

The Development of a Rat Model for Arteriovenous Malformation (AVM) **Gerald J. Cho, Cathy L. Ebert, Brian Richardson, and Jennifer J. Marler**

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Background: Arteriovenous malformations (AVMs) are defects in the circulatory system that result from poorly understood developmental errors during embryogenesis. AVMs can develop at different sites in the body, most commonly in the head and neck, and in the extremities. They can range from focal lesions to extensive Anatomic regions. The only available therapies for treating affected patients are interventional radiology (embolization), sclerotherapy (injection of an agent to induce inflammation and obliteration of affected vessels), surgical resection and reconstruction. Unfortunately, AVMs can often invade deep craniofacial structures, permeate the pelvic tissues, or infiltrate all tissue planes of an extremity, rendering current treatments ineffective and aimed more for palliation.

Purpose: Currently, there are no standard animal models of peripheral AVMs. Dr. Jennifer Marler's laboratory has identified genes that are upregulated and downregulated in AVMs, using microarray analysis of tissue samples taken from affected humans (CCHMC IRD# 03-5-9X Characterization of gene expression in vascular tumors and vascular malformations). These findings predict that certain therapies may be useful in suppressing the progression of AVMs. Our aim is to develop standard rat model that would closely simulate human peripheral AVMs and lend themselves to transgenic studies. In subsequent experiments, we will test three therapies predicted to be effective by microarray analysis.

Methods: All experimental protocols were carried out in Sprague Dawley rats (under a protocol approved by the CCHMC IACUC protocol #4D11089). Six week old female rats were anesthetized with isoflurane using a small animal anesthesia machine, followed by shaving of one groin area. The rats were placed under continuous anesthesia in a supine position on a heating blanket beneath a surgical microscope. The groin was prepped with betadine and draped with sterile drapes. An incision was made along the inguinal crease and femoral vessels exposed. Specifically, 4 groups of rats were created: (1) sham operated control; (2) complete AV fistula; (3) complete AV fistula plus Freund's Adjuvant; (4) complete AV fistula plus bFGF. Specific group interventions were carried out as listed below:

Group 1, sham operated control: Femoral artery and vein was mobilized and clamped for a 35 minute period (amount of time other groups will have their vessels occluded) without further intervention. **Group 2**, complete AV fistula: Following occlusion of the vessels, an end-to-side anastomosis was created with 11-0 nylon sutures using conventional microsurgical technique. **Group 3**, the complete AVF group plus inflammation: Following creation of the anastomosis, 0.05 ml of Freund's adjuvant mixed with Gelfoam® was added to the muscle adjacent to the fistula. **Group 4**, the complete AVF group plus bFGF: 1 µg of bFGF suspended in normal saline and, mixed into Gelfoam® and added to an adjacent muscle.

In all groups, adjacent muscle was closed over the anastomosis with 4-0 monocril. The first dose of pain medicine was delivered while the animal remained anaesthetized. Anesthesia was discontinued and mice recovered in a separate chamber equipped with a heating pad.

To determine whether an appropriate model has been established, the animals were allowed to recover for 4 weeks, and then analyzed through the following methods:

- (1) Laser Doppler: Animals were anesthetized and the vessels evaluated.
- (2) Histology: Animals for histology were be euthanized with pentobarbital. Following measurement of vessel diameter, the vessels will be perfused with heparinized saline followed by perfusion with 4% paraformaldehyde and tissues submitted for histology.

Results/ Conclusions: Gross examination of the AVF 4 weeks post-op revealed a patent AVF, indicating that a viable AVF can be created in the rat femoral vessels. However, gross appearance of the vasculature did not indicate the creation of an AVM. Laser Doppler demonstrated an increase in blood flow at the site of surgical intervention. The increase in blood flow was relatively twice that of the contralateral (unoperated) vessels for all groups receiving surgical intervention. Immunohistochemistry showed that there is an increase in perilipin expression in vasculature distal to the AVF, similar to what is seen in human AVMs. However, the demonstration of this increase in expression in the surgically created rat AVF is a preliminary finding necessitating further experiments given the relatively small n for each group (n=2 for Group 1, n=2 for Group 2, n=4 for Group 3, n=3 Group 4). Staining with anti-CD54/ICAM-1 demonstrated a more intense staining pattern in Group 3 relative to Groups 1, 2, and 4. In human AVM, microarray data indicates an increase in expression of ICAM-1. Preliminary results indicate that the increase in ICAM-1 expression may be a result of an acute inflammatory response to Group 3's treatment with 0.5ml of Freund's Adjuvant, rather than the surgically created AVF's similarity to an AVM, given the relatively low staining density of Groups 2 and 4.