

Dear editor;
Response to reviewers

Authors described the anti-fibrotic effect of sorafenib for epidural fibrosis after laminectomy. This work is interesting, but several issues remained unclear.

Major

1) At six weeks after sorafenib administration, EF and CD105 expression were decreased in sorafenib-treated rats than control rats. As authors mentioned, sorafenib has multiple effects for various molecular targets. Therefore the effects shown in present work remained unclear how the effects are induced. Experiments should be done at various time points (i.e. after several days, after 1 or 2 weeks, and after 6 weeks).

Answer: CD105 is a plasma membrane antigen on the cell surface and indicates microvascularization. In our study, CD105 was not found to have increased or decreased (in the case of decreasing microvascularization, CD105 is expected to relatively decrease). The anti-CD105 antibody was used to determine the degree of epidural fibrosis (EF). Our study clearly explains the effect mechanism of sorafenib. Sorafenib mainly acts through the inhibition of VEGF, and the subsequent prevention of the development of EF. This study was based on the method defined by He et al., together with the review of similar studies in the literature. The literature indicated that the average waiting time in the studies is 4-6 weeks. He et al. found that the inflammatory cell density in the group that received treatment was slightly less than the control only on the 8th day. This study complied with the generally accepted method. As the level of EF on the first 1-2 weeks of a trial will not yield meaningful results, we believe that the studies limited to this timeframe will only lead to the unnecessary use of laboratory animals.

2) The status after laminectomy, the status of surgical wound, wound healing or adverse events should be shown in detail as results.

Answer: No complication or problem is noted in the healing of the surgical wound following laminectomy.

Minor

1) *The data was evaluated by only one pathologist. Authors should mention its independency or reproducibility.*

Answer: The findings were evaluated by a single pathologist in a blinded manner. The used method is explained in detail in the 'Materials and Method' chapter. It should also be noted that the study is reproducible.

2) *Fig.1 might be deleted.*

Answer: Figure 1 shows the mechanism of the development of fibrosis and the mechanism of action of the prevention of EF by sorafenib is demonstrated. It is important regarding the comprehensibility of the study and it should not be emitted from the study.

3) *Fig 2, 3 and 4 should be included in the results section with their explanation.*

Answer: Figures 2, 3 and 4 are included in the 'Conclusion' chapter.

In this manuscript, the authors have examined the effect of sorafenib, a multi-kinase inhibitor, on epidural fibrosis caused by laminectomy. The authors have also investigated the appropriateness of using anti-CD105 and/or anti-osteopontin immunohistochemistry as an alternative method in evaluating the degree of epidural fibrosis. Using 16 male rats, the authors found that topical application of sorafenib substantially prevented the development of epidural fibrosis following laminectomy. They also found that immunohistochemistry using anti-CD105 (but not anti-osteopontin) gave results that are fairly consistent with conventional staging methods in classifying epidural fibrosis. Overall, the work appears to be interesting and the results appear to be convincing. I have several specific comments.

1) *For the immunohistochemistry data shown in Figs. 3 and 4, it would be very helpful to clearly indicate what constitutes a positive staining signal, and what exactly should be counted as a microvessel.*

Answer: These include the evaluation of the newly proliferated vasculature that is stained with CD105, the vessels that appear to be stained in the figures are evaluated as positive. The nuclei that are stained dark brown with osteopontin were accepted as positives and were evaluated and graded using the 10 hpf findings.

2) *The labels on Fig 5 should be enlarged significantly. At the present form, it is very hard to read. The tables within this figure are also very hard to read. The fonts should be enlarged to at least 10.*

Answer: Table 3 clearly demonstrates the findings; and Figure 5, which was a repetition of Table 3 was removed from the study.

3) *Why male rats were chosen? It would be ideal to have same experiments done in female rats as well.*

Answer: There is no specific reason for choosing male rats in the study; however, the majority of the similar studies in the literature have used male rats, which lead to our decision. In addition, due to the cyclical hormonal fluctuations in female rats, it might not have been possible to obtain healthy results.

The manuscript is about an exploratory investigation on the use of sorafenib for preventing spinal epidural fibrosis following laminectomy in rats. The results demonstrated that sorafenib could significantly ameliorate fibrosis score, fibroblast density, inflammatory cell infiltration, and angiogenesis. There are a number of issues regarding the writing of the manuscript:

1) *The superfluous parts:*

a) the second and third paragraphs of the Results, which merely repeat in a long-winded way what has been succinctly written in the first paragraph and clearly shown in Table 3, should be removed;

Answer: The 'Conclusion' chapter was reorganized and the second and third paragraphs were removed.

b) Figures 5A and B, which present the already clear results of Table 3 into two separate, confusing-looking bar charts, are not necessary; and

Answer: Figure 5A-B has also been removed from the study.

c) the paragraph on the use of CD105 in Discussion should be trimmed as the use of this antibody in immunohistochemistry is not novel.

Answer: The paragraphs concerning CD105 in the discussion chapter were abbreviated.

2) *It is not at all clear the whereabouts of the inflammatory cells in Figures 2A and B.*

Replacement with multiple, better labelled photomicrographs is advised.

Answer: Figures 2A and 2B were replaced with microphotographs, and were added to the end of the manuscript as new photographs.

3) *Why are the controls of Figures 3 and 4 having a darker staining background than the sorafenib-treated ones?*

Answer: The contrast difference that appears in the photo is due to the differences in staining, lighting and photographing.

4) *Reference is needed for He et al. of Table 3.*

Answer: Table 3 is the results that are obtained in the study, and there is no need to cite any work.

5) *Wenting et al. should be Ma et al.*

Answer: It was corrected as Ma et al.

6) *The English is good on the whole. There are nonetheless some typos, stylistic and grammatical errors which require more thorough editing. For example, in the sentence "Our results indicated that EF developed to various extents in all control animals, indicating that experimental EF had been successfully produced.", the "had been" should be changed to "was". There are "indicated" and "indicating" in the same sentence. Should the "indicated" be changed to "showed", for example?*

Answer: The article was revised and necessary corrections were made.

Note 1: *Our article was edited by the international editing service and the editing certificate was uploaded to the system. If our article is approved for publication, it can be edited by the editing service of your recommendation.*

Note 2: *The highlight was written and added to the end of the manuscript.*

00698952 comment:

1. Present comment: I am satisfied that the authors have removed the second and third paragraphs, Figures 5A and B and shortened the paragraph about the use of CD105 in response to my comment above.

2. Original comment #2) It is not at all clear the whereabouts of the inflammatory cells in Figures 2A and B.

Present comment: I am not satisfied with the fact that the authors have not shown the inflammatory cells in Figures 2A and B, which the authors wrote about in Materials and Methods, lines 2-3 of p. 10.

Answer: The blue arrow does not show the region with the inflammatory cells but the connective tissue in the HE stained image. The aim in presenting this image is not to show the inflammatory cells but rather to show the tissue composed of collagen. On the other hand, the inflammatory cells are shown in a higher magnification, where the number of the fibroblasts have been counted, grading the fibrosis. The expression in the material and method section, “the inflammatory cells were counted at 40X magnification“, has been changed to “the inflammatory cells were counted at 100X magnification“. This has been included in the manuscript as Figure 2A. The figures named as Figure 2A and Figure 2B in the previous text were changed to Figure 2B and Figure 2C, respectively. The quality of the remaining images were improved.

3. Original comment #3) Why are the controls of Figures 3 and 4 having a darker staining background than the sorafenib-treated ones?

Present comment: The staining background between the control and experimental samples should be similar to make sense of the comparison of the staining intensity

between samples. Obviously, it is not ideal as it is. If there are no better images to substitute those figures, I am prepared to accept them as they are, though with some reluctance. Note that the stars and black arrows are missing from Figure 3.

Answer: *In Figure 3A, 3B, and in Figure 4A, the images of dura, which were indicated with black arrows, was not visualized sufficiently. Therefore, the black arrows were removed from the image. Accordingly, the expressions associated with the black arrows were removed from the respective texts under the figures.*

4. Original comment #4) Reference is needed for He et al. of Table 3.

Present comment: I meant to refer to He et al. of Table 1. I am satisfied that the authors have since added the reference number to He et al. that was not there before.

5. Original comment #5) Wenting et al. should be Ma et al.

Present comment: I am satisfied with the correction.

6. Original comment #6) The English is good on the whole. There are nonetheless some typos, stylistic and grammatical errors which require more thorough editing. For example, in the sentence “Our results indicated that EF developed to various extents in all control animals, indicating that experimental EF had been successfully produced.”, the “had been” should be changed to “was”. There are “indicated” and “indicating” in the same sentence. Should the “indicated” be changed to “showed”, for example?

Present comment: I am satisfied with the correction. As I commented before, the English is good on the whole. I leave it to the authors to decide whether they want to improve the writing further.