Author's response to reviews

Title: From arginine methylation to ADMA: A novel mechanism with therapeutic potential in chronic lung diseases

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Author's response to reviews: see over
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Dear Rhian,

Thank you very much for conducting the review of our manuscript MS: 1810862304232247 “From arginine methylation to ADMA: A novel mechanism with therapeutic potential in chronic lung diseases”. We have found the suggestions of the editor and reviewers most instructive, and we have now performed a significant number of changes within the text, as requested by the reviewers. In short, we have now included two figures, which we believe will be of benefit to the manuscript. We have also performed significant changes to the text, as stated in detail in the rebuttal to the reviewers’ concerns and suggestions. Herewith, please find a revised version of our manuscript, which we hope you will now find suitable for publication in BMC Pulmonary Medicine.

With best wishes from Germany,
For the authors,
Yours sincerely,

O. Eichelberg

Oliver Eickelberg, M.D.
Detailed rebuttal to all reviewers’ comments:

Referee: 1
Comments to Author
Methylation of arginine residues in proteins plays an important role in regulation of protein function. Asymmetric dimethylarginine (ADMA), which is released from methylated proteins during proteolysis, inhibits nitric oxide synthase and elevated levels of ADMA are associated with endothelial dysfunction and atherosclerosis. During the past few years evidence has accumulated that ADMA is also involved in pulmonary hypertension and other chronic lung diseases. This review provides a timely overview of recent findings in this field.

Major compulsory revisions
1. Page 3, second paragraph: the difference between type I and type II PRMTs is described. No reference to PRMT2 is made, although this PMRT may be relevant to chronic lung disease. The authors themselves have published data on specific up regulation of PRMT2 mRNA and protein levels in hypoxic lung tissue, together with increased ADMA levels (Ref 24).
Response 1.1: We appreciate these comments on our manuscript very much. In the revised manuscript, we have now added this information on page 3, 2nd paragraph, line 4ff.

2. Page 3, second paragraph: the authors state that protein methylation, followed by proteolysis, is thought to control methylarginine content in plasma, because free arginine cannot be methylated. I think that protein methylation/proteolysis is indeed the sole source of free intracellular MMA/ADMA/SDMA, but regulation of plasma levels of these compounds is a far more complex process, which also depends on intracellular degradation and export/import from cells, inter-organ transport and renal clearance.
Response 1.2: We agree with Dr. Teerlink in that this process is indeed more complex than initially stated and have now modified this passage on page 3, 2nd paragraph, line 10ff.

Minor essential revisions
1. Page 3, third paragraph: please change “mono- or di-amines” into “mono- or dimethylamine”.
Response 1.3: We have changed this accordingly.

2. Section “Arginine methylation in COPD”, first sentence: Change “It is recently been suggested” into “It has recently been suggested”. In the same sentence I suggest to change “radical oxygen species” into “reactive oxygen species”.
Response 1.4: We have changed this accordingly.
Response 1.5: We have changed this accordingly.

Discretionary revisions
1. Page 4, first sentence: Indeed, no evidence exists that protein-incorporated methylated arginine residues can be demethylated. However, there are enzymes that can deiminate protein-incorporated MMA. Strictly speaking this is not a demethylation reaction, because the end product is not arginine but citrulline. However, this reaction may indirectly interfere with formation of ADMA/SDMA for two reasons. First, MMA is an intermediate product in the formation of both ADMA and SDMA, and therefore deimination of MMA may also reduce formation of ADMA/SDMA. Second, the net effect of methylation/deimination is conversion of protein-bound arginine into citrulline, thereby reducing the amount of potential methylation sites.
Response 1.6: We agree that this information is essential to this review, and we have accordingly added a new passage discussing these findings on page 4, 1st paragraph, line 6ff.

2. Top of page 5: The suggestion that increased arginase activity, resulting in decreased arginine levels, rather than increased ADMA is responsible for endothelial dysfunction in PH, does not necessarily imply that ADMA plays no role. In this context it may be worthwhile to mention the potential relevance of the arginine/ADMA ratio, which reflects the balance between NOS substrate and inhibitor. At low arginine concentrations the inhibitory effect of ADMA may be stronger.
Response 1.7: We have modified our statement accordingly on page 5, 2nd paragraph, line 10f.

3. A recent study has shown that in preterm infants requiring mechanical ventilation, plasma ADMA levels were positively related to the duration of mechanical ventilation, independent of gestational age (Richir et al. Pediatr Pulmonol 2008;43:1161-1166). Although not related to chronic lung disease, this data may be relevant in the context of this review.
Response 1.8: We thank the reviewer for this comment. We have expanded the section on lung fibrosis accordingly, where we believe this article fits in best (page 7, end of 2nd paragraph).

4. It may be worthwhile to mention that elevated ADMA levels have also been associated with PH in patients with sickle cell disease (Haematologica 2008;93:1410-1412).
Response 1.9: We have modified that section accordingly on page 5, 2nd paragraph, line 6.
Referee: 2

Comments to Author

This is a review on the relation of methylarginines such as ADMA (as well as methylarginine generation and metabolism) and pulmonary disease. There are quite a few reviews on Methylarginines and PRMTs/DDAH available, but none of these is focused specifically on pulmonary disease.

There are some points to be considered however:

With reviews it is good style to provide some information regarding search terms for the literature search and the selection criteria for papers to be included or excluded.

Response 2.1: We appreciate these comments on our manuscript very much. We have largely incorporated all manuscripts of relevance to lung disease. As the literature body of this field is modest, we think that inclusion of selection criteria is less relevant in this case.

I strongly recommend to include a figure detailing the generation and degradation of the methylarginines with special regard to interactions with pulmonary pathology. While the text may be limited additional or more comprehensive information may be presented in a table. Therefore, I recommend to present major data (which part of the methylarginine pathway is up- or downregulated in which type of pulmonary disease... and the corresponding ADMA or SDMA concentrations) in a table.

Response 2.2: We agree that this information is helpful to this review, and we have accordingly prepared and added two figures in our revised version: One detailing the generation and degradation of methylarginines, and one outlining the possibilities of ADMA contribution to lung disease, as suggested.

Minor

SDMA is not as inert as frequently reported, it is partly metabolized in vivo (see Ogawa et al. Arch Biochem Biophys 1987).

Response 2.3: We have modified that section accordingly on page 4, 1st paragraph, line 12f.
Referee: 3

Comments to Author

I think that the authors have written an excellent review of the relevance of arginine methylation to ADMA, and of the contribution of increased ADMA in the pathogenesis of pulmonary hypertension, and in respiratory disease generally. These are areas of considerable recent interest at a time when the emphasis has tended to be more in relation to ADMA and vascular disease, particularly coronary disease. The references provided are up to date and the standard of English good. I would recommend acceptance for publication.

Response 3.1: We appreciate these comments by Dr. Wilcken on our manuscript very much, and are grateful for this evaluation. We nevertheless hope that Dr. Wilcken largely agrees that the changes incorporated into the revised version still strengthen our manuscript above its initial form.
Referee: 4

Comments to Author

Major compulsory revisions:

1. There are also metabolites of enzymatic degradation of SDMA described, although little is known about the specific enzymes involved in this metabolism (Ogawa T et al. Arch Biochem Biophys. 1987 252:526-37). The authors should hint to the possibility that enzymatic degradation may also occur for SDMA.
Response 4.1: We have modified that section accordingly on page 4, 1st paragraph, line 12f.

2. Data from transgenic animal models in which DDAH1 is overexpressed cannot support a role for endogenous expression of DDAH1 in ADMA homeostasis, as suggested by the authors. In overexpression models, the activity of the transgene is largely dependent on the promoter to which it is linked in the overexpression vector. Therefore, these studies cannot rule out the possibility that under wild-type conditions, DDAH2 may be more important than DDAH1.
Response 4.2: We have now modified that section accordingly on page 6, 2nd paragraph. We now supply all key references pertinent to transgenic DDAH1/2 animals. A close look at all findings reported, however, still indicates a greater contribution of DDAH1 than DDAH2.

3. Cytokines and growth factors mentioned in the article should be given by their full names, abbreviations may be given in brackets but sole mentioning of abbreviations is not acceptable (e.g. page 6, Wnt = ?).
Response 4.3: We have modified that section accordingly on page 7, 1st paragraph, line 4f.

4. The discussion of the relevance of plasma ADMA levels needs some amendment. In the study by Cardounel, a relationship between extracellular ADMA and NOS activity was constructed by adding ADMA from the extracellular side. By contrast, whenever endogenous plasma levels of methylarginines are measured, this ADMA stems from “spillover” from intracellular protein breakdown, as correctly described by the authors. Therefore, this is a completely different situation: When ADMA is added from the outside, it needs to be taken up by y+ transporters into the cells – knowing that ADMA does not have a terribly high affinity to the y+ transporters this means that only a minute proportion of any dosage applied to the outside of cells is actually responsible for any biological effect that takes place in the cytoplasm. Quite in contrast, any small change in plasma ADMA under conditions of a clinical study may indicate a much greater change in intracellular ADMA, considering that enzymatic breakdown of ADMA in the cytoplasm is the major pathway of ADMA inactivation, and only part of the intracellular ADMA flux is passed to the extracellular fluid. The authors should apply more caution in
discussing the possible relationship between changes plasma ADMA and disease pathology, taking into consideration the above mentioned thoughts. Maybe some of the prospective clinical trials in which the association between minute differences in ADMA and total mortality or cardiovascular event rates in patients with cardiovascular diseases should be cited here.

Response 4.3: We largely agree with Dr. Boger’s assessment, and thought that our initial text highlighted the role of intracellular ADMA for this purpose. We have now modified that section accordingly on page 9, 1st paragraph, line 3f. We have also added a new reference on clinical trials as Ref. 13.

**Minor essential revisions:**

5. In order to better illustrate the complex metabolism of methylarginines, a scheme drawing would be very helpful. Also, the authors might want to devise a graph in which the possible molecular relationships between ADMA biosynthesis and metabolism and lung pathology are depicted.

Response 4.4: We agree that this information is helpful to this review, and we have accordingly prepared and added two figures in our revised version: One detailing the generation and degradation of methylarginines, and one outlining the possibilities of ADMA contribution to lung disease, as suggested.