Peer Review File

A pilot study of genipin cross-linking effects on bullous keratopathy in rabbits

Reviewer: 1

1. Please describe in detail the bullous keratopathy model along with its methodology and validation.
2. Please use these references in the introduction:
   Alternatives to allograft corneal transplantation. Curr Opin Ophthalmol. 2010

Response to Reviewer: 1

1. The bullous keratopathy model has been elucidated.
2. References have been added in the introduction.

Reviewer: 2

The authors aim to investigate the effect of genipin cross-linking on rabbit bullous keratopathy. They declared that genipin cross-linking treatment for rabbit bullous keratopathy could lead not only to remission of corneal edema but also to relief of pain. The hypothesis for this result is that genipin cross-linking could strengthen collagen fibers arrangement in corneal stroma to avoid formation of corneal edema and bullae. Totally the study is interesting and it has some merit. However, some limitations exist and I can’t feel it is acceptable. The most important error is the sampling. In this study, the authors used only 9 experimental animals and were randomly divided into 3 groups. Moreover, what the author presented is not the repeated experimental result. I could not agree the results from the limited sample. Moreover, the result presentation needs to be improved and some figures seem to be missed.

Change in anterior segment is better to be presented in the same manner (Results shown in Figure 1 and Figure 2 should from the same time point).

Just describe about the changes in stress response (without data) is not acceptable.

The figures in histology changes need to be improved. In Figure 3, the epithelium changes among A, B, C and D are not clear. In Figure 4, the lymphocyte infiltration needs to be confirmed by some specific antibody staining. In Figure 5, I cannot find the figure of treat group. As to apoptosis of stromal cells shown in Table 3, the apoptosis rate in treat group seems increased which mean the more injury.

Further large-scale, high-quality studies to confirm these preliminary results would be worthwhile.

Response to Reviewer: 2

Limitations exist in our study, especially the limited animal sample cannot explain the long-term efficacy and safety of the genipin cross-linking for bullous keratopathy. We would like to use more animal models and human cases in future studies to elaborate the safety and effect of genipin cross-linking for bullous keratopathy. As it is difficult to evaluate the pain and stress in animals directly, we considered using their activity, food consumption, temperamental changes, vocal changes to evaluate their pain and stress response indirectly. We hope a reasonable ocular pain scale for animals could be designed in future to evaluate the pain and stress directly. The result presentation has been improved and the figure 5 has been added. We are very sorry that we cannot provide clearer figures in histology as corneas have been sliced into frozen sections. As to the apoptosis of stromal cells, apoptotic cells were occasionally observed in the cornea stroma after cross-linking in treatment group, while more apoptotic cells are also found in riboflavin and
UVA cross-linking. So we think that genipin cross-linking is obviously safer than riboflavin and UVA cross-linking. We hope that these limitations could be improved in future studies to confirm these preliminary results.

Reviewer: 3
1- The reported thicknesses in Table 1 seem to be too high. Did you measure thickness of healthy animals
2- Table 2 shows that the body weight increased from 2.6Kg to 3.5 in one day, can you explain it why?
3- In figure 4, the authors wrote the stroma is regularly arranged, this is not clear.
4- Figure 5 suggests that there should be more than one figure

Response to Reviewer: 3
1. The corneal thickness of right (healthy) eyes was 390.2±6.1 um.
2. Food was withdrawn overnight prior to the operation. Then they were fed normally in the following days.
3. We are very sorry that we cannot provide clearer figures in histology as corneas have been sliced into frozen sections.
4. The figure 5 has been added

Reviewer: 4
The manuscript of “The Pilot Study of Genipin Cross-linking Effect on Rabbit Bullous Keratopathy (IJO-2015-0569)” reported the cross-linking effects of Genipin on rabbit corneas with bullous keratopathy. As well known, Genipin is a common cross-linking reagent in various biomaterials especially in the cross-linking of collagen fibrils, and has widely utilized in bone allografts, porcine corneas, and the Boston keratoprosthesis donor carrier, etc. Moreover, Avila et al reported the similar results in porcine corneas in 2009. This manuscript has little scientific significance but using a rabbit bullous keratopathy model. According to Table 1 in this manuscript, the average central corneal thickness of bullous keratopathy rabbit models was around 2400 μm which is about 6 fold of the normal central corneal thickness of rabbits. The question is why the authors established so drastic an edema corneal model for investigations? As well defined, bullous keratopathy is the late phase condition of endothelial decompensation. What is the clinical significance of this manuscript? Besides, the authors did not tell the readers how the bullous keratopathy model was established in the “Materials” section of the manuscript. Beside of these, the manuscript was casually prepared and the English writing was not canonical and precise at all.

Response to Reviewer: 4
9 bullous keratopathy New Zealand rabbits received endothelium scraping one week before they were enrolled as the animal models. We hope to evaluate the effect and safety of genipin cross-linking in a stable animal model. It is known that it costs bullous keratopathy patients much time for suitable corneal tissue. Thus, several options have been proposed for the bullous keratopathy treatment. Recently ultraviolet (UVA) and riboflavin cross-linking has been a new treatment for bullous keratopathy. Because of their similar biomechanical efficiency, we used genipin cross-linking as a new attempt for the bullous keratopathy treatment. Besides, the bullous keratopathy model has been elucidated in METHOD.
Reviewer: 5
This paper need extensive revision, especially in the method and results.
They should be arranged again.
In the method section, the method for bullous keratopathy model should be elucidated, while the breeding condition could be reduced if there is no special change. The anesthesia method was repeatedly explained. The TUNNEL kit should be illustrated.
The Figure 5 was not consistent with legend.
Response to Reviewer: 5
The method and results sections have been arranged again. The method for bullous keratopathy model has been elucidated, the breeding condition and the anesthesia method has been reduced. The figure 5 has been added.