Smoothelin-B deficiency in mice results in increased thoracic aorta circumferential stretch and mild cardiac hypertrophy

E. Heijman[1], P. Niessen[2], G. van Eys[2], K. Nicolay[1], and G. J. Strijkers[1]

[1]Biomedical NMR, Department of Biomedical Engineering, Eindhoven University of Technology, Eindhoven, Netherlands, [2]Department of Genetics and Cell Biology, University of Maastricht, Maastricht, Netherlands

Introduction

Smoothelin-B is an α-smooth muscle actin-binding protein that is specifically and abundantly expressed in contractile vascular smooth muscle cells (1). Measurements of the arteries of young smoothelin-B knockout (*Smtn-B*$^{-/-}$) mice showed a reduced arterial contractility and relaxation, while a higher mean arterial pressure was found for fully-grown *Smtn-B*$^{-/-}$ mice compared with their littermates (*Smtn-B*$^{+/+}$). In this study, the lumen diameter of the thoracic aorta was measured through the cardiac cycle with a retrospective cinematographic (CINE) MRI sequence (2) *in vivo* in *Smtn-B*$^{-/-}$ and *Smtn-B*$^{+/+}$ mice. In addition, global functional heart parameters of both genotypes were also determined and compared. It was expected that these measurements would provide insight in the adaptations caused by the loss of smoothelin-B function.

Methods and Materials

C57Bl6 mice (age 8 months; weight, 25-35 g) divided in two groups of 7 *Smtn-B*$^{+/+}$ mice and 5 *Smtn-B*$^{-/-}$ mice were scanned to determine the lumen diameter of the thoracic aorta by a retrospective CINE MRI sequence using a 6.3 T/20 cm Bruker MRI system. The retrospective CINE MRI sequence is a modified FLASH sequence with an in-slice navigator echo (2) with the following parameters: Gaussian-shaped RF pulse, 300 μs; flip angle, 30°; repetition time, 5.4 ms; echo time, 2.98 ms; sample rate, 150 kHz; echo position, 40%; navigator echo points, 96; field of view, 2.5 x 3 cm^2; matrix, 256 x 192; in-plane resolution, 110 x156 μm^2; slice thickness, 2 mm; number of repetitions, 700; total acquisition time, approximately 12 min. The slice of this retrospective CINE MRI sequence was planned in such a way that during the cardiac cycle the aorta stayed within the slice and that the readout direction was perpendicular to the long-axis of the thoracic aorta. Twenty time frames of the aorta were retrospectively reconstructed with a customized program *IntraGate* within the *ParaVision* 3.0.2. software. After zero-filling the MR images to a matrix of 512x512 pixels the lumen diameter was manually determined per mouse at a fixed height (see Figure 1).

Global functional heart parameters of 11 *Smtn-B*$^{-/-}$ mice and 11 *Smtn-B*$^{+/+}$ mice were determined from a stack of slices from apex to base. Each slice was acquired with a prospectively ECG triggered and respiratory gated CINE FLASH sequence (2). The parameters of this FLASH sequence were: Gaussian-shaped RF-pulse, 300 μs; flip angle, 15°; TR, 7 ms; TE, 2.1 ms; acquisition window, 1.27 ms; field-of-view, 3 x 3 cm^2; matrix, 192 x 192; in-plane resolution, 156x156 μm^2; slice thickness, 1.0 mm; number of averages, 6; total acquisition time, approximately 5 min per slice. The global functional heart parameters of each mouse were calculated using semi automatic segmentation with the CAAS MRV FARM program (Pie Medical Imaging, Maastricht, The Netherlands). After the MRI session the excised hearts were weighed and expression of atrial natriuretic factor (ANF) and brain natriuretic peptide (BNP) within the myocardium was investigated by quantitative real-time PCR. The mean expression levels of *Smtn-B*$^{+/+}$ mice were set at 1.

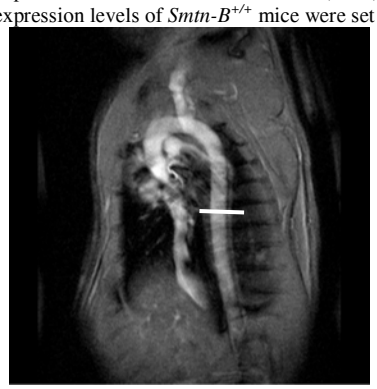

Figure 1: MR image of the aorta of a *Smtn-B*$^{+/+}$ mouse at the end-diastolic heart phase. The white line indicates the position at which the diameter measurement of the thoracic aorta was performed.

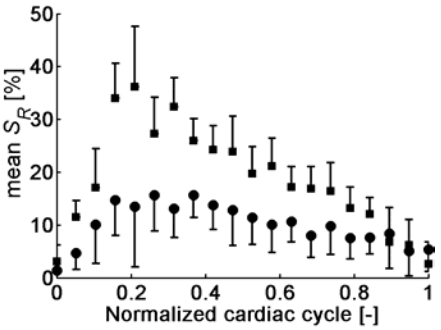

Figure 2: Mean circumferential stretch (S_R) of *Smtn-B*$^{+/+}$ (dots) and *Smtn-B*$^{-/-}$ mice (squares) thoracic aorta during the normalized cardiac cycle with 0 at the begin-systolic heart phase and 1 at the end-diastolic heart phase (mean±SD).

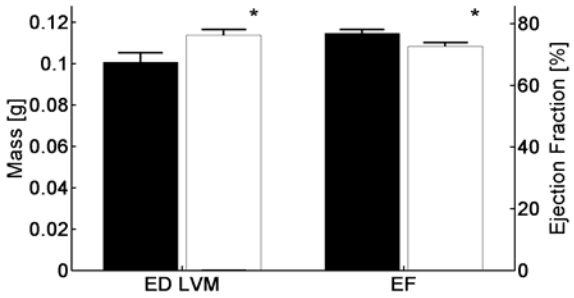

Figure 3: A comparison of global functional heart parameters of the left ventricle between *Smtn-B*$^{+/+}$ (black bar) and *Smtn-B*$^{-/-}$ mice (white bar). ED LVM: end-diastolic left ventricular mass; EF: ejection fraction (mean±SEM ,* P<0.05).

Results

The maximal increase of the lumen diameter of the thoracic aorta was higher (0.40±0.13 mm) for the *Smtn-B*$^{-/-}$ mice compared with the *Smtn-B*$^{+/+}$ mice (0.22±0.04 mm) (P=0.005). The lumen diameter of the thoracic aorta at the end-diastolic (ED) phase was 1.15±0.15 mm and 1.05±0.17 mm for the *Smtn-B*$^{-/-}$ and *Smtn-B*$^{+/+}$mice, respectively. Division of the maximal increase of the lumen diameter by the ED lumen diameter resulted in a maximal circumferential stretch of 39.8±6.3% for *Smtn-B*$^{-/-}$ mice and 19.8±6.3% for *Smtn-B*$^{+/+}$ mice (P=0.014). In Figure 2, the mean circumferential stretch of the thoracic aorta of both mouse groups is plotted after normalization to the minimal lumen diameter in every time frame. It is clearly visible that the mean circumferential stretch was higher for the *Smtn-B*$^{-/-}$ mice compared to the *Smtn-B*$^{+/+}$ mice. Maximum circumferential stretch was reached at approximately the same phase of the cardiac cycle.

Figure 3 shows the ED left ventricular mass (ED LVM) and the ejection fraction (EF), which were significantly different between the *Smtn-B*$^{-/-}$ mice and *Smtn-B*$^{+/+}$ mice (P=0.023 and P=0.020, for ED LVM and EF, respectively). The higher ED LVM was also confirmed by the total heart weight: 0.16±0.01 g for *Smtn-B*$^{-/-}$ mice and 0.14±0.01 g for the *Smtn-B*$^{+/+}$ mice (P=0.045). The relative expressions of both natriuretic peptides were elevated; ANF: 1.39±0.16 (P=0.05), BNP: 1.22± 0.07 (P=0.08) in *Smtn-B*$^{-/-}$ mice compared with *Smtn-B*$^{+/+}$ mice; ANF: 1.00±0.15, BNP: 1.00± 0.13.

Discussion & Conclusions

The elevated ANF levels as well as the higher ED LVM of *Smtn-B*$^{-/-}$ mice indicated a higher mean arterial pressure compared to the *Smtn-B*$^{+/+}$ mice. This could imply that *Smtn-B*$^{-/-}$ mice have a higher systolic blood pressure, which could explain the observed increase in mean circumferential stretch of thoracic aorta. To confirm the higher systolic pressure the stiffness of the thoracic aorta is currently under investigation by measuring the pressure pulse wave velocity inside the aorta.

In conclusion, this study demonstrates that retrospective and prospective CINE MRI methods can be used for phenotyping small differences in the cardiovascular system of smoothelin-B knockout mice. Smoothelin-B deficiency results in an increased mean circumferential stretch of the thoracic aorta, increased ED LVM and decreased EF compared to their wild-type littermates.

References: 1) Renssen SS *et al*, Cardiovasc Res 2002;**55**:850-63
2) Heijman E *et al*, NMR in Biomedicine 2006: **in press**