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Technical Feasibility and Histopathologic Studies of Ethylene Vinyl Copolymer (EVAL) Using a Swine Endovascular Embolization Model

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PURPOSE: To study the safety, technical efficacy, and histopathology of the ethylene vinyl alcohol copolymer (EVAL) embolic mixture in an animal model. METHODS: Microcatheterization of the rete was performed in 29 swine. The clinical, angiographic, and histopathologic consequences of superselective injection of the two principal embolic mixture components (EVAL and dimethyl sulfoxide [DMSO]) were evaluated. Necropsy and standard histologic preparation were used for pathologic analysis. RESULTS: Significant technical difficulties and adverse outcomes occurred. The EVAL mixture was difficult to see under fluoroscopy and prematurely polymerized during one embolization, resulting in catheter occlusion. However, polymerized EVAL did not adhere to the catheter. DMSO damaged the plastic hubs of the microcatheters. Infusions of DMSO always caused immediate, moderate to severe vasospasm and frequently caused either subarachnoid hemorrhage or stroke. Histopathologic findings of both DMSO and DMSO plus EVAL were similar, producing variable endothelial denuding, thrombosis, and disruption of internal elastic lamina in the acute stage. An intense, mixed inflammatory response, organized thrombus, and transmural necrosis with extravasation were seen in the subacute and chronic stages. CONCLUSIONS: Despite having some desirable features as an embolic agent, significant problems were encountered with EVAL; the most important of which is that one of the principal components of the embolic mixture, DMSO, seems to be very angiotoxic.

Index terms: Interventional materials, embolic agents; Interventional materials, liquids; Interventional neuroradiology, experimental; Animal studies


A nonadhesive, liquid embolic agent, ethylene vinyl alcohol copolymer (EVAL), has been used clinically for the treatment of cerebral arteriovenous malformations (AVMs) in Japan. Although preliminary reports of clinical use of EVAL have been favorable in a small number of cases (1, 2), this embolic mixture has not been tested thoroughly in laboratory animals and has been anecdotically implicated in causing serious complications (3). This prompted us to study the safety, technical efficacy, and histopathology of endovascular embolization with EVAL, using the swine endovascular embolization model (4–6).

Materials and Methods

The experiments were performed in accordance with guidelines for the use of laboratory animal subjects in research by the University of California at Los Angeles Chancellor’s Animal Research Committee and the National Institutes of Health. Twenty-nine young adult Red Duroc swine were intubated and placed under general anesthesia by continuous inhalation of 1% to 2% halothane. Prenesthesia sedation was achieved with intramuscular injection of ketamine (150 mg) and xylazine (2 mg/kg). A 6-F vascular sheath was placed into the right common femoral artery using the Seldinger technique.

A tapered 5.5- to 4.0-F guiding catheter was used to select the common carotid artery, through which a Tracker 18 microcatheter/Seeker 14 microguidewire system (Target Therapeutics, Fremont, Calif) was placed coaxially for
superselective catheterization of the ascending pharyngeal artery (ie, artery of the rete). The tip of the microcatheter was placed distal to the pharyngeal branch of ascending pharyngeal artery to ensure that infusions were delivered to the rete only. Angiography was performed by hand injection to ensure proper positioning and to study the anatomic configuration and blood-flow pattern of the rete mirabile. Injection rates were modified on a case-by-case basis to minimize filling of the contralateral rete via the coronal plexus.

Low-viscosity EVAL and dimethyl sulfoxide (DMSO) were kindly provided by Dr Waro Taki of Kyoto University Medical School (Kyoto, Japan) and Dr Hiroo Iwata of the National Cardiovascular Center (Suita Osaka, Japan). This embolic mixture is composed of 5 g of powdered EVAL and 35 g of powdered metrizamide dissolved in 60 g of the organic solvent, anhydrous DMSO (1). A two-stage delivery technique is necessary to perform embolization with this material. This technique consists of first infusing the catheter with anhydrous DMSO, followed by injection of the EVAL embolic mixture.

Two modifications of the originally described two-stage delivery technique (1) for endovascular embolization with EVAL were used. The first modification consisted of using only 1.0 mL of DMSO for infusion before injection of the EVAL embolic mixture. This volume reduction was done to adjust for the smaller infusion volumes needed for the retial system when compared with analogous superselective microcatheterizations in humans with brain AVMs. Because of complications encountered in the early part of the investigation (to be described later), this two-stage delivery technique was modified again (second modification) by infusing only 0.3 mL of DMSO into the dead space of the microcatheter before injection of the EVAL embolic mixture.

A protocol was developed to study certain hemodynamic consequences (assessed by angiography) and histologic changes from superselective injection of the two major components of the EVAL embolic mixture (ie, DMSO and EVAL mixture). Experimental groupings were divided into the following categories: 1) slow injection of EVAL only, 0.5 mL (n = 13); 2) slow injection of DMSO only, 0.8 mL (n = 3); and 3) injection of DMSO (0.3 to 1.0 mL) followed by EVAL (n = 13). Controls consisted of either contralateral retia infused with saline and iohexol or noncatheterized, uninjected specimens. In group 3, aliquots of approximately 0.4 to 0.9 mL of DMSO followed by 0.5 to 0.8 mL of EVAL were injected under continuous fluoroscopic observation.

Three time intervals for follow-up evaluation of hemodynamic and histopathologic consequences of the infusions were used as follows: acute (same day of injection), subacute (10 days from injection), and chronic (28 days from injection). All follow-up studies included bilateral carotid and ascending pharyngeal artery angiography to assess the extent and persistence of occlusion of embolized ascending pharyngeals and retia in the EVAL group and the angiographic architecture of the retial system in the DMSO groups.

Animals were killed by lethal injection of pentobarbital (100 mg/kg). Necropsy was performed immediately. Each rete was carefully exposed and dissected from the cavernous sinus. The specimen was grossly inspected for various postembolization changes, including texture/consistency, thrombosis, extravasation, inflammatory/granulation response, and fibrosis. Each rete was divided into caudal and cephalic portions, which were placed into 10% formalin for fixation. Standard techniques were used for preparing sections of the rete for light microscopy. Sections were stained with hematoxylin and eosin. An experienced neuropathologist evaluated the histopathologic changes in the embolized/infused retia.

Results

Technical and Clinical Evaluation

Infusions of 0.8 mL or more of DMSO always caused severe, rapidly progressive vasospasm in the distal ascending pharyngeal artery and retial arteries (Fig 1). The vasospasm was always noted to progress during a 15- to 20-minute time interval. None of the swine infused with 0.8 mL or more of DMSO survived for interval follow-up. Infusions of 0.5 mL of DMSO usually caused
acute, moderate vasospasm of the ascending pharyngeal and retial arteries (77%), which was also progressive during a 15- to 20-minute period. Follow-up angiography of this group at 10 and/or 28 days usually showed resolution of vasospasm in the distal ascending pharyngeal arteries, although permanent occlusion was noted in two cases (15%). Subtle, small filling defects within the retial arteries were seen in eight animals (62%), which suggested that persistent thrombosis occurred within some of the retial vessels. In four swine in this group (31%) symptoms of lethargy, loss of appetite, and gait disturbance developed 7 to 14 days after infusion of DMSO, which prompted early killing.

Five swine died in association with DMSO infusions. The first two deaths occurred at the start of the experimental protocol (same day) when larger volumes of DMSO were being infused (1.0 mL). In both of these cases, technical failures in delivering the EVAL embolic mixture into the rete occurred because of severe acute vasospasm from the DMSO infusion. After these swines’ prolonged recovery from anesthesia (several hours), seizures, cardiovascular instability, respiratory arrest, and hyperthermia subsequently developed, and they did not respond to resuscitative therapy. The clinical impression was that an acute ischemic event involving the brain stem occurred after embolization, possibly from either severe vasospasm or thrombosis of the basilar artery. Necropsy could not be performed in these first two animals.

The remaining three died within 24 hours after infusion of 0.8 mL of DMSO, in which all swine were found to be hemiparetic and in shock. These findings were consistent with a clinical diagnosis of massive cerebral infarction.

The actual embolization technique was subsequently modified after encountering the above problems. Only 0.3 mL of DMSO was infused into the microcatheter to fill the “dead space” of the microcatheter. The EVAL mixture was then slowly injected continuously with variable application of pressure to the syringe plunger. This second modification in technique enabled consistent delivery of the embolic material into the rete without acute complication. Catheter occlusion usually did not occur, except in one technical failure caused by premature occlusion of the catheter.

The EVAL mixture was found not to be very radiopaque, which produced some difficulties in monitoring the embolization closely under fluoroscopy. Usually, injection of EVAL through the microcatheter could not be seen under fluoroscopy until very late into the embolization, often resulting in abrupt retrograde filling of the ascending pharyngeal artery (53%). In these cases there was usually mild gross reflux beyond the distal tip of the microcatheter. Because of the nonadhesive nature of the polymer, however, no difficulty was encountered in withdrawing the microcatheter after completing the embolization.

Because anhydrous DMSO is an organic solvent, it may cause damage to various plastics (particularly polyurethane and polyvinyl chloride). We found that DMSO caused significant damage to the hub of a Tracker 18 microcatheter and the various plastic stopcocks commonly used during an embolization procedure. This damage was manifest as clouding and cracking of the exposed plastic. Damage to the hub of the microcatheter could be effectively delayed by placing a micro-guide wire introducer through the hub directly into the proximal end of the microcatheter before infusing DMSO. However, controlled delivery of the EVAL mixture required attaching a syringe containing the embolic mixture to the hub of the microcatheter, thus exposing it to DMSO. Because only one bolus of EVAL was needed to embolize the rete, no problems were encountered in this study from the exposure of the microcatheter hub to the damaging effects of DMSO. If more than one bolus of embolic mixture was planned through the same catheter, the attached stopcock needed to be replaced after each embolization (1, 2).

In all technically successful embolizations with EVAL, complete nonvisualization of the caudal and middle portions of the embolized rete was demonstrated by immediate and interval follow-up angiography using selective injections of the ipsilateral and contralateral ascending pharyngeal and common carotid arteries. These findings were interpreted as successful persistent embolization of the ascending pharyngeal artery and the lower portion of the rete itself. In most cases (82%), the cephalad portion of the embolized rete was filled by ipsilateral external carotid collateral branches (eg, ramus anastomoticus) and by contralateral ascending pharyngeal injection via the coronal retial plexus. These findings indicated that little or no embolic material reached the cephalad portion of the embolized rete.
Gross and Histopathologic Findings

Necropsy was performed in three of the five deaths associated with infusions of 0.8 mL or more of DMSO. In all cases, extensive cerebral infarction of the ipsilateral hemisphere and upper brain stem were found (Fig 2). In the 0.5-mL DMSO group, in the four animals in which the above-described symptoms developed, subarachnoid hemorrhage was found to have developed between 7 and 14 days after infusion. The subarachnoid hemorrhage was mostly confined to the basal cisterns and brain stem, compatible with rupture of one or more of the cerebral arteries of the circle of Willis. Gross inspection of the cerebral arteries showed small microaneurysms, presumably caused by angionecrosis (Fig 2).

Retia infused with DMSO frequently had small areas of bluish discoloration in the caudal portions, which most likely represented small thrombi within the retial arteries. In 4 of 13 specimens from group 1 (31%), gross interstitial hematomas were found in the retial adventitia. These hematomas were usually associated with thrombus in the cavernous and basilar dural sinuses.

The caudal retia embolized with EVAL in the acute and subacute phases were spongy and slightly firm in consistency. “Plastic casting” of the distal ascending pharyngeal and proximal retial arteries with bluish material was seen, which was similar to the appearance of latex injection specimens of vasculature. Careful examination of the cut surface of a dissected rete occasionally revealed minute whitish granular material exuding from the retial vessels, which likely represented small EVAL emboli. No gross abnormalities were seen in the cephalad portions of any of the embolized retia.

Chronic-phase embolization of retia with EVAL was notable for shrinkage and retraction of the embolized caudal portions and increased firmness (particularly when compared with the acute-phase embolized retia). In some cases the embolized retia were hard, having the consistency of scar tissue.

Histologic findings of acute-phase specimens infused with 0.5 mL of DMSO (group 1) were frequent areas of partial and subtotal endothelial denuding and acute thrombus within various-size retial arteries in all specimens. Endothelial denuding was also noted in the distal ascending pharyngeal in two of four (50%) specimens (Fig 3). Occasional focal disruptions of the internal elastic lamina within retial arteries were observed in all specimens. In three specimens (75%), areas of patchy transmural, fibrinoid necrosis of retial arteries were seen in addition to the above-described intimal changes (Fig 3). Furthermore, one of these samples showed an interstitial hematoma adjacent to the necrotic retial artery, indicating rupture with extravasation.

In the subacute specimens partial endothelial denuding with intraluminal fibrin deposition and/or organizing thrombus were frequently seen in all specimens. In five of six (83%) subacute DMSO specimens, mild to moderate mixed inflammatory infiltrates with large numbers of eosinophils were seen in occluded vessels, which were found mostly within the lumens and periadventitial portions of the retial arteries. Occasional foreign-body giant cells were observed also in two of six cases (33%). These inflammatory infiltrates occasionally were found to extend into the vessel wall (Fig 4).

All chronic specimens infused with DMSO showed numerous thrombosed retial arteries with intense, chronic endoluminal inflammation that produced an obliterative endarteritis. Numerous large foreign-body giant cells were seen in association with an intense transmural and periadven-
titial chronic inflammatory infiltrate, which produced an obliterative arteritis (Fig 4).

A variety of notable histopathologic changes occurred in retia embolized with EVAL and followed a characteristic pattern of evolution over time. The acute specimens showed medium and large arteries to be either completely filled with a homogenous gray embolic material (ie, complete casting of vessels) or aggregates of embolic material intermixed with acute thrombus (Fig 5). The latter finding was particularly evident in the ascending pharyngeal and large proximal retial arteries. On close inspection, these embolized retial arteries also showed partial or subtotal endothelial denuding and damage to the internal elastic lamina (Fig 5). These latter findings were similar to those seen in retia infused with only DMSO.

The subacute specimens embolized with EVAL showed mild to moderate mixed inflammatory infiltrate, which included many eosinophils. These infiltrates were found predominantly within the embolized lumens and periadventitial portions of the retial arteries, although occasional transmural inflammation was seen also. Complete endothelial denuding and more extensive disruption of the internal elastic lamina were also seen in two of three (66%) specimens. The embolic material occasionally showed darker basophilic staining.
Fig. 5. Two examples of acute-phase retia embolized with EVAL. There is plastic casting of the lumens of larger retial arteries with a bluish gray material (arrows). In many vessels the embolic material is mixed with coagulated blood (open arrows). Hematoxylin and eosin stain: A, ×15; B, ×10.

Fig. 6. Chronic-phase retia embolized with EVAL also show intense endoluminal, transmural, and periadventitial inflammatory infiltrates, which result in an obliterative arteritis. Note the large foreign-body giant cells (arrows) within the endoluminal and transmural inflammation and nearly complete loss of normal angioarchitecture. Hematoxylin and eosin stain: ×10.

All chronic specimens embolized with EVAL showed a significant intensification of the histopathologic changes described in the subacute specimens. Many of the embolized retial arteries showed a moderate to severe chronic inflammatory infiltrate, often including eosinophils, histiocytes, and very large foreign-body giant cells. The inflammatory infiltrates were periadventitial, transmural, and intraluminal (Fig 6). There was often complete destruction of the intima, with no identifiable internal elastic lamina. In most specimens (75%), there was evidence of transmural fibrinoid necrosis produced by an obliterative arteritis.

Discussion

Several mammals, including ungulates of the order Artiodactyla (including swine), have a rete mirabile, which is a plexiform network of interconnected, microarteries in the size range of 50 to 250 μm. This structure is enclosed within the cavernous sinuses at the skull base and is readily accessible by standard coaxial microcatheterization techniques used in humans. The anatomic arrangement and fundamental histologic construction of the rete (7–11) resembles the angiographic architecture of many human brain AVMs, which have a plexiform configuration of the ni-
dus. Thus, the rete has been used as a basic in vivo model of an AVM nidus for testing the efficacy and histotoxicity of new embolic agents and techniques (4–6). These previous studies have shown that the technical behavior of tested embolic agents and/or the histopathologic responses to these materials are very similar to those observed in humans.

This endovascular embolization model has some limitations, however, which could effect the validity of the current study’s findings. First, the model does not simulate high-flow shunt conditions that are typically encountered with brain AVMs, and thus was unable to test the technical efficacy of delivering embolic material under such conditions. In addition, the slower flow within the retial system may have accentuated the apparent histotoxic effects of the embolic material (particularly that of DMSO). This latter limitation, however, probably does not lessen the validity of the findings of this study, because in some of the previously described clinical embolizations the same “slow-flow” conditions are created when using flow arrest with a calibrated-leak balloon catheter (1, 3). Furthermore, it is possible that the small nutrient arteries that may arise from a feeding pedicle to a brain AVM have substantially slower blood flow and thus are theoretically more susceptible to possible accentuated histotoxicity produced by prolonged exposure to DMSO.

A few technical problems were encountered in this study. First, the relatively larger volumes of preembolization infusions of DMSO (which were actually reduced by 50% from the originally described technique of Taki et al [1]) always caused significant acute vasospasm of the ascending pharyngeal artery. This essentially precluded delivery of EVAL mixture into the targeted retial arteries. Consequently, a significant modification of the two-stage delivery technique was required, in which the second modification in technique enabled consistent delivery of the embolic material into the rete without acute complication and usually without technical failure caused by premature polymerization, resulting in occlusion of the catheter (only one catheter occlusion occurred with this technique).

The EVAL embolic mixture was less than optimally radiopaque, possibly because it is only composed of 35% metrizamide. This diminished visibility under fluoroscopy often resulted in precipitous reflux of EVAL proximal to the tip of the microcatheter, because it was difficult to assess the effects of the embolization visually until there was considerable retrograde filling of the distal ascending pharyngeal artery. Although this technical limitation does not pose a problem in terms of “gluing” the microcatheter to the blood vessel, it does pose a potentially significant risk of inadvertent embolization of proximal normal vascular territory if sufficient purchase into the embolized pedicle is not achieved.

As predicted, the anhydrous DMSO used for this embolic material was found to cause damage to some of the plastic materials that are commonly used for endovascular catheterizations (eg, the hub of a Tracker 18 microcatheter and the plastic stopcocks). Although this problem could be effectively countered if a single delivery of the EVAL mixture was planned, solvent damage to the microcatheter hub resulting from repeated embolizations with EVAL could pose risks of technical failure and/or complication (because of thromboembolism or toxic effects of solubilized plastic polymers).

The most important and disturbing finding of this study was related to the apparent angiotoxicity of relatively small volumes of anhydrous DMSO. This study has documented in swine that slow infusions of 0.8 to 1.0 mL of DMSO into the retial system consistently produced severe vasospasm, which often resulted in cerebral infarction. Furthermore, slightly smaller volumes of anhydrous DMSO (0.5 mL) were associated with subarachnoid hemorrhage in 31% of cases. This seemed to be a delayed complication, apparently not showing until 7 to 14 days after infusion. A possible explanation for this complication is that as the DMSO diffused through the cerebral arterial wall, it caused patchy fibrinoid necrosis of the intima and media (as documented on histopathologic evaluation). The chemically damaged vessel wall was then mechanically fatigued from exposure to the usual hemodynamic stresses of the cerebral circulation, which eventually produced rupture with resultant subarachnoid hemorrhage. This explanation is further supported by the observation of extravasation occurring from a retial artery in one of the subacute specimens seen on histologic examination and the interstitial hematomas of retia infused with DMSO on gross inspection.

Because DMSO is a highly diffusible organic solvent with low viscosity, it is capable of reaching very small nutrient branches that may arise from an AVM pedicle when it is injected superselectively. Furthermore, superselective infusion of DMSO into an AVM nidus under flow arrest or
slow-flow conditions (as has been previously described) may produce prolonged exposure of the relatively thin-walled vessels of the nidus to the angiotoxic effects of DMSO. Thus, we suggest that the use of DMSO for intracranial endovascular embolotherapy may pose a significant risk of complications, such as hemorrhage (from rupture of exposed AVM nidus or nutrient arteries) and cerebral infarction (from vasospasm of nutrient vessels).

Careful review of the original references of Taki et al (1) for support of the safety of intraarterial anhydrous DMSO (12–14) showed that these studies always involved intravenous administrations of aqueous solutions of DMSO (ranging from 10% to 50%). The apparent tolerance of the peripheral venous system to injection of diluted concentrations of DMSO does not necessarily translate to an equivalent experience with intraarterial administration of anhydrous DMSO.

The thrombus in the cavernous and basilar dural sinuses in 31% of retia infused with 0.5 mL of DMSO may have been caused by a combination of extravasation from the damaged retial arteries into the adventitia and the intense periadventitial inflammatory reaction of these vessels. This latter process could have extended into the adjacent cavernous and basilar dural sinuses, producing a thrombophlebitis.

The histopathologic changes seen in the vasculature exposed to DMSO only and DMSO plus EVAL were similar to those changes described with endovascular embolization with other commonly used embolic materials, such as cyanoacrylates (isobutyl cyanoacrylate and N-butyl cyanoacrylate), polyvinyl alcohol, and composite mixtures (eg, avitene with or without polyvinyl alcohol and ethanol) (4, 5, 15–18). These similarities include progressive chronic inflammatory infiltrates containing histiocytes and foreign-body giant cells, which occurred within both the lumina and walls of embolized vessels, intimal damage (to endothelium and internal elastic lamina), and mural necrosis.

There were also a few notable differences in histopathologic changes observed in this study from the histopathologic effects of other embolic materials. First, no extravasation of EVAL emboli was seen in any of the specimens, unlike reports with isobutyl cyanoacrylate, N-butyl cyanoacrylate, and polyvinyl alcohol (4, 17, 18). A possible explanation for this finding is that the EVAL polymer is more readily phagocytized and decomposed than these other embolic materials and thus does not undergo a typical foreign-body sequestration/extrusion process. Another observed difference in histopathologic findings was the lack of intramural hemorrhage in embolized retia. We cannot offer an explanation to account for this difference. Finally, our study showed an unusually prominent number of eosinophilic infiltrates in the subacute and chronic specimens infused with DMSO and embolized with EVAL. This finding indicates a possible allergic/hypersensitivity component to the inflammatory reaction.

It is interesting to note that very similar histopathologic changes (including an intense granulomatous vasculitis) occurred in the DMSO-infused and EVAL-embolized retia. This suggests that the exposure of the microvasculature to DMSO (through a combination of preembolization infusions and the constituents of the EVAL mixture) may have a major role in the histopathologic response to this embolic material. Additionally, the findings of large areas of endothelial denuding and internal elastic lamina damage in acute EVAL-embolized retia were similar to those seen with acute DMSO-only infusions, suggesting that these acute-phase intimal changes may be attributable to DMSO.

The histopathologic findings of our study differ somewhat from those of Taki et al (1). In their study no inflammatory changes were noted within the vessel walls of an embolized swine renal artery (time interval unknown) and a 25-day-old embolized cerebral AVM. Prominent endoluminal inflammatory infiltrates with foreign-body giant cells were observed in their AVM specimen, however, as in our study. It is possible that this discrepancy is related to differences in completeness of histologic examination of samples and natural variability in histopathologic response. It is noteworthy that our findings are very similar to those reported by Fukushima et al (3), in which histopathologic evaluation of a cerebral AVM embolized with EVAL showed extensive endoluminal and intramural inflammatory infiltrates containing foreign-body giant cells and foam cells after 4 weeks from embolization. Furthermore, this report described perivascular hemorrhage around the embolized vessels as was found in our current study, suggesting that the vessels ruptured from exposure to the embolic mixture (either from DMSO alone or combined with EVAL). We believe that the similarities in pathohistologic findings between our study and that of Fukushima et al (3) provide further sup-
port of the validity of the endovascular embolization model used.

In conclusion, although EVAL has some physical properties that make it a more desirable embolic agent (ie, nonadhesive and soft polymer), some significant technical problems and limitations with this embolic material were encountered in this study. Most importantly, the solvent, DMSO, used to prevent premature polymerization of the embolic mixture, seems to be highly angiotoxic when injected superselectively into small arteries. This may pose a significant risk for complication when used for intracranial endovascular embolotherapy.

References