In vivo Assessment of Free Radicals in a Mouse Model for Diabetic Cardiomyopathy

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Target Audience: This study will be of interest to diabetes researchers, particularly those investigating the role of free radicals in type I diabetes, as well as basic scientists who are investigating molecular imaging methods.

Purpose

Cardiovascular disease is the primary cause of morbidity and mortality among the diabetic population [1]. One of the characteristics associated with diabetic cardiomyopathy is the generation of free radicals [1]. DMPO (5,5-dimethyl-1-pyrroline-N-oxide) is a nitrone spin trapping compound that can trap and stabilize free radicals, and use of an antibody that recognizes DMPO-trapped radicals can be coupled to a MRI contrast agent (anti-DMPO antibody-albumin-Gd-DTPA-biotin construct) (anti-DMPO probe) to detect in vivo levels of free radicals in a mouse model for diabetic cardiomyopathy.

Methods

DMPO was administered i.p. to diabetic and non-diabetic mice prior to the i.v. administration of the anti-DMPO probe (anti-DMPO-albumin-Gd-DTPA-biotin) (Fig. 1A). T1-weighted MRI (7 T) and T1 maps were obtained to assess *in vivo* levels of DMPO-trapped free radical in cardiac muscles of diabetic (n=5) and non-diabetic (n=5) mice. Some diabetic mice were also administered DMPO and a non-specific IgG probe isotype as a targeting agent control (n=5). Molecular-targeted imaging data were processed using Paravision software (Bruker Biospin). Fluorescence imaging was used to verify the presence of the anti-DMPO probe targeting the biotin moiety, and fluorescence immuno spin trapping was used to verify the detection of DMPO-trapped free radicals in diabetic cardiac muscle tissue. Quantitative measures of malondialdehyde (MDA)-protein adduct concentration (pmol/mg protein) and 3-nitrotyrosine (3-NT) concentration (nM/mg protein) in tissues homogenates from diabetic and non-diabetic mouse cardiac muscle (n=5 for each) were also done.

Results

There was a significant increase in the percent change in MRI signal intensity in diabetic mouse hearts compared to non-diabetic mice ($p<0.01$), both administered DMPO and the anti-DMPO free radical-targeted probe, or diabetic mice administered DMPO and the IgG isotype non-specific probe ($p<0.001$) (Fig. 1B and C). The biotin moiety on the anti-DMPO probe was used to confirm its presence in excised tissue with streptavidin-Cy3 (Fig. 1D). Fluorescence immunohistochemistry also verified the presence of trapped DMPO-free radical adducts. There was a significant increase in MDA-adducts in diabetic mice ($p<0.05$) compared to non-diabetic mice. There was also a significant increase in 3-NT in diabetic mice ($p<0.05$) compared to non-diabetic mice.

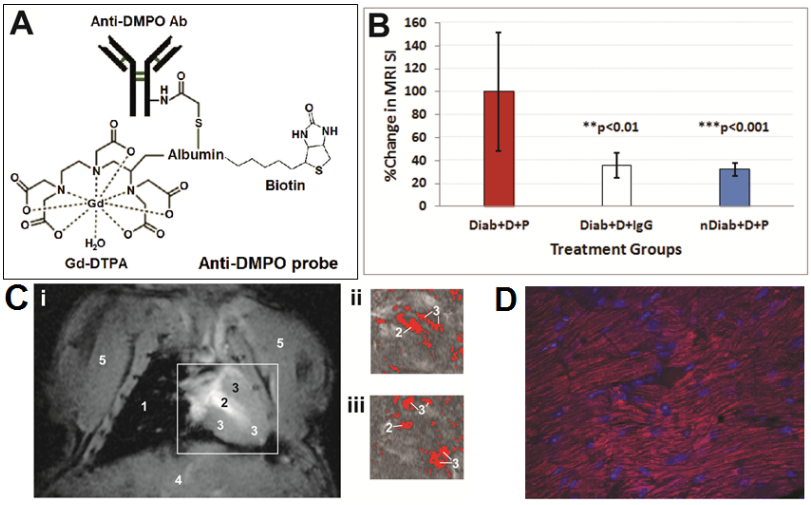

Figure 1: (A) Schematic of anti-DMPO probe. (B) Percent (%) change in MRI signal intensities (SI) in the left ventricle cardiac muscle of mice that were either diabetic (Diab) or non-diabetic (nDiab) and treated with DMPO (D) and either the anti-DMPO probe (P) or an IgG isotype contrast agent (IgG). (C) T_1-w images of STZ mouse hearts with difference images (ii and iii) obtained from 120 min post-administration of anti-DMPO probe minus pre-administration image [raw MR image (i), and thresholded images (ii) and (iii)]. (D) Fluorescence images of streptavidin-Cy3 (red) which binds to biotin moiety of anti-DMPO probe in STZ diabetic mouse cardiac muscle.

Discussion

Diabetic cardiomyopathy associated free radicals were detected in vivo in a STZ-induced mouse model for diabetes using IST combined with molecular MRI targeting nitrone (DMPO) trapped radicals in cardiac muscle. The anti-DMPO antibody was conjugated to the albumin of an albumin-Gd-DTPA-biotin construct. The in vivo results clearly demonstrated the sustained uptake of the anti-DMPO probe in cardiac muscle of diabetic mice, which was significantly increased compared to controls (non-diabetic mice administered DMPO and the anti-DMPO probe, and diabetic mice administered DMPO and a non-specific IgG isotype contrast agent). Verification of the anti-DMPO probe was confirmed in excised cardiac muscle tissue of diabetic mice via targeting the biotin moiety of the anti-DMPO probe with streptavidin-Cy3.

Conclusion

Free radical-targeted molecular MRI can be used to detect *in vivo* heterogeneous levels of free radicals in a mouse model for diabetic cardiomyopathy, and could be utilized to assess the role of free radicals in the pathogenesis associated with either type I or type II diabetes pre-clinical models.

References

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