Cerebral Arterial Lesions Resulting from Inflammatory Emboli

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SUMMARY In order to study the effects of septic embolism on the brain, silicone rubber emboli of various types were injected into the carotid arteries of 35 dogs. Pathologic and angiographic studies were performed to assess the resultant arterial and parenchymal lesions.

Pure silicone rubber emboli (14 dogs) produced occasional intra-arterial thrombosis but no arteritis. Sterile and bacterially contaminated emboli containing a lead-chrome pigment (similar to those used in previous studies of septic embolism) (11 dogs) and pure silicone rubber emboli with transversely oriented canals (10 dogs), after brief placement in a bacterial suspension, were associated with intense inflammatory arteritis. This was accompanied by focal meningitis, subarachnoid hemorrhage, thrombosis, and cerebritis of the underlying cortex. The findings resembled those found in mycotic aneurysm. Aneurysmal dilatation was observed in one postmortem angiogram.

In previous models of mycotic aneurysm, the inflammation attributed to bacterial contamination was probably due to the lead-chrome pigment used.

INJECTION of artificial emboli into the cerebral circulation has often been utilized as a means of producing experimental cerebral infarction. Emboli used have included autologous blood clots,1 steel ball bearings,2 psyllium seeds, pigmented silicone rubber compounds with and without a coating of bacteria,3,4 barium-impregnated silicone spheres,4 and others. These studies usually have reported examinations of the resultant parenchymal lesions but little attention has been given to examination of the arterial lesions produced by the emboli. Molinari and associates,3 in their report describing experimentally produced cerebral mycotic aneurysms, injected pigmented silicone rubber cylinders coated with Staphylococcus into the cerebral circulation. They noted polymorphonuclear leukocyte invasion of the vasa vasorum which they theorized preceded inflammatory infiltration of the arterial wall followed by dilatation and formation of aneurysms. No controls were used with nonpigmented emboli to rule out the possibility of arteritis secondary to the pigment.

The purpose of this study was to evaluate the arterial lesions produced by 3 different types of artificial emboli with or without bacterial contamination.

Methods

Adult mongrel dogs weighing 11.2 to 16.2 kg were anesthetized with intravenous injections of pentobarbital (32 mg/kg of body weight) and, similarly to Molinari's technique, were premedicated with intramuscular injections of 10,000 µ/kg each of procaine and benzathine penicillin G. Under sterile conditions, a longitudinal incision was made in the anterolateral portion of the neck and the common carotid and internal carotid arteries were identified. The internal carotid artery was ligated at its origin and a 16-gauge catheter was introduced into it via a transverse arteriotomy. An embolus was then flushed through the catheter with approximately 5 ml of sterile normal saline solution, after which the artery was ligated distally and the catheter was removed.

Emboli were of 3 types: microfil emboli, solid elastic emboli, and canalated Silastic emboli (fig. 1). These emboli were produced as follows:
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1. A silicone rubber compound containing chrome yellow pigment Microfil MV-122 (Canton Biomedical Products) was molded in polyethylene tubes (1.57 mm inside diameter [ID]) and cut into 6-mm segments. The emboli are subsequently referred to as "Microfil embolus."  

2. A silicone rubber compound consisting of 10 g of Silastic medical-grade Elastomer molding compound (Dow Corning), 30 g Microfil MV diluent (Canton Biomedical Products) (polysiloxane fluid, viscosity 5 cps), and 6 drops catalyst M (Dow Corning) was molded in polyethylene tubes (1.57 mm ID) and cut into 6-mm lengths. The emboli so formed are referred to as "solid Silastic emboli."  

3. The silicone rubber compound used in forming solid Silastic emboli was molded in polyethylene tubes (1.57 mm ID) through which 27-gauge needles had been passed transversely at 2-mm intervals alternately at right angles to each other. Each 6-mm segment had 3 transverse canals (0.4 mm in diameter) each oriented at 90° to its neighbor. The emboli formed are subsequently referred to as "canalated Silastic emboli."  

All emboli were of similar "softness." The Microfil emboli had a greater tendency toward fragmentation during injection and tended to conform more to the size and shape of the artery in which they lodged. All emboli were sterilized with ethylene oxide and aerated for 12 to 24 hours. Contaminated emboli were prepared by placing the emboli for approximately 15 minutes in overnight broth cultures (approximately 10⁶ organisms/ml) of β-lactamase positive Staphylococcus aureus or group D Streptococcus obtained from infected human patients. Neither organism was sensitive to penicillin in the dosages used in this experiment. The animals were sacrificed when moribund (always more than 24 hours after operation) or 6 to 8 days after the operation by a lethal overdose of pentobarbital. Immediately before death, a sample of cerebrospinal fluid (CSF) was withdrawn via cisternal puncture under sterile conditions for bacteriologic culture. The chest was opened and the upper part of the torso and the head were perfused with normal saline via a cannula placed in the aorta, which had been tied off above and below. The calvarium was then removed, the dura was incised, and a swab culture was taken from the surface of the brain surrounding the lodging site of the embolus. After removal of the brain, its major arteries were injected with a barium-gelatin compound, which allowed for later radiographic visualization of the arterial vasculature. The brain was then fixed in 10% formalin.

Results  

The use of 3 types of emboli, each type with and without bacterial contamination, created 6 possible combinations; however, the animals could be separated into 3 main groups on the basis of gross and microscopic pathologic findings. These 3 groups were as follows:

Group A — Microfil Emboli, Sterile and Contaminated with Group D Streptococcus  

Six emboli were infected with group D Streptococcus; another 5 were sterile when injected. All arterial lesions of the major arteries at the base of the brain appeared similar. Grossly, the embolus lodging site was surrounded by opaque exudate, thickened arterial wall and leptomeninges, and subarachnoid hemorrhage for an area about 1 cm in diameter. In 3 contaminated emboli and 1 sterile embolus there were varying degrees of subdural hemorrhage. Four contaminated emboli and 2 sterile emboli extruded from the arteries and were found embedded in adjacent inflammatory tissue or in the subdural clot. Microscopically, both infected and noninfected emboli were associated with severe necrotizing vasculitis (fig. 2A).

All arterial walls were infiltrated by polymorphonuclear leukocytes and in the most severe cases the entire thickness of the wall was completely necrosed. In less severe cases, the internal elastic membrane was fragmented or coagulation necrosis of the media associated with intramural hemorrhage was noted. More distant to the embolus lodging site, inflammation involved the leptomeninges and subarachnoid space and the artery was surrounded by inflammatory infiltrate, which sometimes penetrated into the adventitia of the vessel. In 5 contaminated emboli and 5 sterile emboli, thrombosis was seen in the artery itself or in other vessels lying in proximity to the involved artery. In 1 case, stretching and fragmentation of the internal elastic membrane suggested that dilatation of the artery had occurred. Finally, in most cases, polymorphonuclear infiltration extended into the subjacent cortex, which often showed hemorrhagic infarction. No bacteria were seen on Gram stains in 3 cases.

Cultures of CSF were negative in all cases except 3 that showed a few colonies of Staphylococcus epidermidis, which were considered contaminants. Brain swabs in all cases showed only a few colonies of presumed environmental contaminants (that is, gram-positive and gram-negative bacilli that were not identified and St. epidermidis).

Group B — Sterile Solid and Canalated Silastic Emboli and Contaminated Solid Silastic Emboli  

This group included 11 sterile solid Silastic emboli, 1 solid Silastic embolus contaminated with group D...
Streptococcus, 1 solid Silastic embolus contaminated with *S. aureus*, and 1 sterile canalated Silastic embolus. The latter 2 emboli were recovered from the intercavernous internal carotid artery; all other emboli came from the middle cerebral artery.

No inflammation was evident grossly in any of the animals (fig. 3). Microscopically, slight mononuclear cell infiltration was seen occasionally in the adventitial coat of the artery in which the embolus had lodged (fig. 2B).
FIGURE 5. Right middle cerebral artery in longitudinal section showing evidence of arteritis caused by contaminated canalated Silastic embolus. Note pattern corresponding to transverse canals. (Hematoxylin and eosin; $\times 40$.)

One case showed thrombosis and dilatation in the lumen. In 1 case (sterile solid Silastic embolus), diffuse subarachnoid hemorrhage was noted. We were unable, unfortunately, to locate the precise source of bleeding. Microscopic sections of the artery adjacent to the embolus showed a single break in the internal elastic membrane only.

Cultures of CSF were negative except for one which grew Moraxella sp. and Corynebacterium sp., both considered contaminants. A few colonies of contaminants grew in all brain swabs except 3; in these many colonies of gram-positive cocci other than S. aureus, gram-positive bacilli, or gram-negative bacilli were noted. (These were sterile emboli.)

Group C—Canalated Silastic Emboli
Contaminated With S. aureus

Grossly, in each of the 10 emboli in this group, the lodging site was surrounded by an area of subarachnoid hemorrhage and clouding or thickening of the leptomeninges approximately 1 cm in diameter (fig. 4). In 2 cases there was a subdural collection of blood over the corresponding hemisphere about 1 mm thick. In 2 cases the embolus partially protruded from the artery (possibly having occurred during removal of the brain).

Microscopically, in 9 cases a predominantly polymorphonuclear inflammatory response involved the artery and subarachnoid space adjacent to the involved segment of the artery (fig. 5). This response was similar in all respects to that described for Microfil emboli. Focal meningitis was seen in 9 cases. Intravascular thrombosis was observed in 7 cases and microscopic evidence of dilatation of the arterial lumen was noted in 5.

Gram-positive cocci were observed in 1 of 6 cases in which a Gram stain was obtained (fig. 6). In 3 of the 10 cases, hemorrhagic infarction of the cortex was apparent underlying the lodging site of the embolus. In no case was a heavy growth of S. aureus recovered from brain swab or CSF. In 5 cases, a heavy growth of organisms other than S. aureus was noted. In 2 cases, an organism other than S. aureus was recovered from CSF (one Corynebacteria, one gram-positive cocci not identified), suggesting contamination of the CSF sample.

In 1 case, postmortem barium angiogram showed a saccular dilatation of the middle cerebral artery immediately proximal to the lodging site of the embolus.
Microscopic findings confirmed aneurysmal formation with dilatation of the lumen and segmental breaking up of the internal elastic membrane (fig. 8). In addition, the typical findings of polymorphonuclear infiltration of the arterial wall with necrosis and intramural hemorrhage were seen.

Gross and microscopic pathologic, bacteriologic, and roentgenographic findings for the 3 groups are summarized in the table.

**Discussion**

Emboli manufactured from pigmented silicone rubber compounds have been used in animal models of embolic cerebral infarctions, cerebral mycotic aneurysms, and brain abscesses. In the latter 2 types, bacterial surface contamination of the emboli was implicated in the pathogenesis of the observed lesions.

Certain noxious chemical compounds also have been noted to cause a severe acute inflammatory reaction when introduced into the wall of cerebral arteries or when applied to their outer surfaces, for example, hypertonic saline, nitrogen mustard, and methyl 2-cyanoacrylate cement.

In several reports, arterial repairs with the latter compound were followed by aneurysmal formation in a certain percentage of cases. Since the sole difference between the Microfil and the Silastic emboli used in our study was the lead-chromate pigment in the Microfil compound, our data suggested that this compound was capable of causing a severe inflammatory reaction with formation of lesions closely resembling lesions produced by pyogenic bacteria. Because similar arterial lesions were observed with all Microfil emboli, whether sterile and injected under sterile conditions or contaminated, we concluded that bacteria did not play a role in the pathogenesis. It is more likely that inflammatory cells respond to tissue necrosis caused by the toxic metal compound and that they infiltrate the wall of the artery and contribute to necrosis of the elastica and media, resulting in dilatation of the artery.

The inflammatory response in the leptomeninges and adventitia of the artery occurring in proximity to the primary lesion could be caused by diffusion of the toxic substance or by chemotaxis caused by release of soluble factors from other inflammatory cells.

Pure silicone elastomers, on the other hand, produced minimal tissue reaction. We were unable to produce an inflammatory lesion by contaminating the smooth, nonwettable surface of the Silastic embolus with bacteria, even though it could be demonstrated that bacteria were present on the surface of the em-
bolus before its injection into the artery. Emboli with transversely oriented canals, however, apparently were capable of transporting sufficiently large numbers of bacteria or their products to their lodging site to produce arteritis. While postmortem arteriograms rarely showed dilatation of the involved segment of artery, microscopic findings resembled those seen in mycotic aneurysms.

Our failure to recover bacteria from the CSF suggested that the infection was well contained. Brain swabs obtained at autopsy under nonsterile conditions seemed to be an unsatisfactory method for recovery of the offending organism.

Conclusions

Although bacterial contamination of pigmented silicone rubber emboli has been thought to be responsible for cerebral mycotic aneurysms and brain abscesses in previously reported experimental models, we believe that a lead-chromate pigment in the compound caused a chemically mediated inflammatory response. We designed a new embolus constructed of Silastic medical-grade Elastomer with transversely oriented horizontal canals, which, when placed briefly in a bacterial suspension, caused arteritis when injected into the cerebral arteries of dogs. The arterial lesions caused by the 2 types of emboli were similar and consisted of necrotizing arteritis with varying degrees of focal meningitis, subarachnoid hemorrhage, thrombosis, and cerebritis of the underlying cortex. Roentgenograms in 1 case demonstrated a saccular aneurysm. While silicone elastomers have mechanical properties different from those of infected vegetations, we believe that our model more satisfactorily imitates the natural process of development of mycotic aneurysms.

References

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R A Foote, T J Reagan and B A Sandok

*Stroke*. 1978;9:498-503
doi: 10.1161/01.STR.9.5.498

*Stroke* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0039-2499. Online ISSN: 1524-4628

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