September 18, 2017

World Journal Gastroenterology

Re: Revision Manuscript 35758

Dear Dr. Ze-Mao Gong,

Enclosed you find our revised manuscript entitled "PXR and CAR modulate differently CYP3A-mediated metabolism in early- and late-stage cholestasis", which we submit for publication in World Journal Gastroenterology.

We thank the reviewers for their constructive comments and valuable suggestions. We have revised the manuscript accordingly to their suggestions and the editors' comments and requests. Our responses to the comment are below the reviewer's individual comments.

Reviewer #1

"Data of regulation/ blocking of PXR and/or CAR should be shown. This data seems to be important for their suggestion."

We thank the reviewer for this suggestion. Discussion on published data about the effects of PXR and CAR modulation or deletion in cholestasis have been added in the text (p. 14, lines 18-25).

Reviewer #2

1. "A course of two week and four week was defined as mild and severe state of cholestasis. Although histological examination and hepatic biochemical findings revealed changes in hepatic tissues of wild cholestasis, data of common serum biochemical failed to demonstrate liver injury. Generally, 2-week of bile duct ligation was enough to cause liver injury in rodents."

As shown in Table 2, in our experimental conditions, a tendency toward an increase in liver enzymes was observed in mildly cholestatic rats (particularly evident for AST and alkaline phosphatase), but these tendencies didn't reach statistical significance, probably because of rat inter-individual variability. As clearly demonstrated by the histological analyses (Fig. 1) and discussed in the text (p. 11, lines 3-8 of the Results), fibrous septa and mild alteration of liver architecture were evident 2 weeks after bile-duct ligation.

2. "The levels of bile acids should be monitored."

The levels of bile acids in the livers of sham-operated and cholestatic rats have been measured and added to the manuscript (p. 11, last 3 lines).

3. "In addition to measurement of PXR, CAR, and CYP3A expression, parameters behind these nuclear receptors and enzymes should be examined for comparison."

In order to comply with the Reviewer's request and the suggestions of Reviewers 1 and 3, additional information about the cross-talk and modulation of NRs in the liver have now been added to the discussion (p. 15, lines 6-19). In this manuscript, we mainly focused on cholestasis-induced alterations of metabolism. Therefore, a mechanistic approach analyzing the effect of further NRs, such as the glucocorticoid receptor (GR) and the liver-specific hepatic nuclear factor 4α (HNF4α) (Stanley et al., 2006), on the expression of CAR and PXR is beyond the aims of this work.

Reviewer #3

1. "Since the focus of the paper is on drug metabolism it would be of interest to include data on the expression of CYP3A4."

In rat liver, there are five CYP3A isoforms of which CYP3A1, CYP3A2 and CYP3A18 are male dominant and CYP3A9 and CYP3A62 are female dominant (Woodland et al., 2008). In particular, CYP3A2 and CYP3A1 (whose expressions were measured in this work) are regarded as the most metabolically relevant isoforms in male rats; the former, which is male specific, is the isoform expressed at the highest constitutive level, whereas the latter is the isoform most susceptible to induction (Ghosal et al., 1996; Jan et al., 2006). CYP3A4, the main CYP3A isoform present in human liver, is not expressed in the liver of rats.

2. "Strictly speaking, the authors only demonstrated a correlation between the changes in PXR and CAR expression, not a cause effect relationship. Please change the wording in the discussion on page 12, lines 19-20."

We definitely agree with the reviewer's suggestion and modified the text accordingly (p. 14, lines 4-5).

3. "In discussing the cross-talks of NRs in the regulation of intermediate metabolism, FXR should be mentioned."

We thank the reviewer for his suggestion. A short discussion about FXR role in liver NR cross-talk has been added to the text (p. 15, lines 6-19).

4. "Discussion, last sentence: BA elimination was not a topic of this paper and the relevant transporters have not been studied. Please modify. 5. Abstract: Consider to modify the

conclusions. Promoting drug metabolism may be a target better related to the topic of this paper. There are no data in this paper on BA elimination."

We agree with the reviewer's suggestions and modified the text accordingly (Conclusions of the Abstract, last 2 lines of the Discussion).