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Acute and Chronic Swine Rete Arteriovenous Malformation Models: Hemodynamics and Vascular Remodeling

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BACKGROUND AND PURPOSE: **An acute and a chronic arteriovenous malformation (AVM) model were developed by using the swine rete to study hemodynamics and vascular remodeling. The models were also used to study in vivo polymerization kinetics and the distribution of various N-butyl 2-cyanoacrylate (NBCA) and Lipiodol mixtures.**

METHODS: **In the acute swine AVM model, retrograde flow through the left side of the rete was created by the placement of an endovascular shunt through the ipsilateral ascending pharyngeal artery. In the chronic model, flow was redirected retrograde through the left side of rete and ascending pharyngeal artery by creating an arteriovenous fistula between the ipsilateral jugular vein and the common carotid artery. After a period of at least 6 months, the entire head with the rete was connected to a perfusion loop driven by a peristaltic pump. A total of 30 swine were used for both the acute ($n = 23$) and chronic groups ($n = 7$). Hemodynamic parameters, including the flow and pressure drop across the rete, were recorded before NBCA embolization. Image processing was used on high-resolution radiographs of the explanted retia to measure the total rete length. Measurements of rete vessel calibers were based on histology.**

RESULTS: **The pressure gradients across retia were higher in the chronic model than in the acute model, but they did not reach the level of statistical significance (23.7 ± 12.0 mm Hg vs 15.4 ± 1.4 mm Hg). The rete blood outflow was significantly higher in the chronic model compared with the acute one (139.9 ± 100.3 mL/min vs 32.5 ± 17.6; $P = .03$). The rete length in the chronic model was significantly higher than in the acute model (593.1 ± 39.9 vs 401.3 ± 65.2 pixel; $P < .001$). The average vessel diameter of the rete in the chronic group was 520 μm and 320 μm in the control animals.**

CONCLUSION: **Increased pressure gradients and flow in the chronic swine rete AVM model may be related to increased size and decreased impedance. The resulting hemodynamic changes reflect a true flow-induced vascular remodeling rather than a simple change related to aging and size of the animal.**

If a cure is not achieved, embolization of an arteriovenous malformation (AVM) before surgery is used to reduce the size of the lesion and improve the safety and efficacy of surgery and radiosurgery. Radiation treatment of smaller lesions has a higher cure rate and is associated with lower morbidity. Thus, embolization before a planned radiosurgery has to obliterate AVM pedicles, including the nidus from the periphery toward the center rather than obliterate a pedicle proximally (1). We constructed acute and chronic swine rete models to study complex mechanism of liquid embolization, including the polymerization kinetics of N-butyl 2-cyanoacrylate (NBCA) for different plexiform human brain AVMs under controlled flow-dynamic parameters.

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Methods

The experimental methods of our study were composed of two phases. In the first phase of our study, we constructed a chronic AVM model in the rete mirabile of swine in a preparation similar to the one described previously by Massoud et al (2). In the second phase of the study, we created an acute rete AVM model that uses combined surgical and endovascular techniques (3). All experiments were conducted in accordance with the guidelines set by the local institutional animal care and use committee. Two retia were removed from swine of different ages and sizes, 35 kg and 100 kg. These animals were not

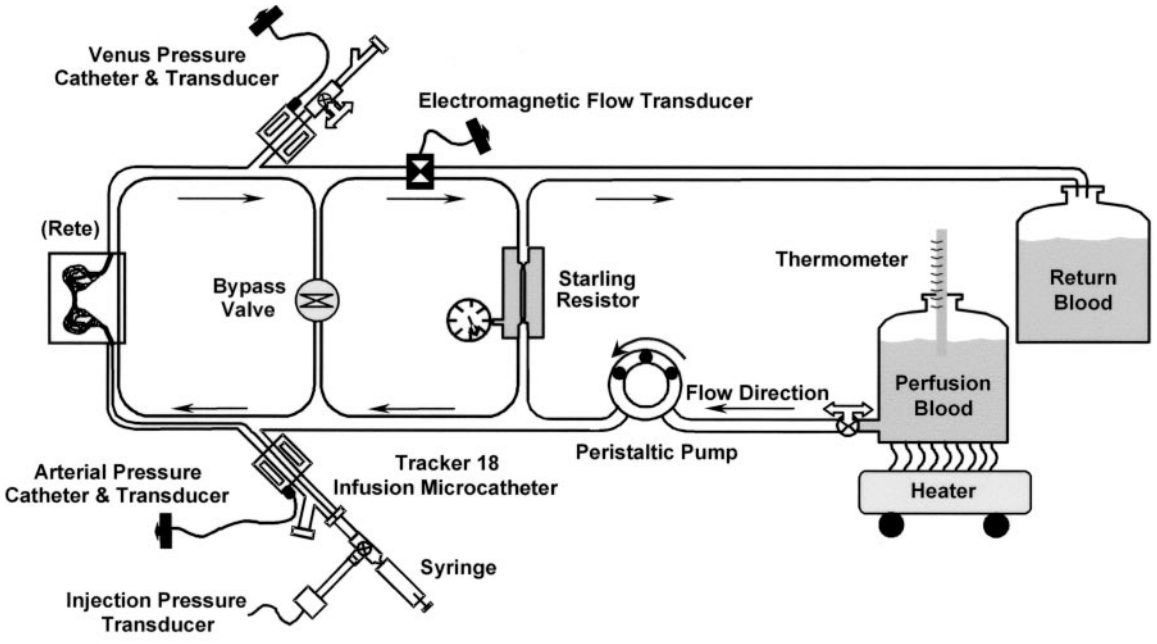

FIG 1. Illustration of the perfusion apparatus used in the chronic swine AVM model.

used for AVM model and did not undergo surgery or an interventional procedure. They served as controls for histology studies.

Preparation of Chronic AVM Model

The surgical preparation was similar to the one described by Massoud et al (2). In brief, sedation was induced by an intramuscular injection of 3.0 mg/kg of Telazol and 3.0 mg/kg Xylazine, followed by 0.02 mg/kg of Atropine to reduce secretion. During surgery, general anesthesia was maintained by spontaneous inhalation of 1.5%–2% isoflurane. After the right side of the neck was shaved and cleaned, a 10-cm-long incision was made to retract the sternocleidomastoid muscle and expose both the right common carotid artery (CCA) and the jugular vein (JV). Vascular clamps were used to isolate a segment of the CCA and the JV. A 3-cm-long incision along the vessel was made on both segments of the isolated area. The dissected areas were rinsed with normal saline solution. Any vasospasm was resolved with a topical application of 2% Lidocaine. After arteriotomy and venotomy, the dissected areas starting from the posterior wall were anastomosed side-to-side. Once the fistula was created, the clamps were removed and the area was rinsed and observed for any leaks. Subsequently, the CCA proximal to the fistula was ligated with suture. The pressure drop due to the fistula was sufficiently large to divert the blood flow in the ipsilateral ascending pharyngeal artery (APA) in a retrograde fashion. The size of the fistula was large enough to ensure its patency. Details of the arteriovenous fistula are illustrated by Siekmann et al (3).

After CCA ligation proximal to the fistula, the dissected tissue and skin were sutured; the animal was monitored during recovery from anesthesia. A single dose of penicillin (8200 U/kg) was administered after surgery to prevent infection. The final step implemented by Massoud et al, whereby the external carotid artery (ECA) was occluded with a detachable balloon for acute embolization, was omitted in our preparation, because the aim in our preparation was to let the animals recover for a long period of time so that an enlargement of the rete vessels would occur. A follow-up angiogram was obtained 2 weeks after surgery to verify a patency of the fistula.

Ex-Vivo Flow Loop for the Chronic AVM Model

Each animal weighed between 180 kg and 250 kg 6–9 months after the construction of the fistulas, at which point the thickness of the cranial bone prevented clear visualization of the rete or the cerebrovasculature by conventional radiography. To circumvent this difficulty, the animals were euthanized before embolization. The cranium was separated from the body; the two CCAs were isolated at the skull base and sutured to plastic barbed connectors by using a 2.0 suture line. The cerebrovasculature was flushed with saline through one of the CCAs to remove residual blood. The bone mass in the frontotemporal region of the head was removed by using a reciprocating electric saw. Care was taken not to damage the CCAs or the rete. Once the bone mass surrounding the rete was reduced, the cerebrovasculature was reperfused with saline to identify leaks from dissected arteries that needed to be seared. This was particularly true for branches arising from the ECAs. In most cases dissected vessels were cauterized, and in some cases pure NBCA was used to seal off additional leaks (4).

The prepared cranium was connected to an ex vivo mock circulation loop shown schematically in Fig 1. The flow loop was perfused with 3–3.5 L of autologous blood. Twenty milliliters of ethylene diamine tetra-acetic acid (at 7.5% concentration) were added per liter of exsanguinated blood, to prevent coagulation. During the experiments, blood was stirred continuously and kept at 37°C by using a laboratory magnetic stirrer/hot plate. A peristaltic metering pump (Cole-Parmer Instrument Co., Chicago, IL) was used to circulate the blood. The circulation loop with two shunts built into it was composed of Tygon and silicone tubes with a .25-inch internal diameter. A Starling resistor composed the shunt that was used to regulate the maximum pressure through the rete and was adjusted to 170 mm Hg. The second shunt in the system consisted of a bypass valve that was used to purge the air from the perfusion loop before connecting the prepared cranium. The blood flow through the rete was measured in the return line by using an electromagnetic flow metering system (transducer model SP75519 and meter model SP2202; Gould Stantham, Oxnard, CA). Blood pressure at the inlet to and outlet of the AVM model was measured by using disposable Deltran

pressure transducers (Utah Medical Products, Inc., Midvale, UT) with an operating range of −50 to 300 mm Hg connected to a demodulator (Tri-Pack model TP8891; Vivitro System, Inc., Victoria, Canada). In addition, during embolization the injection pressure of the glue was recorded with a differential pressure metering system (transducer model DP15–36 and digital transducer indicator model CD223; Validyne Engineering Corp., Northridge, CA). The calibrated signals from all four transducers (three pressures, one flow) were recorded and stored on a personal computer for further off-line analysis. Data were collected by using Virtual Bench 2.0 software (National Instruments, Austin, TX) and a data acquisition board (Model AT-MIO-16XE-50; National Instruments).

Preparation of Acute AVM Model

Siekmann et al described in detail the acute preparation of the AVM model (3). In brief, a large guide catheter was advanced through the left CCA and tightly fit into the root of the left APA. The other side (hub) of the catheter, now serving as a cannula, was opened to atmospheric pressure during embolization. The cannula replaced the surgical procedure involved in creating the AV fistula and provided a sufficiently large drop in pressure to establish retrograde flow through the cannulated side of the rete. A FasTracker 18 (Boston Scientific/Target Therapeutics, Fremont, CA) was navigated through another guide catheter into the middle of the right APA. Superselective angiograms with and without opening the draining catheter to the atmosphere were obtained to document the efficacy of the model.

The microcatheter was flushed with a 5% dextrose solution several times to remove ion-containing contrast or saline. In the meantime, the glue mixture was prepared in a separate clean area and loaded into a 3-mL syringe for injection. A 3-mL syringe was used (rather than a 1-mL syringe) to eliminate the need for syringe exchange during the glue injection and thus provide continues injection of the glue. A total of 23 successful experiments in pigs of mixed sex, 2–17 months old, and weighing 27–108 kg were used for this phase of the study.

Rete Length and Histology

The retia from both AVM models were harvested immediately after embolization. To measure the length of the harvested retia, high-resolution radiographic digital images (1024 × 1024 pixels with a resolution of 10–11 line pairs/mm) were acquired by using a mammography unit with a 0.1-mm microfocal spot (Mammotest/Mammovision unit; Fisher Imaging Co., Denver, CO). Image processing was performed to determine the centerline of each rete. The centerline was determined to be the locus of points obtained by a thinning operation. A midline for each rete was defined that divided it into right and left halves. The midline was determined to be the most slender part of the rete between its two halves. The rete was considered to begin at the first bifurcation of the APA and to be symmetric around the midline. Therefore, the length of each rete was determined as twice the length of the centerline from the first bifurcation of the APA to the midline on the embolized side.

After obtaining the radiographs, the dissected retia were prepared for histology. The retia were fixed in fresh buffered 3% formaldehyde for 2 hours (48°C), rinsed, dehydrated, paraffin-embedded, and radially sectioned (5 μm) onto poly-L-lysine-coated slides (Mt. Washington Scientific, Timonium, MD) as described elsewhere (5). Samples were obtained radially at predetermined angles spanning the entire rete from the left ascending pharyngeal artery to the right. Sections chosen for color staining were processed with the lipid dye Oil-Red-O according to routine protocols. Histology was performed for retia extracted from both AVM models and the controls.

Hemodynamic parameters in an acute and a chronic swine rete AVM model

	Acute Model	Chronic Model	*P* Value
Age (months)	3.9 ± 3.2	12.4 ± 0.5	<.0001
Weight (kg)	46.9 ± 22.7	213.7 ± 25.9	<.001
Pressure grandient (mm Hg)	15.4 ± 1.4	23.7 ± 12.0	.085
Rete outflow (mL/min)	32.5 ± 17.57	139.9 ± 100.29	.030
Rete length (pixel)	401.3 ± 65.2	593.1 ± 39.9	<.001

Statistical Analysis

Statistical analyses were performed by using SPSS 8.0 (SPSS Inc., Chicago, IL), Instat 3.01 (GraphPad Software, Inc., San Diego, CA), and Microsoft Excel 97 (Microsoft, Inc., Redmond, WA). The means of animals age, weight, pressure gradient across the rete, outflow, and mean rete lengths for both the chronic and acute experiments were compared by using an unpaired *t* test with 95% confidence interval.

Results

A comparison between the chronic and the acute experimental models is shown in the Table. In the chronic model, the rete was 48% longer on average than in the acute model (593.1 ± 39.9 vs 401.3 ± 65.2 pixel; $P < .001$). The difference in rete length may be attributed in part to differences in the age and weight of the animals comprising the two groups. In the chronic group, the average age was 12.4 months, and the average weight was 213.7 kg, compared with an average age of 3.9 months and average weight of 46.9 kg for the acute group. The highly significant difference ($P < .001$ by an unpaired *t* test) in mean rete length between the two groups provided the rationale for normalizing the length of each rete by the average value of the rete length of its corresponding group. The average vessel diameter of the rete in the chronic group was 520 μm and 320 μm in the control animals (Figs 2 and 3). Diameters of the vessels measured at various locations of the retia in the controls of different ages, but same species (ie, barnyard pigs) were not statistically different despite substantial difference in the sizes of animals (Fig 3). One of the control animals was nearly three times the size of the other animal, although no differences in the diameter of the rete vessels were noted at any of the locations where histologic sections were acquired.

The mean pressure drop across the rete in the chronic group was about 34% larger than for the acute group (Table). Although we had complete control of the hemodynamic variables proximal to the rete in the chronic experiments, this was not the case in the acute preparation. The acute model was established with a cannula, and the pressure gradient was established as a result of the difference between arterial pressure and the proximal end of the cannula. Statistical comparison between the two groups, however, showed that the difference in the pressure gradients was not significant ($P > .05$).

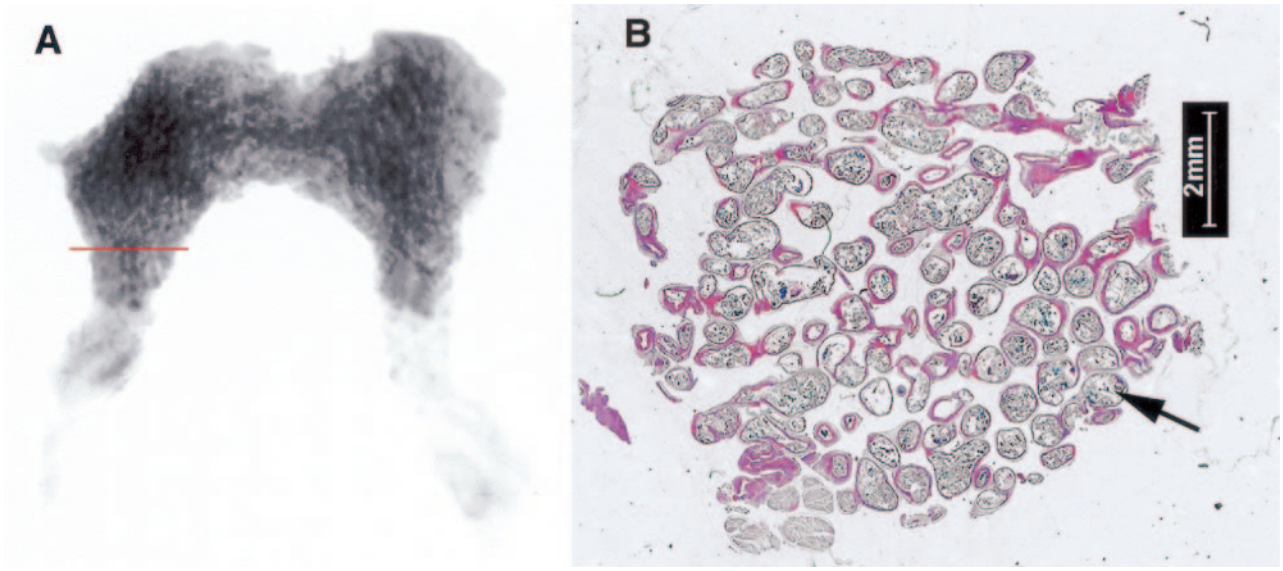

FIG 2. *A,* High-resolution radiograph of a rete in the chronic swine AVM model embolized with a 50/50 + 20 μL acrylate mixture (NBCA/Lipiodol/glacial acetic acid). *Red line* indicates the area of cross-section for histology.

B, Corresponding histology (hematoxylin-eosin stain) for vessel measurements shows most of the arteries of the rete network filled with acrylate mixture (*arrow*).

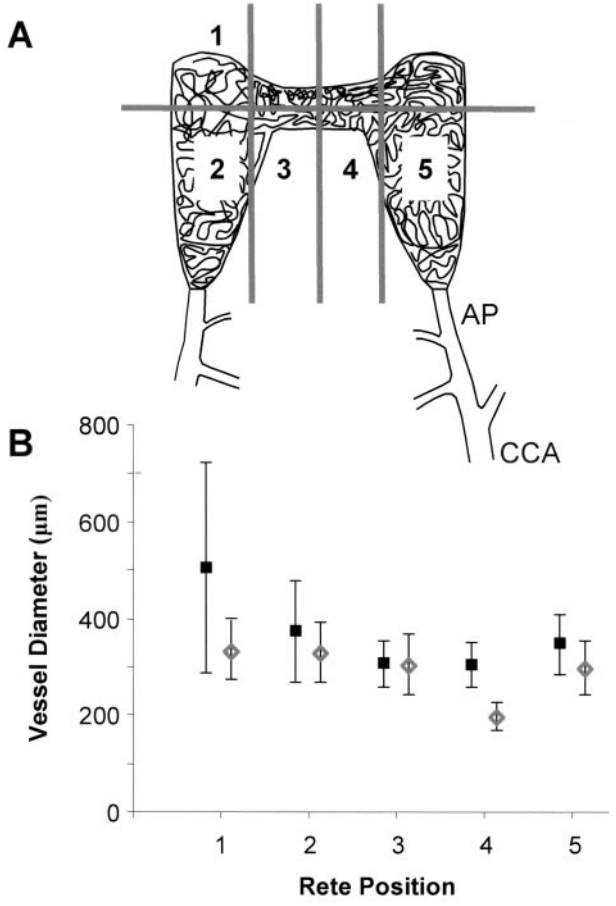

FIG 3. Vessel diameters of nontreated swine retia (controls).

A, Schematic of a rete with section lines demarcating sectors for vessel measurements (AP, ascending pharyngeal artery; CCA, common carotid artery).

B, Graph shows the corresponding vessel diameters in each region for a 35-kg (*diamonds*) and 100-kg (*squares*) swine. Error bars indicate the standard error of mean.

Discussion

The outcome of AVM embolization can be affected by different parameters, making its success an arduous and complex task. Biologic variability of the pathology (ie, the AVM morphology, size, number of feeding arteries, number of draining veins, and size of pedicles) and hemodynamic parameters (ie, pressure gradient across the AVM and blood flow rate through the AVM) can affect the penetration depth of the glue mixture used for embolization. Biologic variability cannot be controlled for an optimized embolization, although adjusting the systematic blood pressure can relatively control hemodynamic parameters.

To study the complexity of embolization with liquid agents and the polymerization kinetics of NBCA, we developed two different swine rete in vivo AVM models. These AVM models mimic a human plexiform AVM with relatively comparable vessel sizes but different architecture and flow dynamic.

In acute cases, the overall volume of the rete was smaller than in the chronic cases, as reflected in the overall amount of acrylate injected. We propose that the increased flow through the chronic model as compared with the acute model is the result of flow-induced vascular remodeling. The substantial difference in mean outflow (more than fourfold higher in the chronic preparation) between the two groups, however, cannot be attributed solely to differences in pressure gradient. A substantially lower resistance to flow through the rete is required to produce such an increase in flow without a substantial increase in pressure drop.

The marked decrease in resistance may be attributed to a combination of the following: loss of vascular tone in the ex vivo preparation can increase vessel diameter and hence reduce resistance; chronic changes in rete hemodynamics were induced surgi-

cally in an attempt to induce ectasia and thereby reduce resistance; the larger and older animals used in the chronic preparation may have developed larger-caliber vessels. Vascular resistance to flow is directly related to path length and, inversely, to the fourth power of diameter. Despite the fact that mean rete length was increased by approximately 32% in the chronic model, the resistance of this model was lower. The dominant factor associated with the reduced resistance to flow in the chronic animals was the increased luminal diameter of the rete vessels.

In their acute study, Massoud et al (6) reported a mean internal diameter of 328 μm for rete vessels (the animals were euthanized on the same day of surgery and embolization) and 490 μm for the mean caliber of rete vessels in their chronic study (the animal was kept alive for 6 months after the surgery and before embolization). It has been shown in rabbits that construction of an arteriovenous fistula produces a large increase in flow associated with a vascular remodeling process that is dependent on matrix metalloproteinases 2 and 9 (7). In our chronic study, the average vessel diameter measured from histologic images was found to be 520 μm (Fig 2), which is larger than the value reported by Massoud et al (6). Retia in our control animals, which did not undergo any surgery or embolization, showed, regardless of their size and age, vessel diameters similar to those found in the acute AVM model (6). For training purposes, however, the acute preparations are less labor-intensive and easier to construct than the chronic model. But both preparations, presenting with different AVM nidus structure and flow properties, are useful for training and can provide realistic in vivo models to study the characteristics of different embolic agents.

Conclusion

Both an acute and a chronic AVM model were constructed by using the swine rete to study NBCA embolization. Flow-dynamic study shows increased pressure gradients and flow in the chronic AVM model, which may be related to increased size and decreased impedance. Our histology study show an ectasia of the rete vessels in the chronic AVM model that reflects probably a true flow-related vascular remodeling rather than an increase in size associated with aging or size of the animal.

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