

ESPS Peer-review Report

Name of Journal: World Journal of Gastroenterology

ESPS Manuscript NO: 3342

Title: Regulation of hepatic EAAT-2 glutamate transporter expression in human liver cholestasis

Reviewer code: 00068482

Science editor: Wen, Ling-Ling

Date sent for review: 2013-04-24 11:02

Date reviewed: 2013-05-17 23:46

CLASSIFICATION	LANGUAGE EVALUATION	RECOMMENDATION	CONCLUSION
<input type="checkbox"/> Grade A (Excellent)	<input type="checkbox"/> Grade A: Priority Publishing	Google Search:	<input type="checkbox"/> Accept
<input type="checkbox"/> Grade B (Very good)	<input type="checkbox"/> Grade B: minor language polishing	<input type="checkbox"/> Existed	<input type="checkbox"/> High priority for publication
<input type="checkbox"/> Grade C (Good)	<input type="checkbox"/> Grade C: a great deal of language polishing	<input type="checkbox"/> No records	<input type="checkbox"/> Rejection
<input type="checkbox"/> Grade D (Fair)	<input type="checkbox"/> Grade D: rejected	BPG Search:	<input type="checkbox"/> Minor revision
<input type="checkbox"/> Grade E (Poor)		<input type="checkbox"/> Existed	<input type="checkbox"/> Major revision
		<input type="checkbox"/> No records	

COMMENTS TO AUTHORS

Najimi et al. reported on the effect of PKC activation on EAAT2 localization and glutamate transport in HepG2 cells. They also studied EAAT2 localization under conditions of obstructive cholestasis in rat and human liver. Transport (substrate uptake assay), immunofluorescence, RT-PCR, and immunohistochemistry studies were performed. EAAT2 expression was found to be up-regulated in liver from rats subject to BDL and in human liver samples with chronic obstructive cholestasis. PMA treatment of HepG2 cells, leading to PKC activation, produced opposite effects. The most relevant and novel contribution of the manuscript is the finding on up-regulation of expression of EAAT2 in liver samples. Treatment with PMA of HepG2 cells failed to demonstrate the mechanism of such up-regulation. Some important issues arise: Major: 1. It is not clear whether EAAT2 is of basolateral or apical localization. In HepG cells the transporter is localized to the basolateral membrane (Fig 4), but the authors state that the background indicates apical localization. Also, what is the localization in immunohistochemistry photos (see Fig 6 and 7)? This is not clear from the images themselves, and no comment was added to the pictures on this specific point. Is EAAT2 abnormally localized to the basolateral membrane in HepG2 cells? If so, then the use of HepG2 cells is not appropriate. In addition, it is not clear how EAAT2 contributes to regulate glutamate intracellular pool and biliary excretion, and how this function depends on apical vs. basolateral localization. All these issues need to be clarified throughout the manuscript. 2. The order of presentation of the contents in the manuscript should be inverted, showing in first place the up-regulation of EAAT2 in liver samples, since this is the most relevant contribution of the manuscript. Secondly, they should present the model used to demonstrate the mechanism involved. 3. It is obvious that treatment of HepG2 cells with PMA is not the right model to explore the



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mechanism responsible for up-regulation of EAAT2 found in liver samples. The authors should discuss alternative mechanisms o better yet, try to find the right model. Incubation of HepG2 cells (expressing NTCP) with cholestatic bile salts such as chenodeoxycholic or cholic acid is surely a more appropriate model. Regarding incorporation of alternative hypothesis to discussion section to explain such up-regulation, could be oxidative stress generated under cholestatic conditions a plausible explanation? Is EAAT2 indeed regulated by oxidative stress? It is known that bile salts accumulated in cholestatic liver and oxidative stress are causally related. Minor: The English needs to be extensively revised all along the manuscript. What is the mechanism of action of PAO? The term externalization refers to insertion?

ESPS Peer-review Report

Name of Journal: World Journal of Gastroenterology

ESPS Manuscript NO: 3342

Title: Regulation of hepatic EAAT-2 glutamate transporter expression in human liver cholestasis

Reviewer code: 00037668

Science editor: Wen, Ling-Ling

Date sent for review: 2013-04-24 11:02

Date reviewed: 2013-06-06 23:35

CLASSIFICATION	LANGUAGE EVALUATION	RECOMMENDATION	CONCLUSION
<input checked="" type="checkbox"/> Grade A (Excellent)	<input checked="" type="checkbox"/> Grade A: Priority Publishing	Google Search:	<input type="checkbox"/> Accept
<input type="checkbox"/> Grade B (Very good)	<input type="checkbox"/> Grade B: minor language polishing	<input type="checkbox"/> Existed	<input type="checkbox"/> High priority for publication
<input type="checkbox"/> Grade C (Good)	<input type="checkbox"/> Grade C: a great deal of language polishing	<input type="checkbox"/> No records	<input type="checkbox"/> Rejection
<input type="checkbox"/> Grade D (Fair)	<input type="checkbox"/> Grade D: rejected	BPG Search:	<input type="checkbox"/> Minor revision
<input type="checkbox"/> Grade E (Poor)		<input type="checkbox"/> Existed	<input type="checkbox"/> Major revision
		<input type="checkbox"/> No records	

COMMENTS TO AUTHORS

This study investigates the regulation of glutamate transporter EAAT2 in liver cells under cholestasis condition. To this end, HepG2 cell culture, liver of bile duct ligated rats, and human cholestatic patients were used. Using a combination of molecular and pharmacological approaches, the authors evidenced that activation of protein kinase C signaling in HepG2 cells decreased both substrate affinity and maximal transport velocity of EAAT2 transporter. Immunofluorescence study evidenced an intracellular translocation of the EAAT2 transporter. Expression of the EAAT2 transporter was instead increased in hepatocytes of rat livers that underwent bile duct ligation, and in human liver specimens manifesting cholestasis. In human samples, the surface expression of the EAAT2 transporter appears to be particularly increased in patient with high level of gamma-glutamyl transpeptidase, as observed in biliary atresia and progressive familial intrahepatic cholestasis type 3. The conclusion of the authors is that a potential cross talk exists between glutamate transporter and bile system. The study is well planned and conducted. The appropriate controls are in place, and the conclusion of the authors is supported by the data reported here. The discrepancy between the results obtained in HepG2 cells and those reported in the other experimental models is not clear. As the authors speculate, this discrepancy may depend on the experimental conditions or models used in the study (i.e. immortalized cells vs. in vivo or ex vivo specimens). No major flaws are noticed in the study. . Minor points: 1. Is there any evidence that under cholestatic conditions or biliary atresia protein kinase C signaling is downregulated in human subjects? 2. Alternatively, could the redox state of HepG2 cells vs. that the human specimens explain the increased expression of gamma-GT and consequently the different expression of EAAT2 in the various experimental conditions?

ESPS Peer-review Report

Name of Journal: World Journal of Gastroenterology

ESPS Manuscript NO: 3342

Title: Regulation of hepatic EAAT-2 glutamate transporter expression in human liver cholestasis

Reviewer code: 02447059

Science editor: Wen, Ling-Ling

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CLASSIFICATION	LANGUAGE EVALUATION	RECOMMENDATION	CONCLUSION
<input type="checkbox"/> Grade A (Excellent)	<input type="checkbox"/> Grade A: Priority Publishing	Google Search:	<input type="checkbox"/> Accept
<input type="checkbox"/> Grade B (Very good)	<input checked="" type="checkbox"/> Grade B: minor language polishing	<input type="checkbox"/> Existed	<input checked="" type="checkbox"/> High priority for publication
<input type="checkbox"/> Grade C (Good)	<input type="checkbox"/> Grade C: a great deal of language polishing	<input type="checkbox"/> No records	<input type="checkbox"/> Rejection
<input type="checkbox"/> Grade D (Fair)	<input type="checkbox"/> Grade D: rejected	BPG Search:	<input type="checkbox"/> Minor revision
<input type="checkbox"/> Grade E (Poor)		<input type="checkbox"/> Existed	<input type="checkbox"/> Major revision
		<input type="checkbox"/> No records	

COMMENTS TO AUTHORS

The authors investigated glutamate excitatory amino-acid transporter 2 (EAAT2) expression in cholestasis. EAAT2 has been extensively investigated before in nervous tissue but this is the first report concerning human liver cell line. Congratulations for the authors for selecting such topic. They investigated the effect of PMA-induced PKC-mediated experimental cholestasis on the EAAT2 expression in HepG2 and demonstrated its down-regulation which was abolished by using PKC inhibitor. On the other hand, they demonstrated EAAT2 up-regulation in rat with ligated bile duct and in human cholestatic liver disease. The authors clearly demonstrated the alteration of glutamate handling by EAAT2 in cholestatic liver disease in humans both at the intrahepatic level as in PFIC and extrahepatic level as in BA. The study design is clear and the methodology used is appropriate and clearly described. They used different methodologies to confirm the results; substrate uptake assay, western blotting, IF, IHC and RT-PCR. Figures are clearly expressing the results in logical sequence that is in accordance with scientific thinking. The conclusion is strongly based on the obtained results from the experiments. Comments to the author: Major 1- EAAT2 expression was focal in PFIC2 (Fig 7B) and membranous in PFIC3 (Fig 7D). The authors need to explain the elevated levels of GGT in PFIC3 and its low levels in PFIC2. This point need to be elaborated. 2- What factors might affect the localization of EAAT2 in human liver cells? Please discuss this point in details. 3- Scale bar is not indicated on the photographs 4- Figure 4A, canalicular localization of MRP2, the figure is not convincing 5- Figure 7, magnification seems to be more than 200x in Fig 7C (more like 400x). please indicate expression by arrows specially in Fig 7A and 7B Minor 1- The authors used DMEM for HepG2 cell culture; was it high or low glucose medium, please indicate the composition of the DMEM, cat. Number and supplier. 2- What was the type of culture plates? Please provide type



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and provider details. 3- In immunoblot analysis, what kind of sample buffer used (reducing or non-reducing?) is the 5% SDS-gel was in-house made or ready-made? Please indicate the provider details in case of ready-made one. 4- During immunohistochemistry, what was the type of secondary antibody, please indicate provider details. 5- What was the statistical software used for data analysis? 6- English language, punctuations and typos need to be revised. 7- The authors sometimes wrote "immunohistochemistry" and others "immune-histochemistry", sometimes "immunostaining" and others "immune-staining", some times "SEM" and others "S.E.M.". Please unify.