Open peer review report 2

Reviewer: Syoichi Tashiro, Keio University School of Medicine, Japan.

Comments to the authors:

Although the entire composition is nice, there are some critical points should be addressed. Particularly, I have a deep concern at Page5, L22-28.

In my opinion, results for MP group seemed not essential regarding western-blotting and immunohistochemistry of NF-H, BDNF, GFAP and CD11b, because Huangqin and Methylprednisolone are different agents and MP is not the theme of this study. Therefore, if they had just mentioned "following assessments were not performed in the MP group, because it is not necessary", it was acceptable for me. But since they have described they had assessed them all in MP group as well, it is better the result to be provided. Because those data further suggest the possibility that Huangqin and MP exert the effect on the similar mechanisms. I haven't heard MP induce BDNF up-regulation.

In this study, authors have investigated how Huangqin flavonoids extraction modify the acute event after the spinal cord injury. Huangqin is one of the most common flavonoids involved in various traditional Chinese medicines not only in China but also in many Asian countries, and also has a number of scientific evidences including anti-inflammatory effects which is considered to be chiefly brought about by Bicalein. Because Bicalein can pass-through the blood-brain-barrier, this effector has attracted attention as a candidate for acute SCI treatment. Authors have orally applied Huangqin to contusive SCI model rats and showed significant recovery in locomotor function and histological and protein expression changes in injured site. This study will proceed our understanding regarding the SCI treatment using Chinese medicine. The entire article is mostly well written, but there are some critical

points should be addressed.

Major concerns

1. Why authors compared the effect of Huangqin to that of Methylprednisolone (MP)?

2. Authors described "no significant difference was observed huangqin group and MP group on the expression of NF-H, BDNF, GFAP and CD11b" (P5 L22). However, because Huangqin and MP are different drugs, the results on NF-H, BDNF, GFAP and CD11b can be different. For example, is it aright that BDNF expression is up-regulated by MP administration? Therefore, the results of MP group should be provided regarding all of these assessments, if it is available. Then, appropriate comparison is also needed in the discussion section.

3. There are some important information missing in Material and Method section.

4. The quality of immunohistochemistry (Fig. 3 and 4) is too poor. High resolution pictures should be needed.

5. The quality of English is not good. This entire manuscript should be edited native English speaker.

Page 2

L36, How many rats are used in this study?

L36, Age of the animals should be provided.

L54, Isn't this dosage too much for humans? How much amount of Huangqin do you use in clinics? Because the densities of flavonoids are different from each country, this information should be clearly described.

Page 3

L4, Citation for your SCI model is needed.

L6, How much amount of MP applied in this study?

L7, When the Huangqin application was started?

L17, How many animals were used in BBB assessments? Please provide the numbers of animals composing regarding each assessment point.

Page 4

L17, Is this method scientifically admitted? Provide citation(s).

Page 5

L26, Please spell out IF and WB.

Page 6

L33, "In other words, flavonoids extraction could down-regulated the GFAP expression at the initial stage, and up-regulated the GFAP expression a few weeks later." Here, aren't "up-" and "down-" opposite?

Discussion

1. The reason why authors compared Huangqin to MP should be described.

2. Authors should summarize the difference between these two agents if authors consider is important.

Figure3

a. Quality should be improved

b.d. Letters on the longitudinal axis and marks for significance cannot be read.

Figure4

b. Quality should be improved

a,b,d. It's better to change the places for GFAP and CD11b, because CD11b is appeared earlier in the manuscript.

b.d. Letters on the longitudinal axis and marks for significance cannot be read.