High-Throughput µMRI Phenotyping of Right Ventricular Mouse Embryonic Abnormalities: Requirement for Intravascular Contrast Enhancement

Y. Z. Wadghiri[1], A. Schneider[1], E. N. Gray[1], O. Aristizabal[1], D. H. Turnbull[1], D. E. Gutstein[1]

[1]New York University School of Medicine, New York, NY, United States

Introduction:

µMRI is a valuable non-destructive screening tool to phenotype mouse mutants during development [1-5]. Imaging of the mammalian cardiac right ventricle (RV) is particularly challenging, especially when a two-dimensional method such as conventional histology is used to evaluate the morphology of this asymmetric, crescent-shaped chamber. Previously, it has been demonstrated that important features of a mutant phenotype affecting the RV can be well characterized at a fine level of resolution with MRI when specimens are fixed and perfused with gadolinium-based contrast agent [6]. Alternatively MRI protocols using endogenous tissue contrast have been used to reduce the specimen preparation time and increase the screening throughput [7]. Here we demonstrate that the use of contrast agent can be critical to visualize the disruption of the architecture of the muscle wall in the embryonic heart in the evaluation of a line of connexin43 (Cx43) mutant mice that are known to have malformations of the RV outflow tract[8].

Design and Methods:

To help delineate the cardiovascular system and facilitate identification of the right ventricle outflow tract (RVOT), embryos at E17.5 were perfusion-fixed with 2% glutaraldehyde/1% formalin through the umbilical vein followed by infusion of the contrast agent BSA-Gd-DTPA mixed in 7% gelatin. MRI was performed on 4 Cx43 conditional knockout embryos (mediated by Pax3-Cre; Cx43-PCKO) and 4 littermate controls using a SMIS console interfaced to a 7T horizontal magnet with 250-mT/m actively shielded gradients (Magnex) and a 25-mmx22-mm (IDxLength) Litz coil (Doty). Images were obtained using a 3D gradient echo sequence (TE/TR/FA = 5-ms/50-ms/35^{O}): 50µm isotropic spatial resolution (Imaging Time=14h35'). The embryo datasets were analyzed with the highest detail through virtual resectioning and maximum-intensity-projections (MIP) using Analyze software (Lenexa, KS). Imaging was followed by paraffin embedding of specimens, histologic sectioning and H & E staining.

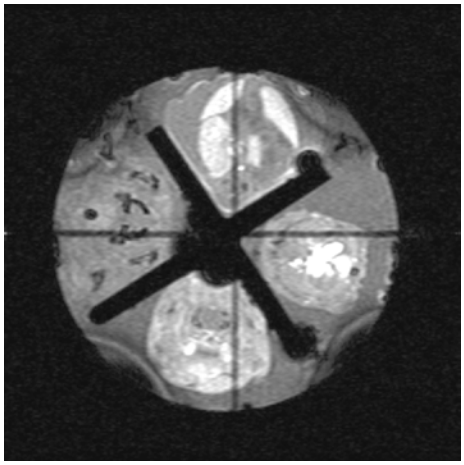

Fig.1: Axial section of a simultaneous 4-embryos imaging setup using a 60-ml syringe holder.

Results and Dicussion:

Imaging of 4 embryos simultaneously allowed for higher throughput than single embryo imaging techniques, while intravascular contrast afforded excellent signal-to-noise characteristics (Fig. 1). All four control embryos had normal appearing right ventricular outflow tract contours, both on histologic sections and by µMRI. Obvious abnormalities in the right ventricular outflow tract were present in only 1 of 4 mutant hearts on histologic sectioning, but abnormal RVOT bulging and trabecular in-growth was seen in 3 of 4 mutants with µMRI. MRI also revealed differences in the nature of the RV walls between controls and mutants which were not evident on histologic sections (Fig.2). Specifically, MRI demonstrated solid, muscular RV walls in the control infundibular regions, while mural contrast infiltration was evident in mutant RVOTs. Furthermore, three-dimensional reconstruction of MR images with orthogonal projections as well as three-dimensional MIPs allow for visualization of the extent of infundibular bulging and other RV abnormalities in mutant hearts (data not shown).

Conclusion:

Right ventricular abnormalities in the Cx43-PCKO mutant mouse embryo can be more reliably visualized with µMRI than with conventional histology. Thus, the use of µMRI with intravascular contrast injection to evaluate late-stage cardiac developmental abnormalities in the mouse embryo can be of substantial value in the characterization of mutant myocardial phenotypes. This protocol should be considered in other mutants that have similar phenotypes and that were analyzed using MRI without contrast agent [7].

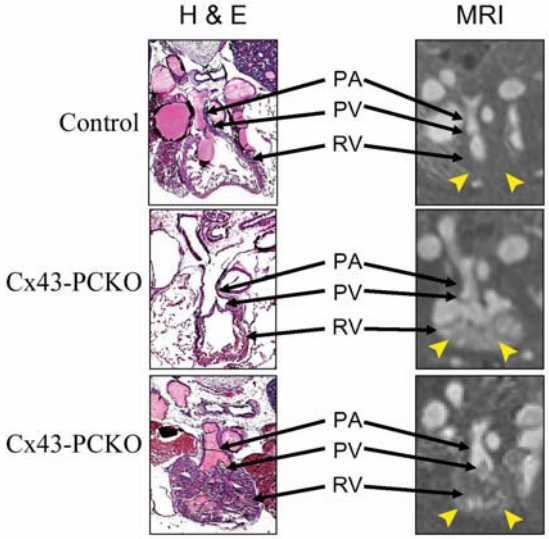

Fig. 2: Comparison of histologic and MR imaging of RVOT control and mutant (Cx43-PCKO) mice. The RVOT of a control embryo has a discrete smooth contour without trabeculation that is clearly evident in MR images (upper yellow arrow). Although histologic sections of the first Cx43-PCKO mutant RVOT appear similar to the control, MR imaging shows clear differences including dilatation of the RVOT and extensive trabeculation (middle yellow arrow). The second Cx43-PCKO is an example of a mutant with heavy trabeculation in which the histologic sections provide a decent match with MR images (lower yellow arrow). PA, pulmonary artery; PV, pulmonic valve; RV, right ventricle.

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