Author’s response to reviews

Title: Anti-inflammatory and Morphologic Effects of Pitavastatin on Carotid Arteries and Thoracic Aorta Evaluated by Integrated Backscatter Trans-esophageal Ultrasound and PET/CT: A Prospective Randomized Comparative Study with Pravastatin (EPICENTRE study)

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Author’s response to reviews: see over
To Reviewer 1

This study showed some flaws: surely the low number of subjects enrolled, as underlined by the authors themselves, but also the choice of the different statin dosage. In this study the authors decided to compare a moderate intensity dosage (Pitavastatin 2 mg) vs a low intensity (Pravastatin 10 mg) (ref: 2103 ACC/AHA guidelines on the treatment of blood cholesterol to reduce atherosclerotic cardiovascular risk in adults Stone NJ et al. J Am Coll Cardiol. 2014). As showed by the results of this study the effects on the lipid profile was certainly more efficacy for Pivastatin than for Pravastatin (table 2). Could be different the results of this study with higher pravastatin dosage?

First, we thank the reviewer for the careful review and constructive comments.

In Japan, the minimum dose of pravastatin is 5 mg/day, maximum dose is 20 mg/day, and intermediate dose is 10 mg/day. The minimum dose of pitavastatin is 1 mg/day, maximum dose is 4 mg/day, and intermediate dose is 2 mg/day. Therefore, we compared the effects of each statin using intermediate dose in Japan.

We added the above information to the “Methods” section.

Thank you for helpful comments.

Minor revisions
1) The use of carotid artery method was not specify in the purpose of the study (page 5)

We corrected the sentence adding the information on carotid artery.

2) Fig 1 first figure on the left Y abscise Changes instead Cchages

We corrected the typographical error. Thank you for helpful comments.
To Reviewer 2

The higher lipid-lowering effect of pitavastatin vs pravastatin has been reported elsewhere: the authors should cite, for instance: Sponseller CA et al, Clin Ther. 2014 Aug 1;36(8):1211-22. doi: 10.1016/j.clinthera.2014.06.009. Nevertheless, I think that the direct comparison in terms of plaque morphology and inflammation is an original aspect of this study and deserves attention. The significant correlation between LDL cholesterol and imaging biomarkers of plaque severity is also worth to be discussed, even though the above mentioned problem of sample size cannot be neglected as far as consensus is concerned. There is some concern on the statistical analysis. I’ve found just a bit surprising that the significance threshold (p<0.05) was reached for the cIBS changes in the pitavastatin (PI) group (-20.1+/−5.0 dB to -19.6+/−7.0 dB) but not in the pravastatin (PR) group (-19.6+/−7.0 dB to -18.9+/−4.4 dB). With such a low number of patient, it would be more convincing if the author can graphically represent their results of cIBS and TBR (e.g. in a scatter-plot and/or box-plot). The authors should refer to the specific comments below to improve their manuscript.

First, we thank the reviewer for the careful review and constructive comments.

According to the reviewer’s comment, we added Figure 3 showing the changes of each parameter from baseline to after 6 months to make readers understand the changes easily. We deleted Table 2 instead. In addition we checked and corrected statistical analyses. We showed mean ± standard deviation in Figure 3 because the data were normally distributed.

Major Compulsory Revision

1) cIBS changes and statistical significance.

I understand all the problems related to the sample size in a clinical study. Of course my first suggestion would be to wait for a higher number of patients, because some reader may think that for N=10/group the study can be underpowered and thus even a p<0.05 doesn’t mean by itself that your results are “robust” (i.e., they could change for small changes of the dataset). If it’s not possible for you to increase the size of your sample (as I imagine), for such a small number of patient it would be more convincing if you could graphically represent your results of cIBS and TBR (e.g. in a scatter-plot). Figure 1 reports the % changes of TBR and cIBS in a pooled form, so it’s impossible for the
reader to infer which points are from the PI group and PR group. I strongly suggest to double-check your statistical analysis.

We checked and corrected statistical analyses. As suggested by the reviewer, we corrected Figure 1 showing the plots in the pravastatin group highlighted by red and showing the plots in the pitavastatin group by blue to make the readers understand the changes easily. Thank you for helpful comments.

2) Measurement of SUV\textsubscript{max} and TBR with 18F-FDG-PET.
Anyway, I’m not sure that the only assessment of the TBR\textsubscript{max} in such a small ROI (1 cm diam) in the carotid artery is enough to assess the whole inflammation burden. It is also well know that tracer quantification in small objects (e.g., plaques < 1cm) is biased by partial volume artifacts. For this reason, I think that also the TBR\textsubscript{mean} (measured as the blood-normalized SUV\textsubscript{mean}) should be reported as calculated on a larger volume of interest covering the arterial bed. Probably your choice of such a small ROI was due to the high residual activity in neighboring pixels due to the short injection-to-imaging time: can you add more comment on this?

We totally agree with you. We added the following sentences regarding the time interval between injection of FDG and imaging and the size of ROI to the discussion section and the study limitations section citing the following the precious report that was suggested by the reviewer.
“In the present study, we obtained PET images using relatively short duration (one hour) after the injection of FDG. There were several studies that employed various durations
between the injection of FDG and PET imaging for the evaluation of atherosclerotic lesions (1 - 3 hours) (9, 12, 18). Studies that employed long duration between the injection and imaging aimed to include the change of proper uptake by macrophages as well as better washout of radioactivity from the blood pool. Contrary, we aimed to include the change of the high residual activity in neighboring pixels due to the short injection-to-imaging time.”

“Fourth, a small size of the ROI chosen for PET quantification in the present study may hinder accurate reproducibility of measurements. Relatively large ROIs should be used to improve the reproducibility of measurements.”


Thank you for very helpful comments.

3) Reproducibility of the ROI selection in PET.
You say that the identification of the target plaque in PET at baseline was done manually by selecting the region with the highest uptake of FDG, whereas you’ve used CT as a reference for TEE. Could you please add more comments on the use of CT plaque images for the selection of your ROI’s in PET? Can you say whether the landmark-based identification of ROIs after six month still leads to the region with the highest uptake of FDG? In other words, in how many cases you can still find the target plaque manually after six months? Again, I think that the small size of the ROI chosen for PET quantification can affect the reproducibility of your measurements, and this is why I suggested to report also TBR_mean in larger ROI’s in the point 2) above.

At baseline, we selected the region with the highest uptake of FDG for the evaluation. After six months later, we selected the same region as that selected at baseline by refereeing CT images. We added the above information to the methods
In addition, we added the following sentences to the study limitation section.

“A small size of the ROI chosen for PET quantification in the present study may hinder accurate reproducibility of measurements. Relatively large ROIs should be used to improve the reproducibility of measurements.”

Minor essential revisions

4) Please add more information on your PET/CT imaging protocol. More specifically, please add info on the reconstruction algorithm (FBP or iterative) and types of correction (attenuation, decay time, etc) which have strong impact on quantification. Add also the total duration of the scan.

We added the following sentences regarding imaging information to the methods section.

“This system can obtain PET and CT images (4.25-mm slice thickness) simultaneously. CT scan was performed for attenuation correction and PET images were reconstructed using iteration algorithm (128 x 128 pixel matrix). Images were obtained over 24 minutes.”

Thank you for your helpful comments.

Discretionary Revisions

(mainly stylistic suggestions)

5) ABSTRACT - Conclusions: “… The pravastatin treatment had less of an effect…”.
Did you mean “was less effective”?

We corrected the sentence. Thank you for your helpful comment.

6) METHODS - PET/CT image acquisition and analysis: “… Patients were injected with 18F-FDG 3.7 MBq (0.1 mCi) /kg…”. Change to “… were injected with 3.7
MBq/kg (0.1 mCi/kg) of 18F-FDG…”

We corrected the sentence. Thank you for your helpful comment.

7) DISCUSSION - 3rd paragraph: “It is know that plasma hs-CRP concentrations are associated with high cardiovascular risk”. Change to “It is known that _high_ plasma hs-CRP concentrations…”(really optional)

We corrected the sentence. Thank you for your helpful comment.

8) You have CT images for all patients for both baseline and follow-up: why not reporting also the Ca (Agatston) score? Perhaps your CT protocol was not diagnostic as it was used only for PET attenuation correction, but I’m just guessing. Could you add some comment on this?

As suggested by reviewer, we did not use Agatston score because the CT protocol was used only for PET attenuation correction. We added the following sentences to the study limitations section.

“We did not use Agatston score for the evaluation of atherosclerosis because the CT protocol was used only for PET attenuation correction. However, process of progression of calcification is gradual and not suitable for the relatively acute change by the effects of statins.”