Cardiac MRI of Magnetically-Labeled Annexin Detects Cell Injury, In Vivo
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BACKGROUND
• Drug-induced, ischemic, and non-ischemic cardiomyopathies are associated with marked cardiac apoptosis.
• Early, non-invasive detection of cardiac apoptosis or cell injury may improve patient outcomes through targeted medical and cell-based therapies.
• Annexin V (ANX), which binds externalized membrane-bound phosphatidylserine, can detect early apoptosis.
• Cardiac MRI exhibits high spatial and temporal resolution and can detect magnetically tagged proteins and cells, in vivo.

HYPOTHESES
• Can ANX, which was previously conjugated to superparamagnetic iron oxide (SPIO), detect cardiac apoptosis/cell injury, in vivo, via T2-MRI?
• Is the in vivo pattern of ANX-SPIO T2* signal loss distinct in oxidative (Doxorubicin) vs ischemic cardiac injury?

METHODS
Generation of ANX-SPIO Conjugate Protein
Purified human ANX V was previously generated using purified human ANXV protein and Ferritin iron oxide (11.0mg Ferrit in eos.) through a series of oxidation/reduction steps.

In Vitro Detection of Apoptosis Using ANX-SPIO
T2-MRI of ANX-SPIO was previously found to identify small populations of apoptotic cardiomyocytes (CMs), Endothelial (FA), and mesenchymal stem cells (mMSCs) in culture, with high specificity and sensitivity.

In Vitro Detection of Apoptosis Using ANX-SPIO
20ug of ANX-SPIO was delivered via tail vein into FVB/n mice 48 hours after myocardial infarction (MI) surgery, and 48 hours after intraperitoneal Doxorubicin (DOX, 25mg/kg) injection. Previous studies of the dose of DOX showed extensive cardiac fibrosis, cell death, and ventricular dysfunction at 1 week. Cardiac MRI evaluated T2* signal loss within the myocardium at 72, 77, and 87 post-ANX-SPIO. All day 2B, MRI’s were performed before and after re-injection of ANX-SPIO. Similarly, Cardiac MRI was performed on DOX-treated animals on days 2 and 7. A 3 Tesla, GE MRI system was used, employing cardiac and respiratory gating, with a custom surface loop receiver coil, designed by Dr. B. and Dr. N. Qualitative differences in T2* signal loss patterns were assessed. All animal work was in compliance with APLAC safety and ethical requirements, APLAC Protocol #17306.

CONCLUSIONS
• ANX-SPIO detects areas of cardiac injury, in vivo.
• The pattern of T2* signal loss created by ANX-SPIO is distinct in DOX-treated versus mMSC treated hearts.
• Future studies:
  • Histopathological confirmation of iron stain, caspase activity
  • Assessment of dynamic changes following therapy

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