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.

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Author's response to reviews: see over
Editors of BMC Pharmacology and Toxicology
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Dear Sir,

MS: 5234360361279637

Thank you for the timely review of our manuscript for publication in BMC Pharmacology and Toxicology. We are pleased to submit the revised version of our manuscript entitled “Magnesium supplementation and high volume hydration reduce the renal toxicity caused by cisplatin-based chemotherapy in lung cancer patients”. 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. 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. This article was checked by native English speaker and corrected the grammatical errors. There is no conflict of interest in this study. Please let us know if you have any additional suggestions or concerns.

Yours sincerely,

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Referee: 1

Major Compulsory Revisions

1. A term of historical prospective cohort study is difficult to understand. This study seems to be a retrospective study.

   Reply:
   Thank you very much for bringing it to my attention. Generally, “A retrospective study” means a case-control study. This study is not a case-control study, but a retrospective cohort study. Retrospective cohort study is also called historical prospective cohort study cited from NCI Dictionary of Cancer Terms (http://www.cancer.gov/).

   In the case of a retrospective cohort study, the investigator collects data from past records and does not follow patients up as is the case with a prospective study. However, the starting point of this study is the same as for all cohort studies. The first objective is still to establish two groups - exposed versus non-exposed; and these groups are followed up prospectively in the ensuing time period. "

   In our study, the starting point was December 2011, and the first objective is to establish three group and these group were followed up in the period of the first line chemotherapy. Therefore, I adopted a historical prospective cohort study as the name of our retrospective cohort study.

2. Page 7, line 124. The authors mention that “these regimens were … conducted in accordance with the Japanese Good Clinical Practice guidelines”.
   Wasn’t cisplatin-based chemotherapy conducted as a clinical practice?

   Reply:
   I agree with Referee 1’s comment.

   Yes, I conducted this study as a clinical practice. I deleted the statement that “and conducted in accordance with the Japanese Good Clinical Practice guide lines”. (old version Page 7, line 126)

3. Please describe a frequency of blood chemistry test.
Reply:
Thank you very much for pointing that out.
We conducted blood chemistry test twice a week in the first cycle of the chemotherapy.
I described this in page 8, line 131 and line 138.

4. Table 3. Frequency of grade 2 to 3 serum creatinine elevation in the high-volume hydration Mg- group was approximately 46%. This frequency is amazingly high, compared with historical data. Is this data correct?

Reply:
I agree with Referee 1’s comment. However this data is correct. We conducted this study as a clinical practice, different from clinical trials. Patient characteristics in our study might be different from those in clinical trials. Most clinical trials were conducted blood chemistry tests once a week. (Furuse et al: J Clin Oncol 1999, 17:2692-2699, Noda et al: N Engl J Med 2002, 346:85-91) We conducted blood chemistry tests twice a week. It is thought that the maximum serum creatinine elevation was caused at an early time point after the dosage, and decreased in a few days. In our study, serum creatinine levels were decreased in a few days from the peak (data not shown). We might catch the detail analysis of serum creatinine progress.

Minor Essential Revisions
1. Page 11, line 203. “Second, In our study” should be changed as “Second, in our study”.

Reply:
Thank you very much for bringing it to my attention.
I changed the word “In” to “in”. (Page 13, line 233)
Referee: 2

Major Compulsory Revisions

1) Page 9, line 165-: Authors argued that "a trend towards a decrease in the post-Ccr compared to the pre-Ccr." However I wonder whether the difference of median value between pre- and post Cr and Ccr was clinically meaningful (Table 4). Furthermore, authors should be more cautious to conclude that "high-volume hydration and Mg" is better than "low-volume hydration with Mg" based on ONLY 17 patients of low volume hydration.

Reply:
Thank you very much for bringing it to my attention.
However, I think that the difference of median values between pre- and post sCr and CrCl were clinically meaningful, because, cisplatin deteriorated the renal function, only in the first cycle of chemotherapy. Cisplatin results in cumulative nephrotoxicity. There is a high possibility that post-sCr may decrease after 2nd or later cycles of chemotherapy. We may prevent the CDDP related nephrotoxicity after 2nd cycles of chemotherapy.
I agree with Referee 2’s comment about conclusion, however, I did not mention that "high-volume hydration Mg+" is better than "low-volume hydration Mg+" clearly. We concluded that low-volume hydration Mg+ regimen can be considered for patients with adequate renal function.

2) Authors did not present and include adverse events other than the renal toxicity. The gastrointestinal toxicity is closely related to oral fluid intake and should be reported and included in multivariate analysis as an explanatory variable.

Reply:
I agree with Referee 2’s comment.
Thank you very much for giving us very useful information.
We re-collected the grade 2 or more anorexia data, and tried to investigate the contribution of grade 2 or more anorexia to ΔCrCl in univariate and multivariate analyses. Grade 2 or more
anorexia was not a predictor of $\Delta$CrCl (p=0.531 and p=0.562, respectively).

I would suggest that antiemetic agents were changed between three historical groups. The low-volume Mg+ group had palonosetron and fosaaprepitant meglumine described in table 1. Therefore, gastrointestinal toxicity in this group may be milder than that in other two groups.

I described this analysis in Patients and Methods section. (Page 8, line 135,136 and Page 9, line 150)

I also added this data in table 3 and 5.

3) Page 6, line 108 and Page 7, line 124: Authors reported "The chemotherapy procedure was explained and informed consent was obtained from all the patients." and "These regimes were approved by the Institutional Review Board for Chemotherapy of Osaka City University Hospital, and conducted in accordance with the Japanese Good Clinical Practice guidelines." However, authors did not declare whether there was an approval of institutional review board to conduct this exploratory analysis using patients' clinical data.

Reply:
Thank you very much for bringing it to my attention.
We mentioned this answer in the referee 1, No. 2 question.
Referee: 3

Major suggested revisions:
1. These data are consistent with the postulate that cisplatin leads to hypomagnesemia which promotes renal injury and support previous findings that Mg supplementation is useful for protecting against renal injury in humans (Willox et al, 1986; Bodnar et al, 2008; Muraki et al, 2012; Hirai et al, 2013; and Yoshida et al, 2014) and in rodents (Solanki et al, 2014). References should include the most recent paper by Yoshida et al in 2014 because this was a large retrospective study of 496 thoracic cancer patients (treated with cisplatin +/- Mg) over 3 years. The authors should compare their dose of Mg (8mEq) with those used in these previous human studies?

Reply:
Thank you very much for giving us very useful information.
I agree with Referee 3’s comment.
Willcox et al. used 16 mEq of Mg sulphate, Bodnar et al. 40 mEq, Muraki et al. 8 mEq, Hirai et al. 20 mEq, and Yoshida et al. 8 mEq. We referred these reports and decided to infuse 8 mEq of Mg sulphate.
I described this information in Background section. (Page 5, line 80-87)
I add the references, the number was 11-13. (Page 16)

2. Please clarify – lung cancer patients (as described in abstract) vs. patients with malignant chest tumors (as described in the methods).

Reply:
I agree with Referee 3’s comment. I took a mistake.
I changed the words “lung cancer patients” to “patients with malignant chest tumors”. (Page 3, line 38)

3. Please clarify ‘electrolyte-containing solution’ line 114

Reply:
We had a simple mistake of the word “solution” to “solutions”. (Page 7, line 120, 121 and 125)

“Electrolyte-containing solutions” include normal saline and one quarter saline solution Soldem 3A (Terumo, Tokyo, Japan) with 35 mEq/l of sodium ion.

I discribed the name in page 7, line 121.

4. Please clarify that serum (vs. urine or plasma) creatinine was measured in the methods section.

Reply:
I agree with Referee 3’s comment.
I added the word “serum” in front of “hemoglobin”, “albumin” and “creatinine”. (Page 7, line 112)
I changed abbreviations for creatinine and creatinine clearance to “sCr” and “CrCl” in our manuscript as the question number 2 of Minor essential revision listed below.

5. Why limit this study to the first round of cisplatin – was the Mg or hydration treatment restricted to the first round of cisplatin only. Cisplatin results in cumulative nephrotoxicity (and that is what ultimately matters for the patient); therefore, one might expect better results in later cycles. Justification should be provided.

Reply:
I agree with Referee 3’s comment.
However, up to later cycles, the various conditions of the patients may affect the renal function, including dose reduction of cisplatin, cancer progression, other adverse reactions and decrease of performance status. We thought that the difference of renal function in the first course was easy to reflect pure influence only for cisplatin. Moreover, our results may predict the change of sCr and CrCl in the following cycles. I hope to investigate the cumulative nephrotoxicity after several courses in the next time.

6. Please define what is meant by Mg-based medications (do you mean Mg-containing medications or medications associated with Mg depletion)-provide
examples.

**Reply:**
Thank you very much for bringing it to my attention.

I mean “Mg-based medication” as Mg-containing medications for purgative.

I changed it “Mg-containing medications for purgative”. (Page 7, line 114 and Page 9, line 150)

I also changed this in table 2 and 5.

7. The % of males in each group are not similar (hi vol+Mg=76%; high vol-Mg=67% and low volume+Mg=94%) – females are proposed to be more sensitive to cisplatin-induced nephrotoxicity. Has this been considered? The authors should address this.

**Reply:**
Thank you very much for bringing it to my attention.

I agree with your comment that in general, females are more sensitive to cisplatin-induced nephrotoxicity. However, in your comment, there are mistakes of % of males in each group. In correct, high volume+Mg=67%; high volume-Mg=76% and low volume+Mg=94%.

The high volume+Mg group had higher rate of female and lower rate of nephrotoxicity than the high volume-Mg group. Mg supplementation may have a much greater impact on the nephrotoxicity than sex. Besides, we investigated that sex didn’t contribute to the $\Delta$CrCl in the univariate and multivariate analyses (p=0.182 and p=0.127, respectively).

I added this data in table 5.

8. The authors should discuss how they decided on the low and high volume protocols. Were there differences in osmolarity in these two protocols?

**Reply:**
I agree with Referee 3’s comment.

Thank you very much for bringing it to my attention.

We decided these protocols based on previous reports. Recent prospective studies reported
the feasibility of low-volume hydration with Mg supplementation. The volume of hydrations were 1,500-2500 ml in their studies.

I described this information in Discussion section. (Page 12, line 209-211)

Osmolarity is an estimation of the osmol concentration of plasma and is proportional to the number of particles per litre of solution; it is expressed as mmol/l. It is derived from the measured Na+, K+, urea and glucose concentrations. However, the term osmolarity has largely been superseded by osmolality, therefore, we use Osmolality for the rest of this article.

Two factors are affecting cisplatin induced renal toxicity; it was Mg and hydration volume. The hydration volume was related to osmolality, and serum and urinary concentration of cisplatin. The cisplatin nephrotoxic injury has been implemented in dose-dependent and cumulative cisplatin levels. In renal proximal tubule cells, the accumulation of cisplatin from the basolateral side was significantly greater than that from the apical side. High-volume hydration reduces the concentration of cisplatin level compared to low-volume hydration. It is suggested that the low plasma cisplatin level reduces renal accumulation of cisplatin and decreases nephrotoxicity. Besides, high volume hydration decrease osmolality in blood and this will result in decrease secretion of antidiuretic hormone, decrease water reabsorption, less concentrated urine, and increase the output of urine. It is suggested that the low urinary cisplatin level reduces renal accumulation of cisplatin and decreases nephrotoxicity. Therefore, the differences in osmolality in these two protocols may be related to nephrotoxicity.

I described this information in Discussion section. (Page 12, line 214-221)

9. One important consideration the authors should address is whether Mg supplementation will also protect the tumors from cisplatin-mediated killing – this could be discussed in the discussion section. Hopefully, the authors are considering long-term studies that would be able to answer the question concerning the effects of Mg on guarding against cisplatin-induced tumor killing.

Reply:
I agree with Referee 3’s comment.
Thank you very much for bringing it to my attention.

Laboratory and clinical findings of hypomagnesemia have been observed in patients treated with high-dose cisplatin.

In low magnesium conditions, cell cycle inhibitory proteins such as p21 and p27 were upregulated, while cell cycle promotion proteins such as cyclins D and E were down-modulated [21]. It is also suggested that there is a peculiar correlation between magnesium availability and vascular endothelial growth factor [22]. It is concerned that low magnesium inhibit tumor cell proliferation and neoangiogenesis. Therefore, Mg supplementation for protecting nephrotoxicity has possibility to promote tumor cell proliferation.

However, the previous prospective clinical trials and retrospective studies also indicated that Mg supplementation did not affect tumor response of cisplatin-based chemotherapy [7, 10, 11]. The association between hypomagnesemia and tumor response of chemotherapy should be investigated in detail in the future.

We added our opinions in Discussion section (Page 12-13, line 222-231) and Conclusion section (Page 14, line 246).

**Minor essential revisions:**

1. Why did the authors choose a historical prospective design rather than a randomized prospective design (if they knew they would be testing three treatment strategies)? Please clarify that this wasn’t a retrospective study. Did the subjects agree to participate in research testing the effects of hydration volume and Mg on kidney injury induced by cisplatin or were they consented to receive cisplatin for the treatment of their lung cancer (according to the current regimen used by the hospital)?

   **Reply:**
   Thank you very much for bringing it to my attention.

   I mentioned this answer in the referee 1, No. 2 question.

   We could not conduct a randomized prospective study, because it became clear that Mg supplementation reduce renal toxicity induced by cisplatin.

   Patients consented to receive cisplatin-based therapy for treatment of their lung cancer according to the current regimen used in the hospital.
2. Standard abbreviations for creatinine and creatinine clearance should be considered (e.g. sCr or Cr for creatinine and CrCl or ClCr for creatinine clearance.

Reply:
I agree with Referee 3’s comment.
I changed abbreviations for creatinine and creatinine clearance to “sCr” and “CrCl” in our manuscript.

3. Is the data shown in Figure 1 the same as the data shown in Table 4 – if they are the same, why show both sets of data?

Reply:
Thank you very much for bringing it to my attention.
I had a mistake of the title of figure 1. Figure 1 shows the comparisons of $\Delta sCr$ (A) and $\Delta CrCl$ (B) between treatment groups. Table 4 shows the comparison pre- to post- sCr, and CrCl in each group.
I changed the title of figure 1 in Results section (Page 10, Line 176) and Figure legends section (Page 17, line 336).

4. Why not combine Table 4 into Table 2 – simply add pre and post Cr values (above and below each other) to keep the data together in a single table?

Reply:
I do not agree with Referee 3’s comment.
To combine Table 4 into Table 2 is very confusing. I think the tables which separate two parts are easy to look.

5. Out of curiosity – why wasn’t serum Mg data available for patients…isn’t that part of standard of care for cisplatin patients?

Reply:
Thank you very much for bringing it to my attention.

Only 1% of total body Mg is in the extracellular fluid. Therefore, serum Mg level may not reflect Mg depletion exactly [23].

I added this information in Discussion section (Page 13, line 237-238), and in Conclusion section (Page 14, line 245).

6. Several grammatical and spelling errors should be corrected (e.g. abstract should be re-written to improve clarity; line 72 nephrotoxic damage; line 75 shortening; lines 168 and 171 higher (instead of increased); line 203 Second in our study; and throughout manuscript the use of respectively requires an ‘and’ between the p values (e.g. p<0.05, p<0.01, respectively – should be p<0.05 and p<0.01, respectively).

Reply:

Thank you very much for bringing it to my attention.

I corrected the mistakes that was pointed out.

In the Abstract section, I deleted “1)” “2)” and “3)” (Page 3, line 39-41), and I added some sentences to improve clarity.

I corrected a number of errors as below.

1. The values in table 5 were changed, because I calculated the contributing factors to ∆CrCl in multivariate analysis again, in order to add the new factors (sex and anorexia). (Page 3, line 49, Page 11, line 188 and table 5)

2. I made some mistakes with the number of references. I corrected them.
   (Page 5, line 81) [9]→[10]
   (Page 5, line 82) [9]→[7]
   (Page 8, line 131) [2]→[2, 11]

Other references were also changed their number because I added new references.

3. I corrected a spelling errors as below.
   “Cockroft-Gault”→“Cockcroft-Gault”. (Page 8, line 139)