Author's response to reviews

Title: Efficacy and safety of inhaled calcium lactate PUR118 in the ozone challenge model - a clinical trial

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Author's response to reviews:

Dear Professor Riccardi,

On behalf of all authors I would like to thank you and the reviewers for carefully evaluating our manuscript and for giving us the opportunity to submit a revised version.

Below please find our point to point response in which we have addressed all questions and comments that were raised by the reviewers. We hope that you will find our response adequate and will consider the revised version of our manuscript for publication in BMC Pharmacology and Toxicology.

Editor:

1. Please include the TRN as the last line of the abstract

Response: The TRN is now included into the abstract (page 2, line 22).

2. Please move ethics and consent statements to the 'Methods' section.

Response: We deleted the ethics and consent statement at the end of the manuscript. In the original version of the manuscript the ethics and consent statement was already included on page 6, line 7-12. A separate sub header was now added.

Reviewer Pierluigi Paggiaro:

We would like to thank Dr. Paggiaro for taking the time to carefully assess our manuscript.
1. The only point that the author should mention in the discussion is the real possibility to modulate with some pharmacologic intervention the acute response to ozone. This stimulus is quite strong, and the potential inhibitory effect of other drugs should be mentioned. Considering the controversial effect of corticosteroids and other drugs on this acute inflammatory response, the probability to significantly prevent acute neutrophilic recruitment in the airways is not particularly high. Previous experience with other drugs should be reported.

Response: We agree with Dr. Paggiaro that ozone can be considered as a strong stimulus to induce the influx of neutrophils into the airways. However, it has been shown in 2 independent studies that the response can be modulated by high doses of inhaled or oral steroids, when dosed just prior to exposure (Ref. 12 and 26). In addition, a CXCR2 inhibitor was shown to be capable to totally inhibit the influx of neutrophils after ozone exposure (Ref. 13). To emphasize this point we added a sentence to the “Discussion” that summarizes the previous experience with other drugs that were tested with the ozone challenge model (starting page 14, line 3).

Doing so, we noticed that ref 12 and 26 were listed twice and corrected the Reference list respectively.

2. Introduction. The potential usefulness of PUR118 in the management of airway diseases is not very clear: if the compound may protect from epithelium damage induced by oxidant or other pollutants, the possibility of modifying airway inflammation when already present is less evident. Therefore, the translation from a positive effect on the ozone-induced acute inflammation in healthy subjects to the potential usefulness in stable COPD is not correct. This should be mentioned in the introduction and probably better expanded in the discussion.

Response: We agree that the ozone challenge model is not a COPD model. But it can be used for proof of concept studies to test the ability and dosage of novel drugs to modulate the influx of neutrophils into the airways. Ozone induced epithelial damage resulting in an up regulation of e.g. IL8 is a potential mechanism for ozone induced neutrophilic airway inflammation. The data of the CXCR2 ozone study (Ref 13) nicely demonstrates that neutrophil influx can be totally inhibited while the IL8 signal barely changes. The processes responsible for airway neutrophilia in COPD are much more complex especially the persistence of neutrophilic airway inflammation after smoking cessation. Nevertheless, the airway neutrophils in COPD subjects also need to migrate into the airways, therefore a compound that acts against epithelial damage and epithelial chemoattractive signaling in the ozone challenge model appears to have a potential to modulate neutrophil numbers in chronic disease processes. Besides the anti-inflammatory effect that was seen pre-clinically, the positive effect on mucociliary clearance suggests an additional potential benefit (which was not the aim to be tested in this study) of PUR118 for the treatment of chronic airway diseases. Therefore an application in COPD or cystic fibrosis is proposed.
even without a direct anti-inflammatory effect. We extended the last sentence of the introduction (page 4, line 9) to clarify the ratio and added 2 sentences to the “PUR118” section of the discussion (page 12 line 23 and page 13 line 4).

3. Methods. Some details in the study design are not clear: a) salbutamol was always administered before ozone exposure? why (considering the the functional airway response to ozone in normals is not particularly relevant, and that salbutamol may modify the acute response to ozone) ? the explanation for that has been done in the discussion, but it should mentioned also here; b) in the three treatment periods, two visits are reported while the days seem to be 3: this is not clear.

Response: a. We added a respective sentence for explanation to the “Treatment” section (page 6, line 21). As Dr. Paggiaro already stated, this was not done to avoid an ozone induced bronchoconstriction. The inflammatory response of ozone was not altered by this treatment either.

b. We thank Dr Paggiaro for making us aware of a mistake in Figure 1 and the description of the design. Figure 1 was edited and the missing pretreatment visit 2 was added. This resulted in a renumbering of visits both in figure 1 and in the manuscript the section “Study design” (page 4). In addition, the ozone challenge visits included the blood sampling 24 h after the challenge. This “visit” was not provided with an own number in order not to overcrowd the study protocol, which, however, was not clearly stated in the figure so far and caused confusion. We hope now to have clarified this issue and apologize for this mistake.

4. Results. a. In my opinion, it is not correct to put the safety data before the efficacy data, this seems to stress that the main outcome of the study was safety, but this can not be obtained with a small number of subjects. b. For the efficacy data, these are reported in a fairly long details, considering that a clear difference in sputum cell counts and other soluble mediators seems very small, in general not significant, and without any consistency. They might be better summarized.

Response: a. We now report the efficacy data prior to the safety data in the results. To be consistent with this sequence we also changed the sequence in the title, the abstract and in the last paragraph of the introduction.

b. Despite the fact that the PUR118 effect on most investigated markers was small or negative we considered it important to report this data. The respective tables are already in the online supplement and not the main body of the manuscript. It would be possible to also move the text that reports the results of WBC, blood neutrophils, monocytes, CRP and CC16 to the online supplement, if the editor feels that the main body of the manuscript should be shortened.

5. Discussion. All the data reported on the effect of PUR118 seem to be obtained in experimental models or in normal subjects, these effects might be different in COPD patients with alterations in the epithelial barrier and lining fluid.
Response: Yes, this is correct. The ozone challenge model in healthy subjects is thought to provide a first proof of concept of novel anti-inflammatory treatment strategies early in the drug development process. Studies in the diseased target population are generally more expensive and especially in COPD they could be more difficult to perform and to interpret e.g. due to coexisting comorbidities. In case of a positive result in this proof of concept study the next step would be to test whether this novel treatment can also be successfully applied to the actual diseased target population.

6. The repeatability of ozone challenge should not be derived from the comparison between baseline and low dose PUR118, but from previous studies showing that the test is reproducible in terms of respiratory function and sputum neutrophil changes.

Response: Dr. Paggiaro is correct in stating that there is already data available with respect to the repeatability of the ozone challenge. Nevertheless we felt it important to show that this was also true in this study. We now added a reference that showed the reproducibility of the model (Ref.27).

Reviewer Seigo Minami:

We thank Dr. Minami for the very thorough review of our manuscript.

Reviewer’s report

Dear Editor

This study was a single-blinded phase Ib proof-of-concept study that evaluated anti-inflammatory effect and safety of inhaled calcium lactate PUR 118 in ozone challenge model. This study showed safety of PUR 118, but failed to show clinically significant effect of PUR 118 on the ozone-induced airway inflammation.

This study is a clinical trial of a new concept of anti-inflammatory drug, and I think this study is interesting for many readers. However, I recommend the authors to revise or clarify the some minor comment described below.

Major Compulsory Revisions

None

Minor Essential Revisions

1. Background, 2nd paragraph, Line 12, page 3; Dose ‘TNFA’ mean TNF alpha?
Response: Yes, this abbreviation is now explained in the text.
Some abbreviations should be defined in the text at first use.
Response: Done, except for abstract.

I Background, 3rd paragraph, Line 4-5, page 4; ‘IL8’ = interleukin 8?, ‘MPO’ =‘myeloperoxidase’? The former abbreviation and definition are shown in Method, ‘Analysis of efficacy and safety endpoint’, Line 7, Page 7.
Response: Yes, this was corrected.

I Methods, ‘Study design’, Line 17, Page 4; ‘ECG’=electrocardiogram? This abbreviation and definition are shown in Method, ‘Analysis of efficacy and safety endpoints’, Line 15, Page 7.
Response: Yes, this was corrected.

I Methods, ‘Subject eligibility criteria and ethics statement’, Line 30, Page 5; ‘FEV1’= Forced Expiratory Volume in one second ?
Response: Yes, this was corrected.

I Methods, ‘Subject eligibility criteria and ethics statement’, Line 1, Page 6; ‘FVC’=Forced vital capacity?
Response: Yes, this was corrected.

I Methods, ‘Analysis of efficacy and safety endpoints’, Line 10, page 7; ‘CC16’ = ‘Clara cell protein 16kDa’?
Response: Yes, this was corrected.

I Result, ‘Safety- adverse events’, Line 21, Page 9; ‘TEAE’ = treatment emergent adverse events?
Response: Yes, this was corrected. Please note that the section safety was moved behind the section efficacy (see comment 4. from Dr Paggiaro)

I Result, ‘Efficacy’, Line 12, Page 10; (IQR) = interquartile range?.
Response: Yes, this was corrected.

I Results, ‘Efficacy’, Line 1, Page 11; WBC=white blood cell?
Response: Yes, this was corrected.

I Table S1; squa. cells=squamous cell?
Response: Yes, this was corrected.

2.I Table S6; IL8 = interleukin 8?, MMP9 = matrix metalloprotease 9?,

3. Some units should be defined in the Tables; Table 4; TCC and WBC 106/mL?
Response: Yes, this was corrected.
Table 6S; TCC 106/mL?
Response: Yes, this was corrected.

4. Methods, ‘Subject eligibility criteria and ethics statement’, Line 13, page 5; ‘Four subjects terminated the study after the first treatment with PUR 118’ This is also described in Results. Thus, this sentence can be omitted.
Response: This sentence was deleted.

5. Methods, ‘Subject eligibility criteria and ethics statement’, Line 18, page 5; ‘Females of child-bearing potential must have negative pregnancy test and agree to use two methods of birth control throughout the study.’ What do you mean by ‘two methods’? For example, ‘condom’ and ‘pills’?
Response: Yes, or condom plus spermicidal gel, condom plus occlusive cap, or IUD plus condom. Two methods mean “double-barrier” which is common standard in early stages of drug development.

6. Methods, ‘Subject eligibility criteria and ethics statement’, Line 17-19, page 5; Why this study paid too much attention to birth control? Was ozone challenge model or PUR 118 worried about teratogenicity?
Response: No, it’s the standard procedure for clinical trials in this phase of drug development.

7. Methods, ‘Subject eligibility criteria and ethics statement’, Line 26-27, page 5; The second exclusion criteria; These abuse test, urine nicotine test and alcohol breath test were performed every visit, or only before entrée?
Response: These tests were only performed during the screening visit.

8. Methods, ‘Exposure to ozone’, Line 25, page 6; the exposure chamber (2.7 x 2.3 x 2.5 m3) # (2.7 x 2.3 x 2.5 m) ?
Response: Actually this does not need correction. Instead of writing 2.7 m x 2.3 m x 2.5 m, we summarized the unit into m3 at the end. 2.7 x 2.3 x 2.5 m = 15.525 m would not be correct.
9. Methods, ‘Sample Size and Power’, Line 2, 4, 5, Page 9; 15%(20%), 99%(99%), 99%(94%) and 89% (67%), Are the percentage numbers in the parentheses standard deviations?

Response: No, we provided the data for both an observed within-subject variability of 15% and (20%), as outlined at the beginning of this rather long sentence. The data for the 20% variability is given in parenthesis.

10. Results, ‘Disposition of subjects’, Line 14, Page 9; ‘two subjects terminated the study after the 11 mg dose treatment period due to unrelated AEs.’ It is difficult to determine whether or not each AE is related to PUR 18. Even though those AEs did not seem to be related to the experimental treatment, I recommend details of those AEs.

Response: The first subjects reported moderate pain in both knees (MedDRA: Musculoskeletal and connective tissue disorders, Arthralgia), the second a mild sore throat (MedDRA: Respiratory, thoracic and mediastinal disorders, Oropharyngeal pain). We added this information to the text (Page 9, line 22).

11. Results, ‘Safety- adverse events’, Line 23-24, Page 9; ‘to be of mild (50) or moderate (26) intensity’ The numbers in the parentheses mean the number of subjects? In other sites, the number of subjects are shown as (n= ).

Response: Yes, this was corrected.

12. Result, ‘Safety- adverse events’, Line 24, Page 9, ‘There was only one severe TEAE (syncope) in one subject with 5.5mg PUR 118’; Did this subject continue the further treatment of 11 and 2.8mg PUR 118?

Response: No, the subjects discontinued the study. (Page 11, line 16)

13. Results, Efficacy; I Like Table 5, Table 4 and Table S1 can be combined? The post-baseline data are overlapped in Table 4 and S1.

Response: Table 4 lists the data for all 24 subjects, while table 5 lists the data of the 12 subjects that completed all visits, therefore these tables should not be combined. Dr. Minami is correct that Table 4 and Table S1 both contain the post baseline ozone data. It would be possible to combine these tables, however, for the price of clarity. We show the effect of ozone challenge in Table 4, while table S1 provides the changes from baseline, which we decided to add to the supplement, as we considered these very detailed data to be of interest for only some readers. Deleting the actual baseline data from table S1 would, in our view,
impede reading this table. We are therefore hesitant to combine the tables.

Table 4 uses standard deviation (SD)? Table 4 does not identify the numbers in the parentheses. On the other hand, Table S1 uses range. They should be standardized.

Response: Table 4 gives median and interquartile ranges. This information was now added. Table S1 and all other tables in the supplement follow the same standard and provide detailed lists of ranges. For easier reading and brevity we decided to use medians and IQR for the tables listed in the manuscript, except for the demographics, where the full range is of greater interest. We are hesitant to change this and would therefore like to leave the final decision on the required format to the editor.

If combination of these two Tables is impossible, because I think that Table S1 seems more important in this study than Table 4, Table S1 should appear in the text.

Response: The reason why we would like to adhere to 2 separate tables is given above. May we allow us to argue against the view of Dr. Minami with respect to the importance of the tables? We assume that the ozone challenge as proof of concept model might not be familiar to all readers of BMC Pharm Tox, therefore table 4 was included to clearly state the effect of ozone on the cellular and biochemical sputum composition.

Table S1 is very detailed, including all potentially relevant information for all subjects that were treated with the three doses. These details are likely to be of interest for only some readers, especially as only small changes in sputum composition occurred. Therefore we would not consider this table to be suited for the main body of the manuscript.

We believe that table 5 provides an easier to read, quick and clear overview of the small effects seen after treatment. It avoids redundant information, lists only the data of those subjects that completed all treatments and it concisely lists the median (IQR) data for all visits and not changes from baseline.

14. Discussion, Reproducibility of the ozone induced airway inflammation, in page 13-14 and Table S6; Dose comparison between baseline and low dose, and between baseline and medium dose really demonstrate the reproducibility? Is there a study that showed no significant change of inflammatory parameters before and after repeated ozone challenges without interventions?

Response: Yes, this reference is now cited (Ref 27), nevertheless, due to the lack of effect of PUR118 the good reproducibility can also be seen in this study.

15. ‘Ethical approval’, in Page 15; this is overlapped in this section and in the Method, ‘Subjects eligibility criteria and ethics statement’, the 3rd paragraph,
page 6. I think that ‘Ethical approval’ section can be omitted. Ethical statement should be separated from the subject eligibility criteria in Method section. If the limit on the number of characters permits, I recommend that the supporting information Ethics/Registration should be moved to the main text in order to inform readers of unusual repeated amendments of protocol and those reasons.

Response: We deleted the sections “Ethical approval and informed consent” at the end of the manuscript, as the same information was already given in the Method section. Here we now created an own sub header for the Ethics statement.

The provided additional information of the Ethics and Registration process is very detailed and specific, therefore we would suggest leaving this as “Additional file”. We now explicitly refer to this file at the end of the Ethics section, so that interested readers could easily use the respective link to direct to this document.

Discretionary Revisions

1. In this study, PUR 118 was not sufficient to reduce ozone-induced airway inflammation. Did PUR 118 have a clinically significant effect of anti-inflammation? Or, was the experimental method insufficient for demonstration of PUR 118’s real effect? Or, were the experimental doses of PUR, based on a mouse model (Discussion, Design, Line 14-16, page 13), clinically insufficient? Why did not this study choose 44mg or 22mg dose of PUR 118 in a previous clinical study? The authors may describe why PUR 118 did not show significant effect in this study.

Response: The study design was based on previous experience with this compound, including preclinical and safety data. Nevertheless some assumptions had to be made e.g. for the duration of treatment, which is the only point that we speculated upon as a potential reason for the small effect of PUR118. Without supporting data we are hesitant to extent the speculation any further in the manuscript.

The ozone challenge model is basically a proof of concept model for the anti-inflammatory potential of a novel compound. As the “clinical effect” of the ozone exposure is generally very small, no clinical benefit was expected in this study. Some subjects respond with cough, or mild chest pain and the effect of ozone on lung function is small as can be seen by the respective figure provided in the additional files.

The ozone challenge model has, on the other hand, shown that it responds to treatment with steroids (Ref 12, 26), a CXCR2 antagonists (Ref 13), or a pan-Selectin Antagonist (PPT 2010), therefore we are confident, that the experimental model worked.

2. Discussion, ‘PUR118’, Line 3-25, page 12; these two paragraphs only explains PUR 118, but does not discuss the study results. The authors can move these
paragraphs into Introduction.

Response: This is correct, however, based on comments by Dr. Paggiaro this section now also refers to the results. This section is thought to provide some more background on the compound that will help interpreting the results and is thought to support the study design itself. In this respect we would like to keep this section in the Discussion. In its present form it would also be too detailed for an introduction and would make the introduction too long.

3. If possible, I hope that, despite of minimal benefit in this trial, the authors describe the next steps and future perspectives of PUR 118 as much as the authors can disclose.

Response: Despite the unequivocal results in the present study, preclinical and clinical studies suggest that PUR118 may have additional clinical benefit in treating other types of inflammation. The following results were recently published for a liquid formulation of PUR118:

The effects of an epithelial barrier protective cationic aerosol on allergen-induced airway inflammation in asthma: a randomized, placebo-controlled clinical trial.

Clin Exp Allergy. 2014 Sep;44(9):1200-3. doi: 10.1111/cea.12383 <a href="javascript:" cref="CitaviPicker10.1111/cea.12383"><img style="border: 0px none;" src="data:image/png;base64,iVBORw0KGgoAAAANSUhEUgAAABAAAAAQCAYAAAAf8/9hAAAAGXRFWHRTb2Z0d2FyZQBBZG9iZSBJbWFnZVJlYWR5ccllPAA ... zF1rezNTYQn9v3DhyVCsjYxXt6t9ZV4f1vgzgZDJIDPcwB2JUjFUU71pU1GUrqvX02FJ2nDU/QkTznP1fHXHBQ4OLfwUYALhaDRT0WgkEAAAAAElFTkSuQmCC" alt="Pick It!" title='Titel anhand dieser DOI in Citavi-Projekt übernehmen'/></a>. Therefore its currently evaluated what additional trials might be needed to advance the PUR118 program.