In Vivo Imaging of Free Radicals in Cardiac Tissues from Diabetic Mice with the use of MRI

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Purpose: Evidence in experimental studies suggests that oxidative stress contributes in an important role in the pathogenesis and pathophysiology of cardiac diseases and diabetes. The opportunity to exclusively observe these oxidative stresses *in vivo* would be beneficiary. This study reports on *in vivo* imaging of protein/lipid radicals with the use of molecular MRI (mMRI) and immune-spin trapping (IST) on diabetic cardiac muscle.

Methods: *STZ-induced Diabetes Model:* C57BL/6J mice (n=20; 6-8 weeks) were treated with streptozotocin (STZ) (100 mg/kg i.p./day for 2 days), and between 4-6 weeks mice were assessed for glucose levels. Severe diabetes was characterized when glucose levels were >300 mg/dl (n=10), and moderate diabetes was considered when glucose levels were between 200-300 mg/dl (n=3). To test for glucose, a drop of blood from the tail was put on a testing strip and read on a Bayer Ascensia Elite XL glucometer. For control groups, (1) non-diabetic mice were given the spin trapping compound 5,5-dimethyl-pyrroline-N-oxide (DMPO) (non-disease control) and administered anti-DMPO probe (n=6), (2) diabetic mice were given DMPO and administered anti-DMPO probe (n=5), or (3) diabetic mice were given DMPO but administered the non-specific IgG contrast agent (contrast agent control) instead of the anti-DMPO probe (n=7). DMPO administration started at 7 weeks following STZ treatment. Mice were administered the anti-DMPO probe at 8 weeks following STZ treatment. DMPO (25 µl in 100 µl saline) was administered i.p. 3 x daily (every 6 hours) for 5 days (i.e. 0.42 µl DMPO/µl saline/day). Mice were initiated administration of DMPO 7 weeks following STZ administration, prior to injection of the anti-DMPO probe. *Synthesis of DMPO-specific MRI Agent:* The contrast agent, biotin-BSA (bovine serum albumin)-Gd-DTPA, was prepared as previously described[1]. Each animal was injected with 200µg anti-DMPO and 100µg biotin-BSA-Gd-DTPA. Non-specific mouse-IgG conjugated to biotin-BSA-Gd-DTPA was synthesized by the same protocol. *MRI and mMRI:* MR experiments were carried out under general anaesthesia (1-2% Isoflurane, 0.8-1.0 L/min O2). MR equipment that was used included a Bruker Biospec 7.0 Tesla/30 cm horizontal-bore imaging spectrometer. Anaesthetised (2% Isoflurane) restrained mice were placed in an MR probe, and their cardiac tissue (heart) were localised by MRI. Images were obtained using a Bruker S116 gradient coil (2.0 mT/m/A) and a 72 mm quadrature multi-rung RF coil. Mice were imaged at 8 weeks following STZ administration. Multiple 1H-MR image slices were taken in the coronal (horizontal) plane using a gradient echo multislice (FLASH (Fast Low Angle SHot); repetition time (TR) 250 ms, echo time (TE) 6 ms, 256x256 matrix, 2 steps per acquisition, 3x3 cm2 field of view (FOV), 1 mm slice thickness) with motion suppression turned on. Mouse tissues were imaged at 0 (pre-contrast) and at 90-100 min post-contrast agent injection. Mice were injected intravenously with anti-DMPO or normal mouse IgG antibodies tagged with a biotin-Gd-DTPA-albumin-based contrast agent (200 µl/kg; 1 mg antibody/kg; 0.4 mmol Gd+3/kg) [25-27]. Relative MR signal intensities were calculated for the selected ROIs, and difference images were obtained between before and 90 min after injection of the anti-DMPO probe or IgG contrast agent.

Results: MRI was used to detect the presence of the anti-DMPO adducts by either a significant sustained increase ($p<0.001$) in MR signal intensity. The biotin moiety of the anti-DMPO probe was targeted with fluorescently-labeled streptavidin to locate the anti-DMPO probe in excised cardiac tissues, indicating elevated fluorescence only in cardiac muscle from mice administered the anti-DMPO probe. As a negative control a non-specific IgG antibody covalently bound to the albumin-Gd-DTPA-biotin construct was used.

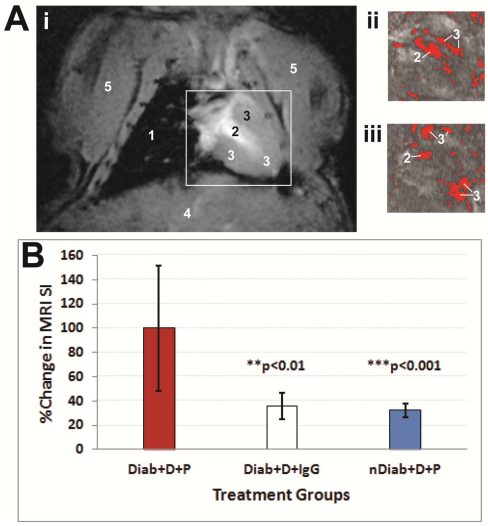

Figure 1: (A) MR image of a diabetic heart (i), with anti-DMPO difference images (2 thresholded slices obtained that correspond to image 'I'; ii and iii) depicting uptake of anti-DMPO molecular MRI probe. Anatomical assignments are: (1) lungs, (2) left ventricular chamber with blood, (3) left ventricular cardiac muscle, (4) liver, and (5) breast muscle. Note anti-DMPO probe in cardiac muscle (3), as well as blood circulation (region 2). (B) Quantitative MRI assessment of immunospin-trapped radicals. Percent (%) in signal intensity (SI) in cardiac tissues from STZ-induced diabetic (highly; >300 & moderate ; 250-300 blood glucose) mice (n=13) after being administered DMPO daily for 5 consecutive days and an IgG-albumin-Gd-DTPA contrast agent (nonspecific control) or the anti-DMPO-albumin-Gd-DTPA probe. As well as non-diabetic (normal; <200 blood glucose) mice (n=5) after being administered DMPO daily for 5 consecutive days and the anti-DMPO-albumin-Gd-DTPA probe. Significant differences (***P<0.01 for IgG control; **P<0.001 for non-diabetic anti-DMPO probe) were found between IgG controls and non-diabetic anti-DMPO administered mice.

Discussion: With the use of mMRI and an anti-DMPO probe specific for DMPO-protein/lipid radical adducts combined with a gadolinium (Gd)-DTPA-albumin-based contrast agent for signal detection, this allowed the detection of signals associated to protein/lipid radicals in the cardiac tissue of diabetic mice.This study has supporting evidence (Fig. 3) that diabetic /cardiac tissue has more radical accumulation, as detected by mMRI and immunospin-trapping, compared to non-diabetic cardiac tissue.

Conclusion: Diabetic mice have more radical accumulation in cardiac tissue, as measured by presence of the anti-DMPO probe and molecular MRI, than control mice. This approach could be used to evaluate the role of free radicals in diabetic disease pathology.

References:

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