Peer-review Comments and Author Responses

Reviewer 1

Title

1. It is a great title. It is concise. It is clear. The journal is already explicit that it is a Mini-review article. Thus, you don't need to add it to your title.

We have deleted this from the title.

Abstract

2. The only thing I'd recommend a change is instead of calling it "Discussion", call it "Conclusion". It needs a conclusion.

We have added a conclusion section.

Introduction

3. Great section. It is concise. It delivers the message.

Thank you, we believe that too.

Method

4. Even though it is mentioned that the inclusion and exclusion criteria are provided in the supplemental document, it is important to add them in this section. Otherwise, it becomes difficult for readers to capture the PICOT of the study. Only when reading the results, it becomes clear what the population is considered.

We added a limited description of the studies for better understanding of the overall inclusion criteria. Unfortunately, we cannot add the complete criteria due to the word count limit.

Results

5. It is clear that when the authors write that the use of the intervention increased or decreased, for instance, a serum marker, it is a statistically significant change. However, it would be nice to see some numbers to understand if it is also a clinically significant change.

Due to the lack of standardized measures and the variable criteria to evaluate changes in each individual study, we decided not to include exact values to ease the reading of the results.

Discussion

6. This is a well-written section. As a limitation, I'd also add that the studies added lack a standardized intervention. Even though they all compared NAC, it is not clear the presentation of the substance nor its dosage used.

We added that the heterogeneity in the exposure type and intervention (dosage, presentation, route of administration) exhort cautionary conclusions, so we didn't consider it necessary to further address it.

7. Table 1 - Add the title on the top of the table instead of below it. Also, it would be cleaner to build it without borders.

We added the title on the top and deleted the borders.

Reviewer 2

8. Please refer to the study's aim in the very last sentence of the abstract introduction.

We incorporated it in the document.

9. Please include a more detailed methodology, including the databases used and the eligibility criteria.

Databases had already been included in the manuscript. We have now also incorporated eligibility criteria.

10. More information should be given about the review's findings, such as some of the biomarkers where acetylcysteine caused an anti-inflammatory response or the main affected pathways.

This information was briefly added in the Discussion section (studies that were included in our review speculated the reduction of inflammation through surrogate variables represented by biomarkers). The main affected pathways for such a decrease of the biomarkers were added briefly in the discussion section, and as these were not the primary interest for our review, it was not extensively detailed.

11. The study's significance, justification, and knowledge gap enclosed in the first paragraph are based on human model contexts, reflecting certain inconsistencies since the study's aim is based on mice and rats models. The relation between the animal model studies and their implications for further human studies is known; however, the delimitation between each model and context should be clear. Given the inconsistency of references, I strongly advise you to include a transition sentence between your research gap statement, and the next and final sentence, which is your study aim. To make the narrative easier to follow, the transition sentence could address the uses of animal models and preclinical trials in studying similar effects related to NAC or inflammatory biomarkers in humans.

We incorporated a transition sentence that explains the relation between our review in animal models and their implications in humans.

12. Please add additional information regarding the search strategy, including eligibility criteria, main search terms used, and search dates. Even though it has been specified in the supplementary material, the methodology section should provide sufficient information to reproduce the review.

This recommendation has been incorporated into the Methods section.

13. The inconsistency between NAC use in humans and animals remains, confusing the discussion's opening sentence. To better explain what the review is trying to say, it might be better to start the first paragraph with animal models or to leave it just as it is written and add transitional sentences between the first and second paragraphs

Even though we kept the existing syntax, we clarified the distinction between human and animal model studies and their respective results throughout the discussion.

14. No comments. Well written. I would add a sentence to remark on the context of pre-clinical studies conducted on rats and mice to maintain the logical sequence between the mini-review scope, aim, and conclusions.

Indeed, we have now highlighted the context of animal models in the conclusions.

15. Since the criteria for including articles on rats or mice have already been set, you could consider taking "human studies" off the list of exclusion criteria.

Although we don't mention this criterion in the main document, we modified the list of exclusion criteria in the supplemental document following the recommendation.

16. Table 1. The sample size of some of the studies needs to be specified. Could you please include it?

We have now included the reported sample size where feasible.

Reviewer 3

17. Is there a checklist like STROBE (observational studies) for evaluating preclinical studies?

The ARRIVE guidelines 2.0 are a checklist tool to assess the findings of animal studies.

18. How did the authors analyze the quality of the included studies? Due the methodology of studies be so heterogeneous, is there a better methodology for evaluating biological markers and experimental models?

We used the adapted version of the Cochrane Risk of Bias tool developed for animal experimental models: the SYRCLE Risk of Bias tool.

19. When searching for data, if instead of being a mini-review it were a systematic review, what kind of database would you be searching for? How to find studies that are not present in the main databases. Mainly to find related studies not indexed on other platforms. Could this approach be better or worse for the research question? Could reliability be compromised by small studies?

The mini review is supposed to give a short overview/update on research in a certain area. If it was a systematic review, we would have tried to retrieve every possible source to provide a very complete overview on this research. Thus, we would have checked more databases like Web of Science, Animal Study Registry, Google scholar, etc. Further, we would have searched gray literature via conference abstracts or presentations, asked experts in the field for relevant literature, and checked reference lists of chosen articles. In principle, it is better to also include gray literature because it can be beneficial against publication bias. However, as said before, it depends on the purpose of the article how extensive the search should be.

20. There are few issues in the studies: Steerenberg et al. (2004) - The authors found different results in different results – mainly in winter (confounder?). Shima et al. (2006) - The limitations of transferring results from them to mice lungs or human lungs exposed by inhalation as respect the difference between the cells constituted in peritoneal cavities and those in lungs, and the sensitivity of cells in mice and human to oxidative stress (translational medicine?).

Indeed, there are limitations to the studies, especially since they are of preclinical nature and therefore preliminary when it comes to thinking about these mechanisms in humans. We have added a comment to the limitations section.

21. In the Results, there is no mention of the preclinical study category: "hypothesis generating" (exploratory) and "hypothesis testing" (confirmatory). Can the 19 studies be separated into exploratory and confirmatory? These are all preclinical studies that follow a hypothesis testing (confirmatory) design. However, that does not preclude the studies from finding possible new hypotheses. We do not consider this classification as relevant to the light of results found since it does not make an impact. Table 1 already includes the variables/classification by which we mean to compare our included studies. Is there an optimal assessment of Air Pollution? Is it standardized? Are there other ways to evaluate ambient pollution than diesel exhaust particles (DEP), EHC-93 urban particles (Ottawa dust) and others?

The Air Quality Index (https://www.airnow.gov/aqi/aqi-basics/using-air-quality-index/) is a standardized governmental approach to assess how polluted the air currently is or how polluted it is forecast to become. Methods to simulate air pollution are not within the scope of your review and are detailed in https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6756763/.

22. Why in some studies did researchers not use more than one inflammatory marker such as C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), ferritin, fibrinogen, tumor necrosis factor alpha (TNF-α) or other biomarkers in the studies? What kind of

confounders can be found? Imprecision in measuring actual exposures to pollutants, high variability of pollutants in urban atmospheres, personal behaviors (in the case of humans – e.g.: smoking, occupation, local socioeconomic and environmental factors).

The choices of inflammatory markers of researchers from the included studies are out of the scope of this review. We took the available information. This limitation has been addressed in the manuscript. And we can not speculate on the reasons why they did not choose more biomarkers since there could be many, one of them being that they were trying to solve their own specific hypotheses. As this is a review of animal studies, the confounding variables in humans such as smoking, occupation or other factors can not be explored properly and any conclusion should be made with caution as this was not the objective of this review.

23. In the Conclusion the authors explained about assumptions for new studies. Is there a better approach to perform novel studies? Kind of air pollution? Best inflammatory markers? How do you translate preclinical studies to clinical studies?

The measurement of inflammation using markers stated by the authors is the common practice. There are some markers such as CRP, ESR, free iron and homocysteine that have not been used in the studies included in our study. The best and exact approach until date is biopsy; however, this is limited to preclinical studies as such invasive methods probably will not be considered in future clinical studies. We encourage comparison against placebo for future studies. We have added it in the conclusion as to direct future studies.