Author's response to reviews

Title: Short-term glutamine supplementation decreases lung inflammation and the receptor for advanced glycation end-products expression in direct acute lung injury in mice

Authors:

Yin C Chuang Dr (chuangkenneth@hotmail.com)
Huey M Shaw Dr (mei@mail.chna.edu.tw)
Chi C Chen MS (ccomm2@yahoo.com.tw)
He J Pan MS (camille3935@hotmail.com)
Wei C Lai MS (laiweiji@hotmail.com)
Hui L Huang Dr. (cherry85@mail.chna.edu.tw)

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Author's response to reviews: see over
Reviewer: Ruud Veldhuizen

Thanks so much again for your comments. We also ensured that revised manuscript conformed to your journal style and giving a point-by-point response to the concerns. The constructive criticism provided has allowed us to significantly improve our manuscript and hope that the reviewer will find the changes undertaken satisfactory.

Major comments:

In the revised manuscript Dr Yin-ching Chuang and colleagues have addressed the majority of my previous concerns, at least in their cover letter. However many of the concerns I raised previously are still of concern when reading the revised manuscript (i.e. no, or insufficient changes were made to the revised manuscript. See the details in my previous review.

Comment 1) values of unchallenged mice. Some comments in text regarding this limitation (and or reference to previous studies) is warranted.

Response:

We agree with your opinion. It has been added as suggested. (Line 264 - 269 of the revised version)

" To elucidate the anti-inflammatory effect of GLN in ALI-challenged mice, we did not analyze BALF cytokines and lung mRNA expression in both unchallenged groups because we found that the levels of these cytokines of unchallenged controls were very low or undetectable in our previous study (data not shown). The other reason was to reduce this experimental cost and assay load, which caused this experimental design flaw in our studies. Therefore, the possible effects of GLN discussed below focus on the ALI-challenged condition in mice. "

Comment 2) The method of killing. It has been changed to "The mice were sacrificed by sodium pentobarbital asphyxiation 3 hrs after ALI challenge." I don’t think asphyxiation is the correct term for killing using sodium pentobarbital.

**Response:**

We apologize for this writing error. You are right. The drug was given by intraperitoneal injection (50 mg/kg). It has been corrected in Line 120-122 and 127-128.

The change of our revised manuscript is showed as bellow:

" Twelve mice in both groups were killed with **intraperitoneal injections of sodium pentobarbital (50 mg/kg)** after 3 h of ALI challenge."

"After 10 days of feeding, 21 mice in both groups were weighed and anesthetized with sodium pentobarbital (intraperitoneal [IP] injection) before inducing ALI by acid and LPS aspiration."

Comment 3 and 4) animal model: The authors have provided a reply to my comments, but no adequate change was made in the revised manuscript; some discussion on the advantages, disadvantages and limitations of the experimental model is warranted. For survival studies the details should be included in the text.

**Response:**

We agree your concerns. It has been revised (Line 245-252) and showed as bellow:

" In our previous studies, we used an animal model of severe ALI by both direct acid and LPS challenge for imitation of clinical cases of direct gastric fluid reflux and bacterial pneumonia. Our previous results indicated that RAGE, TNF-α, and IL-6 expression was significantly higher than that of the unchallenged mice at the early stage (3 and 6 h of injury), but all mice died 48 h after ALI challenge (data not shown). Using this severe ALI model, we speculated that GLN could reduce the
expression of these cytokines at an early stage, consequently improving the outcome of ALI. Therefore, the aim of the present study was to determine whether short-term GLN supplementation had a protective effect in the early stages of injury."

For survival studies, it has been added as suggested. (Line 122-124 of the revised version).

" For survival studies, an analgesic was not provided after ALI challenge. All of the mice were housed individually in a quiet, comfortable environment after ALI challenge. Then, the mice were observed once every h for occurrence of mortality."

Minor Comment 5) n-values in the survival study still don’t make sense to me. The numbers on the graph (such as 33%) would suggest 9 animal per group. The 8 animals per group the authors continue to list in the paper can mathematically not result in the values on the graph.

Response:
Thanks for your very careful review of our paper. You are right. The survival study was performed on nine mice in two groups. Mice were randomly assigned to a control group (n=31) and a GLN group (n=31). The number of mice were shown as bellow:

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>GLN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unchallenged mice</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>ALI-challenged</td>
<td></td>
<td></td>
</tr>
<tr>
<td>for biochemical analysis</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>for the test of survival rate</td>
<td>9</td>
<td>9</td>
</tr>
</tbody>
</table>

. I am sorry to let you so confuse. It has been corrected (Line 120).

" After ALI challenge, nine mice in both groups were used for survival rate assessment. "
Quality of written English: Needs some language corrections before being published.

Response:
We have corrected my manuscript by a native English teacher and shown a proofreading certification as below.

Thank you again for your good comments. ^_^