

List of Responses

Comments 1- in MATERIALS AND METHODS: Histologic evaluation section:

a) Collection of blood and chemistry tests should not be included here.

Response: We have re-written this sentence. “Histologic evaluation” is replaced with “Biochemic and histologic evaluation”.

b) the duration is written as (48 h), should be (48 hours).

Response: We have revised this section. “48 h” is replaced with “48 hours”, and “1 h” is replaced with “1 hour”.

c) the Masson Stain could be written as (the Masson's trichrome Stain).

Response: “Masson Stain” in histologic evaluation and figure legends is replaced with “Masson's trichrome Stain”.

d) Histopathological examination: Language editing should be done for this section.

Response: We have re-written this paragraph. “The different areas for measurement of liver fibrosis, including intrahepatic bile duct and hepatic parenchyma, were compared with respect to the model duration in Figure 4. The area of hepatic fibrosis and fibrotic thickness of the bile duct increased significantly over time. The highest proportion of hepatic fibrosis (12.58%) could was found in the week 6 group, outnumbered the remaining groups ($p < 0.01$). The fibrotic thickness of the bile duct

was the highest (54.73 am) in the week 6 BDL group, compared with that of other groups ($p < 0.01$). ” is replaced with “All mice were sacrificed after the MR examination under general anesthesia to procure their livers and collect blood at defined time points, as mentioned above. Blood was collected to measure clinical chemistry parameters, including alanine aminotransferase (ALT) and aspartate transaminase (AST) levels. Liver specimens were soaked in a 4% phosphate-buffered formaldehyde solution for 48 hours, fixed using paraffin, and transversely sectioned. Liver tissue sections were soaked in a water bath and baked for 1 hour at 70 °C. The baked sections were then stained with the Masson's trichrome Stain Kit (G1006, Servicebio, Wuhan, China) and observed under an Olympus microscope (DP72, Olympus Corporation, Tokyo, Japan). The rate of liver fibrosis and fibrotic thickness of the bile duct were analyzed using ImageJ software, which was defined by the area of positive staining with blue color divided by the total area of the background.”.

Comments 2- Discussion section:

a) Pathophysiology of liver fibrosis should briefly be discussed.

Response: We have added discussion of pathophysiology of liver fibrosis in discussion section. “The BDL model has been widely used to study cholestatic liver injury and the subsequent fibrosis that follows.” is replaced with “The BDL model has been widely used to study cholestatic liver injury and the subsequent fibrosis that follows. Surgical BDL can induce the strong proliferation of bile duct cells, while the variable activation of oval cells (i.e. hepatic progenitor cells) depends on additional

liver damage, leading to extensive bile duct reactions, cholestasis, portal inflammation, and rapid establishment of bile duct fibrosis. This model was first established in rats and then successfully applied to rabbits and mice. ”

b) Other studied tools for non-invasive assessment tools of liver fibrosis in the literature should be mentioned in brief especially the radiologic tools.

Response: We thank the reviewer for invaluable suggestions. We have added discussion of non-invasive assessment tools in discussion section. “ In our study, standardized protocols with strict guidelines were followed, thereby enabling us to establish a mouse model of BDL, successfully. In addition, MRI was utilized in postoperative evaluation to noninvasively image and depict the changes in morphological and functional processes. Thus, our results revealed that the BDL mouse model combined with multiparameter MRI is an innovative way to investigate liver fibrosis, and it induces a complex cascade of changes that can be observed clearly on MRI images.” is replaced with “Non-invasive liver fibrosis evaluation, including serum tests, ultrasound elastography, and magnetic resonance elastography (MRE), overcome many limitations of liver biopsy and, therefore, are now incorporated into specialist clinical practice. These are valuable for ruling out advanced fibrosis or cirrhosis; however, each individual test cannot be fully predictive when used alone^[10]. In this study, MRI was utilized in postoperative evaluation to noninvasively image and depict the changes in morphological and functional processes. Our results revealed that the BDL mouse model combined with

multiparameter MRI is an innovative way to investigate liver fibrosis, and it induces a complex cascade of changes that can be observed clearly on MRI images.”

Comments 3- Figures. a) Is figure 1 original from this study?

Response: Yes, figure 1 is our original picture. J-Y. L took the photo during the operation, and Y-H. L made the schematic diagram.

b) in figure legends, you can use abbreviations.

Response: We have re-written the figure legends of fig. 2, “bile duct ligation” is replaced with “BDL”, and “magnetic resonance cholangiopancreatography” is replaced with “MRCP”.