3D Analysis of Cerebral Vascular Patterning in Mouse Embryos with Contrast Enhanced Micro-MRI

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Introduction.

The mammalian vascular system develops as a complex, but apparently stereotypical network of blood vessels required for embryonic and postnatal growth and viability. Analyzing the three-dimensional (3D) aspects of embryonic vascular patterning is currently difficult, which limits our understanding of normal development and impedes our ability to analyze abnormal patterning in genetically-engineered mice. Traditional histological methods are inherently two-dimensional, and reconstructing 3D images of the vasculature is very difficult and seldom ever attempted. A major advantage of micro-MRI is that whole embryo data are acquired as a single 3D volume, which can be displayed and analyzed using volumetric analysis software. Previously, it has been shown that MRI of fixed mouse embryos can be used to analyze cardiac and great vessel anatomy, using either inherent contrast or contrast-enhancement after perfusion-fixation with a paramagnetic contrast agent [1-5]. In this study, we evaluated the potential of contrast-enhanced micro-MRI to facilitate quantitative analysis of the embryonic cerebral vasculature using 3D analysis software.

Methods.

The perfusion-fixation protocol is similar to that described previously by Smith and co-workers [1-2], with some modifications to selectively enhance venous or arterial vessels. Briefly, each embryo was surgically extracted, maintaining the vascular connections to the placenta and warmed in phosphate buffered saline (PBS). Heparin containing PBS (5,000 units/L) was perfused into the umbilical vein, followed by a fixative perfusion (2% vol/vol glutaraldehyde/1% formalin in phosphate buffer at 300milliosmoles/liter). The contrast agent, BSA-DTPA-Gd containing 1-mM Gd, dissolved in a 10% (wt/vol) gelatin solution, was then perfused through either the umbilical artery or umbilical vein. The contrast solution was colored to enable monitoring of the perfusion, stopping immediately after the head changed color, or continuing until the contrast solution had perfused the entire embryo. After perfusion-fixation, mouse embryos were mounted in a syringe phantom surrounded by Fomblin perfluoropolyether (Solvay Solexis). 3D T1-weighted gradient echo (TE=5ms; TR=50ms; Flip Angle=35°; FOV=$(25.6\text{mm})^3$; Matrix=512^3; Isotropic resolution=50μm; Total imaging time=14 hr, 35 min) images were acquired on a 7T SMIS system, imaging multiple embryos simultaneously in overnight scans [4, 5]. Image segmentation, maximum intensity projection (MIP) and 3D analyses were performed using Analyze software (Mayo Clinic).

Results.

T1-weighted imaging demonstrated complete vascular perfusion of Gd-DTPA-BSA contrast agent through the umbilical vein, and enhancement of the entire cardiovascular system that highlights the larger venous vessels (Fig.1a). Enhancement more selective for cerebral arteries was achieved by perfusion of contrast agent through the umbilical artery, stopping as soon as the head changes color (Fig. 1b). The modified perfusion-fixation protocol enabled analysis of cerebral arterial anatomy over a wide range of embryonic stages (Fig. 2), from embryonic day (E)11.5 to E17.5 (E0.5 is defined as noon of the day after overnight mating). To assess the feasibility of quantitative analysis of vascular patterning, 3D analysis was performed on MIP images of the basilar and vertebral arteries in the posterior brain, using the Tree Analysis function of Analyze software. These preliminary results demonstrate developmental stage-dependent changes in vascular patterns, with minimal variability in the lengths between vascular branch points and the angles between branches at each developmental stage (Fig. 3).

Conclusions.

Contrast-enhanced micro-MRI, combined with 3D image analysis, shows great potential for the study of vascular development in mouse embryos. Previously published contrast perfusion-fixation MRI protocols were found to highlight larger venous blood vessels. Modification of the perfusion-fixation protocol enabled selective enhancement of the cerebral arterial system, allowing for effective analysis of either the arterial or venous cerebral vascular systems. Preliminary quantitative measurements support the hypothesis that the vasculature develops in a stereotypical pattern, with little variability at each developmental stage.

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References.

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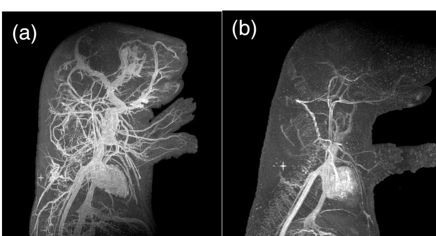


Fig.1. 50μm isotropic MRI volume rendered maximum intensity projections of E17.5 wildtype embryonic vasculature. (a) Full vascular perfusion. (b) Partial vascular perfusion. Notice the difference in vascular enhancement achieved by partially perfusing embryos with Gd-DTPA-BSA in (b).

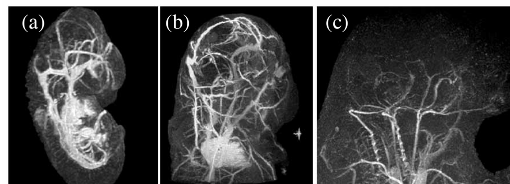
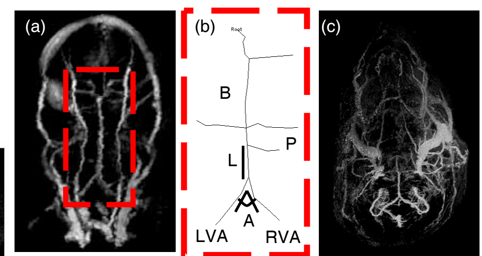


Fig.2. Maximum Intensity Projections of E11.5 (a); E15.5 (b) and E17.5 (c) wildtype embryos. Note full vascular perfusion in (a) and (b) and partial vascular perfusion in (c).



	E12.5 (n=3)	E17.5 (n=3)
A (°)	80.57 ± 8.83	62.70 ± 15.00
L (μm)	533.5 ± 77.5	1166.5 ± 76.5

Fig.3. Maximum Intensity Projections of E12.5 (a) and E17.5 (c) wildtype embryos. (b) 3-D skeletal structure of basilar artery (BA) in (a). A = Angle between vertebral arteries (LVA;RVA) in degrees. L = Length between vertebral artery and first pontine artery (P) in μm.