BAISHIDENG PUBLISHING GROUP INC
8226 Regency Drive, Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242 Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com http://www.wjgnet.com

ESPS PEER REVIEW REPORT

Name of journal: World Journal of Gastroenterology
ESPS manuscript NO: 14271
Title: Early activated hepatic stellate cell-derived molecules reverse acute hepatic injury
Reviewer code: 02527554
Science editor: Su-Xin Gou
Date sent for review: 2014-09-29 15:50
Date reviewed: 2014-10-15 10:32

CLASSIFICATION	LANGUAGE EVALUATION	RECOMMENDATION	CONCLUSION
[ ] Grade A: Excellent	[ ] Grade A: Priority publishing	Google Search:	[ ] Accept
[ ] Grade B: Very good	[Y] Grade B: Minor language polishing	[ ] Existing	[ ] High priority for publication
[ ] Grade C: Good	[ ] Grade C: A great deal of language polishing	[ ] No records	
[Y] Grade D: Fair		BPG Search:	[ ] Rejection
[ ] Grade E: Poor	[ ] Grade D: Rejected	[ ] Existing	[Y] Minor revision
		[ ] No records	[ ] Major revision

COMMENTS TO AUTHORS

Chang et al. had set up an experiment to show that early activated Hepatic Stellate Cells had protective effect on the acute hepatic injury. Overall, the experiment design and rationale is fine, and the manuscript is well written. However, there are certain concerns needed to be considered. 1. According to the result of this study, molecules from the culture medium of HSC(5d) was able to improve the serum level of liver enzyme as well as better survival curve after ALI. This only explained that HSC had certain protective effect of hepatic injury. However, the title indicated early activated HSC could "reverse" acute hepatic injury. This might be a little bit over-reading. 2. The authors had done a nice protein assay to show different molecules existed in the culture medium between HSC(5d) and HSC(P3). However, it would be great the authors could also show the dynamic expression of these significant molecules during each passage of HSC.

BAISHIDENG PUBLISHING GROUP INC
8226 Regency Drive, Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242 Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com http://www.wjgnet.com

ESPS PEER REVIEW REPORT

Name of journal: World Journal of Gastroenterology
ESPS manuscript NO: 14271
Title: Early activated hepatic stellate cell-derived molecules reverse acute hepatic injury
Reviewer code: 00068292
Science editor: Su-Xin Gou
Date sent for review: 2014-09-29 15:50
Date reviewed: 2014-10-15 11:54

CLASSIFICATION	LANGUAGE EVALUATION	RECOMMENDATION	CONCLUSION
[ ] Grade A: Excellent	[ ] Grade A: Priority publishing	Google Search:	[ ] Accept
[ ] Grade B: Very good	[ ] Grade B: Minor language polishing	[ ] Existing	[ ] High priority for publication
[ ] Grade C: Good	[Y] Grade C: A great deal of language polishing	[ ] No records	[ ] Rejection
[Y] Grade D: Fair	[ ] Grade D: Rejected	BPG Search:	[Y] Minor revision
[ ] Grade E: Poor		[ ] Existing	[ ] Major revision
		[ ] No records	

COMMENTS TO AUTHORS

This study investigated the effects of paracrine molecules secreted by hepatic stellate cells (HSCs) at different activation stages on liver function index in mice with acetaminophen-induced acute liver injury. Several comments and suggestions are listed as the following: 1. Please add page number throughout the manuscript. 2. Please write the details regarding the housing environment condition such as temperature and humidity and the purchasing company, brand name, form (chow diet or powdered diet), and the major compositions of laboratory food for mice, as well as how the surgery was conducted in the Materials and Methods section. 3. Please add the detailed information regarding the environment and medium compositions for growing HSCs in the Materials and Methods section. 4. Please write the full name of the terms demonstrated in the text in the first time before using the abbreviations, such as DMEM, FBS, and alpha-SMA in the Materials and Methods section. 5. Please define "blank conditioned medium" clearly for the control group in the Materials and Methods section. 6. Please add the methods and detailed information for Figure 3 including the vehicle in the control group to demonstrate the effects of HSC lysate on the survival rate in the Materials and Methods section. 7. Student's t-test was used for comparisons between two groups with normal distribution in this study, however, it is not suitable for the comparisons between two groups when the study design has more than 2 groups. Please consider other more appropriate statistical methods (any multiple comparison tests for normal distribution) for multiple comparisons after one-way analysis of variance. 8. The authors has demonstrated "A P-value less than 0.05 was

considered statistically significant", however, P<0.24 and P<0.37 showed in the Results section should not be statistically significant. 9. Please add body weight and food intake data for mice in 3 groups in the Results section. 10. The authors determined the survival rate, the activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), and pathological observations for liver necrosis and immune cell infiltration by H&E staining to assess liver injury after administration of conditioned medium of HSCs. However, there are no any biochemical parameters for inflammation. Please add some comments in the Discussion section. 11. Please quantitate the expression level of alpha-SMA in HSCs in Figure 1. 12. The authors showed that "infusion of HSC lysate provides a trend toward increased survival in APAP-induced ALI" in Figure 3, however, the P value was more than 0.05 stated in the legend of figure 3. Please revise the statement regarding the survival did not differ significantly among the five groups or specify the exact P value to provide the evidence that there was a trend toward increased survival after infusion of HSC lysate. 13. The authors demonstrated that "HSC-CM (5d) contained significantly more MCP1, MIP-1γ, HGF, IL-10 and MMP-2, which might correlate with the greater inflammatory inhibition and therapeutic activity of HSC-CM (5d) in ALI" in the last sentence of the Results section, however, the authors did not determine any inflammatory markers in mice after the induction of acute liver injury in this study. Therefore, please revise the sentence according to the evidence of this study. 14. Some of the results were re-stated in the Discussion section, and little discussion was shown in the Discussion section. Please add more discussion regarding the comparisons between the previous and present studies as well as the possible mechanisms. 15. Please correct the grammar errors, typos, and punctuation marks, and polish the writing throughout the manuscript before further publication.

BAISHIDENG PUBLISHING GROUP INC
8226 Regency Drive, Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242 Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com http://www.wjgnet.com

ESPS PEER REVIEW REPORT

Name of journal: World Journal of Gastroenterology
ESPS manuscript NO: 14271
Title: Early activated hepatic stellate cell-derived molecules reverse acute hepatic injury
Reviewer code: 02497108
Science editor: Su-Xin Gou
Date sent for review: 2014-09-29 15:50
Date reviewed: 2014-10-15 12:56

CLASSIFICATION	LANGUAGE EVALUATION	RECOMMENDATION	CONCLUSION
[ ] Grade A: Excellent	[Y] Grade A: Priority publishing	Google Search:	[ ] Accept
[Y] Grade B: Very good	[ ] Grade B: Minor language polishing	[ ] Existing	[ ] High priority for publication
[ ] Grade C: Good	[ ] Grade C: A great deal of language polishing	[ ] No records	
[ ] Grade D: Fair		BPG Search:	[ ] Rejection
[ ] Grade E: Poor	[ ] Grade D: Rejected	[ ] Existing	[Y] Minor revision
		[ ] No records	[ ] Major revision

COMMENTS TO AUTHORS

Summary In this study, the authors designed to investigate the protective effect of activated HSC-derived molecules in the regulation of liver function against APAP-induced acute liver injury. According to the author's present results indicated that differences in morphology, phenotype, and protein expression reveal between initiated HSCs and perpetuated HSCs. Initiated HSC-derived molecules protected hepatocytes against cell death and increased the survival rate of mice exposed to APAP-induced acute liver injury. Over all, the results are presented clear. In addition, the conclusion is supported by the results. Some minor comments are suggested for the manuscript. Comments 1. The dose of cells administered was 2 × 106 per subject. The majority of experiments were performed with the optimal cell mass of 2 × 106 cells. Please provide additional information for the selection of dosage. 2. Acute liver injury was induced by intraperitoneal injection of APAP in phosphate-buffered saline (PBS) at the dose of 750 mg/kg. How to make sure the procedure is successful or not. 3. Initiated HSCs attenuate acute hepatocyte injury; however, perpetuated HSCs may induce in hepatic fibrosis. Please provide the additional information.

BAISHIDENG PUBLISHING GROUP INC
8226 Regency Drive, Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242 Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com http://www.wjgnet.com

ESPS PEER REVIEW REPORT

Name of journal: World Journal of Gastroenterology
ESPS manuscript NO: 14271
Title: Early activated hepatic stellate cell-derived molecules reverse acute hepatic injury
Reviewer code: 02539179
Science editor: Su-Xin Gou
Date sent for review: 2014-09-29 15:50
Date reviewed: 2014-10-06 01:45

CLASSIFICATION	LANGUAGE EVALUATION	RECOMMENDATION	CONCLUSION
[ ] Grade A: Excellent	[ ] Grade A: Priority publishing	Google Search:	[ ] Accept
[ ] Grade B: Very good	[Y] Grade B: Minor language polishing	[ ] Existing	[ ] High priority for publication
[Y] Grade C: Good	[ ] Grade C: A great deal of language polishing	[ ] No records	
[ ] Grade D: Fair	[ ] Grade D: Rejected	BPG Search:	[ ] Rejection
[ ] Grade E: Poor		[ ] Existing	[ ] Minor revision
		[ ] No records	[Y] Major revision

COMMENTS TO AUTHORS

Major criticisms: 1. The morphologic and phenotypic changes of hepatic stellate cells activation are well-known and the data are not closely relevant to the aim of the study. 2. The number of HSCs that were transplanted to the acute liver injured (ALI) mice, and the source, vehicle, concentration of cellular lysates of HSCs that were injected to the ALI mice must be introduced in detail. The protein concentration and the concentration of specific growth factors, such as HGF in the conditioned medium of HSCs should be detected or verified. 3. The source of HSCs were from mice, the protein array system was however for detecting human cytokines, as it was mentioned in the methodology. 4. There are differences between HSC-CM (condition medium) and “paracrine molecules”, the term shouldn’t confuse each other throughout the paper.

BAISHIDENG PUBLISHING GROUP INC
8226 Regency Drive, Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242 Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com http://www.wjgnet.com

ESPS PEER REVIEW REPORT

Name of journal: World Journal of Gastroenterology
ESPS manuscript NO: 14271
Title: Early activated hepatic stellate cell-derived molecules reverse acute hepatic injury
Reviewer code: 02860670
Science editor: Su-Xin Gou
Date sent for review: 2014-09-29 15:50
Date reviewed: 2014-10-06 18:22

CLASSIFICATION	LANGUAGE EVALUATION	RECOMMENDATION	CONCLUSION
[ ] Grade A: Excellent	[ ] Grade A: Priority publishing	Google Search:	[ ] Accept
[ ] Grade B: Very good	[Y] Grade B: Minor language polishing	[ ] Existing	[ ] High priority for publication
[Y] Grade C: Good	[ ] Grade C: A great deal of language polishing	[ ] No records	
[ ] Grade D: Fair	[ ] Grade D: Rejected	BPG Search:	[ ] Rejection
[ ] Grade E: Poor		[ ] Existing	[ ] Minor revision
		[ ] No records	[Y] Major revision

COMMENTS TO AUTHORS

In this interesting article Chang and colleagues tested the capability of hepatic stellate cells (HSC) to prevent or ameliorate hepatic necrosis process after APAP-induced acute liver injury(ALI) in vivo. In particular the authors compared cells lysates from HSC coltured for 5 days after isolation (HSC5d) versus activated HSC, after passage 3 (HSC3p). The injection of these cells lysates in mice influenced hepatic regeneration after ALI. In particular freshly HSC prevented hepatocytes necrosis, while no protection was observed in control group or activated HSC injected mice. Furthermore the Authors performed a protein array to evaluate any difference between lysate form HSC 5d and HSC 3p, founding that only 7 proteins are different. In particular HSC 5d produce more MCP-1, mmp2, IL-10, MIP-1g and HGF while HSC 3p produced more Fas-ligand and SCF. The work is very interesting and the observation of Chang et colleagues are intriguing, but in order to have a more complete work the authors should answer the following points. The experiments performed by Chang and colleagues are very interesting and point the attention of different effect of HSC at different stage of differentiation; would be interesting to test the effect of freshly isolated HSC lysates in their model. It is well known that the culture of primary HSC on plastic support it's per se a stimulus able to differentiate them to activated HSC or myofibroblasts; so 5 days could be a transition point in the HSC differentiation, using freshly isolated HSC could be used as "basal level". As reported by figure 2 HSC5d treated mice start die after 24 h and continued till 72 h, but the authors reported hepatocytes necrotic analyses only at 24h, could be interesting to evaluate immuohistochemistry

analyses of mice at 48, 72 and 96h to have a better understanding of the entire process. The Authors reported no inflammatory infiltrate in liver of mice treated with HSC5d lysate at 24h, have the Authors evaluate infiltrate at different time point such as 48, 72 or 96? The HSC5d could delayed the recruitment? Would be very interesting if Authors evaluated the role of one or all the factors in protective effects of HSC5d lysate, because this paper reported interesting observation but no mechanisms. In particular, its curious that HSC5d produced more MCP-1 and MIP-1g and this resulted in less leukocytes recruitment. Furthermore, Would be interesting to evaluate what will happen if the Authors treated the HSC5d lysate with specific neutrolizing antibodies to MCP-1 or MIP-1g prior the administration to mice. Minor points References are quite old Legend of figure 5 reported "HSC-CM (5d) treatment improves hepatocellular necrosis and immune cell infiltration in APAP-injured liver tissue" while the figure clearly showed a decreased necrosis.

BAISHIDENG PUBLISHING GROUP INC
8226 Regency Drive, Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242 Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com http://www.wjgnet.com

ESPS PEER REVIEW REPORT

Name of journal: World Journal of Gastroenterology
ESPS manuscript NO: 14271
Title: Early activated hepatic stellate cell-derived molecules reverse acute hepatic injury
Reviewer code: 02461636
Science editor: Su-Xin Gou
Date sent for review: 2014-09-29 15:50
Date reviewed: 2014-10-13 11:05

CLASSIFICATION	LANGUAGE EVALUATION	RECOMMENDATION	CONCLUSION
[ ] Grade A: Excellent	[Y] Grade A: Priority publishing	Google Search:	[ ] Accept
[Y] Grade B: Very good	[ ] Grade B: Minor language polishing	[ ] Existing	[ ] High priority for publication
[ ] Grade C: Good	[ ] Grade C: A great deal of language polishing	[ ] No records	
[ ] Grade D: Fair		BPG Search:	[ ] Rejection
[ ] Grade E: Poor	[ ] Grade D: Rejected	[ ] Existing	[Y] Minor revision
		[ ] No records	[ ] Major revision

COMMENTS TO AUTHORS

The work entitled "Early activated hepatic stellate cell-derived molecules reverse acute hepatic injury" by Chang et al. describes the protective effect of conditioned medium from early activated HSCs on hepatocytes in injured liver from acetaminophen injury. The work is novel and not described before. The findings point towards an in vivo protective component of HSCs during early activation. There are only few points of concern that I have for this work: Major concern: The paper would have much more value if the authors were able to provide further mechanistic insight into the role of HSC conditioned medium on hepatocyte growth in vitro. The group has shown that conditioned medium from day 5 HSCs can release growth promoting factors in vivo that could slow down liver injury. One of the factors shown was HGF. If the authors could show in a co-culture system that conditioned medium from day 5 HSCs leads to growth of hepatocytes in culture, that would really add more novelty to the work. I think co-culture systems are possible to do easily and growth could be checked by bromodeoxyuridine assays (BrDU) or any other assay such as tritiated thymidine assays. After they check growth promoting phenotypes, this co-culture system could allow them to further check what pathways in hepatocytes are induced by culturing them with HSCs and this could become a subject of future study. Minor changes: Label for figure 1 says MSA instead of SMA Figure 4B: Statistical analysis of number of experiments/ standard deviation not provided for the graph.

BAISHIDENG PUBLISHING GROUP INC
8226 Regency Drive, Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242 Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com http://www.wjgnet.com

ESPS PEER REVIEW REPORT

Name of journal: World Journal of Gastroenterology
ESPS manuscript NO: 14271
Title: Early activated hepatic stellate cell-derived molecules reverse acute hepatic injury
Reviewer code: 01805584
Science editor: Su-Xin Gou
Date sent for review: 2014-09-29 15:50
Date reviewed: 2014-10-11 19:56

CLASSIFICATION	LANGUAGE EVALUATION	RECOMMENDATION	CONCLUSION
[ ] Grade A: Excellent	[ ] Grade A: Priority publishing	Google Search:	[ ] Accept
[ ] Grade B: Very good	[ ] Grade B: Minor language polishing	[ ] Existing	[ ] High priority for publication
[Y] Grade C: Good	[Y] Grade C: A great deal of language polishing	[ ] No records	
[ ] Grade D: Fair	[ ] Grade D: Rejected	BPG Search:	[ ] Rejection
[ ] Grade E: Poor		[ ] Existing	[ ] Minor revision
		[ ] No records	[Y] Major revision

COMMENTS TO AUTHORS

The manuscript of 'Early activated hepatic stellate cell-derived molecules reverse acute hepatic injury' investigates the protective effect, and also the underlying mechanisms, of early activated hepatic stellate cells (HSCs) against APAP-induced acute liver injury in C57BL/6J mice. According to the experimental observations, the culture supernatant of early activated HSCs (HSC (5d)), rather than that of permanent activated HSCs (HSCs (P3)), is sufficient to improve the aminotransferase levels and pathological disorders (hepatocellular necrosis, immune cell infiltration) by tail vein injection. Protein array further uncovers the significant difference in chemokines and growth factors, including MCP1, MIP-1γ, HGF, IL-10, MMP-2, and SCF, between culture supernatants of HSC (5d) and HSCs (P3). In contrast to its fibrosis-inducing effect in chronic liver injury, HSC is then suggested to play the beneficial role in, at least a certain kind of, acute liver injury. Major comments 1. The effect of early activated HSCs against acute liver injury has been tested, and confirmed, in the APAP-induced animal model. Unfortunately, these obtained evidences are relative weak to the reviewer. Firstly, only one dose of culture supernatant is employed in this study. Does it work in a dose-dependent manner? Moreover, the therapeutic components of culture supernatant has not been extracted and subjected to identification. It's familiar to us that the secretion of chemokines and growth factors, which are suggested to serve as the therapeutic components, may be affected by the viability of HSCs. This problem can even fail the repetition of experiment. Even if the protective effect of early activated HSCs is mediated by the differentially secreted chemokines and growth factors, more than 6

candidates still remain a problem. Do all chemokines and growth factors exert the beneficial action? As to the reviewer, HGF and IL-10 seem to contribute to the therapeutic potential of early activated HSCs. Verification, however, is still needed by in vitro study. 2. Is there anyone who has ever used the methods concerning about HSC-CM and in vivo injection? Please list their articles in the reference. According to the knowledge of reviewer, HSC-CM should be processed by 0.22 μm filtration to avoid the bacterial contamination. Injection of HSC-CM without filtration probably leads to infection and sepsis. Minor comments 1. The title of manuscript seems to be confusing and ambiguous to the reviewer. Alternatively, another concise and plain one is suggested. 2. There are limited mice (four animals) in each group. Taken into account of the death rate (as shown in Fig 4), the statistical analysis may not be persuasive. 3. There are lots of grammar mistakes and informal English in the text, such as 'Initiation HSCs and perpetuation HSCs were observed by microscope' (page 2) and 'Our goal was to determine if HSCs at different activation stages had different effects on APAP-induced ALI'. Improvement by native speaker may be a best solution. 4. The Abstract of text provides reviewer with little information. For example, 'Different morphologies and phenotypes were observed between initiation HSCs and perpetuation HSCs'. Furthermore, the conclusion of Abstract is redundant.

BAISHIDENG PUBLISHING GROUP INC
8226 Regency Drive, Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242 Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com http://www.wjgnet.com

ESPS PEER REVIEW REPORT

Name of journal: World Journal of Gastroenterology
ESPS manuscript NO: 14271
Title: Early activated hepatic stellate cell-derived molecules reverse acute hepatic injury
Reviewer code: 02439215
Science editor: Su-Xin Gou
Date sent for review: 2014-09-29 15:50
Date reviewed: 2014-10-08 22:06

CLASSIFICATION	LANGUAGE EVALUATION	RECOMMENDATION	CONCLUSION
[ ] Grade A: Excellent	[ ] Grade A: Priority publishing	Google Search:	[Y] Accept
[Y] Grade B: Very good	[Y] Grade B: Minor language polishing	[ ] Existing	[ ] High priority for publication
[ ] Grade C: Good	[ ] Grade C: A great deal of language polishing	[ ] No records	
[ ] Grade D: Fair	[ ] Grade D: Rejected	BPG Search:	[ ] Rejection
[ ] Grade E: Poor		[ ] Existing	[ ] Minor revision
		[ ] No records	[ ] Major revision

COMMENTS TO AUTHORS

1, Experiments must have been conducted with appropriate replication, but the author did not indicate that the experiments were repeated and got similar results. 2, What does "At 5 days" In the first paragraph of RESULT mean? 3, There is no "1" or "2" in the fig. 5 ("1: centrilobular vein, 2: portal vein" in the fig legend). 4, Figure 1-"A:HSC(24h)", "B:HSC(5d)" and "C:HSC(p3)" should be changed to "A: HSC (24h)", "B: HSC (5d)" and "C:HSC (p3)". 5, Figure 3- "HSC(5d)" and "HSC(p3)" should be changed to "HSC (5d)" and "HSC (p3)". 6, Figure 5- "HSC -CM(5d)" and "HSC -CM(p3)" should be changed to "HSC-CM (5d)" and "HSC-CM (p3)".