Peer Review File

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Revision Report

First of all, I would like to express our sincere gratitude to the reviewers for their comments. These comments are all valuable and helpful for revising and improving our manuscript, as well as the important guiding significance to our researches. We have studied comments carefully and have made correction which we hope meet with approval. Revised portions are marked in red in the revised version, The summary of corrections and the responses to the reviewer's comments are listed below.

Summary of the revision:

- **Section 2:** Upon incorporating the recommendations of reviewers, we determined that hydrogen peroxide, as an important component of Fenton's reaction, is highly involved in the production of hydroxyl radicals in lipid peroxidation and plays an important role in preterm preemy-associated encephalopathy

- **Section 3:** To enhance the article's readability and illustrate the role of iron metabolism abnormalities in HIBD, we further elucidate the role of blood transfusion and iron supplementation in anemia in preterm infants and the consequences of this approach.

Responses to reviewers (original comments by reviewers are in blue color)

Reviewer A

1. Comment 1: Abstract: The heading states “Background and Objective”, however, no objective or goal is stated.

1. Reply 1： We have modified our text as advised(see Page2,line33),which has been emphasized in red. “We hope to clarify the mechanism of ferroptosis in hypoxia-associated brain injury , inhibit the relevant targets of ferroptosis in hypoxia-associated brain injury to guide clinical treatment, and provide guidance for the subsequent treatment of disease-related drugs. ”

1. Changes in the text: Page2,line33

2. Comment 2: Page 3: delete the heading “Introduction”.

2. Reply 2: We have modified our text as advised(see Page3,line56)

2. Changes in the text:Page3,line56

3.Comment 3: Maintain consistency with the acronyms (e.g., GPX4 or Gpx4).

3. Reply 3: We have modified our text as advised(see Page6,line155;Page6,line156), which has been emphasized in red. “The necessity of GPX4 is underscored by the embryonic lethality of GPX4-null mice and the survival challenges exhibited by neural-specific GPX4 knockout neonatal pups[37, 38]. Given that GPX4 enzymatic activity requires glutathione, ferroptosis susceptibility is influenced by GSH levels [38].”

3. Changes in the text: Page6,line155;Page6,line156

4. Comment 4: PUFA was spelled out on page 4, line 20, and on page 6, line 120. Maintain consistency in definition.

4. Reply 4: We have modified our text as advised(see Page7，line178), which has been emphasized in red. “…, as well as PUFA content in lipid metabolism,”

4. Changes in the text:Page7，line178

5. Comment 5: MDA was spelled out on page 8, line 250 and page 9, line 268.

5. Reply 5: We have modified our text as advised(see Page10,line265), which has been emphasized in red. “…, producing MDA—a cytotoxic compound that can induce cross-linking and polymerization of vital macromolecules such as proteins and nucleic acids.”

5. Changes in the text:Page10, line265.

6. Comment 6: Same comment for other acronyms such as HIF and PHD.

6. Reply 6: We have modified our text as advised(see Page2,line25), which has been

emphasized in red. "…, disruptions in hpoxia-inducible factor-prolyl hydroxylase (HIF-PHD) axis,". We have modified our text as advised(see Page6,line167), which has been emphasized in red. "HIFs are transcriptional activators induced by hypoxic stress,". We have modified our text as advised(see Page13, line378), which has been emphasized in red. "HIF-1, a key transcription factor, serves as the principal regulator of oxygen equilibrium." We have modified our text as advised(see Page7, line169), which has been emphasized in red. "PHD are a family of non-heme, iron-dependent dioxygenases that require oxygen, α-ketoglutarate, and divalent iron ions for catalytic activity [44]." We have modified our text as advised(see Page14,line380), which has been emphasized in red. "This metabolic shift is governed by HIF, with its stability under low oxygen conditions being managed by the PHD enzymes, particularly three isoforms (PHD1–3) [91]. "

6. Changes in the text: Page2,line25;Page6,line167;Page13,line4378;Page7,line169;Page14,line380.

7.Comment 7: In the conclusions, the authors repeated what they did, but did not provide an overall conclusion and take-home message.

7. Reply 7: We have modified our text as advised(see Page14,line 397), which has been emphasized in red. "Furthermore, we review therapeutics that target ferroptosis for HIBD and CIRI treatment, we conclude that deferoxamine could provide neuroprotection by chelating iron ions and maintaining iron homeostasis; 17β-estradiol can treat HIBD by reducing lipid peroxidation, and the neuroprotective agent UBIAD1 can also reduce lipid peroxidation and protect the nerve. As for amino acid metabolism disorders, treatment with xanthoxanthin, galpinein, chamomilin and melatonin reduced GPX4 expression and inhibited neuronal ferroptosis; The modulators of HIF-PHD axis represented by cardamonin can protect the nerves through HIF-PHD. Panax notoginsenoside R1, Caspase-12 inhibitor and other drugs can reduce neuronal ferroptosis by inhibiting endoplasmic reticulum stress. Taken together, these results suggest that iron homeostasis, reducing lipid peroxidation, amino acid metabolism disorders, reducing endoplasmic reticulum stress and regulating HIF-PHD axis can reduce neuronal ferroptosis. Thus, we hope to provide ideas for the clinical treatment of hypoxia-associated brain injury. "

7. Changes in the text: Page14，line397

8.Comment 8: The authors mainly focused their review on ferroptosis in neonatal brain injury. While it is clear that iron plays a key role in ferroptosis, it is well known that hydrogen peroxide is highly involved in the Fenton-Haber-Weiss reaction that leads to the production of the hydroxyl radical in lipid peroxidation. More discussion about this process is needed, focusing on the preterm infant.

8. Reply 8: We have modified our text as advised(see Page5,line127), which has been emphasized in red. "It is well known that hydrogen peroxide plays an important role in the occurrence of Fenton reaction.The presence of hydrogen peroxide with ferrous iron generates hydroxyl radicals that oxidize PUFA to alkyl radicals (L•). L• reacts with oxygen molecules to produce lipid peroxyL groups (LOO•), which results in the accumulation of lipid peroxidation products (LOOH)[26], phospholipid hydroperoxide (P-LOOH) production can lead to structural damage of cell membrane." We have modified our text as advised(see Page9,line257),which has been emphasized in red. "Preterm brains are particularly vulnerable to ROS attack because they are rich in polyunsaturated fatty acids but low in antioxidants [63]. Newborns are in a state of high oxidative stress during pregnancy, delivery and postpartum, and they experience an environmental transition from intrauterine hypoxia to postnatal hyperoxia (during the process from intrauterine environment to extrauterine environment, the fetus changes from intrauterine environment of 20-25 mmHg PO2 to 100 mmHg PO2), resulting in increased ROS production[64]."

8. Changes in the text：Page5，line127;Page9,line257

9. Comment: Another important fact that was excluded is the role of blood transfusion and iron supplementation in preterm infants for anemia. Blood transfusion and iron supplementation causes increased non-transferrin bound free iron levels in preterm infants. This makes iron more available to react with hydrogen peroxide which is generated by dismutation of superoxide anion.

9. Reply 9: Thank you very much indeed for your comments. In response to your suggestion above, we agree with the comments you provided that transfusion and iron supplementation can occur with excessive iron accumulation beyond the binding capacity of the major transferrin proteins, which in turn allows for elevated levels of reactive oxygen species. We have added to the article (see Page 8, line 228) the reasons why preterm infants are susceptible to anemia and why repeated transfusions and excessive iron supplementation due to anemia are susceptible to the further

development of the Fenton-Haber-Weiss reaction, and have highlighted it in red as: With advancements in perinatal medicine, the incidence of HIBD in term infants has significantly decreased, making HIBD in preterm infants a major clinical issue [59]. Preterm delivery interrupts intrauterine extramedullary hematopoiesis prematurely; concurrently, bone marrow hematopoiesis in these infants is underdeveloped compared to that in term infants, rendering them less capable of adapting to rapid postnatal growth and development. Additionally, maternal iron acquisition is limited before the eighth month of gestation but increases thereafter; premature birth, therefore, reduces the neonate's iron reserves. Consequently, blood transfusions and iron supplementation are considered necessary to manage anemia in preterm infants [60]. However, frequent transfusions and excessive iron supplementation can lead to iron overload, surpassing the body's transferrin binding capacity and resulting in an excess of non-transferrin-bound iron. This excess iron then participates in the Fenton-Haber-Weiss reaction with hydrogen peroxide, produced through superoxide anion disproportionation, as previously described [61].

9. Changes in the text：Page8，line228

Reviewer B

Comment 1: **Title**

Please add “Narrative Review” in the title as requested by the Narrative Review Reporting Checklist.

Reply 1: We have revised the title as requested. We have modified our text as advised(see Page1,line2-3),which has been emphasized in red.

Changes in the text: Page1,line2-3.

Comment 2: **Main text**

Narrative Review should organize the main text in Introduction, Methods, main body, and Conclusions. Please revise the subtitle.

Reply 2: We have modified our text as advised(see Page3,line60),which has been emphasized in red.

Changes in the text: Page3,line60.

Comment 3: **Table 1**

Table 1 was not cited in the main text, please indicate where to cite.

Reply 3: We have modified our text as advised (see Page3,line80-81), which has been

emphasized in red.
Changes in the text: Page3,line80-81.

Comment 4: **Figure 1**
Figure 1 was not cited in the main text, please indicate where to cite.
Reply 4: We have modified our text as advised (see Page14,line397-399), which has been emphasized in red.
Changes in the text: Page14,line397-399.