Author's response to reviews

Title: Magnesium supplementation and high volume hydration reduce the renal toxicity caused by cisplatin-based chemotherapy in lung cancer patients; a toxicity study.

Authors:

Takako Oka (takako_mix@yahoo.co.jp)
Tatsuo Kimura (kimutats@med.osaka-cu.ac.jp)
Tomohiro Suzumura (tsuzumura@msic.med.osaka-cu.ac.jp)
Naoki Yoshimoto (yoshimoto_fuucyan@yahoo.co.jp)
Toshiyuki Nakai (shintokumaru1984@mail.goo.ne.jp)
Norio Yamamoto (norisuke-1020@softbank.ne.jp)
Kuniomi Matsuura (kuniomi@med.osaka-cu.ac.jp)
Shigeki Mitsuoka (micchan@msic.med.osaka-cu.ac.jp)
Naruo Yoshimura (y-naruo@sc4.so-net.ne.jp)
Shinzoh Kudoh (shinzohykudoh@med.osaka-cu.ac.jp)
Kazuto Hirata (kazutoh@msic.med.osaka-cu.ac.jp)

Version: 9 Date: 4 October 2014

Author's response to reviews: see over
Dear Sir,

MS: 5234360361279637

Thank you for the second review of our manuscript for publication in *BMC Pharmacology and Toxicology*. We are pleased to re-submit the revised version of our manuscript entitled “Magnesium supplementation and high volume hydration reduce the renal toxicity caused by cisplatin-based chemotherapy in patients with lung cancer”. We thank all the reviewers for their comments and suggestions that have clearly strengthened the paper.

In the attached pages, we address the issues raised by the reviewers and indicate where changes have been made to the manuscript. This article was re-checked by native English speaker and corrected the grammatical errors. We also attached the certification of English proofreading at the end of this letter.

We believe we have sufficiently addressed all substantive critiques and look forward to your acceptance of these changes. All authors have read and approved the manuscript. There is no conflict of interest in this study. Please let us know if you have any additional suggestions or concerns.

Yours sincerely.
Tatsuo Kimura M.D.
kimutats@med.osaka-cu.ac.jp
Department of Respiratory Medicine,
Graduate School of Medicine,
Osaka City University,
1-4-3, Asahi-machi, Abenoku Osaka
545-8585, Japan
phone number : 81-6-6645-3916
Fax number : 81-6-6646-3917
Reviewer’s report:
2. Are the methods appropriate and well described?
The authors state that the subjects agreed to the cisplatin therapy – did they agree to the various magnesium/hydration protocols? Can the authors confirm that the subjects consented to be treated with the magnesium/hydration protocols? Were these Mg/hydration regimens approved by the IRB?

Reply:
Thank you for your indication.
The Mg/hydration protocols were approved by the Institutional Review Board for Chemotherapy of Osaka City University Hospital. The chemotherapy procedure was explained and informed consent was obtained from all patients. Since this was a historical prospective cohort study, the difference from the previous Mg- protocol was explained to patients in high volume Mg+ group, and the patients in low volume Mg+ group were informed about the difference from the previous high volume Mg+ protocol.

I described this information in Treatments section and moved the sentence ‘The chemotherapy procedure was explained and informed consent was obtained from the patients’ from Patients section to Treatments section. (Page 8, line 129-133)

3. Are the data sound?
The data presented are sound but very limited by assessing only after the first round of cisplatin chemotherapy. The major problem with cisplatin is that the damage is cumulative with every cycle and few patients would ever only receive a single treatment. This is a major limitation of the study. Because this data should be available in the subjects’ charts – it does not seem reasonable to include it. The authors state that ‘our results predict the change of sCr and CrCl in the following cycles – why not be sure of this instead of guessing?
Reply:

I agree with Referee 3’s comment.

Knowledge of sCr in the following cycles would be important. We did, however, analyze the number of chemotherapy cycles and major reasons for treatment discontinuation (Supplementary Table 1).

**Supplementary table 1.**

<table>
<thead>
<tr>
<th>Cisplatin chemotherapy administered</th>
<th>the number of chemotherapy cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>high-volume hydration Mg-</td>
<td>41</td>
</tr>
<tr>
<td>high-volume hydration Mg+</td>
<td>27</td>
</tr>
<tr>
<td>low-volume hydration Mg+</td>
<td>17</td>
</tr>
</tbody>
</table>

In the high-volume Mg- group, the most common event leading to discontinuation was renal toxicity (n = 9, [22%]), followed by generalized weakness (n = 5, [12%]). On the other hand, in the high- and low-volume Mg+ groups, it was seen that none and 1 patient, respectively, discontinued cisplatin treatment due to renal toxicity. The reasons for discontinuation in these groups were other adverse reactions, switching to another regimen, and cancer progression.

We also analyzed the sCr of each group at the end of subsequent cycles (Supplementary Table 2).

**Supplementary table 2.**

<table>
<thead>
<tr>
<th>The comparison between pre and end of sCr in each group.</th>
<th>pre sCr (mg/dl)</th>
<th>end of sCr (mg/dl)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>high-volume hydration Mg-</td>
<td>0.75</td>
<td>1.05 (0.64 to 0.92)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>high-volume hydration Mg+</td>
<td>0.71 (0.58 to 0.84)</td>
<td>0.75 (0.66 to 0.86)</td>
<td>0.08</td>
</tr>
<tr>
<td>low-volume hydration Mg+</td>
<td>0.72 (0.68 to 0.78)</td>
<td>0.81 (0.72 to 0.89)</td>
<td>0.01</td>
</tr>
</tbody>
</table>

The median values of sCr at the end of therapy in the high-volume Mg- and low-volume Mg+ groups were significantly increased compared with pre-sCr using the paired t-test and Wilcoxon matched-pairs signed-rank test (p < 0.001, and p = 0.01, respectively). Moreover, in the high-volume Mg- group, the median value of sCr at the last cycle was significantly higher than after the first cycle (p = 0.004) (data not shown). Thus, Mg supplementation with high-volume hydration may have a much greater impact on renal toxicity caused by cisplatin.
5. Are the discussion and conclusions well balanced and adequately supported by the data?

See number 3. Also, with respect to assessing Mg status – the authors indicate that they did not include the subjects’ serum status because serum Mg is only 1% of the body’s total Mg – however, this is used clinically to determine which patients should receive Mg supplementation during cisplatin treatment. Despite its poor correlation, serum Mg reflects Mg status due to acute changes and is clinically relevant. Therefore, this information should be included in the manuscript because it should be revealed whether serum Mg levels could be used as a guide for providing Mg treatments.

**Reply:**

I agree with Referee 3’s comment that “serum Mg reflects Mg status due to acute changes”.

It is known that serum hypomagnesemia reflects Mg depletion, but serum Mg is only 1% of the total Mg present in the body [23]. In some cases, levels of serum Mg may not be decreased even in the presence of Mg depletion. Cisplatin treatment is one such case, and Mg supplementation is needed even in the absence of serum hypomagnesemia.

I described this information in Discussion section. (Page 13, line 233-236)

I changed the word ‘hypomagnesemia’ to ‘depletion of Mg’ or ‘Mg depletion’. (Page 3, line 36, Page 5, line 78, Page 11, line 198, Page 11, line 203, Page 12, line 221, Page 13, line 228, and Page 14, line 257)

8. Do the title and abstract accurately convey what has been found?

The abstract and methods section should remove the term: ‘chest tumors’ and used the term lung cancer (to be consistent with the title, background, discussion and patient population.

**Reply:**
I agree with Referee 3’s comment.
I changed the term ‘chest tumors’ to the term ‘lung cancer’. (Page 3, line 38 and Page 6, line 103)

9. Is the writing acceptable?
No, the manuscript requires significant re-writing (mostly grammar/sentence structure) to improve readability. For example: “Willox et al revealed THAT 16mEq magnesium (Mg) supplementation WAS beneficial.” “It is concerned that low Mg inhibit tumor cell proliferation and neoangiogenesis. Therefore, Mg supplementation for protecting nephrotoxicity has possibility to promote tumor cell proliferation.” – these are just a couple of examples where grammar and sentence structure hinder readability of the manuscript.

Reply:
Thank you very much for bringing it to my attention.
This manuscript and title were edited and corrected by an experienced proofreader who was a native speaker of English, again. These changes were indicated by red letter in the revised version. I also corrected the mistakes that was pointed out in question 9. (Page 5, line 78-79 and Page 13, line 225-226)
CERTIFICATE OF ENGLISH PROOFREADING

Date: October 3, 2014

Honyaku Center Inc. certifies that the manuscript entitled “Magnesium supplementation and high volume hydration reduce the renal toxicity caused by cisplatin-based chemotherapy in patients with lung cancer patients: a toxicity study” has been edited and corrected to the highest standards.

Neither the contents of this manuscript nor the author’s intentions have been altered in any way. This manuscript has been edited and corrected by an experienced proofreader who is a native speaker of English and who is under the direct supervision of Honyaku Center Inc.

Youhei Wada
Deputy General Manager
Osaka Sales Division 2