In vivo Cardiac MRI Development for Studying Zebrafish Models of Myocardial Disease

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Introduction

Zebrafish's ability to regenerate cardiac muscle makes it a good model for cardiovascular research. External development of the fish facilitates creation of mutant and transgenic lines. The zebrafish heart is two-chambered, but many studies have proved it to be highly relevant to human cardiovascular function and genetics[1].These characteristics along with low maintenance costs and the availability of large base of established knowledge on development biology and genetics of zebrafish attracts more researchers towards zebrafish studies. Unlike embryos, adult zebrafish are pigmented, so optical studies are restricted to the early developmental stages. The goal of this study is to explore the efficacy of MRI in *in vivo* cardiac imaging of adult zebrafish heart. Since the available MRI literature in zebrafish heart is very limited[2], ex vivo studies are carried out to study the structure of the heart in high resolution. Finally, we have demonstrated cardiac cine MRI in *in vivo* zebrafish heart using retrospective sequence with navigator echo.

Methods

Ex vivo study: An adult zebrafish (14.5 months old) was fixed and stained for 2 hours using a 20:1 volume ratio of 4% paraformaldehyde and PBS: GD solution. It was then stabilized and stored in 15 ml of 200:1 PBS:GD solution prior to imaging. For MR imaging the fish was suspended in a 15 ml tube of Fluorinert™ FC-70(3M Company, St. Paul MN). It possesses anatomic structures similar to that observed in live fish and does not suffer from motion artifacts observed in *in vivo* imaging. The high resolution *ex vivo* scans were obtained using a T1-weighted FLASH sequence (T_R = 152 ms; T_E = 2.8 ms; FA = 30°; FOV = 1.8cm x 1.8 cm; matrix = 256 x 256; in-plane resolution 70 µm; navgs = 8). The *ex vivo* images were used to study the anatomical details of heart and to standardize the scan acquisition geometry to localize the heart.

In vivo study: A 14.5 month old adult wild type zebrafish was anesthetized in 0.005% MS-222 (Tricaine, Sigma) in water for three minutes. The fish was then transferred to a closed chamber filled with water and anesthetic. The posterior half of the fish was secured between two soft sponges inside the chamber, preventing movement and allowing free gill movement for respiration and an unobstructed view of heart. The MRI imaging was done in a Bruker Biospin Avance 500MHz 11.7T vertical bore magnet (Bruker Biospin, Karlsruhe, Germany) and a 20mm diameter volume coil. 2D FLASH sequences (sagittal and axial) are used to localize the heart. As prospective ECG and respiratory gating was not possible, retrospective gating was used. Cine images of the heart along the ventricle's short axis are obtained using a 2D FLASH sequence with a navigator echo (IntraGate, Bruker) using retrospective CINE MRI (T_R = 16ms; T_E = 3ms; FA = 10°; FOV = 1.8cm x 1.8 cm; matrix = 256 x 256; in-plane resolution 70 µm; number of repetitions = 300; scan time = 10 min). The continuous slices covering the heart with slice thickness of 0.25 mm are obtained. In our studies we have observed that the fish can survive in this closed chamber for approximately 90 minutes.

Results

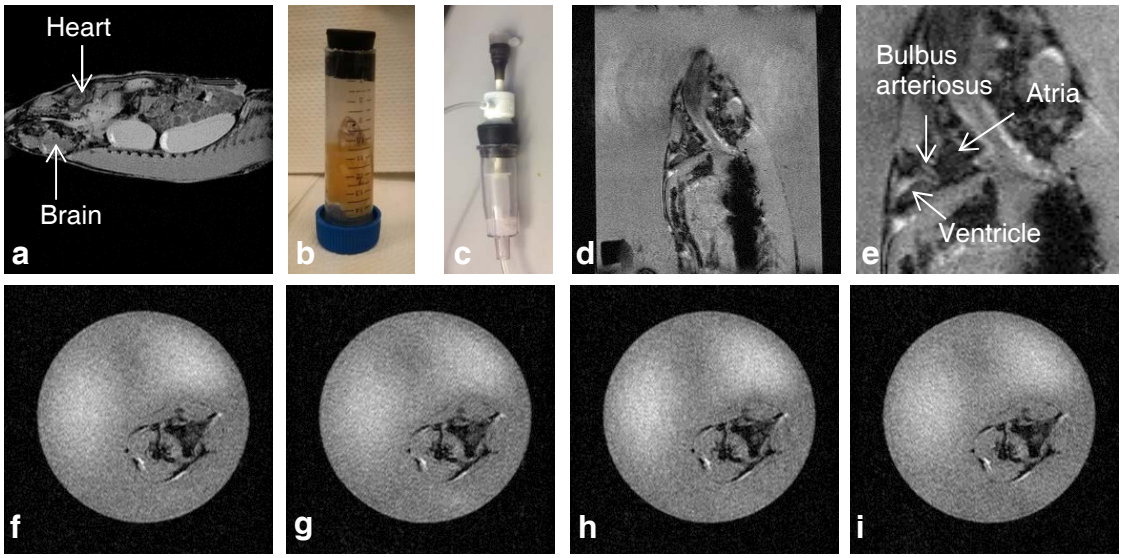

Figure 1 a) *ex vivo* sagittal view, b) *in vivo* imaging set up, c) flow chamber d) *in vivo* sagittal slice e) enlarged view showing two chambers of heart along with bulbus arteriosus f-i) *In vivo* cardiac cine scans obtained along the short axis of the ventricle shows the heart motion through various cardiac phases

Conclusion To our knowledge this is the first report of cine cardiac MRI in *in vivo* zebrafish heart. Retrospective gated cine MRI is used to acquire cardiac images in *in vivo* zebrafish heart. Future studies will involve longitudinal cardiac MRI studies using flow chamber and comparing it with the data from ultrasound studies.

References

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