Reviewer's report

Title: TIP peptide inhalation in experimental acute lung injury: effect of repetitive dosage and different synthetic variants

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Reviewer: Tyler J Wellman

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Review of “TIP peptide inhalation in experimental acute lung injury: effect of repetitive dosage and different synthetic variants”

Major compulsory revisions:

1. Motivation: After reading the abstract and background, it remains unclear if the authors want to determine if there is a dose-effect relationship, or whether repeated applications of a given dose will show further benefits. These are not the same question. Although both questions are probably relevant, the authors should make it more clear what their goal was, and how this goal relates to potential near-future trials in patients. The repetitive application may be more relevant to ARDS patients who are in the ICU for several days, since smaller doses could perhaps be used at periodic intervals, reducing any possible side effects.

2. Significance of effect of repeated TIP-A administration: I believe the data more likely point to a lack of an effect of the second TIP-A administration. Firstly, the changes in EVLWI over 6 h were exactly the same in the SD and RD groups, implying no difference with the repeated dose. Second, in Figure 2, which shows changes over time in EVLWI, there were differences between the groups at baseline and 3h, when the groups had not yet been treated differently. These initial differences are on the same order of magnitude of the difference existing at 6h. This is not very convincing evidence for an effect of repeated dose. Some normalization to the initial EVLWI is necessary, I believe, to show a difference from 3-6h in the RD group.

3. Lack of control (placebo) group: While it is recognized that the authors previously published data showing that TIP-A reduces lung water content in comparison to a placebo, the lack of a control or placebo group in this study is a limitation. Firstly, it remains unclear if TIP-B would reduce lung water content compared to a placebo, since these were never compared. Thus, it cannot be definitively concluded that TIP-B is as effective as TIP-A. Additionally, without data from a control group in this study, it is impossible to determine the relative importance of the first and second doses of TIP-A. It could be that the SD-TIP-A causes 80% of the lung water reduction, and the RD-TIP-A only confers another 20% reduction. Since there is not control group to compare to, however, it is not possible to determine this. Do the authors have control data they could present?
Minor Comments:

Abstract:

Background:
- the “a” in “a” pulmonary edema is not necessary
- it is not clear what the abbreviation EVLWI stands for
- what was your reasoning for wanting to test repetitive TIP inhalation? To test for long-term effects? To test for a dose response relationship? A brief mention here would motivate the study better.

Results:
- what is “the second TIP dosage”. Is this a higher or lower dose, or the other TIP variation? Not completely clear.

Conclusions:
- I am not sure if fact that repetitive application reduced LW more implies that there is a dose-dependency. It could be that the clearance of TIP is rapid, so the repetitive administration simply maintained an effective concentration for clearance of LW from alveoli. This would be different from the idea of a higher concentration having a greater affect, as implied with a dose-dependency relation.

Background
- Pulmonary edema is not always the first pathophysiologic sign, as discussed in the mentioned reference. During infection-related ARDS, neutrophil accumulation and activation may occur first, and lead / contribute to edema. Perhaps rephrase.
- widespread is a single word
- In the sentence containing “reduces microvascular permeability leading to oedema formation”, I believe you mean that the permeability leads to edema formation, but it may be read as the application of TIP leads to edema formation, which is incorrect. I would suggest rephrasing to be more clear.
- “An application of TNF…” maybe should be the start of a new paragraph.
- “has not yet” rather than ‘yet has not” sounds better
- “In succession to a study that approved…“ I think demonstrated may be a better word than approved. Approved in regard to drugs tends to imply approval by regulatory authorities.
- Hypothesis 2: You say repetitive doses will “increase this effect”, but you do not actually specify what the effect will be in hypothesis 1, you just say that there will be an effect. Please be more explicit with your expectations.

Methods:
- Page 6, paragraph 1: Can you cite any previous studies that used this same two-hit model of ARDS, and showed that it was a “better” representation of the
human disease?
- Page 6, paragraph 2: by “solved” do you mean “dissolved”?
- Page 6, last paragraph: the V/Q symbols have a semi-colon after them, and the dot is off-set. Maybe it is a conversion problem
- Page 7, first paragraph: Again, what does EVLWI stand for (I presume WI = water index)? Also, what machine did you use to measure this?
- Page 7, para 1: please describe the computation of AFC, rather than just referencing it. This is a critical parameter for your study.

Results:
- Page 8, para 1: “amounts of fluid that was not extractable” - “was” should read “were”. Also, not necessary to say “respectively” in next sentence if you give the group names, as it currently is written.
- Were there no statistical differences between the recovered fluid in each group? This seems implicit, but you may want to say it explicitly.
- Why didn’t the authors study a group where just the vehicle was administered by nebulization at 0 and 3h, ie, a control group? Maybe this was studied in one of the previous studies? Regardless, comparing the current data to such a control is necessary to establish that SD TIP-A and SD TIP-B reduced lung water more than just reabsorption in itself would.

Discussion:
- “Direct correlations between EVLWI and the amount of nonaerated lung tissue respectively V;#/Q;# distribution have been characterised.” There are grammar problems here.
- “Therefore, the present data cannot differentiate, if these two mechanisms are associated or occur merely simultaneous.” Again, check grammar.
- Page 13, limitations: “we passed on an additional placebo group that would merely repeat this previous work without an impact on this study’s two hypotheses.” I don’t really agree with the lack of need for a placebo group in this study. Particularly for the first hypothesis, a placebo group is necessary to establish the specific effects of TIP-A and TIP-B on AFC. Even if you showed that TIP-A reduces lung water content, and here you show that TIP-B is not significantly different from TIP-A, it cannot necessarily be extrapolated that TIP-B would reduce lung water more than a placebo. If you have control data from that previous study under the same conditions, why not show it here for comparison?
- Do you think that redistribution of perfusion following TIP administration could influence your measurements of EVLW? This is possible, as show in “Redistribution of pulmonary blood flow impacts thermodilution-based extravascular lung water measurements in a model of acute lung injury” by Easley et al.
- Kinetics of TIP-A in the lungs: It would be very helpful to know how quickly the TIP peptides are cleared from the lungs after administration. If it occurs within 3 hours, which seems plausible, then the second administration would not increase
the dose already in the lungs, but would simply maintain a more constant concentration over the full 6 hour study. If it is not cleared quickly, the second dose would simply increase the concentration such that the results would indicate whether there is a dose-response relationship. Is it possible to measure the peptide concentration in the lungs at 3h, or over time in general, for example using bronchoalveolar lavage? This would give a much clearer picture of how the TIP peptide affects lung water content over longer time frames, and what the best strategy for administration should be.

Table 1: “No relevant intergroup differences” is a sentence fragment.

Figure 2: The error bars are not consistently displayed. Please correct.

Figure 2: Differences between the SD and RD groups were present at all time points studied, though perhaps without significance at 0 and 3 h. Did the authors test for differences in EVLWI between SD and RD at those time points? From the data, it appears that the difference at 3h is similar to the difference at 6h, but the IQR may actually be higher at 6h. Thus, it would be surprising if there were to be no statistical difference at 3h, but one existed at 6h. But almost certainly, the difference between the SD and RD groups (TIP-A) does not get significantly larger from 3-6h. This seriously questions the relevance of the effect of RD that is claimed at 6h, since the starting point for these groups was not exactly the same. A more fair way to analyze these data would be to look at % changes in EVLWI relative to baseline in each group. Did the authors look at this? I would guess that the differences between SD and RD would disappear after such normalization, calling into question the effectiveness of the second administration.

Level of interest: An article of importance in its field

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

Statistical review: Yes, and I have assessed the statistics in my report.

Declaration of competing interests:

I declare that I have no competing interests.