Author’s response to reviews

Title: Human Aldose reductase inhibitory activity of selected Kampo formulations

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Author’s response to reviews: see over
Dear Editor:

We thank for the constructive comments and suggestions from the reviewers on our paper entitled “Aldose reductase inhibitory activity of selected Kampo formulations” (MS: 2002719569128019). According to these comments, we revised and improved the manuscript. We hope that these changes will be satisfactory.

With best regards,

Yours sincerely,

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Reviewer #1: The paper described the aldose reductase inhibitory activity of some selected Kampo formulations, which are now clinical used for the treatment of diabetes complications.

It is quite interesting that all these Kampo formulations showed AR inhibitory activity, since AR has been paid much attention as an effective molecular target for the treatment of diabetes complications.

1) In the paper, the description of the comparison with epalrestat, is a little hard to understand. I suggest if the author should give a Potency Index (daily dose/IC_{50}) in Table 1.

**Answer:** We thank the suggestion. According to this valuable comment, we presented the inhibitory activity by IC_{50} also in µUnit/mL for comparison of their potency on an actual dose basis.

2) As an associated issue, even Chotosan showed the most potent activity among the tested samples, it seems weak than epalrestat by 30 folds. The authors should discuss how to consider the clinical usage of Chotosan by the viewpoint of AR inhibition.

**Answer:** We thank the reviewer for the constructive suggestion. Since Chotosan showed weaker hAR inhibitory activity than epalrestat, it is difficult be prescribed independently only by the viewpoint of AR inhibition. However, taking the advantage of lower side effect and multi-function property of Kampo formulation, a combination therapy maybe favorable for their clinical applications. We add this consideration in the revised manuscript.

3) A minor point: the authors should point out they have used human aldose reductase in this study. Abbreviation of hAR should be better than AR.

**Answer:** Revised according to the comment.

Reviewer #2:

1) As authors describe in Results&Discussion (ref. 23), similar study on inhibitory effects of Kampo formulations on aldose reductase have been reported. Authors should
cite the previous reports in the introduction. And then differences from the previous
studies should be described.

**Answer:** We thank the suggestion. According to this valuable comment, we cite the
literature in the introduction section (new Ref No. 19). And the differences between the
present study and the previous studies were compared and described in the section of
result and discussion.

2) Conversion to doses: It is interesting. But, because bioavailability of Kampo
formulation is generally unclear, the reviewer think that the calculation of 'the amount
of AR inhibition in single dose in µM' might be unreasonable.
For example: polyphenolic constituents can inhibit the aldose reductase, but their
absorption ratio from the intestinal tracts are usually low.

**Answer:** We thank the comments from the reviewer. As also pointed out by reviewer 1,
conversion to doses was confused for comparison. We improved this point by expressing
the IC\textsubscript{50} using µUnit/mL. Also, we totally agree with the reviewer that bioavailability
must be taken into consideration for the clinical application of Kampo formulations.

3) In this manuscript, discussion is depended on enzyme inhibition by extracts only.
Authors should examine the effects of Kampo formulations using cells, tissues or
experimental animals as possible as, or find new active constituents.
Then this manuscript can be more interesting.

**Answer:** We thank for the critical comments from the reviewer. As pointed out, further
detailed biological and chemical investigation is required for better understanding the
action of Kampo formulations used in this study. Actually, an investigation
incorporating this point is in progress now, and will present elsewhere.