Author’s response to reviews

Title: Liver injury monitoring, fibrosis staging and inflammation grading using T1rho magnetic resonance imaging: An experimental study in rats with carbon tetrachloride intoxication

Authors:

shuangshuang xie (xiess1989_happy@163.com)
hanxiong qi (2812298227@qq.com)
qing li (liqyzx@163.com)
kun zhang (13516258019@163.com)
longjiang zhang (1637929809@qq.com)
yue cheng (chengyxss2015@163.com)
wen shen (shenwen66happy@126.com)

Version: 3 Date: 26 Nov 2019

Author’s response to reviews:

Dear editors and reviewers,

Thank you very much for reviewing our manuscript entitled “Liver injury monitoring, fibrosis staging and inflammatory grading with T1rho MR imaging: an experimental study in rats with carbon tetrachloride intoxication” (BMGE-D-19-00187R2). The comments are exceptionally valuable, inspiring, and constructive for our work. We rejoiced at your decision in the letter dated Oct 29, considering a major revision required of our manuscript, addressing all the issues brought up by the reviewers.

Below, I will detail how we revised our manuscript in order to address each of the comments in the original decision letter.

A. Response to suggestions of editors

We have revised our manuscript according to the journal style and improved the English language of it.

B. Response to comments of reviewer 1
Comments-1. It showed that liver T1rho values of rats with same inflammation grades showed no difference among different fibrosis stages, and only T1rho values of different inflammation grades with fibrosis stage 3 had significant difference. Can you please show the values of T1rho of different inflammation grades with fibrosis stage 3?

Author’s response:

Thanks for your kind suggestion. We have added the values of T1rho of different inflammation grades with fibrosis stage 3 in Results (Page 2 line 28).

Comments-2. As the authors mentioned in the manuscript, a rat model of liver fibrosis may not reflect the pathologic changes of the human liver accurately. The authors should discuss more about the merit of T1rho MRI in clinical detection or diagnosis of liver disease according to the published literatures and also the new findings in this paper.

Author’s response:

Thanks for your kind suggestion.

In clinical studies, most studies (including study from our group) have only investigated the value of T1rho in assessing liver fibrosis, and only two studies investigated the relationship between liver T1rho values and inflammatory activity. In liver fibrosis, except one study (Takayama et al. 2015), other studies have got positive results. In inflammatory activity, both the two clinical studies have got a negative result, which was inconsistent with animal studies.

In our study, we investigated the value of T1rho in diagnosing liver fibrosis and inflammatory activity at the same time, and also controlled the fibrosis stages or inflammatory activity to investigate their impact on liver T1rho values separately. Our results showed that both liver fibrosis and inflammatory activity had impact on liver T1rho values, but inflammatory activity had a greater impact on liver T1rho values than fibrosis, which was not mentioned in all previous studies. In chronic liver disease, the more severe the degree of liver fibrosis usually combined with the higher inflammatory activity. So, when T1rho was used to diagnose liver fibrosis, the impact form inflammatory activity should be considered.

The merit of T1rho MRI in clinical detection or diagnosis of liver disease according to the published literatures has been mentioned in Background (Page 1 line 22-30), and I also discussed these in Discussion (Page 2 line 22, 29-30, Page 3 line 1-6, 14-16). The discussion of the new findings in this paper were listed in Discussion (Page 3 line 29-30, Page 4 line 1-3).

Comments-3. The authors investigated the impact of liver fibrosis and inflammation on liver T1rho values by using CCl4-induced liver injury in rats. Can T1rho be used as a non-invasive imaging technique for evaluating liver fibrosis of inflammation? Can you determine the diagnostic accuracy of T1rho for significant fibrosis or inflammation by using ROC (receive operating curve) analysis?
Author’s response:

Thanks for your question and kind suggestion.

Depending on our results, T1rho can be used as a non-invasive imaging technique for evaluating liver fibrosis of inflammation.

We have added the diagnostic accuracy of T1rho for significant fibrosis or inflammation by using ROC analysis in Results (Page 3 line 9-15) and Discussion (Page 2 line 19-20, Page 3 line 10-11).

C. Response to comments of reviewer 2

Comments-1. T1rho MR imaging has already been widely studied in human. Authors should explain more about the necessity of using the rat model in the present study. Other rat models for liver fibrosis (such as BDL model) should be mentioned in the discussion part. Differences between animal fibrotic models and human liver fibrosis should be discussed.

Author’s response:

Thanks for kind suggestion.

Some clinical studies have used T1rho MR imaging to evaluate liver fibrosis, liver cirrhosis and liver function, but few studies with liver pathological results. In addition, all previous experimental and clinical studies evaluated the correlation between fibrosis stages or inflammation grades and liver T1rho values separately. In experimental study, the pathological results can be got easily. So, we use a rat model to explore previously unresolved or controversial issues. The necessity of using the rat model in the present study has been mentioned in Background (Page 1 line 24-28, Page 2 line 9-17).

In previous animal studies associated with liver T1rho MRI, only two studies used BDL model. BDL model has the feature of high success rate and reproducible histological change course of the liver, while the inflammatory reactions associated with this model is mild. In our study, we plan to investigate the value of T1rho MRI in detecting liver fibrosis or inflammation activity at the same time. CCl4-induced diffuse liver fibrosis is associated with a greater extent of inflammation. So, CCl4 model was selected. We have mentioned BDL rat model in Discussion (Page 1 line 19-22).

We have added the discussion of differences between animal fibrotic models and human liver fibrosis in Discussion (Page 2 line 22, 29-30, Page 3 line 1-6, 14-16).

Comments-2. Authors should provide detailed serological data of these rats, at least information about inflammation activities, and analyze correlation between inflammatory indexes and T1rho results.
Author’s response:

Thanks for this professional question.

Before this experiment study, our group recruited 54 patients with chronic liver disease, and analyzed the relationship between liver T1rho values and laboratory indexes related to liver function and fibrosis (As shown below. These data have not been published yet.). The results showed that liver T1rho values only correlated with PCIII (rs=0.448) and IV-C (rs=0.444).

Considering the relatively low correlation between liver T1rho values and laboratory indexes, and the test results may be affected by many factors, we didn’t get serological data of these rats.

In the future, we are planning to explore the correlation between inflammatory indexes and T1rho results in a large clinical cohort.

Correlation between liver T1rho values and laboratory indexes

<table>
<thead>
<tr>
<th></th>
<th>rs</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT</td>
<td>-0.069</td>
<td>0.649</td>
</tr>
<tr>
<td>AST</td>
<td>0.065</td>
<td>0.669</td>
</tr>
<tr>
<td>ALP</td>
<td>0.231</td>
<td>0.123</td>
</tr>
<tr>
<td>GGT</td>
<td>0.248</td>
<td>0.096</td>
</tr>
<tr>
<td>TP</td>
<td>0.152</td>
<td>0.314</td>
</tr>
<tr>
<td>ALB</td>
<td>-0.134</td>
<td>0.374</td>
</tr>
<tr>
<td>A/G</td>
<td>-0.049</td>
<td>0.745</td>
</tr>
<tr>
<td>TBIL</td>
<td>-0.123</td>
<td>0.415</td>
</tr>
<tr>
<td>D-BIL</td>
<td>0.122</td>
<td>0.430</td>
</tr>
<tr>
<td>I-BIL</td>
<td>-0.180</td>
<td>0.243</td>
</tr>
<tr>
<td>PCIII</td>
<td>0.448</td>
<td>0.019*</td>
</tr>
<tr>
<td>IV-C</td>
<td>0.444</td>
<td>0.020*</td>
</tr>
<tr>
<td>HA</td>
<td>0.273</td>
<td>0.169</td>
</tr>
<tr>
<td>LN</td>
<td>0.099</td>
<td>0.624</td>
</tr>
</tbody>
</table>
Comments-3. Find better staining pictures.

Author’s response:

Thanks for your kind suggestion. We have re-stained these specimens, and shown the new pictures (Figure 4, 5).

Comments-4. Figure 1 contains 110 rats.

Author’s response:

Thanks for your careful check. The pathology results demonstrated that a small number rats showed fibrosis in stage 1 to 3. As a result, 10 rats were supplemented into the fibrosis group and sacrificed at week 2 after the injection of CCl4. In total, 110 rats were used in this study. This has been mentioned in Results (Page 1 line 3-5).

We have changed the total number of rats in figure 1 to 110.

Comments-5. Typing errors.

Author’s response:

Thanks for your careful check. We have modified all typing errors.

Dear editors and reviewers,

We would like to express our most sincere gratitude for the carefully, patiently and constructive comments. They are extremely helpful for our work. We have made substantial revisions according to these comments. We hope the above responses can address all of your questions properly. If you have any further questions, please do not hesitate to contact us.

Again, thank you very much and looking forward to hearing from you soon!

Sincerely yours,

Wen Shen, MD, PhD, Professor

No.24 Fu Kang Road,
Nan Kai District,
Tianjin, China, 300192
Tel: +8615900209418
Fax: +86-022-23626501
Email: shenwen66happy@126.com