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Evaluation of Three Embolic Agents in Pig Rete

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In ongoing research into potential embolic agents, three substances were evaluated for their effectiveness in occluding the pig rete. Selective injection of these agents produced varying degrees of occlusion, with the most effective a microfibrillar collagen hemostat in a 33% ethanol solution. Angiostat, a collagen particulate, did not produce rete occlusion, and all pigs injected with it developed adverse effects, including coma and apnea. Tisseel, a tissue sealant, produced partial rete occlusion, but was uniformly difficult to manage through long catheters, and its use was discontinued.

Microfibrillar collagen hemostat with ethanol appears to have the greatest potential as an embolic agent in low-flow structures.

The introduction of smaller catheters, superselective catheterization, and intraprocedure monitoring with digital fluoroscopy has led to improved efficiency, control, and safety of embolization. Many embolic agents are currently available; however, the ideal agent, combining ease of handling, rapidity of solidification, and safety, has not yet been found.

The pig rete (Fig. 1) was chosen as the site to evaluate three potential embolic materials. This interarterial vascular network simulates a small arteriovenous malformation with feeders; predominantly, the internal carotid artery (called the ascending pharyngeal artery by some authors [1]), a nidus (the rete itself), and a distal draining vessel (the internal carotid artery). The pig rete is easily accessible at autopsy, enabling pathologic correlation. Rete vessel size in our pig population was 70–275 μm, with a mean of 154 μm.

The following embolic agents, which can all be injected as liquid suspensions, were evaluated: (1) microfibrillar collagen hemostat (MCH),* (2) glutaraldehyde cross-linked collagen (GAX),† and (3) fibrin sealant (Tisseel).‡

MCH is a topical hemostatic agent prepared from denatured bovine collagen [2]. It is fibrillary, with diameters similar to blood fibrin, and this serves as a matrix for platelet aggregation and clot formation. MCH was first described as an embolic agent in 1978 [3]; however, recanalization occurred and it was subsequently suggested for preoperative use [4]. It is potentially antigenic, because of the presence of bovine collagen, but animal and human studies have not demonstrated any significant effects, though in animals there was a rise in antibodies to bovine serum components.

GAX is an immunologically inert collagen particle, derived from bovine skin [5, 6]. The particle size is 4.9 (SD = 2) × 72 (SD = 40) μm. It produces occlusion in vessels of 25–250 μm by filling them with particles.

Tisseel is a tissue sealant that contains bovine aprotinin (a polypeptide, obtained from animal organs, which inhibits proteinase and kallikrein), human factor XIII, fibrinogen, albumin, plasma fibronectin, and plasminogen activated with bovine thrombin in calcium chloride. When mixed, this produces a viscous solution that sets [7].

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* Avitene, Alcon Laboratories, Fort Worth, TX 76134.
† Angiostat, Target Therapeutics, San Jose, CA.
‡ Immuno Industriestraβe 72, Vienna, Austria.

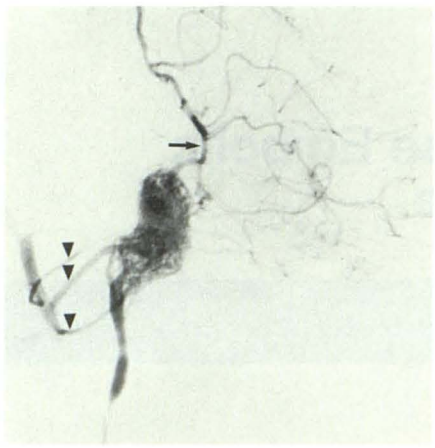
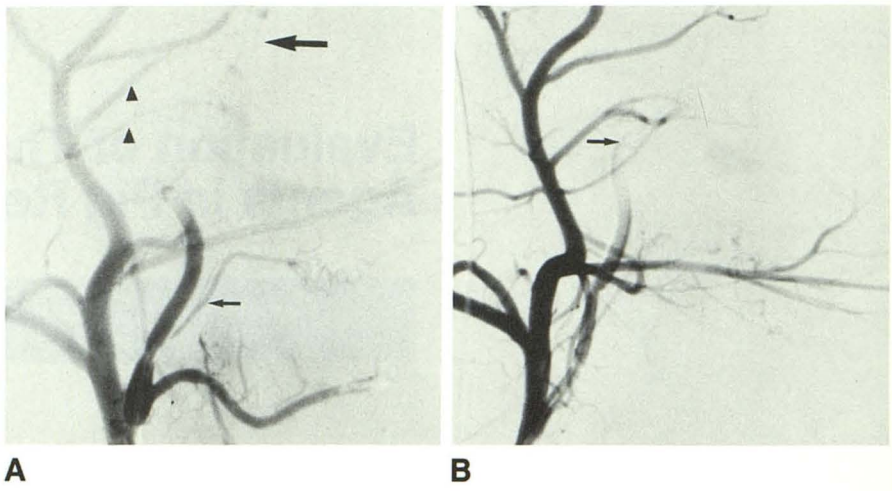


Fig. 1.—Selective internal carotid angiogram shows filling of pig rete. *Arrow* = distal internal carotid artery. Note external carotid branches (*arrowheads*).



A B

Fig. 2.—*A*, Immediately after embolization, rete of internal carotid artery is occluded with MCH and ethanol. Note meningeal branch, which anastomoses with vertebral artery (*small arrow*). Faint filling of distal internal carotid artery (*large arrow*) by external carotid collaterals (*arrowheads*). External carotid artery fills by reflux.

B, 2 weeks after embolization there is continued occlusion of proximal internal carotid artery (*arrow*).

Materials and Methods

Domestic swine weighing 20–40 lbs (9–18 kg) were used; the animals were sedated with 20 mg/kg ketamine and 2.5 mg diazepam intramuscularly, and subsequently anesthetized with halothane and nitrous oxide. Via percutaneous transfemoral catheterization, a 6-French introducing catheter was positioned in the common carotid artery, and the internal carotid artery was selectively catheterized coaxially with a 4-French catheter. In some cases a 5-French catheter was used without the need for a coaxial system. An attempt was made to place the catheter in the internal carotid artery distal to a small meningeal branch that was found to anastomose with the vertebral artery.

Initial superselective angiograms were obtained by using nonionic contrast material (iohexol,[§] 300 mg I/ml), MCH as a 10 mg/ml solution with 33% ethanol added (since previous studies have shown recanalization with MCH alone), GAX (7 mg/ml) with or without 33% ethanol, or Tisseel (as supplied—thrombin 500 solution). Embolic agents were injected in 1-ml aliquots until occlusion was produced.

If the animal survived, it was restudied at least 2 weeks later. The previously embolized carotid artery was restudied, and the contralateral rete was embolized to serve as an acute model. The animal was sacrificed, the brain and rete removed, and gross and microscopic neuropathology obtained. The sections were stained with hematoxylin and eosin and Masson's trichrome.

Laboratory bench testing was also performed to evaluate the size and type of catheter through which these materials would pass.

Results

Group 1: MCH and Ethanol

In all five animals studied in this group there was radiologically complete occlusion of the rete (Fig. 2) after injection of 1 ml of the mixture. On sectioning, acute changes in retia (Fig. 3) consisted of plugging of vessels with slightly hyaline material and thrombus, with most of the vessels occluded. The few that were patent were in the anterior portion of the rete and were believed to be due to external carotid feeders that were not embolized.

Chronic changes in five retia (Fig. 4), studied 2–6 weeks postembolization, consisted of fibrotic changes in the luminal thrombus, with only minimal evidence of early recanalization. There was a marked perivascular inflammatory infiltrate of lymphocytes, though this was not as marked as that seen after bucrylate injection. Only one infarct was seen; this was in the cerebellum and was likely due to injection below the internal carotid-vertebral anastomosis. No MCH was seen in leptomeningeal vessels.

Clinically, the pigs were unaffected by the injection of MCH and ethanol. Prior to this study, injections of similar volumes and concentrations of ethanol into pig internal carotid arteries had devastating results (all died). This provided clinical evidence that MCH and ethanol did not pass through the rete.

Group 2: GAX (with and Without Ethanol)

There were five animals in this group, and only one survived for later study. Initially, GAX and ethanol were injected, but this solution passed right through the rete and did not produce occlusion, although there was slowing of flow in two retia. Owing to the nonocclusion of the rete, ethanol and GAX passed into the brain with devastating results—two pigs both developed apnea, which necessitated sacrificing them. Thinking that this might be an ethanol effect from our prior experience with ethanol, we injected only GAX particles, but the effect was similar, with apnea in one pig and no radiologic evidence of rete occlusion. A second pig survived the initial injection, only to develop apnea after the second injection. The third pig went into a coma after it was injected. At

[§] Omnipaque, Winthrop Laboratories, Sterling Drug Ltd., Aurora, Ontario, Canada L4G 3H6.

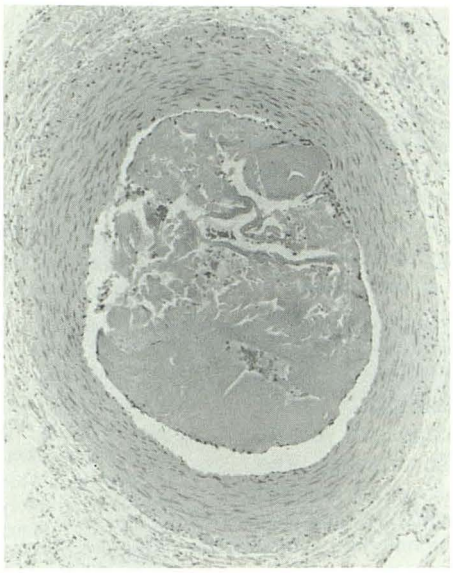


Fig. 3.—Lumen of rete vessel immediately postembolization with MCH and ethanol shows hyaline material and thrombus. (×240 magnification).

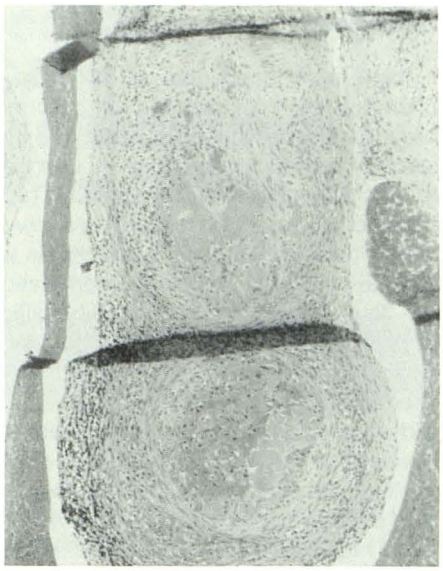


Fig. 4.—Rete at 6 weeks postembolization with MCH and ethanol. Hyaline material remains in lumen; inflammatory cells are in wall; there is no recanalization. Space between material in lumen and wall is fixation artifact.

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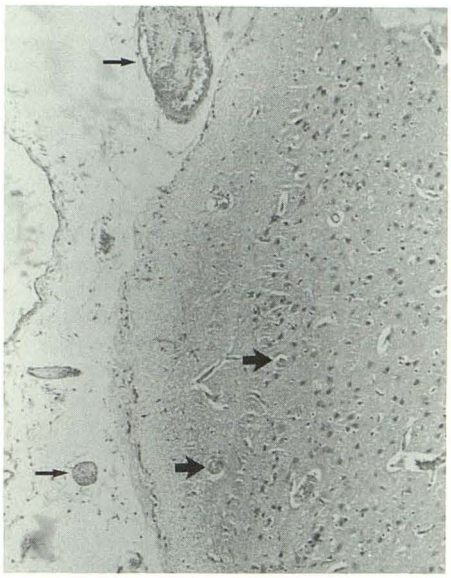

Fig. 5.—Occipital cortex showing multiple small (*thick arrows*) and large (*thin arrows*) leptomeningeal artery occlusions with particles of GAX (×240 magnification) (GAX without ethanol).

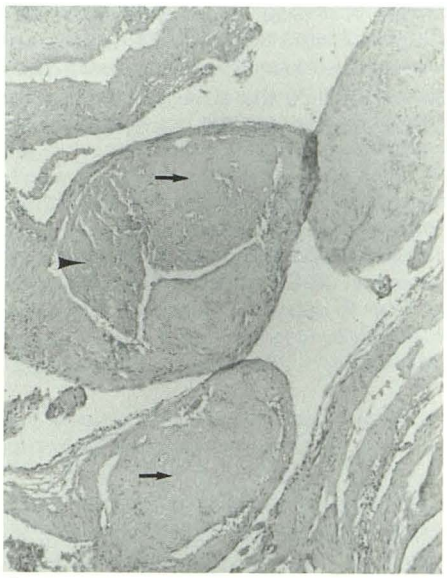

Fig. 6.—Rete with clot (*arrowhead*) and GAX (*arrows*) in lumen (×240 magnification).

5 6

autopsy, there was no evidence of diffuse cerebral edema in any of the pigs. On histologic section, GAX was seen in multiple leptomeningeal vessels (Fig. 5). Sections of the rete did show some GAX in the vessels, but these were not occluded (Fig. 6).

Group 3: Tisseel

Only two animals were studied with this agent. Although other researchers [8] have injected Tisseel through 3-French catheters, we could not inject it through catheters smaller than 5 French. The time required for this agent to form into gel when injected through long catheters was very unpredictable; however, it did eventually produce partial rete occlusion. Because Tisseel was unpredictable and difficult to work with, the study of this agent was discontinued. Unfortunately, no pathology is available in the two retia that were partially embolized.

Laboratory testing showed that MCH and TAX could both be injected through catheters of size 2 French or greater. However, MCH did not pass through calibrated leak balloons while GAX could be injected through them with minimal difficulty. Concentrations of MCH greater than 20 mg/ml often blocked the 2-French catheters; GAX did not show such problems. Tisseel could only be injected through 5-French and greater catheters. Again, this latter agent was not comprehensively studied because of its unpredictability in vivo.

Discussion

There is no doubt that of the three agents, MCH with ethanol is the best potential embolic material. We did not follow up pigs for extended periods to see whether recanalization of the rete does occur—the longest period of follow-up was 6 weeks, with no significant recanalization seen at this time. However, the pig rete is a low-flow structure. Until a high-flow AVM model is found that is easy to develop and use, the evaluation and future modification of this mixture in high-flow, or fistulous, AVMs will be difficult. The fact that it cannot be injected through calibrated-leak balloons puts some constraint on its use in intracranial AVMs, but the recent introduction of 2-French catheters with steerable or torque guidewires enables superselective catheterization of vessels previously achievable only with flow-directed catheters. The Tracker[‖] catheter, which is one of these, allows the material to be injected without difficulty, provided that the concentration of MCH is maintained below 20 mg/ml.

The fact that GAX passes through even a low-flow model such as ours makes it a less than ideal agent. Larger particle sizes may improve its usefulness, but this will need to be evaluated. We are not certain why the pigs developed so many problems related to its injection. It is likely that these were all due to small-vessel occlusion and diffuse hemispheric ischemia, since the absence of diffuse edema in the brains would tend to exclude some sort of toxic effect. Studies evaluating GAX in peripheral arteries (e.g., dog internal iliacs) or capillary networks (e.g., liver) [3] reported good occlusion.

While Tisseel may be useful as a topical sealant, the difficulties in injecting it through the types of catheters used in percutaneous embolization make it impractical as an embolic material.

In conclusion, MCH with ethanol is an effective embolic agent, combining ease of handling and injection with effective small-vessel occlusion. However, the long-term occlusive effects of the agent (greater than 6 weeks) are still unknown.

[‖] Target Therapeutics, San Jose, CA.

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