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Name of Journal: *World Journal of Gastroenterology*

Ms: wjg/2012/032558

Title: Diammonium glycyrrhizinate reduces portal hypertension in isolated portal perfused rat livers with chronic hepatitis

Reviewer code: 0006937

Science editor: Nan - Nancy

Date sent for review: 2012-03-19 13:00:08.0

Date reviewed: 2012-03-31 00:11:20.0

CLASSIFICATION	LANGUAGE EVALUATION	RECOMMENDATION	CONCLUSION
<input type="checkbox"/> Grade A (Excellent)	<input type="checkbox"/> Grade A: priority publishir	Google Search:	<input checked="" type="checkbox"/> Accept
<input type="checkbox"/> Grade B (Very good)	<input checked="" type="checkbox"/> Grade B: minor language polishing	<input type="checkbox"/> Existed	<input type="checkbox"/> Reject
<input checked="" type="checkbox"/> Grade C (Good)		<input checked="" type="checkbox"/> No records	<input type="checkbox"/> Withdraw
<input type="checkbox"/> Grade D (Fair)	<input type="checkbox"/> Grade C: a great deal of language polishing	WJG Search:	
<input type="checkbox"/> Grade E (Poor)	<input type="checkbox"/> Grade D: rejected	<input type="checkbox"/> Existed	
		<input checked="" type="checkbox"/> No records	

COMMENTS

CONFIDENTIAL COMMENTS TO EDITOR:

The aim of this study is to investigate the effect of diammonium glycyrrhizinate (DG) on portal hypertension portal hypertension of rat with carbon tetrachloride (CCl₄)-induced chronic hepatitis demonstrated for 84 days by measurements of the ascetic volume, serum biomarkers, portal pressure in vivo, splenic index, pathological changes and deposited collagen in liver. The expression of inducible nitric oxide synthase (iNOS) in liver was assessed by immunohistochemistry and quantified by morphometry. The isolated portal perfused rat liver (IPPRL) was performed at d0, d28, d56, and d84 in the progress of chronic hepatitis. After constriction with phenylephrine, the cumulative geometric concentrations of DG were added into the recirculating perfusate. The iNOS in portal triads was regressed with the area under the curve (AUC) of DG to relax portal vein. The results show that the EC₅₀ values of DG were positively correlated with the durations of chronic hepatitis. More macrophages infiltrated into the portal triads and expressed more iNOS as advanced portal hypertension. The AUC of DG was positively correlated with the amount of iNOS in portal triads. DG indirectly reduced portal hypertension in IPPRL with chronic hepatitis. The possible mechanisms related to the inhibition of peroxonitrite released from macrophages next to terminal portal venules.

Comments:

This is a very interesting experimental study conducted in an animal model of (CCl₄)-induced chronic hepatitis. However, the data is not very conclusive for the purpose the experiment it is



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designed for. The authors agree that the effects they have demonstrated in this model is indirect. The manuscript will need to be revised from the current version for English Editing and for clear representation of Figures 2 and 4. Figure 3 will look better if quantitation of pathological data are separated from the figure itself.

Recommendation.

Resubmission with Edited version for additional review.

Name of Journal: *World Journal of Gastroenterology*

Ms: wjg/2012/032558

Title: Diammonium glycyrrhizinate reduces portal hypertension in isolated portal perfused rat livers with chronic hepatitis

Reviewer code: 0052966

Science editor: Nan - Nancy

Date sent for review: 2012-03-19 13:00:08.0

Date reviewed: 2012-03-27 00:56:17.0

CLASSIFICATION	LANGUAGE EVALUATION	RECOMMENDATION	CONCLUSION
<input type="checkbox"/> Grade A (Excellent)	<input type="checkbox"/> Grade A: priority publishir	Google Search:	<input checked="" type="checkbox"/> Accept
<input type="checkbox"/> Grade B (Very good)	<input checked="" type="checkbox"/> Grade B: minor language polishing	<input type="checkbox"/> Existed	<input type="checkbox"/> Reject
<input checked="" type="checkbox"/> Grade C (Good)		<input checked="" type="checkbox"/> No records	<input type="checkbox"/> Withdraw
<input type="checkbox"/> Grade D (Fair)	<input type="checkbox"/> Grade C: a great deal of language polishing	WJG Search:	
<input type="checkbox"/> Grade E (Poor)	<input type="checkbox"/> Grade D: rejected	<input type="checkbox"/> Existed	
		<input checked="" type="checkbox"/> No records	

COMMENTS

CONFIDENTIAL COMMENTS TO EDITOR:

Overall I think the meritorious but it should be corrected as I describe below.

General Comments

The investigators studied the affect of diammonium glycyrrhizinate (DG) on portal hypertension in rates with CCl₄ induced hepatitis. There were several findings, including: (1) The rat portal hypertensive model was demonstrated by pathological changes as noted in the study, (2) The EC₅₀ of DG at d0 was 10x at d28, d56, and d84, showing DG be more effective in portal hypertension. (3)The expression of iNOS disappeared gradually in lobules, but strengthened continuously in portal triads along with the progress. The amount of iNOS in portal triads was positively correlated with the area under the curve of DG to reduce portal hypertension. This study therefore is suggestive that DG can



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reduce portal hypertension. Such action may explain the efficacy of Chinese medical herbs in treatment of patients with chronic hepatitis. It suggests that discovery of more effective candidates targeting for macrophage-derived iNOS or NADPH oxidase in portal triads may be possible.

Overall the study is meaningful and thorough. However, I have some reservations. Most importantly, it must be corrected at some point for grammatical error. Another concern is that the origin of various equations should be explained and described. Also I am wondering about the fluctuations in some parameters in measurements from d0, to d28, d56, and d84 – the authors should provide explanation. Finally, a concern I have is how the CCL4 model in rats would relate to clinical cases of hepatitis and portal hypertension. This should be explained in more detail. I also provide in the Specific Comments section some additional suggestions.

Specific Comments

P2, p3, etc.

EC50 is not defined nor explained in the manuscript.

P10 bottom

‘(2) Cellular Quantification: The iNOS-IHC-OD per volume (Table 3) in portal triads at d28, d56 and d84 were significantly increased by 19.39%, -20.00% (decreased in fact),’

Explain why the decrease. Explain fluctuations in other parameters.

P11

‘ $y = -0.3691 + \frac{0.29847}{[1 + 10(4.75212 + 0.09841x)]}$ (R=0.9975, P<0.01),
 $y = -0.1162 + \frac{0.09902}{[1 + 10(9.064 + 0.8616x)]}$ (R=0.9942, P<0.01),
 $y = -0.03321 + \frac{0.02189}{[1 + 10(3.547 + 0.3404x)]}$ (R=0.9994 ...’

Explain the equations in more detail. Cite.

P11 bottom

‘In the present study, we used the portal hypertension model in rat with CCL4-induced chronic hepatitis to investigate ...’

How does the CCL4-induced hepatitis in the rat relate to clinical hepatitis patients with portal hypertension? What correlations are there in the literature?



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Name of Journal: *World Journal of Gastroenterology*

Ms: wjg/2012/032558

Title: Diammonium glycyrrhizinate reduces portal hypertension in isolated portal perfused rat livers with chronic hepatitis

Reviewer code: 0034696

Science editor: Nan - Nancy

Date sent for review: 2012-03-19 13:00:08.0

Date reviewed: 2012-04-16 13:14:42.0

CLASSIFICATION	LANGUAGE EVALUATION	RECOMMENDATION	CONCLUSION
<input type="checkbox"/> Grade A (Excellent)	<input type="checkbox"/> Grade A: priority publishir	Google Search:	<input checked="" type="checkbox"/> Accept
<input type="checkbox"/> Grade B (Very good)	<input checked="" type="checkbox"/> Grade B: minor language polishing	<input type="checkbox"/> Existed	<input type="checkbox"/> Reject
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<input type="checkbox"/> Grade D (Fair)	<input type="checkbox"/> Grade C: a great deal of language polishing	WJG Search:	
<input type="checkbox"/> Grade E (Poor)	<input type="checkbox"/> Grade D: rejected	<input type="checkbox"/> Existed	
		<input checked="" type="checkbox"/> No records	

COMMENTS

CONFIDENTIAL COMMENTS TO EDITOR:

I recommend this paper requires major revise.

In this manuscript, the authors examined whether diammonium glycyrrhizinate reduces portal hypertension and found that indirectly reduced portal hypertension. They suggested that the possible mechanisms related to the inhibition of peroxynitrite released from macrophages next to terminal portal venules. This manuscript is potentially interesting. However, the authors should perform following studies in order to address these issues and increase the impact of this manuscript.

Introduction

Is ref 10 associated with diammonium glycyrrhizinate?

Fig 1 is not necessary and should omit.

Fig 2 is not necessary and should omit too.

Results

In 3.1-3.4, authors should describe the interpretation of these results.

In figure 3, authors should precisely determine which cells were stellate cells or macrophages by immunohistochemistry. They described the "More macrophages infiltrated into the portal triads and expressed more iNOS as advanced portal hypertension" in the abstract. However, they did not show any evidence that macrophages are the main source of iNOS.



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For the analysis of glycyrrhizinate action, authors should describe in figure or table because these are main findings of this paper.

What will be happen by in vivo administration of DG after CCl₄ treatment?

Fig 4 is not necessary. This is just an imagination.