

December 30, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 14465-review.doc).

Title: MRI of the Pancreas in Streptozotocin-Induced Diabetic Rats: Gadofluorine P and Gd-DOTA

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The manuscript has been improved according to the suggestions of reviewers:

1 Format has been updated according to the given formats and the correction requested by the Editor throughout the text: short running title was remade; in our institution, animal studies underwent Institutional Animal Care and Use Committee approval of protocol, but not Institutional Review Board approval.

2 Revision has been made according to the suggestions of the reviewer

(1) Reviewed by 00503608

1. One does wonder why the various contrast agents were evaluated in a linear fashion rather than using additional groups. This would probably bolster the argument that Gadolinium P is superior to Gd-DOTA for the diagnosis of DM. This was addressed briefly in the discussion, but perhaps this could be expanded upon. It is entirely feasible that the changes identified may have been associated with the timing of the MRI evaluation rather than the agent used.

We thank a reviewer for the comment. We added our limitation in the discussion (page 17). We added supplementary data. MR imaging which was selected for comparison of Gd-DOTA and Gadolinium P was obtained in day 4, day 5. In the supplementary figure, blood glucose level was elevated above 450mg/dL from day 3 showing plateau. If MR imaging obtained earlier than we did, more part of MR signal change may depend on timing.

2. Please discuss the clinical implications in much greater detail. How will this test be helpful and who would we use it for? It is hard to believe that an x-ray would be superior to blood testing for the diagnosis of diabetes, although it is a very interesting idea. What are the cost considerations? Would there be any role for the evaluation of other pancreatic disease (chronic pancreatitis and pancreatic cancer) that have a known association with DM?

We thank a reviewer for the comment. We added details in the discussion (page 16- 17).

(2) Reviewed by 00503542

This study seems to have tried to compare two MRI enhancers to detect early onset of type 1 diabetes mellitus (DM) in rats. The aim of this study seems quite reasonable and potentially important. However, the authors seem to have failed to draw a scientifically robust conclusion because of several reasons as follows.

1. As authors mentioned in the text, two enhancers are tested at different time points, which make direct comparison impossible. Results of two enhancers can be compared in control rats, because there may not be significantly different conditions in normal pancreas at the different time points. However, comparison cannot be regarded as proper at the different time points with different glucose levels in diabetic rats. This observation should have been done at the same time point in different animal groups after the same DM-induction.

We thank a reviewer for the comment. Other reviewer mentioned the same thing. We added our limitation in the discussion (page 16- 17) and supplementary material. Details are in the reply of reviewer 00503608 above.

2. From the description of the introduction, it is concerned that the authors may not have a right definition of “blood flow” of a tissue. Most references they cited reported increased vascular permeability or vascular dysfunction in the diabetic islets. However, in such situation, microsphere technique of blood flow measurement (for example, ref. #9) may show false positive increase because of congestion that accumulates more blood to the inflammatory tissue. However, this is not a true increase in blood flow. Tissue blood flow is estimated based on the blood volume that comes in to and goes out from the unit tissue volume in a unit time duration.

We thank a reviewer for the comment. We deleted the first “blood flow” in the first paragraph in introduction. And, we deleted the reference “Carlsson PO, Sandler S, Jansson L. Pancreatic islet blood perfusion in the nonobese diabetic mouse: diabetes-prone female mice exhibit a higher blood flow compared with male mice in the prediabetic phase. *Endocrinology* 1998; 139(8): 3534-3541 [PMID: 9681505 DOI: 10.1210/endo.139.8.6153]” in that sentence. We changed the second “blood flow” in the first paragraph in introduction into “vascular permeability”

3. Again, as the authors mentioned in the text, the DM model employed in this study is difficult to be regarded as that of typical type 1 DM, because of the lack of classic insulinitis.

We thank a reviewer for the comment. We deleted “type 1” in the aim and conclusion in the abstract, the last paragraph in the introduction, the last paragraph in the discussion.

4. The histological parameters of islet diameter and islet number may not be suitable because, from Fig. 7, atypically long and narrow tissues seem to be regarded as islets. This should be examined by more specific staining like insulin staining. From the photographs like Fig. 7, islet area may be a better parameter.

We thank a reviewer for the comment. We agree with your suggestion. This is a weak point in our study. However, the histologic analysis was done by a board certified pathologist. In our next study, we will follow your suggestion.

5. From the above consideration (#2) of increased vascular permeability together with the result shown in Fig. 9, the negative correlation of SI and islet number may reflect an important, but yet unproven finding of less islets in the pancreas with more extravasation of the enhancer.

We thank a reviewer for the comment. We added more explanation in the second paragraph in the discussion and contrast media part in material and method with a reference [Ni Y. MR Contrast Agents for Cardiac Imaging. In: Bogaert J, Dymarkowski S, Taylor AM, Muthurangu V, editors. Clinical Cardiac MRI. Berlin Heidelberg: Springer-Verlag, 2012: 31-51]. As we mentioned the second paragraph in discussion and contrast media part in material and method, the blood concentration of Gd-DOTA decreases rapidly after injection from both elimination and extravasation out of the vasculature. However, Gadofluorine P can re-circulate in the blood binding to albumin due to its nonspecific protein binding

property. The elimination half-life of Gadofluorine P is about six times greater than that of Gd-DOTA. In rabbits, the plasma elimination half-life of Gadofluorine P is around 2 h, and Gadofluorine P is almost completely eliminated from blood/plasma within 24 h after intravenous injection. In rats, the biodistribution of Gd-DOTA was a distribution half-life of 3 min and an elimination half-life of 18 min. Then, Gadofluorine P can extravasate in regions of increased vascular permeability and bind to extracellular protein in the interstitial space. Therefore, less islets in the pancreas with more extravasation of the enhancer in the pancreas of diabetic rats with Gadofluorine P. We also prove the accumulation of Gadofluorine P in the pancreas using LA-ICP-MS.

6. The authors' attempt to minimize the number of used animals is important. However, more important thing is to plan experiments properly to draw scientific conclusion in order to make good use of experimental animals.

We thank a reviewer for the comment. This is limitation of our study. In our next study, we will follow your suggestion.

7. "progeoglycans" (page 13, line 18) should be "proteoglycans".

We thank a reviewer for the comment. The word is corrected.

(3) Reviewed by 00503540

1. I have no idea why the diabetic pancreas was enhanced more prominently by gadofluorine P comparing with normal pancreas. This study is not well designed to prove the mechanism. Histological assessment is not sufficient because there is only HE data. Immunostaining for vascular network using vWF or CD31 is necessary. Is the reason for the

enhancement is reflected on hypervascularization of the pancreas or leakage of the contrast agent due to destruction of the vascular network? Please have additional examinations to reveal that.

We appreciate your professional comments. We agree with your opinion of immunostaining for vascular network using vWF or CD31, and this is limitation of our study. But, we demonstrated the extravasation and accumulation of Gadofluorine P in the pancreas of diabetic rat using LA-ICP-MS. We added more explanation in the second paragraph in the discussion

2. STZ-induced Diabetes is NOT the model for type 1 DM. If the authors use the word "type 1 DM", please change the model to spontaneously induced diabetic rat. Or, should change the word to "drug-induced diabetic rat".

We thank a reviewer for the comment. Other reviewer mentioned the same thing. We deleted "type 1" in the aim and conclusion in the abstract, the last paragraph in the introduction, the last paragraph in the discussion. The short running title of our manuscript was changed into "Gadofluorine P enhanced MRI in Streptozotocin-Induced Diabetes"

Thank you for your time and consideration of our manuscript for a possible publication in the World Journal of Gastroenterology.

Sincerely yours,

A handwritten signature in purple ink, consisting of stylized, overlapping loops and strokes.

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