Decreasing patchy fibrosis using combined CaMKII inhibition and anti-fibrotic eplerenone treatment tempers arrhythmogenesis in chronic pressure overloaded mice

Authors:
H E Driessen¹, MSC Fontes¹, M Brans¹, L Van Stuijvenberg¹, MA Vos¹, TAB Van Veen¹, ¹University Medical Center Utrecht, Medical physiology - Utrecht - Netherlands,

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Background/Introduction
Elevated activity and expression of Ca2+/calmodulin-dependent protein kinase II (CaMKII) is a hallmark of pathophysiological cardiac remodelling. This, together with increased fibrosis deposition, disturbs calcium homeostasis, electrical coupling, cellular excitability, and tissue integrity leading to increased arrhythmia vulnerability. Previously, we showed that acute CaMKII inhibition is antiarrhythmic in dogs and rabbits. However, in mice with chronic pressure overload, chronic CaMKII inhibition did not reduce arrhythmogenesis or fibrosis formation, although it preserved conduction characteristics and Cx43. In a different study, we showed that aldosterone antagonism exerts anti-fibrotic effects in physiologically aging mice, making it a tempting strategy to combine with chronic CaMKII inhibition.

Purpose
To investigate a combination therapy of CaMKII inhibition with the aldosterone antagonist eplerenone to prevent fibrosis and subsequent arrhythmias in a mouse model of chronic pressure overload.

Methods
To induce chronic pressure overload, transverse aortic constriction was performed on wild type (WT) mice, or mice with cardiac expression of autocamtide-3-related-peptide (AC3I, CaMKII inhibitor). Eplerenone (200mg/day, E) treatment started 1-week post-surgery. After 12 weeks, arrhythmogeneity was tested on Langendorff-perfused hearts. Levels of total, patchy, and interstitial fibrosis were determined using new custom made ImageJ protocols.

Results
Arrhythmia inducibility was tempered, but not significantly suppressed; AC3I 21%, AC3I-E 7% and WT-E 15%. Total tissue fibrosis levels proved to be equal among groups; AC3I 4,3%, AC3I-E 3,2% and WT-E 4,7% (fig 1A). AC3I showed a slight decreased patchy fibrosis compared to WT-E (2,2 vs 3%), a clearer decrease in patchy fibrosis was found in AC3I-E compared to WT-E (1,2 vs 3% p=0,08) (fig 1B). Interstitial fibrosis did not differ among groups; AC3I 2,6%, AC3I-E 2,3% and WT-E 2,4%. Total fibrosis was equal in mice with and without arrhythmias (5,5 vs 3,8%, fig 1C), patchy fibrosis was significantly less in mice without VT/VF; 1,9 vs 3,9% (fig 1D, p<0,05).

Conclusion
Addition of eplerenone to chronic CaMKII inhibition didn’t decrease total fibrosis, but appears to slightly decrease patchy fibrosis. This possibly suggests a small effect of eplerenone in preventing patchy fibrosis in a pathological setting. Tempered arrhythmogenesis corresponds with significantly decreased patchy fibrosis in non-inducible mice, confirming a connection between arrhythmia vulnerability and level of patchy-fibrosis. Lack of a more pronounced anti-fibrotic effect could be due to the relatively short duration of treatment, the timing of
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