Reviewer’s report

Title: Expression of Matrix Metalloproteinases-2 and aquaporin-1 in corneoscleral junction after angle-closure in rabbits

Version: 1 Date: 16 Jul 2018

Reviewer: Sarah Atkinson

Reviewer's report:

While this is an interesting paper, there are some major changes and clarification needed to it before publication. These are as follows:

1) With regard to numbers of controls vs. experimental numbers, there needs to be some evidence provided that the small number of controls is a sufficient number for comparison. It might be useful to look at the ARRIVE or PREPARE guidelines for conducting animal studies. Was a power calculation performed to ensure that the number of controls used was adequate?

2) Throughout the manuscript, numbers of animals used for histology, immunofluorescence and real-time measurements is unclear, perhaps it would be useful to provide a flow chart or table of what happens to the experimental and control animals following operational procedures?

3) Numbers of animals used for the sub-divided groups appear to be quite small and therefore are unlikely to yield meaningful results, however, perhaps with clarification of exactly how many animals are used (previous point) this may be addressed. With sub-divided groups based on down-stream methods and time points there may not be sufficient numbers to answer the scientific question described within this manuscript. Therefore, justification is needed by the authors for the animal numbers used.

4) Can the authors comment on why they believe only 20 operations were successful? Is it possible to compare those that had 2 operations and those that had 1 operation and use them as equivalent within the experiment?

5) Were all animals of similar age and sex?

6) Why did the authors use this particular model of angle closure? Have previous uses of this model produced increases in IOP similar to humans?
7) At what point were eyes enucleated?

8) Why are the sections used for staining so thick (4mm), is this an inaccuracy in the measurement stated within the methods?

9) There are no antibody controls mentioned within the methods or presented within the results? This would raise concerns that the staining shown can not be determined to be specific, particularly as it does not appear that a blocking step was used before the application of primary antibody.

10) More detail needs to be provided within the methods eg. what microscope was used to image staining? How was H&E staining performed? What was the source of the DAPI? Were control eyes stained (these are shown in results but details are needed within the methods)?

11) It appears that only 2 eyes were used for real-time, this is a small number? Also how many control/normal eyes were used with this method?

12) Sybr green was used for real-time PCR, from the manuscript, it is unclear if the proper validation controls for this method of measurement have been used eg. melting curves and standard curves.

13) No information on concentration of RNA/cDNA used.

14) No primer sequence is provided for GAPDH.

15) Within the methods, it appears that the ELISA samples are taken from different tissue compared to real-time and histology samples, is this the case? and if so why?

16) was a significant difference shown for IOP between experimental and control groups?

17) TIMP2 can function as an inhibitor and activator of MMPs, so it might be a bit simplistic to say it is inhibiting MMP2? More discussion is needed to explain the increase in both TIMP2 and MMP2.
18) Abbreviations and full terms are used interchangeably throughout the manuscript eg. TM and trabecular meshwork, consistency in terms would be useful.

19) Are there any other likely reasons for the increase in MMP2 alongside those stated within the manuscript?

20) Have the authors any evidence for irreversible TM damage occurring at 1month post-operative? Have they used a stain or other method to detect TM damage eg. AS-OCT?

21) Figure 1, need to say what the arrows represent within the figure legend.

22) Figure 3a - black lines within figure, not clear what these are, need to include some labelling/arrows to indicate structures within images. The different timepoints don't appear to show the same area of tissue, however, this may be clarified with labelling.

23) Figure 3b - same points need addressed as 3a with regards to labelling structures showing the equivalent tissue area at each timepoint, no antibody controls shown, therefore hard to determine if staining shown is background or specific staining.

24) Not clear within figure 4 what statistical analysis comparisons were performed, this needs to be indicated within figure or figure legend. It's not clear what is meant by 3 experiments within the figure legend, as the surgery all seems to have been performed at the same time, should this be 3 replicates of real-time PCR?

25) Figure 5, similar comments to figure 4, were 3 experiments performed? What is statistical significance based on? There doesn't seem to be any conditions which are significant at p<0.01, therefore this does not need to be included in the figure legend.
Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.
No

Does the work include the necessary controls?
If not, please specify which controls are required in your comments to the authors.
No

Are the conclusions drawn adequately supported by the data shown?
If not, please explain in your comments to the authors.
No

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I recommend additional statistical review

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