Dear Editor and the reviewer,

Thank you very so much for comments and suggestions for our manuscript, and we believe your valuable advice has greatly improved our manuscript. We have examined comments carefully and have made corrections. The main corrections in this paper and the response to the editor and reviewer's comments are as follows;

Response to the comments made by a reviewer .（Reviewer'scode:00505382）

1. considering late deaths (e.g. one death after 7h in the control group), stretching the follow up from 8 to 12 or 24 hours somehow modify the results?

- Thank you for your comment. As you mentioned, it was relatively short time to evaluate a survival rate in this study. In our previous study, beta antagonists worsen the recovery from severe hemorrhagic shock within 5hours. So, we examined it in more than five hours in this study. Examination in the long time may be a future research theme more.

2. pH and HR figures would benefit from an adjustment of Y-axis scale

- Thank you for your comment. As you mentioned, we adjust Y-axis scale and changed pH and HR figure. We corrected HR figure of Fig.2 (page22) and PH figure of Fig.3(page24).

3. TNF-alfa figure is difficult to interpretate because of the overlap of error bars, and could be rearranged

- Thank you for your comment. As you mentioned, it is difficult for interpretation, so we corrected TNFα figure of Fig4 (page26).

Response to the comments made by a reviewer . (Reviewer'scode:02488399)

1. Excellent research with interesting study design, and results.

-Thank you for your review and comment. We really appreciate your high evaluation of our study.

Response to the comments made by a reviewer .（Reviewer'scode:00506276）

Major comments:

1. MR antagonists were administered in food. When food was withdrawn before the experiments? If the experiments were performed during the light period, is it possible that the activity of MR antagonists expired before exsanguination which could explain the negative results?

- Thank you for your comment. As you mentioned, it is important that we consider about the activity of MR antagonists. In this study, rats were fed until just before experiment. Rats lose blood within two hours after the meal end.

2. It should be specified how much blood was removed from the animal during the experiment.

-Thank you for your comment. As you mentioned, it is important that we describe removal blood volume. We appended this in the section of experimental protocol at page 6 line 9/10 as follows.

"Removal blood volume were 13±0.4ml, 13±0.5ml, and 13±0.4ml in the control group, SPL group, and EP group, respectively."

3. Page 7: there is the discrepancy regarding time of blood collection for TNF measurement between line 4 and lines 5/6.

-Thank you for your comment. As you mentioned, it is important that we describe in detail. We corrected this in the section of experimental protocol at page 6 line 16/21 as follows.

"And then PH, Lactate, BE and Hb were measured immediately by The ABL800 FLEX blood gas analyzer. Furthermore, arterial blood samples (1.5 mL) were obtained before HS and at 2, 4, and 5 h after HS recovery to measure plasma tumor necrosis factor (TNF)-α. The TNF-α concentrations were measured using enzyme-linked immunosorbent assay (ELISA) kits (Boster Biological Technology, Pleasanton, CA, USA)."

4. Results section (page 8): it should be clearly described how blood pressure changed in eplerenone-treated animals vs. other groups.

-Thank you for your comment. As you mentioned, it is important that we describe about change of SAPs. We appended this in the section of result at page 9 line 6/9 as follows.

"SAPs of the control group decreased more in comparison with SAPs of the MR

antagonists treatment group. SAPs of the EP treatment group did not decrease very much in comparison with the SAP of the control group."

5. The method of lactate measurement should be described.

-Thank you for your comment. As you mentioned, we corrected this in the section of experimental protocol at page 6 line 16/21 as follows.

"And then PH, Lactate, BE and Hb were measured immediately by The ABL800 FLEX blood gas analyzer. Furthermore, arterial blood samples (1.5 mL) were obtained before HS and at 2, 4, and 5 h after HS recovery to measure plasma tumor necrosis factor (TNF)-α. The TNF-α concentrations were measured using enzyme-linked immunosorbent assay (ELISA) kits (Boster Biological Technology, Pleasanton, CA, USA)."

6. Why IL-1beta, IL-6 and ICAM-1 concentration were not measured in the blood? mRNA expression in the liver may not be representative for these inflammation markers. For example, IL-1beta level may be more dependent on inflammasome processing of its precursor rather than on mRNA expression.

-Thank you for very valuable comment. As you mentioned, IL-1beta, IL-6 and ICAM-1 concentration in the blood may be important. We need more removal blood to measure serum cytokine during the follow-up after the hemorrhagic shock. So we measured only the TNF α concentration. However the TNF α density was lower than it of a past report in this study. Therefore, we evaluated cytokine production in the liver at an early stage in addition.

7. That eplerenone improved blood pressure should be discussed. In addition, it should be discussed why EPL but not SPL had this effect on blood pressure.

-Thank you for very valuable comment. As you mentioned, it is important that we discuss about the reason why blood pressure was maintained in rats of EP group. So, we appended this in the section of discussion at page12 line 7/18 and research perspectives at page 15 line 8/11 as follows.

"In this study, SAPs of the EP treatment group did not decrease in comparison with the SAP of the control group, however it did not affect the survival rate. In a previous study, Kajihara et al. showed that blood aldosterone and cortisol levels were rapidly increased after hemorrhage in dog. After recovery of hemorrhagic shock, arterial blood pressure and blood cortisol decreased, however the blood aldosterone level remained relatively

high. Gregory et al. showed that serum corticosterone was stimulated in hemorrhagic shock model of rats. The blood corticosterone and aldosterone in rat can be related to the blood pressure change after the hemorrhagic shock. However, we did not evaluate the change of the blood pressure and the relations of blood aldosterone and corticosterone, in this study. The difference between EP and SPL is selectivity to MR. So further studies are needed to estimate blood corticosterone and aldosterone in hemorrhagic shock by using rats which MR antagonists were administered" in the section of discussion.

"The present study, SAPs of the EP treatment group did not decrease in comparison with the SAP of the control group, So further studies are needed to evaluate relations of blood corticosterone or aldosterone and blood pressure in hemorrhagic shock by using rats which MR antagonists were administered" in the section of research perspectives.

Minor comments:

1.Page 3 Abstract/Conclusions: there is no evidence of anti-inflammatory effects of MR antagonists in this study.

-Thank you for your comment. As you mentioned, there is no evidence of anti-inflammatory effects of MR antagonists in this study. So, we corrected this in the section of Abstract/ CONCLUSION at page 3 as follows.

"Pretreatment with MR antagonists did not improve mortality or cytokine responses in the liver after HS recovery in rats"

2. The abbreviation "BGA" should be explained when used the first time.

-Thank you for your comment. As you mentioned, the abbreviation "BGA" should be explained. So, we corrected this in the section of MATERIALS AND METHODS /Statistical Analysis at page 8 line 20 from "BGA" to "blood gas analysis (BGA)".

Other changes:

1.We appended zip code of each institution at page1.

2. As to the "animal care use statement", we corrected sentence at page2.

3.We rewrited section of article highlights at page 13/15.

4.We appended reference No 29 and No 30 at page 20.