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Fax: +1-925-223-8243
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PEER-REVIEW REPORT

Name of journal: World Journal of Gastroenterology
Manuscript NO: 36223
Title: β-arrestin 2 attenuates lipopolysaccharide-induced liver injury via inhibition of TLR4/NF-κB signaling pathway mediated inflammation in mice
Reviewer's code: 02791367
Reviewer's country: Reviewer_Country
Science editor: Ke Chen
Date sent for review: 2017-09-12
Date reviewed: 2017-09-18
Review time: 6 Days

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

COMMENTS TO AUTHORS

This paper shows that β-arrestin 2 protects against LPS-induced hepatocyte injury and hepatic inflammation via inactivation of TLR4 and NF-κB pathways. Major comments: 1. Authors have used very high dose of LPS (5mg/kg). Can they justify the use of that high dose of LPS. 2. Does β-arrestin 2 directly protects hepatocytes from LPS-induced cell death, like inhibiting hepatocyte apoptosis or favoring hepatocyte proliferation? It is possible that absence of β-arrestin 2 impairs hepatocyte proliferation and therefore facilitates injury. 3. Fig 1: Authors have shown hepatocyte apoptosis by histological scoring. Staining for TUNEL or cleaved caspase 3 will be preferred to demonstrate hepatocyte apoptosis. 4. It was not clear how β-arrestin 2 protected against TLR4 signaling. Minor comment: 1. Correct the spelling for the word Expression in the legend of Figure 4. 2. In the methods authors shows that they used 3 strains of mice, β-arrestin

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2-WT, β-arrestin 2-KO and the C57BL6/J. Did they really use the C57BL/6J mice in any of these studies? Otherwise please correct it.

Dear reviewer 02791367,

Thank you for your hard work and kindly advices firstly. The followings are the answers.

(1) Major comments 1: Authors have used very high dose of LPS (5mg/kg). Can they justify the use of that high dose of LPS.

Answer：Yes. This is a wildly used dose in the literature. For example, El Kamouni S, El Kebbaj R, Andreoletti P, El Ktaibi A, Rharrassi I, Essamadi A, El Kebbaj MS, Mandard S, Latruffe N, Vamecq J, Nasser B, Cherkaoui-Malki M. Protective Effect of Argan and Olive Oils against LPS-Induced Oxidative Stress and Inflammation in Mice Livers. Int J Mol Sci. 2017 Oct 19;18(10). pii: E2181. doi: 10.3390/ijms18102181.

(2) Major comments 2: Does β-arrestin 2 directly protects hepatocytes from LPS-induced cell death, like inhibiting hepatocyte apoptosis or favoring hepatocyte proliferation? It is possible that absence of β-arrestin 2 impairs hepatocyte proliferation and therefore facilitates injury.

Answer：We have found that LPS does not induce apoptosis, injury or inflammatory factors secretion in primary hepatocytes isolated from C57BL/6J mice in a recent study not mentioned here. The original β-arrestin 2+/- heterozygous C57BL/6J mice were gifted from Dr. Robert J Lefkowitz Duke University Medical Center, Durham, NC. The absence of β-arrestin 2 has not showed impaired hepatocyte proliferation or apoptosis in the phenotype examination period when our lab firstly received the animal.

(3) Major comments 3: Fig 1: Authors have shown hepatocyte apoptosis by histological scoring. Staining for TUNEL or cleaved caspase 3 will be preferred to demonstrate hepatocyte apoptosis.

Answer：Staining for TUNEL was presented in the new figure 1 now.

(4) Major comments 4: It was not clear how β-arrestin 2 protected against TLR4 signaling.

Answer：More studies are still needed to clarify the exact mechanism and we will try to do it in the future.

(5) Minor comment 1: Correct the spelling for the word Expression in the legend of Figure 4.

Answer：Thank you! It is done.

(6) Minor comment 2: In the methods authors shows that they used 3 strains of mice, β-arrestin 2-WT, β-arrestin 2-KO and the C57BL6/J. Did they really use the C57BL/6J mice in any of these studies? Otherwise please correct it.

Answer: As a matter of fact, we only use the β-arrestin 2-WT C57BL/6J mice and

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β-arrestin 2-KO C57BL/6J mice for experiments, they are the off-springs of the β-arrestin 2+/- heterozygous C57BL/6J mice. Thank you!

Sincerely yours,
Xiuqing Wei

PEER-REVIEW REPORT

Name of journal: World Journal of Gastroenterology
Manuscript NO: 36223
Title: β-arrestin 2 attenuates lipopolysaccharide-induced liver injury via inhibition of TLR4/NF-κB signaling pathway mediated inflammation in mice
Reviewer's code: 00038362
Reviewer's country: United States
Science editor: Ke Chen
Date sent for review: 2017-09-12
Date reviewed: 2017-09-24
Review time: 12 Days

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

COMMENTS TO AUTHORS

In this article the investigators determine the influence of beta-arrestin on LPS-induced liver injury using knockout mice and in vitro using RAW264.7 cells. Both in vivo and in vitro results show that absence of beta-arrestin aggravates LPS toxicity and release of cytokines. The role of beta-arrentin in inflammation and inflammatory responses are well known. The absence of beta-arrestin is known to reduce the infiltration of immune cells into site of injury. Also, beta-arrestin null mice developed more severe arthritis.

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Therefore, this findings are not surprising. Analysis at a single time-point (4 hrs) was not justified and seems insufficient, strong mechanistic data documenting the role of TRL-4 is lacking and furthermore, the practically of inhibiting beta-arrestin as a therapeutic target must be handle with caution due to the role of this molecule in controlling the function and activity of many other signaling pathways.

Dear reviewer 00038362,

Thank you for your hard work and helpful advices firstly. The followings are the answers.

Comments: Analysis at a single time-point (4 hrs) was not justified and seems insufficient, strong mechanistic data documenting the role of TLR-4 is lacking and furthermore, the practically of inhibiting beta-arrestin as a therapeutic target must be handle with caution due to the role of this molecule in controlling the function and activity of many other signaling pathways.

Answers: We have performed the time course of LPS-induced liver injury in C57BL/6J mice and found that time-point (4 hrs) was the best observation time-point in a preliminary experiment, however the data was not shown here. We will try to do more studies to present more strong mechanistic data documenting the role of TLR-4 and the mechanism of how beta-arrestin 2 regulate TLR4/NF-kappa B pathway in the future. We will agonize beta-arrestin 2 in macrophage and Kupffer cell indirectly through agonizing some G protein coupled receptors such as GPR120, GPR43 and GPR41 which are not wildly expressed in various types of cells. Direct agonist of beta-arrestin 2 is not available now and will not be preferred since beta-arrestin 2 is universally expressed in various types of cells. However, beta-arrestin 2 can be a therapeutic target.

Sincerely yours,
Xiuqing Wei