Reviewer's report

Title: Ruscogenin ameliorates diabetic nephropathy by its anti-inflammatory and anti-fibrotic effects in streptozotocin-induced diabetic rat

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Reviewer: LEE-TIAN L. T. CHANG

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Major Compulsory Revisions

1. The qualities of all IHC data are not consistent. Same glomeruli size and outlook should be observed in same treatments in different figures (Fig. 1-4). There are no scale bars to normalize these problems. Poor backgrounds were noticed in all IHC figures. Authors should use arrow or arrowhead to point out their positive areas. I cannot observe the PAS signs in Fig.1. It looks like H&E stain. Figure 1 of following reference, J Mol Hist (2009) 40:107–115, can demonstrate an apparent difference of PAS stain on glomeruli between normal and diabetic rats. If possible, arrows to point mesangial expansion areas are essential. Ruscogenin decreased MCP-1 and ICAM-1 expression are good point to link mild macrophages infiltration in glomeruli. A linkage of figure 2 and 3 with a double stain on ED-1 cells and MCP-1/ICAM-1 nephron cells will confirm the anti-inflammation effect of ruscogenin. A direct data to prove ruscogenin inhibit chemotaxis will prove a strong result on this study.

2. As authors' mention, reference 14 and 15 provide ruscogenin showed similar results on anti-inflammatory activity through regulating NFkB and ICAM-1. It meant novelty did not demonstrate in this study. At least, author should point out this anti-inflammatory activity of ruscogenin was proved first time in diabetic nephropathy. Similar in vivo data on NFkB response and macrophage infiltration after ruscogenin treatment were also published by International Immunopharmacology 16 (2013) 7–16. Again, an advanced mechanism study will help us to understand the potency of ruscogenin on clinical usage.

3. Rosiglitazone, a PPARr agonist, was used to be a control in this study. In section of result and discussion, authors also discuss the difference between ruscogenin and rosiglitazone. Ruscogenin did not own anti-hyperglycemic activity, but show similar renoprotective effect as rosiglitazone (Table 1). Rosiglitazone is not a drug used in T1D patients. Am J Physiol Renal Physiol 295: F1071–F1081, 2008, this paper proves that rosiglitazone is not only an insulin sensitizer; it reduces diabetic nephropathy by depressing oxidative injury. Whether or not to complete a further investigation of ruscogenin influence on PPARr, a data of direct renoprotective function of ruscogenin will be more helpful. Otherwise, rosiglitazone control will not link to the renoprotective effect of ruscogenin. Because ruscogenin cannot lower hyperglycemia and HbA1c, a test to combine insulin and ruscogenin on STZ-mice will confirm the clinic potential of ruscogenin.
Minor Essential Revisions
1. Error typo was noticed in this manuscript. Glomerularbrosis (page 12) should be corrected to glomerular fibrosis. A detailed screen is necessary.
2. Word sizes of each figure should be consistent.
3. Table 1 should a dose-dependent manner of ruscogenin. But, photos of all figures did not demonstrate the dose-dependent results. Authors should provide supplementary data or talk it in section of discussion.

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.