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Title: Matrine promotes neural circuit remodelling to regulate motor function in a chronic model of spinal cord injury

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COMMENTS TO AUTHORS

Strength of the study:
The study represents a continuation of previous work to evaluate the effects of matrine in acute SCI. The study is well-written with novel data and recent developments are fairly presented. The manuscript covers the topic in an objective and analytical manner. Results of this study is significant to the field of neuroregeneration, particularly in the context of chronic SCI.

Limitations of the study:

The study is weakened by the absence of a control & sham groups to assess the degree of recover by the administration of matrine (see major concerns section). In sum, this work brings a novel pharmacological approach to the treatment of chronic SCI. Considering the limited options in the actual medical management patients suffering chronic SCI, the current work presents a valid effort to advance and promote new strategies in spinal cord neuroregeneration. Therefore, the publication of this article is encouraged, upon the proper address of each concern listed above.

The study aims to investigate whether matrine (an alkaloid activator of extracellular heat shock protein 90) ameliorates chronic spinal cord injury (SCI) in mice. The authors claimed that continuous treatment with matrine at 28 days after injury was able to increase the density of neurofilaments H (NF-H marker), synapsis (bassoon marker) as well as an increase in motor neurons (choline acetyltransferase marker) in a chronic SCI mice group. The study is overall well-written and brings and interesting novel mechanisms associated to SCI. Moreover, it seems a continuation from a previous solid work on the effects of matrine in acute SCI (Tanabe et al 2018). The manuscript is sound and fairly well-written. The material and methods are clearly described. However, some concerns listed below should be addressed before the study can be considered by publication.

Major concerns:

- Based on the data presented, the authors have well demonstrated a progressive improvement in motor recovery in the SCI+matrine mice group in comparison a SCI+vehicle group. However, the study was not properly designed to evaluate how much the treated group recovered from a non-SCI condition (Control and/or Sham group). The authors are invited to explain why these experimental groups were not included in this study.
- In page 10, line 167-168 as well as in page 11, line 184 the author refers to observed long fiber like structures and extended axons in the matrine group (figure 3). However, geometry of the transversal spinal cord sections presented in figure 3A can only demonstrate increase in axonal density. May be the authors can consider rephrase these sentences or include supplementary representative longitudinal sections of both mice groups to properly show the readers these findings.

- The presence (or not) of side-effects by the administration of matrine has not been described in this study.

Minor concerns:

In the Abstract section:

- Page 1, line 11: "... whether matrine ameliorates chronic SCI mice" may be rephrased as "...whether matrine ameliorates chronic SCI in mice.".

- Page 1, line 17: the authors may correct the word "lumber" to lumbar.

In the Introduction section:

- Page 4, line 55: may be to authors can rephrase the sentence "...Although the previous our study clarified.." to "... Although our previous study clarified .."

In the Material and Methods section:

- Page 4, line 68-69: please specify the genetic background and catalog number of the mice used for these experiments.

- Page 5, line 81: the authors may correct the word "gropes" to groups.

- Page 5, line 81: please clarify the sentence "...Based on the score of the BMS and the BSS at 28 days after injury, the SCI mice were assigned to two gropes; vehicle and matrine" as in the current form the text seems confusing. Were animals under SCI randomly assigned to vehicle and matrine groups and then BMS and BSS scores collected in both groups?

- In page 7, lines 113 and123: for clarification purposes the authors may consider to properly edit the underlined sentences or just remove them.

In the Discussion section:

- Page 10-11, line 180-181: please rephrase and clarify the sentence:" Our previous study demonstrated that matrine is a new pharmacological type of drug, HSP90 chaperon activator, and ameliorated acute SCI.".

- Page 11, line 184: please clarify "Matrine extended axons in the spinal cord (Figure 3) and increased synaptic density on motor neurons innervating hindlimbs (Figure 4)..." It seems part of the sentence is
missing.

- Page 11, line 196: the authors may correct the word "lumber" to lumbar.

- Page 13, line 218: please rephrase the last sentence: "… effectiveness of matrine in chronic SCI is still limited, the incompleteness may be resolved by combination with other therapies …

In the Figure section:

- Figure 3A has a high level of background which does not allow to see the differences in axonal densities, particularly between vehicle and matrine at the caudal side (presented in the quantitative analysis of figure 3D)

- Figure 4A legends at the top looks deceiving. Might be the authors should preserve the channel color of ChAT and Bassoon at the right and the merge figure at the right. The authors may correct the label "MARGE" to MERGE. For completeness of results, the authors may include the quantifications of ChAT markers in both groups as well.