Peer Review File

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Reviewer 1:

Comments:

The article titled "A Narrative Review: Murine models of age-related macular degeneration for drug evaluation: comparison of sodium iodate and lipid peroxide" is a partial review of the preclinical methods for animal AMD models. The work is of interest to compare and understand the different models and their affectations depending on the animals to which the damage is applied and the routes of administration.

1.However, it is not a complete review as quite a few models are missing (especially models of genetic alterations) as well as other models of chemical damage. I recommend adding information in this regard, at least in summary form.

Reply1*:* We totally agree with the reviewer that AMD model is a very large topic, and this manuscript only covers models generated by sodium iodate and lipid peroxide as indicated in the title. Follow reviewer's suggestion, we provide the summary description of genetic AMD models in the text and Table 1, and revised the title to better reflect the scope of this review.

Change in the text:

*(Old Line, 36-37) To date, researchers have investigated and developed several non-genetic AMD murine models (**Table 1**). but we do not…….*

*→ (New Line, 37-43) To date, researchers have investigated and developed genetic (**Table 1**) and non-genetic AMD murine models (**Table 2**). The genetic mouse model includes juvenile macular dystrophy, metabolic pathway, inflammatory and oxidative stress genes. The table 1 displays that inflammation is essential part to drive disease progression especially into the wet form. The phenotypes of all these genetic models get severer by light, fat diet and/or laser. However, we do not……*

2.On the other hand, I think it would be advisable for the article to be written in an impersonal way, it gives it a much more scientific and professional character.

Reply2*:* Following reviewer's suggestion, we have revised text description in an impersonal manner.

***Change in the text**:*

(Old, Line 10-15) Here, I describe the detailed procedures and the differentiated pathological features of murine retinal degeneration models induced by sodium iodate and lipid peroxide (HpODE), as preclinical study platforms. I further discuss the application benefit of scleral-choroid/RPE/retina whole mounts to observe the degeneration of the outer retina and the infiltrated microglia, as well as other assessment methods for translational studies.

→ (New, Line 10-17) In this review, non-transgenic models, especially, murine retinal degeneration induced by sodium iodate and lipid peroxide (HpODE) are discussed as preclinical study platforms. Detailed procedures and the differentiated pathological features of them are described. Further, the benefits of using scleral-choroid/RPE/retina whole mount preparation for observing the degeneration of the outer retina and the infiltrated microglia as well as other assessment methods are described and discussed for translational studies.

(Old Line 23-24) I refer GA as the Dry form and CNV as the Wet form of AMD.

→ (New Line 25) GA is referred as the Dry form and CNV as the Wet form of AMD.

(Old Line 50-53) Here, I describe, compare and discuss the details of murine retina degeneration models induced by sodium iodate or lipid hydroperoxide HpODE as well as provide the standard protocols used to induce retina degeneration with these methods.

→ (New Line 59-62) The purpose of this review is to describe, compare and discuss the details of murine retina degeneration models induced by sodium iodate or lipid hydroperoxide HpODE and provide an example of the practical protocols commonly used to induce retina degeneration with these methods.

(Old, Line 112-116) Based on my experience and studies of retina degeneration caused by sodium iodate and HpODE, I describe here the practical approach for methods. Sodium iodate (10 to 50 mg/kg) were peritoneally injected into the mice of BALB/C and C57BL/6J (8-10 weeks, 20-30 g) or SD (8-10 weeks, 200-350g) rats for the observation of retinal degeneration,

→ (New, Line 122-124) Sodium iodate (10 to 50 mg/kg) were peritoneally injected into the mice of BALB/C and C57BL/6J (8-10 weeks, 20-30 g) or SD (8-10 weeks, 200-350g) rats for the observation of retinal degeneration,

(Old, Line 229-234) Here, I discuss ERG as a non-invasive method and the invasive assessment of retinal thickness, photoreceptor loss, outer nuclear layer folds, RPE loss, subretinal microglia infiltration, and expression of inflammatory, oxidative and cell death genes. Moreover, I discuss the benefit of the sclerochoroid/RPE/retina whole mount application to observe the horizontal view of the disorganized subretinal and outer nuclear layers (AES-21-27 this issue), and the infiltrated subretinal microglia.

→ (New, Line 239-246) In this section, ERG as a non-invasive measurement is described, and the invasive assessment of retinal thickness, photoreceptor loss, outer nuclear layer folds, RPE loss, subretinal microglia infiltration, and expression of inflammatory, oxidative and cell death genes are discussed. Moreover, the benefits of the sclerochoroid/RPE/retina

whole mount application to observe the flat view of the disorganized subretinal and outer nuclear layers (AES-21-27 this issue), and the infiltrated subretinal microglia are discussed.

3.Some figure of the different animals and / or routes of administration would be appreciated.

Reply3*: We provide a figure (Figure 1) of administration routes, and the figure legends*

Change in the text:

(Old, Line58-60) via the administration routes of tail or femoral vein, retro-orbital venous sinus, intraperitoneal, subretinal and/or intravitreal injections.
→ (New, Line 70-72) via the administration routes of tail or femoral vein, retro-orbital venous sinus, intraperitoneal, subretinal and/or intravitreal injections (Figure 1).
→ (New, Figure legend) Figure 1. Schematic diagrams illustrate chemical administration routes of tail (A), femoral vein (B), retro-orbital venous sinus (C), intraperitoneal (D), subretinal (E), intravitreal (F) injections.

4.Likewise, it should be noted that the methodology used by the author for his experiments is put in a different section, not followed by the summary of the models.

Reply4*: The practical methodology was/is in a different section with model summary: Introductory description of sodium iodate and lipid peroxide models is at Section2, and the practical protocol description is provided at Section 3. Further the assessment methodology is provided at Section 4. We maintain the current format and sections.*

Reviewer 2:

Comments:

1. There is not a single animal model of AMD because it is a multi-factorial disease involving multiple genetic susceptibilities and multiple non-genetic factors. The author does not discuss these factors. A thorough discussion of all possible AMD-like models is not provided.

Reply1*: We provide the summary description of genetic AMD models in the text and Table 1. However we do not review thoroughly the genetic and non-genetic AMD models, because this review is designed to provide methodology description of sodium iodate and HpODE AMD models in a practical way, rather than thorough description of all AMD models.* We revised title to better reflect scope of this review.

Change in the text:
*(Old Line, 36-37) To date, researchers have investigated and developed several non-genetic AMD murine models (**Table 1**). but we do not…….*
*→ (New Line, 37-43) To date, researchers have investigated and developed genetic (**Table 1**)*

*and non-genetic AMD murine models (**Table 2**). The genetic mouse model includes juvenile macular dystrophy, metabolic pathway, inflammatory and oxidative stress genes. The table 1 displays that inflammation is essential part to drive disease progression especially into the wet form. The phenotypes of all these genetic models get severer by light, fat diet and/or laser. However, we do not……*

2. The sodium iodate model has previously been suggested to mimic features of AMD. This suggestion is also proposed in the current manuscript. However this model is deficient.

***Reply 2**: We provide the comparison and discussion on the models of SI and HpODE in the section of discussion and conclusion.*

Change in the text at lines:
(New Line, 365-373): Among the AMD models, sodium iodate model has been most widely studied and used for the translational study, and the different dosages and administration routes of sodium iodate provides different aspects of the phenotypes. On the other hand, the sodium iodate model is acute, and might be hard to study the intermediate/progressing form of AMD, whereas HpODE AMD model is more slowly progressed than sodium iodate model and finally includes CNV. However, HpODE was only studied in SD rats and albino rabbits via a route of subretinal injection. Thus, additional studies will be valuable for translational studies for relating human AMD progressing.

3.The title of the paper is not consistent with the content. A comprehensive assessment of animal models of AMD is not provided.

***Reply 3**: The title of the paper has been revised.*

***Change in the text**:*
(Ttile, Old) A Narrative Review: Murine models of age-related macular degeneration for drug evaluation: comparison of sodium iodate and lipid peroxide
→ (Title, New) Comparison between sodium iodate and lipid peroxide murine models of age-related macular degeneration for drug evaluation- a Narrative Review

4. It is incorrect to state that light damage models do not involve damage/death to RPE. It is well known that there are light exposure conditions that damage RPE.

***Reply 4**: We added blue light RPE degeneration in the text.*

***Change in the text** :*
(Old, Line 44-45) but light-induced retina damage is limited to photoreceptors, and does not involve the retina pigment epithelium (RPE)
→ (New, Line 49-53) Depending on the source of lamps and wave lengths of lights, light

induces damage of either photoreceptors or RPE, or both: usage of fluorescent lamps has induced photoreceptor damage rather than RPE, whereas usage of light-emitting diode (LED) lamps and source of blue light induce RPE degeneration

5. Line 367. 'For example, FAF is associated with 365 increasing lipofuscin (62), infiltrated microglia (3, 63), photoreceptor rosettes (63- 366 65) and migrating RPE cells (63), which can be observed and studied in the animal, but not in the human eye.' This statement is not correct and/or understandable.

Reply 5*:We have updated the text.*

Change in the text

(Old Line 365-368) For example, FAF is associated with increasing lipofuscin, infiltrated microglia, photoreceptor rosettes and migrating RPE cells, which can be observed and studied in the animal, but not in the human eye.

→ (New Line 385-388) For example, FAF is associated with increasing lipofuscin, infiltrated microglia, photoreceptor rosettes and migrating RPE cells, of which association can be fully dissected in the animals, but not in the human eyes.