A New Canine Model of Proximal Internal Carotid Embolism

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SUMMARY A new model of internal carotid artery embolism was developed using autologous clot. The clot was prepared by incubating blood at room temperature for 2 hours to inactivate plasminogen activators and then refrigerating it at 4°C for 22 hours. The purpose of the experiment was to devise a model of the intravascular lesion and not of stroke itself. The dog was chosen as the experimental animal since it has a maxillary-carotid artery which permits collateral flow beyond proximal internal carotid artery occlusions. A volume of clot measuring 0.25 to 0.30 cc was sufficient to occlude the artery for 48 hours in 80% of the animals without causing major strokes. We have used this model to study clot radiolabeling and suggest it may also have application for evaluating thrombolytic drugs.

Although a number of models of cerebrovascular problems exist which have permitted investigation of various aspects of the disease, many are invasive and too "artificial" for the study of the detection and treatment of thromboembolic occlusions. We, therefore, initially developed a canine model of cervical carotid thrombosis which was used to study the fibrinogen uptake test (FUT). The current report concerns the development of a new canine model of internal carotid occlusion by emboli formed from autologous clot, which though it is prepared in vitro, consists only of blood components.

Thrombi, the source of emboli, have been extensively evaluated, and the varying and complex structure of white arterial thrombi and red venous thrombi, as well as the inhomogeneity within each group, have been noted. Attempts have been made to create blood clots closely mimicking "natural" thrombi by both in vivo and in vitro techniques. Simpler methods to produce an autologous clot with optimal strength using blood coagulated in a test tube have been tried but without complete success.

The strength of a clot is based on a variety of factors involved in its production and destruction. Clot strength is related to clot retraction which is caused by the contraction phase of viscous metamorphosis of platelets. The amount of retraction is reflected by the volume of clot compared to the original volume of blood. The firmness of a clot is described more specifically by its modulus of shear elasticity which can be measured elegantly by thromboelastography, but grossly by simple observation. Retraction progresses over time but is counteracted by autoclytic dissolution of the clot.

The literature suggests that plasminogen activators which can lead to clot lysis are inactivated at room temperature, but that clot retraction is promoted by storage at 4°C. The characteristics of undiluted, unaltered clotted whole blood have not been studied under various conditions of time and temperature. The dog is especially suited as the experimental animal for this model because of the presence of the maxillary-carotid anastomosis (fig. 1). This collateral vessel allows occlusion of the proximal internal carotid artery without development of a major cerebral infarction.

Methods and Results

Preparation of Clot. Clot retraction and lysis were studied by keeping canine blood at room temperature for varying times to inactivate plasminogen activators and then incubating it at 4°C for varying times to promote clot retraction. This was to determine the optimal conditions for producing the firmest clot.

Clots were prepared under four different circumstances. Specimens of blood were drawn from 6 to 10 mongrel dogs for each study. Tubes of blood were prepared by placing 3 cc of blood into a sterile 75 X 16 mm test tube. A short sterile wooden applicator stick was inserted into each tube, which was sealed with a sterile rubber stopper. For one group, the tubes were refrigerated immediately and for the other 3, blood was incubated for 1, 2 or 3 hours at room temperature and then refrigerated.

Clot retraction and strength were evaluated on individual specimens examined over a 48-hour period. Clot retraction was studied by removing the applicator stick, together with the adherent clot, and then measuring the residual volume. The size of the clot was 3 cc minus the residual volume. The clot's strength was judged by how strongly it adhered to the applicator stick and how well it resisted attempts to fragment it. The ability of a clot to maintain its configuration was also used as an indicator of strength.

Results have shown that preparation of the clot by initially incubating blood at room temperature for 2 hours and then storing it at 4°C for 22 hours yielded an essentially optimal clot for an embolus (fig. 2).
meglumine diatrizoate-sodium diatrizoate (Renographin-76, Squibb). The internal carotid artery was easily seen, as were its intracranial branches (fig. 3). The maxillocarotid anastomosis could often be seen, but its course was most obvious on selective internal carotid injections (fig. 4).

Clot prepared as outlined was decanted into a 1 cc syringe, and various amounts of clot were injected. The clot was positioned just beyond the origin of the internal carotid artery by flushing with Renographin, and common carotid angiograms were repeated to verify occlusion. A volume of 0.25 to 0.30 cc was sufficient to cause internal carotid artery occlusion. At times post-embolization angiograms demonstrated the maxillocarotid anastomosis filling from the external circulation (fig. 5). This was true even if it had not been well seen on common carotid injections before embolization. Although some animals developed mild strokes, this did not correlate well with the presence or absence of an obvious maxillocarotid anastomosis,
presumably indicating that small bits of clot passed into the intracranial circulation and caused infarction. A greater amount of clot tended to cause severe strokes or death (fig. 6). Clots persisted at least 48 hours in 12/15 (80%) dogs.

Discussion

A new model of cervical carotid embolism has been developed which has several advantages and a number of possible applications.

This model is simple and requires commonly used equipment and minimal manipulation. The clot can easily be produced with sterile technique. We feel that an 80% success rate in producing internal carotid occlusion is acceptable.

This model may have an application in several areas. One is for use in radiolabeling studies, which is how we have successfully employed the model. Second is to evaluate thrombolytic therapy, drugs for which, such as urokinase, have been released by the Federal Drug Administration, and have been shown to be effective for the treatment of pulmonary embolism. Urokinase was shown to be safe when used in patients with stroke although there were many problems with this study. The study of the effects of urokinase on emboli in a model such as ours would be helpful in evaluating the drug in detail.
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References


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