Peer Review File

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Reviewer Comments

Comment 1: For all figures, the authors need to make clear that the groups were control, chemotherapy, and chemotherapy + LIPUS. Currently it is not clear what the treatment groups were and when the measurements were taken relative to treatment initiation. Figures 4 and 6 are especially confusing, since it only shows control (no treatment) and LIPUS. It's not clear whether any of these groups had chemotherapy. Figure 6 needs to indicate the level of factors in control (untreated animals) vs any chemotherapy-induced animals.

Reply 1: Thank you for your suggestion. I will answer your question here. First of all, chemotherapeutic drugs enable us to establish a model of myelosuppression, not a treatment. The SD rats used in this experiment were injected with chemotherapeutic drugs such as paclitaxel and carboplatin. Therefore, the LIPUS group is the SD rats irradiated with LIPUS after the establishment of the model, and the control group is the SD rats treated with sham irradiation (no energy output during LIPUS irradiation) after the establishment of the model. We collected samples before injection and 4th, 7th, 9th, 11th, 14th and 18th after injection. After injection of chemotherapeutic drugs for 4 consecutive days, the rats were treated with LIPUS for 7 days. Thank you for pointing out the problem in figure 4 and figure 6. It is true that our labeling is not accurate. The day 0 data in the figure are collected before the injection of chemotherapeutic drugs, that is, the data of normal rats (this set of data represents rats that have not been injected with chemotherapeutic drugs). The other two groups (control group and LIPUS group) were injected with chemotherapeutic drugs. The day 0 data in figure 6 represent the factor levels of normal rats (untreated rats).

Changes in the text: We have modified our text as advised. (see Page 4, line 99; see Page 5, line 103, 104): In order to establish a model of myelosuppression, all rats

were injected intraperitoneally according to the dose of paclitaxel (10 mg/kg, Aladdin Reagent Co., Ltd, Shanghai, China) plus carboplatin (16 mg/kg, Aladdin Reagent Co., Ltd, Shanghai, China) dissolved with physiological saline for 4 consecutive days. The rats were randomly divided into two groups: control group (injecting drugs+ no energy output from LIPUS device., n = 40) and LIPUS group (injecting drugs+LIPUS, n = 40).

Comment 2: It is not clear whether the images in Fig 5 were representative or how the data from these images could be quantified or evaluated. Some measurement should accompany these images.

Reply 2: Thank you for your suggestion. We used H&E staining results and electron microscopy to observe the effect of LIPUS on bone marrow. Quantitative analysis was mainly conducted by HE staining, while electron microscopy was mainly used for qualitative analysis to observe bone marrow tissue aggregation.

Changes in the text: We added some sentences according to your suggestion. (see Page 10, line 228): The results were analyzed by H&E staining and scanning electron microscopy. In normal rats, bone marrow tissue was gathered to form a colony (Fig. 5a). However, after drug administration, the adherent cells are significantly reduced and less gathered (Fig. 5b). On the 11th day, the cells began to re-form new small colonies, but the number of attached cells did not increase (Fig. 5c). In contrast, on the 11th day, cells in the LIPUS group were abundant and large colonies were formed (Fig. 5d). It showed that LIPUS promoted the adhesion of bone marrow cells and helped the bone marrow cells to form colonies.

Comment 3: The authors should use "euthanized" instead of "sacreficed".

Reply 3: Thank you for your advice. We have corrected it in the manuscript.

Changes in the text: It has been done as required. (see Page 6, line 140): To investigate the change of bone marrow, the rats (n = 6) were euthanized on the 0th, 4th, and 11th day, the right hind limb hair was shaved and the skin was disinfected.

Comment 4: The "0th" day is not standard English; please use "day 0".

Reply 4: Thank you for your reminding. We have modified this part to the manuscript.

Changes in the text: We have modified our text as advised. (see the full text): we have changed "0th" day to "day 0".

Comment 5: The method for scoring the bone marrow should be stated more clearly.

Reply 5: Thank you for your advice.

Changes in the text: We have modified our text as advised. (see Page 6,7, line 144-152):After dehydration, decalcification, embedding, sectioning and staining, the morphology of hematopoietic tissue was surveyed under optical microscope (BX51, Olympus, Germany) with the same magnification and 10 visual fields were observed for each tissue specimeneach tissue specimen was observed with the same 10 visual fields. Bone marrow tissue sections (10 images per sample) were quantitatively analyzed by Image J (IBM, Armonk, NY, USA) software, and the proportion of hematopoietic tissue in the above samples was calculated.

Comment 6: The writing regarding the effect of LIPUS on SCF is unclear; first it states that there was a difference, then it states that there was not. The authors appear to be trying to describe the differences between the chemotherapy, chemotherapy + LIPUS and control groups. This section needs to be rewritten.

Reply 6: Thank you for your advice. First of all, as explained in comment one, we don't have a chemotherapy group, and chemotherapy is our modeling method. In

addition, we make a comparison in terms of time within the group and then between groups, so there is no contradiction.

Changes in the text: We have modified our text as advised. (see Page 8, line 189-196): On the 11th day after chemotherapy, the level of SCF in LIPUS group and control group was significantly higher than that on the 4th day respectively. ($P < 0.05$ = .However, on the 11th day, there was no significant difference in SCF between the LIPUS group and control group($P > 0.05$, Fig.2).