A model of brain abscess: septic homologous blood clot emboli in rats

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Brain abscesses in rats were produced by intra-arterial injection of septic homologous blood clot emboli. The production rate was 100% and the histopathological features closely resembled those seen in other animal models and in spontaneously occurring brain abscesses in humans. This small-animal model may be useful for systematic study of the development of brain abscesses as well as for evaluation of various therapeutic procedures.

KEY WORDS • brain abscess • experimental model • septic embolus • infection • rat • Staphylococcus aureus

Various animal models of brain abscesses have been described in the literature. One type involves the direct inoculation of bacteria into the brain which simulates brain abscesses that occur following chronic otitis media or penetrating head injury. Various species of animals, including small animals such as rats, have been used for producing this type of brain abscess. Another type is termed a "metastatic brain abscess" and is often seen in patients with bacterial endocarditis, lung abscesses, or cyanotic congenital heart disease: that type has been created in dogs and monkeys by introducing septic emboli. However, there is no small-animal model for the study of metastatic brain abscess, which is the more common type of brain abscess in humans.

In order to study the pathogenesis of metastatic brain abscesses, as well as to evaluate drug therapy more effectively, a small-animal model is needed that allows a comparative study of various parameters at selected points in a large number of animals. It has been demonstrated experimentally and clinically that brain necrosis is a prerequisite for brain abscess formation. Kudo, et al., recently reported a small-animal model of cerebral infarction involving the injection of aseptic homologous blood clot emboli into rats; they suggested that the model was applicable for the study of brain abscesses. We have attempted to create brain abscesses using a model of cerebral infarction with intra-arterial injection of septic homologous blood clot emboli.

Materials and Methods

Thirty-two Wistar strain male rats, weighing 200 to 300 gm each, were used in this study. The preparation of the septic homologous blood clot emboli and technical procedures of embolization are the same as described by Kudo, et al., to produce cerebral infarction. Animals were anesthetized with ether and then 0.1 ml of blood was obtained by cardiac puncture and stored at room temperature for 48 hours for clot formation. The clots were separated from the serum and were fragmented by injection through a No. 26 needle into normal physiological saline. The latter step was repeated three times until the clot fragments were about 100 μ in size.

At the time of embolization, the animals were again anesthetized with ether. The left common carotid artery was surgically exposed and the external carotid artery was temporarily occluded. A suture thread was placed around the common carotid artery. The septic emboli suspended in physiological saline were injected into the common carotid artery while the thread was kept taut. After injection, the site of injection was sealed with surgical adhesive. The external carotid artery was reopened and the suture thread removed to reestablish blood flow. The surgical site was closed. No antibiotics were administered.

The bacterial organism used was Staphylococcus aureus (Smith strain). Bacterial incubation was carried...
out in sterilized brain-heart infusion culture medium for 24 hours, and $1 \times 10^9$ colony-forming unit (cfu)/ml was obtained by centrifugation of the culture medium at 8000 rpm for 30 minutes. The blood clot emboli and bacteria were mixed together at the time of embolization, then 0.2 ml of saline containing bacteria ($1 \times 10^9$ cfu/ml) and clot fragments made from 0.1 ml of blood was injected manually over about 3 seconds.

**Results**

No operative deaths occurred. Postoperative symptoms and signs varied from one animal to another, depending upon the extent of the infarction. They included narrowing of the eye fissure and pallor of the eyeball on the affected side, licking or biting, circling, tilting of the head, limping, decreased spontaneous activity, and seizures. Seven of the 32 animals died within 2 days after embolization because of severe brain edema due to injection of the septic emboli. There were no significant cerebral hemorrhages in these seven animals, and the infarcts were primarily of an anemic type. The remaining 25 animals showed a rapid improvement in clinical conditions by the 5th postoperative day. The animals were anesthetized and killed by exsanguination on Days 2 (two rats), 3 (two rats), 5 (five rats), 8 (one rat), 9 (one rat), 10 (five rats), 14 (three rats), and 15 (six rats). Cultures of the abscesses were carried out in three animals on Days 5 and 15, and all grew *Staphylococcus aureus*. No recurrence of focal signs or signs of expanding intracranial lesions were noted during the course of the experiments.

Pathologically, abscesses were found in all animals. The gross features of the lesions varied with the different time intervals. The lesions were observed externally or on cut surfaces of brain and were more or less hemorrhagic. The lesions were scattered and multiple, with the most common site being in the distribution of the middle cerebral artery. The contralateral hemisphere was occasionally affected, with the abscesses usually microscopic in size.

Histologically, 6-hour-old lesions showed massive necrosis of the tissue and recent thromboemboli with bacteria. Two-day-old lesions were characterized by florid vasculitis, heavy neutrophilic infiltration, and hemorrhage in the necrotic areas. There were also many scattered bacterial colonies. Five-day-old lesions were composed of a central necrosis with bacterial colonies, heavy neutrophilic collection, numerous foamy macrophages, and vascular and fibroblastic proliferation with multifocal recent and old hemorrhages. Foamy macrophages often phagocytosed the bacteria. By Day 15, the abscesses were well delineated from the surrounding tissue by concentric proliferation of fibroblasts. The collagenization of the fibrous capsule, however, appeared weak. Lymphocytes and plasma cells were present, with proliferation of pleomorphic microglia and reactive fibrous astrocytes in the periphery of the lesions. In almost all animals the leptomeningeal spaces and lateral ventricles were infected (Fig. 1).

**Discussion**

Our results demonstrate that the intra-arterial injection of septic homologous blood clot emboli provides an excellent model from which to study the formation of metastatic brain abscesses. The small-animal model has advantages such as easy management, economy, and use of common experimental animals. This model would make it possible to do a systematic correlative study of different parameters at selected times in a large number of animals. The clinical symptoms and signs as well as the topographic distribution of brain abscesses were similar to those described by Kudo, *et al.* The pathological features were similar to those described as a prototype of brain abscess using various methods and animals and also simulated spontaneous brain abscesses in humans.

Molinari, *et al.*, found that animals with septic emboli infected with virulent pathogens such as *Staphylococcus aureus* died of subarachnoid hemorrhages from ruptured mycotic aneurysms within the first few days, rather than of abscess formation. It is interesting to consider why the animals in this experimental paradigm, in spite of having *Staphylococcus aureus* as the causative organism and not receiving antibiotics, sur-

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**Fig. 1.** Gross photographs of paraffin-embedded sections. *Left:* Five-day specimen showing brain abscesses with central necrosis and hemorrhage. *Right:* Fifteen-day specimen showing brain abscesses with clear delineation of fibrous capsule formation.
Experimental brain abscess in rats

vived and formed abscesses with no early deaths due to subarachnoid hemorrhage. The reasons remain unclear at the present time.

Our success in brain abscess formation using *Staphylococcus aureus*, a very common virulent bacterium, as a causative organism suggests that the use of the embolic model of cerebral infarction first described by Kudo, et al., with various organisms, even including fungi, may allow the study of other types of brain abscesses. In spite of advanced surgical techniques, improved diagnostic procedures, and more effective antibiotic treatment, mortality due to brain abscesses is still high.4 We hope, therefore, that our model will form the basis for the development of more effective therapy of brain abscesses.

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References