Inhibition of ASK1 Enzymatic Activity Does Not Protect Heart From Angiotensin-II Induced Hypertrophy

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Introduction

In the diseased heart, cardiac hypertrophy acts as an initial compensatory mechanism to preserve cardiac function. This cardiac remodeling, however, is in itself a risk factor which ultimately leads to decompensation and heart failure. Angiotensin II (Ang-II) is an oligopeptide that has been shown to induce hypertension and cardiac hypertrophy and has been used to study the mechanisms underlying hypertrophy in the murine model [1]. Mitogen-activated protein kinase (MAPK) signaling cascades have been proposed to play an important role in the onset of cardiac hypertrophy [2]. Apoptosis signal-regulating kinase 1 (ASK1) is a MAPK kinase kinase that has been shown to relay signals to c-Jun N-terminal kinase (JNK) and p38 MAPK. Given that these kinases play a putative role in the progression of cardiac hypertrophy, there is potential therapeutic benefit of targeting these pathways via ASK1 inhibition [3]. ASK1 deficient mice (ASK1-/-) have been shown to have reduced Ang-II-induced hypertrophy compared to wild-type mice [4]. However, whether the reduction in hypertrophy was due to the interruption of ASK1 enzymatic activity or by an alternate mechanism (i.e. protein scaffolding) is not known. In this study, the effect of directly interrupting ASK1 kinase activity on Ang-II-induced hypertrophy was examined in ASK1 mutant knock-in mice (ASK1 mKi). The mutation was designed to allow expression of a "kinase dead" ASK1 protein.

Methods

Osmotic pumps were implanted subcutaneously in ASK1 wild type (ASK1-WT, n=6) and mutant knock-in (ASK1 mKi, n=6) mice to deliver 2mg/kg/day Ang-II. Animals were scanned weekly for a period of four weeks using a 9.4T small animal vertical-bore magnet (Bruker Biospec, Billerica, MA). Gradient-echo bright-blood scout images (TR=50ms, TE=2.7ms, 128X128 matrix, 3X3cm FOV, NEX=4) were acquired in the coronal and sagittal plane to determine the long axis of the left ventricle. Transverse, ECG-triggered, gradient echo bright-blood cine images (TR=12ms, TE=2.7ms, 128X128 matrix, .195X.195X1 mm voxels, 8 slices, FOV, NEX=4, 10 movie frames, 15ms frame delay) through the short axis were acquired from the base to the apex of the heart (Figure 1). Two additional groups of age-matched control mice (n=3 for both ASK1 wild-type and mKi) were scanned in the fourth week. At the end of four weeks, all animals given Ang-II were sacrificed and cardiac tissue wet-weights were recorded.

Cardiac functional parameters for the left ventricle (LV) were calculated from MR images using the Analyze (Biomedical Imaging Resource, Mayo Clinic, Rochester MN) software package. Indexed left-ventricular mass (LVMI) was determined by the difference between the epicardial volume and the cavity volume multiplied by the specific gravity of the myocardium (1.05 g/cm^3) normalized to body weight. The ejection fraction was calculated by dividing the difference in the LV cavity volume at end diastole (EDV) and end systole (ESV) by the end diastolic volume.

Results and Discussion

Comparison of LVMI and ejection fraction revealed no significant difference between the wild-type and mutant knock in groups (Figure 2) over the four week period studied. From baseline to four weeks of Ang-II infusion, there was a decrease in ejection fraction (6.5% WT, 6.9% mKi) and a significant increase in LVMI (47.7% WT, 37.8% mKi, P<.01) for both groups. In addition, there were similar differences in LVMI (51.6% WT, 57% mKi) and ejection fraction (8.36% WT, 8.26% mKi) between both Ang-II infused groups and their corresponding age-matched controls at week 4. Comparison of LVMI MRI measurements and tissue wet weights showed a strong correlation for both groups (r =.98 WT, r =.91 mKi).

While published studies have shown reduced cardiac remodeling in an ASK1 knock-out rodent model of cardiac hypertrophy, our results indicate that inhibiting the kinase activity of ASK1 by disrupting the ATP binding domain has no effect on alterations of cardiac function induced by Ang-II infusion. The present results do not rule-out the possibility that ASK1 may participate in kinase-independent hypertrophic signaling, perhaps via protein-protein scaffolding [5].

References

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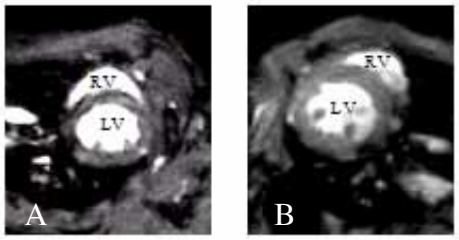

Figure 1. True-transverse bright-blood functional cardiac images through the left (LV) and right (RV) ventricle at baseline (A) and four weeks of Ang-II (B). Left-ventricular hypertrophy is evident after four weeks of Ang-II infusion compared to the pre-infusion baseline condition.

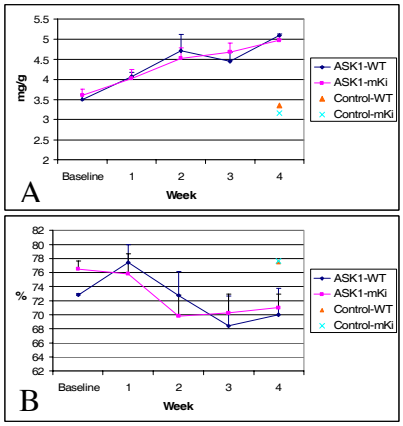

Figure 2. Comparison of cardiac parameters in ASK1 wild-type (ASK1-WT), mutant knock-in (ASK1 mKi) and their respective age-matched controls (Control-WT, Control-mKi). Unlike ASK1 deficient mice reported in the literature, the mutant knock in mice showed no difference in increase of cardiac mass (A) and decrease of ejection fraction (B) after four weeks of Ang-II infusion.