Reviewer's report

Title: Spironolactone ameliorates endothelial dysfunction through inhibition of the AGE/RAGE axis in a chronic renal failure mouse modelRunning title: Mineralocorticoid receptor antagonist, advanced glycation end products, chronic renal failure

Version: 0 **Date:** 28 May 2019

Reviewer: Jesper Bech

Reviewer's report:

The study by Wang et al reports the effects of in vivo treatment with spironolactone or AGE-breaker (ALT-711) in a rat model of non-diabetic CKD on renal function, vascular reactivity (endothelial- and non-endothelial dependent). From the in vivo model, immunehistochemistry studies were performed in vascular tissue, ie. distribution of eNOS, P-eNOS and anti-RAGE. In parallel, a range of in vitro studies were performed on HAEC's to elucidate on the potential mechanisms of action of spironolactone with emphasis on the interaction with the AGE/RAGE axis. In these studies, the authors used cell viability assay, Western blots and confocal microscopy.

General comments

The study is well designed and clearly reported. The authors are able to demonstrate significant effects of spironolactone in vivo on AGE-related effects and effects on vascular reactivity. The extension of these observations to cell-derived studies using HAEC's supports the notion, that important aspects of the clinical effects of spironolactone perhaps could be explained by the proposed upregulation of SIRT3 and NOX.

Specific comments

In the title and a few places in the text (p. 23, line 15), the authors refer to "a mouse model".....the study was performed on Sprague-Dawley rats.

The authors suggest, that there was no difference in the in vivo effects of spironolactone vs. ALT-711 on renal function based on analysis of the BUN-results. However, looking at the creatinine data (a better estimate of GFR...?) suggests, that creatinine levels rose by approx. 107 % in Groups 2 and 3, but only 22 % in Group 4 (spironolactone). This seems to be a quite significant difference and may suggest a differential effect of ALT-711 vs. spironolactone on renal function. This should be clarified by the authors, i.e. whether this represents a power issue ? In line with this - the levels of BUN could reflect other issues than renal function (protein intake, diuresis etc...)

The in vivo study might improve by reporting blood pressure data (those were measured according to the methods section) and body weights of the animals

Why did the authors not include a group of animals subjected to combination treatment (ALT 711 + spironolactone)? This would have provided more information about the in vivo importance of the suggested pathways.

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