Author's response to reviews

Title: Expression of Matrix Metalloproteinase-12 in Aortic Dissection

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Author's response to reviews: see over
Dear Dr. Shipley and Prof. Edward N Baker,

Thank you very much for your letter and the comments about our paper. We have checked the manuscript and revised it according to the comments. We have carefully considered the comments and have revised the manuscript accordingly.

1. The domain structure and activation of MMP-12 should be explained in the introduction. There are references throughout the paper to the 54 kDa zymogen, and active 45 kDa and 22 kDa species. A diagram showing the pro-region, the catalytic domain and the hemopexin domain would help a great deal, and it should be explained that loss of the pro-region gives the 45 kDa species, while loss of the hemopexin domain then leaves the 22 kDa catalytic domain.  

Yes, The diagram (figure 1) and relative words have been added in text.

2. The statistical significance of the results must be better documented. For example, Figure 5 gives the fluorescence enzymology results. An activity level is given for each sample, but it is not stated whether this is a single measurement or the mean value from multiple measurements on the same sample. What is the experimental error for each sample? Error bars should be shown. Also, although the experimental do seem to show higher levels than the control, the mean value and standard deviation should also be stated for each group.

Yes, It is true! Error bars has been added. The activity of MMP-12 was assessed using a peptide-based FRET assay. Fluorescence intensities were recorded using a Fluoroskan Ascent fluorimeter at an interval of 5 min over a period of 1 h.

3. Some of the figure captions require more explanation. In Figure 1, it should be stated what kind of gel this is. Also, what are the diffuse black bands above and below the PCR bands?

Yes. It has been added. PCR amplifications were analyzed by electrophoresis using 1% agarose gel stained with Gelview. The diffuse black bands
above and below the PCR bands is the background of Gelview, a safer nucleic acid dye alternative to Ethidium Bromide.

In Figure 3, what is the “positive comparison”? Is it recombinant MMP-12 after activation, and if so, how was it activated?

Yes, Revised. It should be “positive control”. Recombinant MMP-12 is expressed in E. coli as inactive zymogens (54 kD). After refolding, zymogens is quickly activated through autolytic cleavage of the propeptide domain to yield a proteolytically 45 kD form, which then breaks down to the common 22 kD form.

Figure 4, the zymogram, seems to be of very poor quality, and I am not sure what I am meant to be seeing here. I can the white bands in the control lane 9, at 45 kDa and 22 kDa, but the experimental lanes look very confusing and it is hard to see anything clearly. I think this figure could be omitted.

Yes, Figure 4 and relative words in text have been deleted.

Figure 5. What are the activity units? Also, as noted above, error bars should be given for each sample (if multiple measurements were made). If each bar represents only a single measurement, this should be stated, together with an explanation of why multiple measurements were not made.

Yes, the activity units is Relative Fluorescence Unit (RFU). Error bars has been added.

4. There are many places where literature citations should probably be given, but are not.
   p.3, line 14. “68.2% of patients died in 48 h”. Where do these figures come from?
   p.4, line 10. “it can cause extracellular matrix metabolism disorders”. Citation?
   p.4 line 10. “Previous studies have shown…..” What previous studies? Citation?
   p.15, lines 5-6. “MMPs …play an important role in the formation of AD”. Citation?
   p.15 lines 15-16. “Other research shows…..plaque rupture”. Citation?

Yes. The references have been added.

5. PCR primers are given on p.7, and are stated as corresponding to sequences from exon 5 and exon 7. In Figure 1 and in the text (p.12) the PCR product is 250 bp. What residues of MMP-12 does this correspond to?

Yes, “The primer sequences correspond to residues Asp211 to Pro292 in the catalytic domain of human MMP-12” has been stated in text.
6. On p.13, it is stated that MMP-12 had its highest activity in 20 mM CaCl2. Is this an expected result? If not, can the data be shown?

Yes. a new figure (figure 5) has been added and the data has been shown in this figure.

7. What is MMP inhibitor v? Surely it has a proper name? This is important to the paper and should be better described. What kind of inhibitor is it? Are there literature citations?

Yes, “MMP Inhibitor v (Merck KGaA Frankfurter Str. 250, 64293 Darmstadt, Germany) is an orally active non-peptidyl hydroxamate compound that acts as an effective broad-spectrum inhibitor against MMP-2, -3, -8, -9, -12, -13.” Has been added in text.

8. In the discussion (p.16) it is stated that for damaged aorta walls, MMP-12 (not MPP-12) expresses at high levels and is activated into active 45 kDa and 22 kDa forms, but for the control 54 kDa and 45 kDa forms but not the 22 kDa form are found. But one cannot say that “this shows” that MMP-12 plays a key role in aorta wall damage. This is not a logical conclusion as written.

Yes, it has been revised in text, a typical figure (figure 4) has been provided.

Minor essential revisions
1. As a general comment, units for experimental quantities should be consistent, and there should always be a space between a number and its unit. As an example of the inconsistency in units, on p.9 we have 15 minutes (correct unit is min); 1 hour (correct unit is h, which is used correctly later).

Yes, revised.

2. Writing. There are too many problems with the writing for me to list them all. The authors should get help from a native English speaker or an English writing service if possible. The following examples are not a complete list. Abstract. In some places this is written like a recipe (“Select 12 cases….”; “Use reverse transcription…””) but in other places it is written as a description of what was done or what was found (“Continuous variable were compared….”; MMP-12 in the aorta walls was expressed….”). Be consistent! The latter style is the correct one.

Yes, revised. The manuscript has been revised by a native English
speaker.

p.3 line 2. Sentence does not make sense. Should perhaps be: “For AD patients, expression of MMP-12 in than aorta wall was higher than in serum”.

Yes, revised.

p.3 line 7. These data provide evidence (not evidences) that MMP-12 may…

Yes, revised.

p.3 line 8. might be of potential relevance (not perspective)…

Yes, revised.

p.3 line 16. In recent years, morbidity has increased. (Not uptrend).

Yes, revised.

p.4 line 3. “The basis pathological change”. Basic? Also “Matrix metalloproteinase.. should begin a new sentence.

Yes, revised.


Yes, revised.

p.4 lines 8-9. “that can guarantee the….and avoid the organisational structure damage”. I do not understand this sentence at all.

Yes, revised.

p.4 line 16. “Inhibit MMPs mediates aortic…”. This sentence also needs to be rewritten.

Yes, has been rewritten

p.4 line 21-22. “..is a member of the MMP family, and is also called macrophage flexible proteae. Its major substrate is artery elastin.”

Yes, has been rewritten

p.5, lines 6-7. “The structure and characteristics of AD and abdominal aortic aneurisms are not the same, however, and there have been no relevant studies of MMP-12 expression in acute AD”.

Yes, has been rewritten.

p.5, lines 9-13. This last paragraph of the introduction is very important in explaining why this study matters. It needs to be rewritten, as I cannot understand what the authors wish to say.

Yes, has been rewritten.

p.6 lines 5-6. “…without aorta wall damage who underwent coronary artery bypass surgery. Samples were taken by punching a hole in the aortic wall tissue with a hole punch.”

Yes, has been rewritten.

p.6 line 18. Start new sentence: “The recombinant protein expressed…” Could also refer to new figure showing MMP-12 domain organisation.

Yes, a new figure (figure 1) and relative words have been added.

p.10 line 4. Sample should be “sample” (lower case) – similarly on line 10. “volumes” (not volums).
Yes, revised.
p.10 line 18. What is R & B?

Yes, revised.
p.10 last line. “…were pipetted into the wells, which were then incubated and washed”.

Yes, revised.
p.11, line 1. “In the color reaction with TMB, the TMB first turned blue due to peroxidase catalysis, and then turned yellow in the acid conditions”. Is this correct? I found the sentence hard to understand.

Yes, has been rewritten
p.11 line 3. “The optical density (OD) was determined using…”

Yes, revised.
p.11 line 7. “Aortic tissues….were harvested”

Yes, revised.
p.12 lines 13, 15. zymogen (not zymogene). Also p.13 line 6.

Yes, revised.
p.12 line 13. “with molecular weight 54 kDa and activated MMP-12 with molecular weight 45 kDa”. Also p.12 lines 15, 16, and p.13 lines 4, 5, 7.

Yes, revised.

Yes, has been rewritten
p.13, line 10. “During the determination of experimental conditions, it was found that…."

Yes, revised.
p.13, line 18. “Determine the activity of MMP-12…”. Rewrite: “When the activity of MMP-12 in the two groups was compared, MMP-12 was found to have higher...”

Yes, has been rewritten

p.14, line 16. “…are membrane necrosis in cystic, fibrosis and elastic fibers brittle.” This needs rewriting. What is meant by “membrane necrosis in cystic”? And I presume “elastic fibers brittle” means “increased brittleness of elastic fibers”.

Yes, has been rewritten
p.15, lines 7-9. This description of MMP-12 processing should be completely rewritten, perhaps along the lines: “MMP-12, also known as matrix metalloproteinase-12, is a member of the MMP family. It is secreted in the form of its 54 kDa inactive zymogen, and is then processed (by what?), by removal of the N-terminal pro-region to give the active 45 kDa proteinase. Further processing, by removal of the C-terminal hemopexin domain, gives the active 22 kDa form.”

Yes, has been rewritten
p.15, line 9-10. As written, this sentence implies that elastin degrades most
elements of the ECM. Is this correct? If not, rewrite.

Yes, has been rewritten

p.15 line 15. What is meant by “the result of atherosclerotic lesions foam cells”? Rewrite or correct.

Yes, has been rewritten

p.16, line 3. Inflammable cell immersion? Do you mean inflammatory cells?

Yes, revised.

p.17 line 16. Again the statement that elastin degrades the ECM. Is this correct?

Reference 13 refers to the function of MMP-12, not elastin, I think.

Yes, revised.

p.18 line 2. Using the recombinant MMP-12…..

Yes, revised.

We hope these will make it more acceptable for publication. If you need any other information, please contact me. Thanks again for the excellent and professional revision of our manuscript.

Best regards,

Yours sincerely,

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