Reviewer's report

Title: Cyclosporine-A Attenuates Retinal Inflammation by Inhibiting HMGB-1 Formation in Rats with Type 2 Diabetes Mellitus

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Reviewer: Lola Mugisho

Reviewer's report:

This manuscript evaluated the effect of cyclosporin-A therapy in type 2 diabetes. The authors propose that cyclosporin-A can protect against retinal changes in diabetes in an HMGB1 dependent manner. While the manuscript was generally well-written, the following major concerns need to be addressed.

Major comments:

* The experiment comprised of 2 parts. In the first part, the effect of cyclosporin A was evaluated in normal and diabetic patients. In the second part, only normal, healthy rats were subjected to treatment. This is confusing because the authors aimed to evaluate the effect of HGMB1 activation in diabetic not normal rats. Could the authors explain the rationale for this experimental design?

* For the second part of the study where HMGB-1 was injected intravitreally, do the authors have any proof that the HMGB-1 was taken up by the retina? Is the expression profile of HMGB-1 discussed in figure 2 likely to be similar to the uptake?

* DMSO was used for control in the first part of the study. However, DMSO is a well-known cytotoxic agent. Were there any toxic effects of DMSO observed during the study? What concentration of DMSO was used?

* In Figure 1, the authors described the presence of oedema within the GCL layer in the diabetic group. Could the authors comment on whether this is the usual pathology seen in DR patients and how reliable it is to assume the presence of oedema from H&amp;E images?

Other comments

ABSTRACT

* Methods - line 52/53 "Another part of normal wild-type rats was subjected to…"
  
    • Reword - Another group of normal wild-type rats were subjected to?
BACKGROUND

* Line 41 - parentheses are needed for IL-1b & TNF-a section, eg "...inflammatory cytokines, namely interleukin-1b... (TNF-a), are elevated in...

* Line 49/50 - reword "recent researches" - researches is not grammatically correct
  o "Recent research" or "Recent studies"

METHODS

* Intravitreal treatment of Cs-A/HMGB-1
  
  • Line 26/27 - Reword "Two parts of experiments were designed in this study." to say "This study comprised of two experimental parts"?

  o Line 49 - "none-diabetic" change to "non-diabetic"

* Immunohistochemistry & Western Blot
  
  o Line 32 - "(Abcam)" - need more of the specifications etc, eg dilution, catalog number, company address

RESULTS

* Animal characteristics
  
  o Line 54/55 - "P<0.01" - p-values for significant should all be lower case & italics
    
    • Ie "p < 0.01" - repeats throughout paper/figure legends

* Pathomorphological alternations of retinal tissues is not grammatically correct. Please rephrase appropriately

* Line 4 - "HE staining" - please rephrase.

DISCUSSION

* Line 6 - reword - "proved" - eg showed
Line 6 - not sure that you can say that it had "an inhibitory effect on diabetes-caused retinopathy" as retinopathy was not measured directly in this study. Please rephrase appropriately.

Line 6/8 - reword - "Similarly, inhibitory effect of Cs-A was showed in vitro". Change to "Similarly, an inhibitory effect of Cs-A was shown in vitro"

FIGURES

* Figure 1
  o Require comments to explain the larger ONL seen in C & D versus A & B
  o The authors suggest that treatment seen in figure 1D is better than in figure 1C, however this is unclear from the images. Is there any quantification to support this statement?

* Figure 2
  o Figure - symbol chosen to indicate significance for DM comparison is not clear. Could the authors consider the use of "+" instead?
  o Representative images in figure 2A-D do not match the quantification data presented in figure 2E.

* Figure 3
  o As the column bars have been differentiated through shading, it would be better to add a legend and remove the treatments names from the x-axis. This is consistent for all similar figures.
  o The y-axis is presented as "pg/mg" however this is not adequately described in the figure legends. Therefore, is this in pg per mg of retina or the entire eyeball, for instance?

* Figure 4
  o The methods suggests that the western blot analysis was carried out in comparison to the actin antibody. However, the figure suggests that the housekeeping gene used was GAPDH. Could the authors please clarify?

Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.
Yes

**Does the work include the necessary controls?**
If not, please specify which controls are required in your comments to the authors.

Yes

**Are the conclusions drawn adequately supported by the data shown?**
If not, please explain in your comments to the authors.

No

**Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?**
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

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