Longitudinal functional and structural characterization of inducible heart specific SOD2 knock-out mice by cardiac MRI

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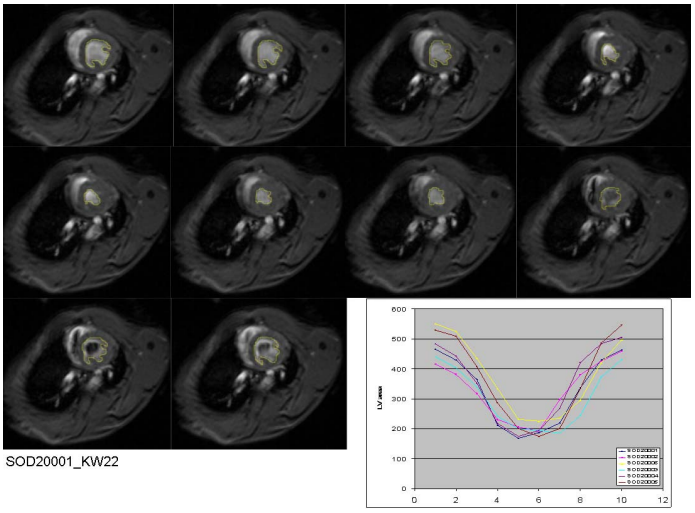
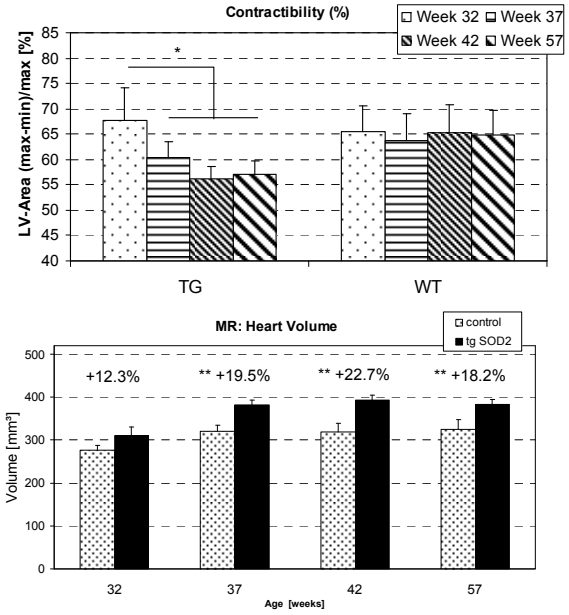
Introduction

Oxidative stress and mitochondrial dysfunction are key components in many diseases, where the mitochondrion is a major source of reactive oxygen species (ROS), which are produced during oxidative phosphorylation in the respiratory chain [1]. Of all ROS the superoxide radical (O_2^-) is the major component and under normal conditions reduced to H_2O_2 by the mitochondrial form of superoxide dismutase (SOD2). Although the heart can potentially use a variety of energetic substrates, it mainly relies on fatty acid oxidation, which is carried out in the mitochondria. In this context, heart failure essentially reflects the inability of the heart to perform work and has been associated with energetic failure in the mitochondria. Hence, mitochondrial dysfunction will have a severe impact on cardiac function. A direct test of mitochondria-related heart disease can be realized by increasing the endogenous oxidative stress by down-regulating compensatory mechanisms. Consequently, a mouse model with a deactivated SOD2, which develops a dilated cardiomyopathy and a biochemical phenotype, was generated [2, 3]. Here we show the phenotypic characterisation of a new transgenic mouse model (Fsod2H), in which SOD2 was inactivated in the heart by the inducible expression of the cre gene under the control of a tamoxifen-inducible heart-specific Myh6 promoter. By this strategy the SOD2 gene modified to carry two loxP sites between exons 1 and 4 is knocked down specifically in the heart in an inducible manner. In-vivo imaging performed in these animals from 32 - 57 weeks of age (i.e. 21 to 46 weeks post induction) and compared to non-induced controls animals revealed pathological changes in heart size and function.

Animal Handling and Methods

MRI data were acquired on a Biospec 47/40 scanner (Bruker BioSpin, Ettlingen, Germany) at 4.7 Tesla. Mice were anaesthetized through continuous inhalation of 1.2-1.5% isoflurane (in 70:30 N_2O:O_2). Following a T_1-weighted set of pilot images, cardiac imaging was performed using the IntragateFLASH protocol (Bruker Biospin) where retrospective gating is carried out with a navigator echo. Consequently, no respiratory and/or cardiac gating was required for the MR imaging. Heart images were acquired in orientations: pseudo short axis, short axis, and 4-chamber view with the following parameters: TR 8 ms, TE 3 ms, flip 10 deg, 300 repetitions, FOV 40 mm x 40 mm, slice thickness 1.2 mm, 10 images per cycle. In addition, an anatomical T_1-weighted FLASH image stack was acquired to evaluate the total volume of the heart: TR 160 ms, TE 3.5 ms, flip 30 deg.
All MR data sets were analyzed using the freeware platform ImageJ. Contractibility of the left ventricle is given as the difference between maximum and minimum area normalized to the maximum area [4]. Heart volume was calculated by measuring the area per slice multiplied by slice thickness.





Above: Navigator-retrospectively gated cardiac imaging of a mouse heart and absolute area of the left ventricle (see insert). ROI given in the image.
Right top: Age-dependent change in contractibility of the left ventricle
Right bottom: Age-dependent change in total heart volume measured by MR

Results

Cardiac images obtained by the Intragate protocol are presented above, where the insert shows the absolute area of the left ventricle over one heart beat as delineated by the ROI (yellow line) in the MR images. The graph on the top right side shows the age-dependent change of ventricular area with respect to the maximum area, i.e. the so-called contractibility of the heart. Compared to the initial time point a significant reduction of contractibility was observed for the late time points for tg SOD2 mice, whereas no effect was observed in wildtype mice. In addition to a reduced cardiac function an increase in total heart volume was measured (graph, bottom right) for all time points between the two groups. Over time the difference in cardiac volume increases and appears to reach a maximum at the last two time points.

Conclusion

Overall, MRI allows for longitudinal quantitative assessment of functional and structural changes in the mouse heart and may, therefore, allow the evaluation of therapeutic approaches using antioxidative compounds.

References

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