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Laboratory Study

Comparison of an animal model of arteriovenous malformation with human arteriovenous malformation

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ABSTRACT

This study assessed the blood flow and histological changes of an animal model of arteriovenous malformation (AVM) over 84 days in 71 rats, and compared the histological findings to 17 specimens of human AVM. Carotid–jugular fistula blood flow positively correlated with time. The maximum flow rate occurred at 42 days, at which time the nidus was considered mature and was histologically similar to human AVMs. Morphological similarities between the model and human AVM vessels included heterogeneously thickened walls, splitting of the elastic lamina, thickened endothelial layers, endothelial cushions, lack of tight junctions, loss of endothelial continuity, endothelial–subendothelial adherent junctions, and luminally directed filopodia. These findings support the theory that vascular changes in human AVMs are secondary to increased flow and provide a basis for using this model in studies of AVMs.

1. Introduction

Central nervous system arteriovenous malformations (AVMs), as a leading cause of stroke and death in children and young adults, impose a significant burden on society. Although many AVMs can be treated successfully with surgery or radiosurgery, a significant group of patients have large and deep lesions that are untreatable. In addition, patients treated with radiosurgery remain at risk of hemorrhage during the latent period between treatment and AVM obliteration.[1]

Further advances in the treatment of AVMs are likely to involve molecular and cellular strategies to enhance the efficacy and safety of radiosurgery or endovascular treatment. Development of such strategies will require a model of AVMs that mimics the hemodynamic, histological, molecular, and ultrastructural characteristics of human AVMs, which are all different to those of the normal vasculature.[2–4] A rodent AVM model mimics the hemodynamic characteristics of AVMs in that there is a large arterial input, a "nidus" of branching and reconnecting vessels, and coalescence of the vessels in the nidus into a single draining vein.[1,4] A method has been developed to administer radiosurgery to the model nidus, making this an attractive model for studying molecular strategies to enhance the thrombotic response to radiosurgery.[4]

The main aim of this study was to further investigate the morphological characteristics of the animal AVM model, and to compare these directly to human AVMs. Demonstrating morphological similarity between the model vessels and human AVM vessels would add support to the use of this model to study strategies for enhancing radiosurgery for AVMs. An additional aim was to investigate the hemodynamic characteristics of the model, particularly with reference to the vessel wall stresses created by the increased flow through the model AVM.

2. Materials and methods

2.1. Human AVMs

Patients gave informed consent for the use of tissue, and ethical permission was given by the Human Ethics Committee of the South Eastern Sydney Area Health Service, Sydney, Australia. AVM specimens were surgically removed from 17 patients (13 presenting with hemorrhage and four with seizures). These patients had received neither radiosurgery nor embolization. Normal human control tissue was obtained from three patients without cerebral structural lesions who were undergoing surgery for epilepsy control and from one patient dying from a non-neurological cause. Fresh specimens were processed for ultrastructural study as reported.[5–7] Parallel specimens were embedded in paraffin for histological analysis.[1,4]

2.2. Animal model formation

Following ethical approval from the Animal Care and Ethics Committee (University of New South Wales, Sydney, Australia),

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fistulas were created by anastomosing the caudal end of the left external jugular vein to the side of the common carotid artery in 71 male Sprague-Dawley rats (mean mass ± standard deviation [SD], 364 ± 12 g) (Fig. 1). This arrangement creates a feeding artery (common carotid), arterialized vein (external jugular), a nidus (branches of the external jugular vein that reconnect at the skull base), and a draining vein (sigmoid and transverse sinus).[1,4] At 1, 3, 7, 21, 42 and 84 days following fistula creation, the right and left carotid arteries, the right jugular vein and the arterialized fistulous vein (or left jugular vein in control rats) were exposed under general anaesthesia. Flow in the feeding segment of the jugular vein was measured as described below. At each time, 3 to 6 rats underwent blood flow studies and histological analysis. Control animals had the same vessels exposed and the proximal carotid artery, distal carotid artery, and jugular vein clamped for 25 minutes to mimic the surgical conditions in rats with fistulas.

After completing the blood flow measurements, rats were perfused with phosphate buffered saline and the vascular structures were removed. Tissue for ultrastructural analysis was then processed with an identical procedure as the human AVMs. Tissue for histological analysis was fixed with 4% paraformaldehyde/5% sucrose, and embedded in paraffin.[1,4]

2.3. Morphological analysis

Animal sections embedded in paraffin were cut (10 μm) and stained with Masson-trichrome for evaluation of smooth muscle fibers, intercellular fibers and collagen. Human AVMs and animal tissue paraffin sections were stained with hematoxylin and eosin (H&E) using standard histological methods for evaluation of general vascular structure.

Transmission electron microscopy was used to evaluate vascular wall components. Structural features of similar sized vessels were examined by three observers blinded to the nature of the specimen, and photographed, using 50 electron micrographs per epoxy block, and six epoxy blocks per specimen. Ultrastructural findings on electron micrographs represented more than 90% of vessels in the specimen, and were viewed by three observers blinded to the sample type.

2.4. Animal model blood flow measurements

Blood flow was measured in the carotid artery before and after fistula creation, and in the jugular vein after fistula creation, using a flow probe ([series 1 or 2RB] Transonics Systems, Ithaca, NY, USA) connected to a transit time perivascular flowmeter (T420, Transonics Systems).[1] Blood flow, arterial pressure, and pulse rate were recorded simultaneously through a data acquisition system (PowerLab/8sp System, ADInstruments, Castle Hill, NSW, Australia).

Data are expressed as the means ± standard error (SE) (number of experiments). Statistical differences among different groups were determined by the unpaired Students *t*-test and analysis of variance or the Mann-Whitney nonparametric test if variances were unequal.[8]

3. Results

3.1. Human AVM nidus

Human AVMs consisted of a mass of abnormal arteries and veins with no intervening normal brain tissue (Fig. 2). Vessel size and wall thickness were heterogeneous. There was splitting of the elastic lamina, a variably thickened endothelial layer, endothelial cushions, increased collagen and lymphocyte infiltration in the vessel walls. Transmission electron microscopy revealed an incompetent blood-brain barrier in nidus vessels and in perinidal capillaries. There was a discontinuous endothelium (Fig. 3A), no pericytes (Fig. 3B), no basement membrane and no astrocytic foot process (Fig. 3B). Endothelial cells (ECs) showed fenestrated processes (Fig. 3A), numerous filopodia (Fig. 3A), lysosomes, and cytoplasmic vesicles (Fig. 3B). Gap junctions and adherent junctions were observed between ECs (Fig. 3C). Uncharacterized subendothelial cells were observed.

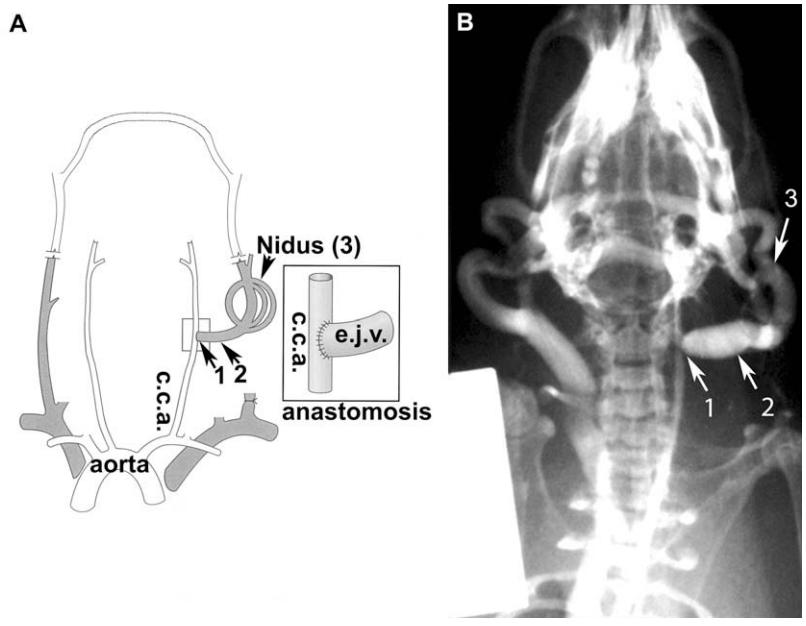

Fig. 1. (A) A schematic showing the arteriovenous fistula (AVF) of the rat arteriovenous malformation model. The normal primary outflow for intracranial venous blood is the external jugular vein (e.j.v.) via the posterior facial vein and the vein from the transverse sinus. The left external jugular vein was ligated at the confluence of the subclavian vein and an end-to-side anastomosis was performed on to the left common carotid artery (c.c.a.). (B) A representative angiogram 21 days after creation of an AVF in rats showing portions of the fistula: 1 = proximal fistula, 2 = arterialized jugular vein, 3 = nidus.