Reviewer's report

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

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Reviewer: Ruud Veldhuizen

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In this manuscript Dr Yin-ching Chuang and colleagues investigate the effect of short-term glutamine feeding on lung inflammation RAGE expression in acute lung injury in mice. To accomplish this they use an injury model of acid and LPS instillation in mice followed by a variety of serum, lavage and tissue measurements. The glutamine is provided in the diet for 10 days before the initiation of injury. Although a significant amount of work is presented, I have a number of major concerns.

Major

1) The manuscript misses essential non-injured control groups in the data presented in figures 1 - 3. The authors have included some unchallenged controls in the data tables 3-5, similar controls should be provided for the remainder of the data to provide a perspective how the changes observed are relative to unchallenged animals. One could also argue that the controls should undergo all of the procedures except for acid/lps administration, rather than being simply unchallenged animals.

2) The method of killing the animals by CO2 asphyxiation is not appropriate for lung injury studies. This method of euthanasia can cause lung edema and hemorrhage and thus impact the outcomes of the study.

3) The animal model is complicated. It consists of both LPS and acid instillation, both of which may have independent as well as synergistic effects on the lung. Utilizing such complicated model, it would need to be well characterized to be able to interpret the data provided. Unfortunately, the information provided is very limited. More information on some of the following: lung histology, lung compliance, inflammatory cell infiltrates, blood gas values and other physiological measurements would be needed to better establish the relevance of this model in the context of ARDS. See the paper by Matute-Bello et al, (Matute-Bello et al on behalf of the Acute Lung Injury in Animals Study Group. An Official American Thoracic Society Workshop Report: Features and Measurements of Experimental Acute Lung Injury in Animals. Am J Respir Cell Mol Biol Vol 44. pp 725–738, 2011), for more information.

It should also be noted that a strong rationale for utilizing this specific model is
4) I also have to question the animal ethics in the survival studies. It is simply described as a “survival study”. The details are important here; how frequent were animals monitored, were they provided with analgesic, were there criteria for euthanasia?

5) The manuscript, especially the introduction, is poorly referenced. Numerous sentences, including ones that contain statements like: "in numerous studies", "it has been shown", "previous studies", are not referenced. Examples: Line 53-55, 59-61, 61-63, 63-65, 68-69, 76-78, etc. An example in the methods is line 137-138.

Minor:

- The authors use the term Acute Lung Injury when referring to patients but should probably switch to the new definition of Acute Respiratory Distress Syndrome in which the term ALI is discouraged.
- The manuscript will need some thorough proofreading
- The administration of acid: was this in water or saline or another buffer?
- The results on weight gain of the lung or not useful, the method to investigate this is wet/dry weight ratio.
- N-values are confusing!! On the one hand the author state that 8 mice per group were used as unchallenged controls (line 104), yet tables 3-5 state n=10-12. Similarly, the aforementioned survival study was performed on 8 mice in each group (line 106). Yet the discussion states survival rates of 11 and 33%, both mathematically impossible with an n=8.
- Figure 2: why is RAGE expressed in mg/ml, but all others in mg/mg. Incidentally, in the expression of this data, mg/ml is probably more appropriate considering that the protein levels in the lavage would change due to edema. At any rate, recovered lavage fluid and protein levels should probably be provided.
- Figure 3, L missing in the label of Graph A

**Level of interest:** An article of limited interest

**Quality of written English:** Needs some language corrections before being published

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests