery. A Gram stain was used when inflammation was suspected from gross findings. Photographs were taken of the fresh brain, of intact brain after fixation, and of representative gross sections. After injections of barium into the arterial vasculature, roentgenograms were taken in the basal, lateral, and often anteroposterior projections to demonstrate the opacified vasculature.

Grading of Lesions

Subdural hematomas were apparent on removal of the calvarium and were always located unilaterally. Their thickness was estimated and graded as follows: grade 0, none; grade 1, 2 mm or less; and grade 2, more than 2 mm. A subdural clot estimated to be 3 mm thick had a volume of 4.5 to 5 ml. Subarachnoid hemorrhage was graded as follows: grade 0, none; grade 1, localized to one hemisphere; and grade 2, diffuse, occurring over both hemispheres. The amount of hemorrhage in lesions visible in gross sections was graded as follows: grade 0, bland or white infarct; grade 1, petechial hemorrhages less than 1 mm in diameter; grade 2, areas of confluent hemorrhage 1 to 4 mm in diameter; grade 3, areas of confluent hemorrhage 5 to 20 mm in diameter; and grade 4, areas of confluent hemorrhage more than 20 mm in diameter. Gross lesions were graded by consensus between two of the investigators (T.J.R. and R.A.F.), whereas the microscopic findings were evaluated by a neuropathologist (T.J.R.) who did not have knowledge of the treatment given.

Results

Arterial Lesions

A minimal mononuclear cell infiltration of the adventitia of the artery was seen with the sterile Silastic emboli. With the Microfil or contaminated Silastic emboli, an intense arteritis was seen with mainly a polymorphonuclear response.

Anticoagulation

Regulation of the prothrombin time was difficult because 1) extreme variations in times occurred among dogs in response to warfarin and coumarin, 2) the anticoagulant effect was enhanced by factors linked to the operation, and 3) the use of pentobarbital for anesthesia, through induction of enzymes, tended to increase anticoagulant requirements during the postoperative period. Thus, if a dog was maintained on the dose of anticoagulant that was "therapeutic" during the preoperative period, the prothrombin time would be excessively prolonged from 16 to 48 hours after the operation and then would decrease to less than the therapeutic level. This necessitated a complex dosage schedule in which the dog received one-half of the estimated therapeutic dose on the day of operation and about twice the therapeutic dose on the day after operation, after which the dose was gradually reduced daily and stabilized at about 110 to 120% of the original therapeutic dose on the seventh postoperative day. No statistically significant difference was noted among the groups in the average prothrombin time.

Clinical Severity of Stroke

The clinical severity of the strokes varied greatly (table 1). Dogs with grade 4 strokes were found only in groups 4, 5, and 6 (anticoagulated contaminated Silastic and both Microfil groups).

Subdural Hemorrhage

The severity of subdural hemorrhage varied among each of the 6 groups (table 2). An excess of subdural hematomas occurred in groups 5 and 6 (Microfil groups) and in group 4 (anticoagulated contaminated Silastic group). No subdural hemorrhages occurred in groups 1 and 3 (sterile Silastic groups).

The data were further tabulated, and the variables were compared individually. In dogs with no inflammatory arterial lesions, there was no significant difference between dogs that received anticoagulants (group 1) and dogs that did not (group 3). In dogs with
Inflammatory arterial lesions, the incidence of subdural hemorrhage tended to increase in dogs that were anticoagulated (groups 4 and 6) in comparison with dogs that were not (groups 2 and 5); this increase was of borderline significance (0.1 > P > 0.05). When all anticoagulated dogs were studied, the incidence of subdural hemorrhage was significantly increased in dogs that had inflammatory arterial lesions (groups 4 and 6) as compared with dogs that did not (group 3) (table 3). In the absence of anticoagulants, there was a mild increase in the incidence of subdural hemorrhage in dogs with an inflammatory arterial lesion; the increase was not significant.

**Subarachnoid Hemorrhage**

A localized (usually 1 to 2 cm in diameter) area of subarachnoid hemorrhage was a constant finding in dogs with Microfil or contaminated Silastic emboli. There was no difference between anticoagulated and nonanticoagulated groups. A generalized subarachnoid hemorrhage was noted in one dog that had received a sterile Silastic embolus.

### Intraparenchymal Lesions

**Gross Observations.** Several distinctive types of intraparenchymal lesions were observed. Deep white matter-basal ganglia infarcts (fig. 1) were the most prevalent lesions, and their development was considered to be secondary to occlusion of small penetrating branches of the middle cerebral artery. Cortical infarcts distal to the embolus lodging site (fig. 1 A and B) were observed in a few dogs. These infarcts may have been caused by secondary factors such as edema, herniation, and subdural hemorrhage, as well as by obstruction of flow in superficial vessels by the embolus itself. Cortical infarcts adjacent to the embolus lodging site (fig. 1) were noted most frequently in groups 5 and 6 and were most often hemorrhagic. The lesions occurred to a lesser extent in groups 2 and 4. Small hemmorhages of the hypothalamus (fig. 1 B) were infrequently observed. These were considered to be due to the transient lodging of emboli at the junction of the internal carotid artery and the circle of Willis, with subsequent distal migration of the embolus.

Lesions associated with noninflammatory emboli were rarely hemorrhagic (table 4). When the groups were compared according to presence or absence of an inflammatory arterial lesion, there was a statistically increased incidence (P < 0.01) of hemorrhage in the dogs with inflammatory lesions (table 5). However, in dogs with inflammatory arterial lesions, there was no statistically increased incidence of hemorrhage in the presence of anticoagulation (table 5).

In most dogs, the hemorrhagic deep parenchymal lesion was in continuity with a hemorrhagic lesion at the embolus lodging site. When only lesions that were clearly discontinuous with lesions at the embolus lodging site were considered, there was little difference among the groups.

**Microscopic Findings.** Arteritis, focal meningitis, thrombosis, dilatation of the arterial lumen, and focal cerebritis were common findings with Microfil or contaminated Silastic emboli; this remained true when anticoagulants were administered (table 6). With sterile Silastic emboli, arteritis and meningitis were not observed, and only 1 dog had intra-arterial thrombosis.

### Postmortem Angiographic Findings

The most common finding was simple occlusion of the proximal portion of the middle cerebral artery of the involved side. In virtually all dogs, reconstitution of the distal middle cerebral artery was seen via corticomeningeal anastomoses from adjacent anterior and posterior cerebral circulations (fig. 2).

In 3 dogs, a rather poorly delineated saccular out-
FIGURE 1. Gross sections of dog brain. A and B showing cortical infarcts (white open arrows), deep white matter-basal ganglia lesions (narrow solid arrows), and hemorrhagic lesion at embolus lodging site (thick solid arrows). C showing deep white matter-basal ganglia lesion (open arrow) and hypothalamic hemorrhage (thick solid arrow).

Pouching was seen at the embolus lodging site; the outpouching appeared to represent an aneurysm. One of these occurred in an anticoagulated dog with a Microfil embolus, 1 in a nonanticoagulated dog with a contaminated Silastic embolus, a third in an anticoagulated dog with a contaminated Silastic embolus (fig. 3). The three aneurysms were all confirmed microscopically (fig. 4).

Bacteriologic Findings

β-Lactamase-producing S. aureus organisms in heavy growth were recovered in 5 dogs in group 4 only. In all groups, a heavy growth of organisms other

TABLE 4 Hemorrhage into Deep Parenchymal Lesions in Dogs With Cerebral Embolization*

<table>
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<td>1</td>
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<td>8</td>
</tr>
<tr>
<td>Total</td>
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<td>6</td>
<td>5</td>
<td>6</td>
<td>3</td>
<td>54</td>
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*Excludes hypothalamic and cortical lesions distal and adjacent to embolus lodging site (see text).
†See text for description of groups.
‡See text for description of grades.

TABLE 5 Hemorrhage Into Deep Parenchymal Lesions in Dogs With Cerebral Embolization

<table>
<thead>
<tr>
<th>Groups*</th>
<th>Hemorrhage</th>
<th>Absent</th>
<th>Present</th>
<th>Total</th>
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</thead>
<tbody>
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<td>19</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td>1 and 3 (noninflammatory)</td>
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<td>1</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>34</td>
<td>20</td>
<td>54</td>
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</table>

Inflammatory arterial lesion present

<table>
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<tr>
<th>Groups*</th>
<th>Hemorrhage</th>
<th>Absent</th>
<th>Present</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 and 5 (nonanticoagulated)</td>
<td>9</td>
<td>11</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>4 and 6 (anticoagulated)†</td>
<td>8</td>
<td>8</td>
<td>16</td>
<td></td>
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<tr>
<td>Total</td>
<td>17</td>
<td>19</td>
<td>36</td>
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</tr>
</tbody>
</table>

*See text for description of groups.
†Difference from nonanticoagulated dogs was of borderline significance (0.1 > P > 0.05).
than \textit{S. aureus} was frequently encountered, presumably due to contamination at the time the culture was taken. \textit{\beta}-Lactamase-positive \textit{S. aureus} organisms were not recovered from the cerebrospinal fluid.

**Discussion**

The control of indices of blood coagulation was not optimal in this study. The prothrombin time was elevated during the early postoperative period because of potentiation of anticoagulant effect by surgery and was difficult to regulate.

An important finding was the increased incidence of subdural hemorrhages in the presence of an inflammatory arterial lesion, as well as a further statistically significant increase in the incidence of subdural hemorrhage in the anticoagulated dogs when an inflammatory arterial lesion was present.

The occurrence of subdural hemorrhages with arterial wall rupture in the dog is an interesting finding. Our observations suggest that in the dog the arachnoid membrane is relatively delicate and closely applied to the wall of the artery. An inflammatory

| Table 6: Microscopic Findings at Embolus Lodging Site in Dogs With Cerebral Embolization |
|-----------------------------------|--------|--------|--------|--------|--------|
| No. of dogs                      |
| Total                            | 12     | 10     | 9      | 8      | 11     | 8      |
| With arteries examined           | 6      | 10     | 3      | 8      | 11     | 8      |
| With arteritis                   | 0      | 10     | 0      | 8      | 11     | 8      |
| With meningitis                  | 0      | 9      | 0      | 8      | 11     | 8      |
| With thrombosis                  | 1      | 8      | 0      | 7      | 10     | 5      |

*See text for description of groups.
process involving the artery invariably affected the adjacent leptomeninges. We theorize that the arachnoid membrane was so weakened that it was unable to contain the hemorrhage, which then ruptured through to the subdural space. A similar mechanism was proposed by Wright et al.\textsuperscript{10} to explain subdural hemorrhages resulting from ruptured congenital aneurysms. The paucity of reported cases of subdural hemorrhage in humans with mycotic aneurysm could reflect a species difference, with selective vulnerability to subdural hemorrhage in the dog.

Localized subarachnoid hemorrhage was an invariable finding with Microfil or contaminated Silastic emboli.

There was a statistically increased incidence of hemorrhage into deep parenchymal lesions in the groups with inflammatory arterial lesions. However, this probably reflected the spread of subarachnoid hemorrhage from the embolus lodging site, possibly along the course of the penetrating vessels. Anti-coagulation did not increase the incidence of this type of lesion.

The predominance of completely bland (non-hemorrhagic) infarctions with sterile Silastic emboli would seem to contradict the commonly held opinion that embolic infarcts are hemorrhagic infarcts. Many investigators\textsuperscript{11} believe that the reestablishment of flow via collateral vessels or the breaking up of an embolus with reflow is responsible for the hemorrhagic component of embolic infarctions. In this model, the embolus blocked the origin of small, penetrating, presumably "functional end-arteries" and was not displaced from its original lodging site. This may explain the lack of hemorrhage in our experimental model.

The chemical inflammatory response evoked by the Microfil emboli was similar to that caused by contaminated Silastic emboli and is probably responsible in part for the arterial lesions observed by previous investigators.\textsuperscript{12} While abscesses have reportedly been produced by Microfil emboli,\textsuperscript{13} we did not observe any such lesions. We did observe cavitation within the infarcts and, with Microfil emboli, spread of inflammation via the Virchow-Robbin spaces to deep within the parenchyma.

The failure to consistently produce mycotic aneurysms of the naturally occurring type observed in humans was not unexpected, because, in most cases, the segment of the artery that was inflamed was also protected from the distending effects of blood pressure by the occluding embolus. Nevertheless, histologically, there are many similarities between the lesions that we observed and naturally occurring mycotic aneurysms. The 3 radiographically demonstrated my-
Cortic aneurysms that we observed indicate that these lesions can be produced by either Microfil or contaminated Silastic emboli.

The failure to recover viable S. aureus organisms from the lesions produced by contaminated emboli is disturbing. Also, there was no correlation between positive cultures and early death due to subdural hemorrhage. It is unlikely that the small doses of penicillin used in this study were capable of inhibiting the organism, which was clearly penicillin-resistant. Nevertheless, Molinari et al. 12 have demonstrated in a similar model that what appeared to be an “inadequate” dose of penicillin greatly modified the outcome of the experiment.

Conclusions

Embolization of major cerebral arteries of the dog resulted in strikingly different lesions, depending on the inflammation-producing potential of the embolus and the presence or absence of anticoagulation. 1. With noninflammation-producing emboli and no anticoagulation, bland parenchymal infarcts were observed and no subdural or subarachnoid hemorrhages were seen. 2. With noninflammation-producing emboli and anticoagulation, similar findings were observed, that is, bland infarcts and no subdural or subarachnoid hemorrhages. 3. With inflammation-producing emboli and no anticoagulation, hemorrhagic infarcts were often seen, as well as subdural hemorrhages, while localized subarachnoid hemorrhage was always present. 4. With inflammation-producing emboli and anticoagulation, hemorrhagic infarcts were often seen and most of the dogs suffered subdural hemorrhages. Localized subarachnoid hemorrhage was a constant finding.

From our experimental model, we conclude that embolization with an inflammatory embolus was associated with a significantly increased incidence of subdural hemorrhage in anticoagulated dogs. A similar trend (not statistically significant) was noted in nonanticoagulated dogs.

Although caution must be exercised in applying the observations of this model to the clinical situation, our findings suggest that, in humans, anticoagulants should be avoided, if possible, in situations in which inflammation-producing emboli might lodge in the cerebral arteries, such as in infective endocarditis.

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