Author's response to reviews

Title: The effect and mechanism of reduced beta2-glycoprotein I on aortic matrix metalloproteinases and tissue inhibitor matrix metalloproteinases in diabetic mice

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Author's response to reviews: see over
Prof. Givvimani
Thank you for your report.

However, I think that your report is not relevant to my manuscript. My manuscript is a study about reduced β2-glycoprotein I protect aorta of diabetic mouse by MMPs/TIMPs. It is not about ventricular septal defect (VSD). In my manuscript, I did not research on TIMP-3 and BNP.

I know you are a great expert of MMPs/TIMPs. I read many references and try to answer your comments.

1) In my manuscript, I didn’t do research about TIMP3. In my research, TIMP-1 is an inhibitor to MMP9, TIMP-2 is an inhibitor to MMP9. From reference, I know TIMP-3 is an inhibitor to MMP2.

2) Ventricular septal defect (VSD) is not discussed in my research. On the TIMPs during VSD, from PubMed, I only find 2 references.

In this paper, the author discussed the TIMP-3,MMP-2,MMP9 in VSD. Interaction between MMP-2, MMP-9 and TIMP-3 may contribute to atrial ECM remodeling of atrial fibrillation. In addition, many studies also appear that the association of MMP-2, MMP-9 and TIMP-3 in heart diseases, heart remodeling and cardiocytes function properly, and supported that MMP-2, MMP-9 and TIMP-3 may play important role in VSD.


In this paper, the author discussed the MMP2,MMP9 and TIMP with VSD with or without transposition of the great arteries (TGA). The results were MMP2 was lower in VSD with TGA then VSD alone. The MMP9 and TIMP were no statistically significant differences between groups.

About TIMP-4, from references, I found TIMP-4 are multifunctional protein. TIMP-4 can induce apoptosis in cardiac fibroblast cells in vitro, as well as inhibiting invasion and migration.TIMP-4, like TIMP-2, is able to interact with pro-MMP2 via its C-terminal domain, and it is also a potent inhibitor of MT1-MMP. The expression pattern of TIMP-4 is distinct from that of the other TIMPs and shows very restricted localization to neural, cardiac(newborn and postnatal day 7 in female CD-1 mice), breast and muscle tissues. Cardiovascular expression of TIMP-4 may be significantly increased in restenosis in response to vascular balloon injury.

3) MMP2 and MMP9 are both gelatinases. In my research, The mRNA and protein of MMP2 and MMP9 of aorta both reduced after treated with reduced β2-GP I compared with diabetic controls. The results were consistent. About VSD, I do not know the results. From Reference 2, I know MMP2 was lower in VSD with TGA then VSD alone. The MMP9 has no statistically significant differences between groups.

Prof. Yasuda
Thank Prof. Yasuda for reviewing my manuscript.
1) Major Compulsory Revisions

(1) My experiment was approved by Ethics Committee of Tianjin Medical University. 4 groups of 20 mice were used to do H&E staining, Sudan IV staining, real time PCR and western blot. 20 mice in each group were suitable in order to gain good results and have statistics meaning. In diabetic control groups and normal control groups also do those experiments. So they also need 40 mice (single 20, twice 20, respectively).

(2) I have intensively proofread the manuscript with the help of native Englishman.

(3) I have adjusted the figures and made them clear. I also added the explanation in the legends.

(4) I decided to do the statistical analyses among four groups because it has statistic difference by ANOVA, then compare each two group by LSD. I would add the results among four groups in manuscript.

(5) β2 GP I can form oxLDL/β2 GP I or oxLDL/β2 GP I /CRP in body. This will consume some LDL-c, so in single dose, LDL-c in β2 GP I group was lower than that in diabetic control. In our experiment, except normal group, mice in other groups were all given high sugar high fat diet, so the blood lipid would continues rising. But we only gave twice β2 GP I. The level of blood lipid rising was more than consuming of β2 GP I with ox-LDL. So in complex dose, LDL-c in β2 GP I group was not lower than that in diabetic control (P > 0.05).

(6) In figure 2, in diabetic control group, mice in single dose were given (8+1+3) weeks high sugar high fat diet in single dose, while mice in complex dose was given (8+1+6) weeks high sugar high fat diet. We didn't treat dyslipidemia, so atherosclerotic area of diabetic control mice in complex dose should be more severe than that in single dose.

(7) Figure 3, I only wanted to display the morphological changes in different groups because I did not do special staining for cells, such as F4/80 for macrophages, CD34 for endothelial cells. I can add in future research. Thank your suggestion.

(8) Mouse's aorta was long enough, so I chose 10 samples from each group to do real time PCR and western blot. I will indicate the number of evaluated samples in Figure 4, 5, 6 and text.

(9) In the left panel of Figure 6, it was the mRNA of p38MAPK in different groups. I have got the statistical significance in reduced β2 GP I treated group with diabetic control group in mono dose and complex dose. In the right panel, in ordinate, it was the ratio of phosphor-p38MAPK/p38MAPK. The ratio could reflect the real changes of p38MAPK. So in right, there is not total p38MAPK alone. Protein changes is similar with mRNA in complex dose, not in mono dose. I think the difference between changes of protein and mRNA have some reasons: 1) mRNA changes can different with proteins. Many steps between transcription and translation. 2) reduced β2 GP I acted on p38MAPK making protein changes need more time. So twice reduced β2 GP I have good effect. This also indicated reduced β2 GP I must multiple treat and can get more aortic protective effects.

2) Discretionary Revisions

In my experiment, I found that reduced β2 GP I can reduce LDL-c. In our research group, others also found that reduced β2 GP I can reduce LDL-c (Zhang R, Zhou SJ, Li CJ, et al. C-reactive protein/oxidised low-density lipoprotein/β2-glycoprotein I complex promotes atherosclerosis in...
diabetic BALB/c mice via p38mitogen-activated protein kinase signal pathway[J]. Lipids Health Dis. 2013; 12:42.). The real mechanism is still to be examined. I will continue to study in the future.