In utero MRI of cerebral vascular development in mice

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Introduction

Vascular system development involves a complex, three-dimensional branching process that is critical for normal embryogenesis. In a previous study [1], we developed a contrast-enhanced perfusion method to selectively enhance the cerebral arteries in fixed mouse embryos and demonstrated that Gli2 mutant mice lack a basilar artery, a key arterial input to the posterior brain regions. However, imaging studies of Gli2 and many other mutant mice with vascular defects are limited because mice do not survive postnatally. Extending vascular imaging to an in utero setting with potential for longitudinal vascular development studies is an exciting possibility. However, in vivo MRI scans routinely result in undesirable image artifact due to subject motion. In this study we utilized an in utero imaging method that we described previously [2], which corrects for motion using an interleaved gating acquisition and serial comparison of rapidly acquired 3D images. We demonstrate the potential of this method by examining vascular development in utero in E17.5 wildtype and Gli2 mutant mice. We show that the in vivo methods produce high-quality images of the embryonic cerebral vasculature and are able to detect the basilar artery phenotype in Gli2 mutants.

Methods

A modified Cartesian 3D gradient-echo sequence was used for this study [2]. In this sequence, respiratory gating data were detected from an unencoded gating signal interleaved with imaging data. Repeated, serial 3D volume images (with limited SNR) were acquired and registered together during reconstruction to eliminate shifts in embryo position during the scan. Registrations were performed based on tools produced by the Montreal Neurological Institute (MNI_AutoReg: http://www.bic.mni.mcgill.ca/software/mni_autoreg) [3,4] with a coarse, manually-drawn mask covering the embryo brain and corresponding blood vessels. All MRI data were collected on a 7.0T magnet using a Bruker Biospin Avance II console. For our initial investigation, we used a 125-µm isotropic resolution (matrix size 192x110x72, field-of-view 24x13.75x9.0, TE/TR = 3.75/35 ms, 16 degree flip angle, 28 repeats, for a total scan time of 2hrs 16mins). For imaging, pregnant mice were anesthetized with isoflurane and placed on their side with one of the uterine horns sitting above a custom setting with potential for longitudinal vascular development studies is an exciting possibility. However, imaging methods for visualization of the embryo vasculature. We clearly discerned the major vascular inputs into the developing brain including the basilar and the carotid arteries in wildtype embryos. In vivo 3D T2*-weighted MR brain also showed an absence of the basilar arteries and other alterations in the structure and density of blood vessels in Gli2 mutant embryos, characteristic of the phenotype described previously in our ex vivo experiments.

Conclusions

With the use of gating and image registration methods, we have shown the acquisition of in utero 3D images of vascular development. We have also demonstrated the in vivo visualization of a deletion of basilar arteries in Gli2 mutants. Taken together, our results show great potential for in vivo 3D micro-MRI to analyze the development of cerebral vasculature as well as any phenotypes associated with genomic manipulation in mutant mouse embryos. This may present important opportunities for investigating embryonic lethal mouse phenotypes, permitting longitudinal observation of vascular development or measurement of vascular perfusion in vivo.

Acknowledgements

This work was supported in part by NIH grant R01 HL078665.

References


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Fig 1: In vivo T2* micro-MRI slices (top two panels) and minimum Intensity Projections of E17.5 wild type and Gli2-/- mutant embryos. Note the absence of the basilar artery (red box) and the reduction in the size of the cerebral blood vessels in Gli2-/- mutants. Labels: basilar artery, BA; carotid artery, CA. Scale bar = 300µm