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Dear Editor,

Thank you for considering our work for publication in the *World Journal of Stem Cells*. Our manuscript #65857 "***Modulating poststroke inflammatory mechanisms—novel aspects on mesenchymal stem cells, extracellular vesicles and microglia***" has been revised according to the reviewers' suggestions. All authors have read and approved the final version of the manuscript.

We have addressed the concerns risen by the reviewers point by point, and the revised text passages are highlighted in grey throughout the paper.

Again, thank you for re-considering our work for publication in the ***World Journal of Stem Cells.***

Yours sincerely,

Thorsten R. Doeppner (on behalf of the co-authors)

Point-by-Point answers to reviewers

Reviewer #1:

1.) I really appreciate your contact in resolving the review of this paper, titled Modulating poststroke inflammatory mechanisms—novel aspects on mesenchymal stem cells, extracellular vesicles and microglia. The review of manuscript NO 65857, I have already finished the preview of the current manuscript. More comments information, you will find information below:

Thank you for this encouraging comment regarding our work.

2.) "When activated after stroke, microglial cell activation includes four distinct phenotypes such as ramified, intermediate[p1], amoeboid, and round phenotypes [26]. The ramified microglia refer to the resting state, whereas intermediate state microglia have larger cell bodies and shorter bumps. The amoeboid microglial cell body, on the contrary, is larger and displays shorter bumps or even no bumps at all, similar to round microglia that are found in the lesion center [26]. Based on these morphological characteristics and their secretion patterns, microglia are characterized as M1 type or M2 type." It should be combined with the expression of specific markers of M1 or M2 here for a better macrophage characterization.

Many thanks for your careful review and positive comments. We also would like to combine the morphology of microglia with the expression of specific markers of M1 or M2 here at the beginning of writing this review. However, current information is limited, and no markers corresponding to the specific morphology of microglia are available.

3.) I have a word document, download attached file in word please.

Many thanks for your constructive suggestion. We have downloaded this file, read it thoroughly, and revised the article comprehensively based on your comments. Revised paragraphs are marked in light grey in the paper.

Reviewer #2

1.) The Manuscript by Monzur et al. reviews the “novel aspects on mesenchymal stem cells, extracellular vesicles and microglia on poststroke mechanisms”. Information collected in the manuscript entitled “Modulating poststroke inflammatory mechanisms —novel aspects on mesenchymal stem cells, extracellular vesicles and microglia” are in a logical sequence with appropriate analysis with figures and table to that contain data to inform the readers. The manuscript builds upon previous important research that is appropriately referenced. The data from this manuscript does move the canon of knowledge forward and may be considered by the Top 10-20 % of the research field. The manuscript is novel and interesting to warrant publication in “World Journal of Stem Cells” after minor revision.

Thank you very much for taking the time to review our manuscript. Your positive comments affirm our research efforts.

2.) The Abstract section are too long. It should be concise and comprehensive.

We have now deleted some contents in this section and revised the text accordingly (Page3/Line 35-52).

3.) Please mention the novelty of this review in the “introduction section”.

We have now added the novelty of this review in the “introduction section” (Page 6/Line108-111).

4.) The characterization of mesenchymal stem cells must be mentioned in more detail. For this reason, you can use and refer the following paper which explain it elaborately and completely: Isolation, culturing, characterization and aging of adipose tissue-derived mesenchymal stem cells: a brief overview. Brazilian Archives of Biology and Technology. 2016; 59.

This is an interesting aspect. Indeed, biological characteristics of both MSCs and their corresponding EVs greatly depend on the tissue source and the preparation of MSCs. Although the main focus of this review was not cell culturing of MSCs etc., we have briefly mentioned this aspect in the revised paper (page10-11/Line204-220)

5.) Also, telomere length shortening is related to neurodegenerative disease, stroke inflammatory and aging. It could be better discussing about the telomere shortening as clinical perspective in the text of the manuscript. You can use and refer the following papers:

Telomere shortening as a hallmark of stem cell senescence. Stem cell investigation. 2019;6. Telomere length: a potential biomarker for the risk and prognosis of stroke. Frontiers in neurology. 2019 Jun 13; 10:624.

This is indeed an interesting aspect. Indeed, telomere length shortening may be related to inflammatory stroke. We therefore reviewed the included studies again. However, so far, no literature has reported that MSCs or MSC-EVs can change telomere length and thus affect the activation of microglia. We therefore omitted this aspect in the present review, which is already quite long anyway.

Reviewer #3

1.) The authors reviewed recent articles investigating the roles and mechanism of MSC and MSC-EVs on microglial activity after ischemic stroke, especially the M1 M2 dynamics. The article is well structured. First, the authors discuss the pathophysiology of ischemic stroke and the role of microglia, then discuss the therapeutic role and the molecular mechanism of MSC and MSC-EV treatment. - The entire article focused on the mechanism of how the MSC and MSC-EV affect microglia.

Thank you for this comment.

2.) Figure 2 should include important signaling pathways/mechanisms on microglial polarization mentioned in the article. An alternative solution is to merge figure 1 and figure 2 together and summarize the molecular impacts of MSC and MSC-EV on microglial.

Thank you for this comment. Figure 1 already describes the signalling pathways of the process of polarization of the M2 phenotype, whereas figure 2 provides an overview as to how MSCs promote neurogenesis and neurological recovery. Inhibition of microglia activation is only one part of the role of MSCs and MSC-EVs in the treatment of ischemic stroke. We would overemphasize the importance of microglia inhibition and neglect the role of other pathways, if we merged figure 1 and figure 2.

With regard to the summarization of the molecular impacts of MSCs and MSC-EVs on microglia, we used a lot of paragraphs to describe the mechanism of MSCs and MSC-EVs on microglial activation after ischemic stroke. In addition, we have made Table 2 to fully illustrate the mechanisms of MSCs and MSC-EVs on microglial activation. We therefore did not prepare an extra figure in this respect.

3.) Line196-Line 204 Regarding BBB disruption, I suggest adding some recent studies about the mechanism: TNFa (Chen et al. Cell Death & Disease, 487 (2019)) microglial phagocytosis (Haruwaka et al., Nature Communications, 5816 (2019))

We have now added some contents to describe the mechanisms regarding BBB disruption based on two recent studies (Page9/Line 185 -191).

4.) Some format and grammar issues: Citation should come before the punctuations, e.g. "[42]," instead of ",[42]". Typos like: "[98, 124].." Line385-390 multiple tenses were used in one paragraph. Please recheck the format and grammar. I recommend accepting this article after minor revision.

We have revised the manuscript according to your suggestions. We read the article repeatedly and polished the language of the article. In addition, one of the reviewers also polished our article comprehensively. I hope that the language of our article can meet the requirements of the World Journal of Stem Cells now.

Reviewer #4

1.) The manuscript by Xin et al first described the different roles of two different phenotypes of microglia in the treatment of ischemic stroke, suggesting that changing the activation status of microglia might be an interesting stroke treatment approach. In recent years, MSCs and EVs have been reported to affect post-stroke inflammatory response, but the exact mechanism of this treatment is still unclear. In this review, the authors summarize the interaction between MSCs and MSCS-EVs with activated microglia, and elaborate on the inflammatory targeting mechanism by which MSC-EVs exert therapeutic effects. The logic of this paper is clear and the structure is rigorous.

Thank you very much for this comment regarding our work.

2.) But one of the deficiencies is that the therapeutic effect of MSCs and MSC-EVs is mainly described in this paper by promoting the M2 polarization of microglia, which leads to the change of various signalling pathways. However, it is not appropriate to place MSC, MSC-EVS and microglia in the same position in the title of this paper, so it is suggested to be modified.

The precise role of MSCs and their corresponding EVs is not yet known with regard to their therapeutic potential under stroke conditions, nor is the precise role microglia known under such settings. This fact has already been correctly stated by the reviewer in his first comment. The title that we have chosen does not imply any sort of hierarchy and tries to merge some aspects of the complex pathophysiology of stroke. Herein, we first focused on the role of microglia in inflammatory stroke, and then on the therapeutic role of MSCs and MSC-EVs in stroke. We emphasized that MSCs and MSC-EVs can inhibit the activation of microglia and change the polarization of microglia, which may be helpful in achieving the therapeutic effect. In line with this, other reviewers are in favor of the present title. Hence, we would like to stick to the title chosen.

3.) Besides, this article illustrates the mechanism of MSC-EVs regulating microglia activity involving cytokines, neurotrophic factors, transcription factors and microRNAs. However, the microRNA mentioned in this article is not reflected in the figure. It is suggested to add this part in the figure.

We have now added the microRNAs in the Figure 1 as suggested.

Reviewer #5

1.) The review is very exhaustive and precise. It clearly describes all the "partners" of the question. The title reflects the main subject and figures and tables help to follow the description.

Thank you for this positive feedback from your side.

Comments from the editor

1.) The authors did not provide original pictures. Please provide the original figure documents. Please prepare and arrange the figures using PowerPoint to ensure that all graphs or arrows or text portions can be reprocessed by the editor;

We have now prepared the figures using PowerPoint to make sure that all content is able to be reprocessed. We will upload it with this revised manuscript.

2.) PMID and DOI numbers are missing in the reference list. Please provide the PubMed numbers and DOI citation numbers to the reference list and list all authors of the references. Please revise throughout

We have revised the reference list accordingly.

3.) Please obtain permission for the use of picture (s).

We confirm that all our figures are original. All cartoon pictures paid purchase from biorender.com.