Author’s response to reviews

Title: Effects of semicarbazide-sensitive amine oxidase inhibitors on morphology of aorta and kidney in diabetic rats

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Author’s response to reviews:

To Reviewer1

Dear expert:

Thank you for your letter and for your comments concerning our manuscript entitled “Efficacy of semicarbazide-sensitive amine oxidase inhibitors in the prevention of diabetic vascular complications” (BEND-D-19-00025). Those comments are all valuable and very helpful for revising and improving our paper, as well as the important guiding significance to our researches. We have studied comments carefully and have made correction which we hope meet with approval. Revised portion are marked in red in the paper.

The main corrections in the paper and the responds to your comments are as flowing:

1. "AG and 2-BEA are common low-toxic SSAO inhibitors" is a clerical error. It has been changed to "AG is a common low-toxic SSAO inhibitor", 2-bromoethylamine is a synthetic chemical, toxicity The role remains to be explored. Therefore, this study attempts to use a cheap and low-toxicity drug AG for research, which is expected to have a high research significance. Aminoguanidine has been found for centuries and has been used in the phase III clinical trial of diabetes. In the trials (including ACTION trial, ACTION II trial), there was no difference in the incidence of adverse drug reactions between the low-dose treatment groups and the placebo group [1, 2].

2. SSAO can be oxidatively dehydrogenated to produce FA, H2O2. Our experimental group tried a variety of methods to measure the level of formaldehyde, and failed to detect the difference. The reasons for this were as follows: 1) There are many ways to produce formaldehyde; 2) The concentration of formaldehyde in rats is low. The sensitivity of the current detection method is low and cannot be detected. Differences in formaldehyde levels; 3), species differences. Previous studies have shown that 2-bea can inhibit the increase of SSAO activity in the process of experimental
atherosclerosis in New Zealand rabbits, reduce the concentration of formaldehyde, and reduce the formation of atherosclerotic plaque. Previous studies have shown that 14c radioisotope labeled methylamine in vivo, observed the residual amount of 14c in each tissue, confirmed that 14c labeled methylamine was converted to formaldehyde by SSAO deamination, and generated Formaldehyde rapidly binds irreversibly to tissue proteins. Our follow-up study will use isotopically labeled 14c-methylamine to indirectly detect the difference in formaldehyde concentration between ssao oxidative deamination [3], and further detect H2O2 levels.

3. The activity of SSAO in diabetic rats was significantly increased. Many studies showed that it was significantly associated with diabetic vascular complications. The SSAO activity of diabetic rats was significantly increased in this study. The inhibition of SSAO can significantly improve the aortic and renal morphological changes in diabetic rats. The mechanism is as follows (Fig. 1): inhibition of SSAO oxidative deamination reduces the production of toxic substances such as aldehydes and h2o2. This study only initially investigated the effects of SSAO inhibitors on aorta and kidney injury in diabetic rats. The treatment, post-study still needs to explore in detail the detailed molecular mechanism.

Figure 1 toxic products produced by oxidative deamination of SSAO(see in the General information)

4. Diabetic vascular complications include macrovascular diseases such as aortic atherosclerosis and microangiopathy such as diabetic nephropathy. The experimental period of this study is short. The pathological morphology of aorta and kidney in DM group has not shown typical diabetic nephropathy and main The pathological changes of atherosclerosis, the subject of this study is not accurate, has been modified into the effect of semicarbazide-sensitive amine oxidase inhibitor on the morphological changes of aorta and kidney in diabetic rats.(lines 2 and 3, page 1).

5. The results shown in Figure 2 and Figure 3 have been modified into column graphs.

6. According to the editorial requirements, the experimental results of NC+AG and NC+2-BEA on et-1 and no have been supplemented. The current research lacks the results of NC+AG and NC+2-BEA in Figure 4 and Figure 5, because the SSAO activity and formaldehyde concentration in the NC group were low. It was difficult to measure the plasma SSAO activity and formaldehyde concentration in the NC group after the SSAO inhibitor. After administration of SSAO inhibitor in NC group, there was no significant difference in pathological results of blood glucose, body weight, no, et-1, aorta and kidney compared with NC group, and no serious adverse events occurred.

7. The discussion about model of type 1 diabetes mellitus have been removed. (page 10)

8. We have revised the incorrect terms (signal, line 26, page 2; expect, line 28, page 2; suicidal, line 50, page 3).

9. The incomplete sentence on the first line of the page 4 has been revised.

10. The plasma ET-1 level was higher in the DM + 2-BEA group than in the DM + AG group.

Once again, thank you very much for your comments and suggestions.

Best Wishes

references:
To Reviewer 2

Dear expert:

Thank you for your letter and for your comments concerning our manuscript entitled “Efficacy of semicarbazide-sensitive amine oxidase inhibitors in the prevention of diabetic vascular complications” (BEND-D-19-00025). Those comments are all valuable and very helpful for revising and improving our paper, as well as the important guiding significance to our researches. We have studied comments carefully and have made correction which we hope meet with approval. Revised portion are marked in red in the paper.

The main corrections in the paper and the responds to your comments are as flowing:

1. According to the expert opinion, the concentration of streptozotocin used to establish the type 1 diabetes model has been added to the abstract. (lines 12 and 13, page 2).
2. According to the expert opinion, the title, abstract, and conclusion have been revised to "the influence of aortic and renal morphology of type 1 diabetic rats".
3. Diabetes vascular complications have been described in the background and conclusions (aortic atherosclerosis, coronary artery disease, increased risk of cerebrovascular disease, peripheral vascular disease), microangiopathy (diabetic nephropathy, diabetic retinopathy, Diabetic neuropathy, diabetic cardiomyopathy). The experimental period of this research project is short. The pathological morphology of aorta and kidney in the DM group has not been typical of the pathological changes of diabetic nephropathy and aortic atherosclerosis. Some of the contents are not accurate and have been modified to be "semicarbazide sensitivity." Effects of amine oxidase inhibitors on morphological changes in aorta and kidney of diabetic rats. (lines 2-3, page 1; lines 5-6, page 2; lines 6-7, page 3, lines 2-4, Page 4, lines 24-26, page 13)
4. T1DM model established in our study was successful and easily performed, along with high stability. Aortic macrovascular complications and changes in renal diabetic nephropathy can occur early in the model. In our experiment, the rat aorta was used to study the morphological changes of diabetic macrovessels, mainly due to the ease of collecting thoracic aorta, and the aortic atherosclerosis changes in the early stage of the model. The renal arteries and carotid arteries were not selected because of the lack of adequate literature. (lines 1-6, page 11).
5. The method of taking the aorta has been described in detail.(lines 9 to 15, page 6).
6. Methods for detecting plasma NO levels have been described in detail on the detection of major indicators.(lines 2 and 9, page 7).
7. for the typo, has been modified to plasma SSAO. (Figure 4a)
8. Figure 2 and Figure 3, according to the Eliane Hiromi Akamine revision proposal, has been modified to a column chart.
9. The IC50 of AG to inhibit SSAO is lower than to inhibit NOS, many studies have shown that the dose for AG to inhibit NOS is mostly 100mg/kg/d. In this study, 25mg/kg/d AG was intraperitoneally injected, At the same time, another high-selective SSAO inhibitor 2-BEA improved the ET-NO homeostasis in diabetic rats. The role of SSAO oxidative deamination in diabetic vascular dysfunction was demonstrated.(lines 9, 10, 11, lines 12 - 15, page 13)
10. SSAO expression in the nc group was low, significantly increased in the dm group. In the experiment, DM+AG and DM+2BEA, the aorta was not significantly different from the NC group in morphology (light and electron microscopy).
11. It has been modified in the discussion section. AG and 2-BEA have certain functions to regulate
the dynamic balance of ET-NO system and improve endothelium-dependent vasodilation in diabetic rats. This experiment shows that AG and 2-BEA inhibition at 8 weeks SSAO oxidative deamination improves pathological changes in aorta and kidney of diabetic rats. (lines 17, 19, page 13)

12. It has been revised in the conclusion. (lines 24 and 26, page 13)

Once again, thank you very much for your comments and suggestions.

Best Wishes