Journal of Clinical and Translational Research
Peer review process file 04.201801.001

Post-hepatectomy liver regeneration in the context of bile acid homeostasis and the gut-liver signaling axis

Lianne de Haan, Sarah van der Lely, Anne-Loes K. Warps, Quincy Hofsink, Pim B. Olthof, Mark J. de Keijzer, Daniël A. Lionarons, Lionel Mendes-Dias, Bote G. Bruinsma, Korkut Uygun, Hartmut Jaeschke, Geoffrey C. Farrell, Narci Teoh, Rowan F. van Golen, Tiangang Li, Michal Heger

Corresponding authors:
Tiangang Li, Department of Pharmacology, Toxicology & Therapeutics, Kansas University Medical Center, University of Kansas, Kansas City, United States

Michal Heger, Department of Experimental Surgery, Academic Medical Center, University of Amsterdam, Amsterdam, the Netherlands

Handling editor:
Yao Liu
Membrane Biochemistry and Biophysics, Utrecht University, the Netherlands

Review timeline:

Received: 22 February, 2016
Editorial decision: 21 March, 2016
Revision received: 24 August, 2017
Editorial decision: 25 January, 2018
Revised received: 25 January, 2018
Editorial decision: 25 January, 2018
Published ahead of print: 16 February, 2018

1st Editorial decision

Date: 21-Mar-2016

Ref.: Ms. No. JCTRes-D-16-00007
Post-hepatectomy liver regeneration in the context of bile acid homeostasis and the gut-liver signaling axis
Journal of Clinical and Translational Research

Dear Dr. Heger,

Reviewers have now commented on your paper. You will see that they are advising that you revise your manuscript. If you are prepared to undertake the work required, I would be pleased to reconsider my decision.

For your guidance, reviewers' comments are appended below.

If you decide to revise the work, please submit a list of changes or a rebuttal against each point which is being raised when you resubmit your work.

Your revision is due by Apr 20, 2016.

To submit a revision, go to http://jctres.edmgr.com/ and log in as an Author.
You will see a menu item call Submission Needing Revision. You will find your submission record there.

Yours sincerely

Yao Liu
Editorial Board Member
Journal of Clinical and Translational Research

Reviewers' comments:

Reviewer #1: Authors present a review article describing the role of bile acids in liver regeneration. Authors include underlying mechanisms triggered by different cell types responsible for liver growth and how bile acid homeostasis impact on such complicated mechanisms. In addition, authors link each chapter to clinical implications in this field. Though this is an extended review and very well written, some issues should be addressed:

1) Authors describe underlying mechanisms extensively. Unfortunately, some very important things may get lost though the style and structure of the manuscript. Therefore, I would suggest including some graphic illustrations on important mechanisms, cells, receptors, etc.

2) The well written text would gain more reputation, if authors include bullet sentences or provide short summaries. Selection and to highlight references of special interest, could also help the reader to focusing on important findings and publications.

3) Authors connect basic mechanisms in each chapter to the human situation. For the reader it would become easier, if authors could summarize the main translational aspects in a separate chapter at the end. Furthermore, the reader would appreciate a summary or table including the most recent and important studies completed or recruiting patients or even planned in this field.

4) A separate short chapter including future implications at the end of the review could also be of interest.

Reviewer #2: This overview deals with the physiology of bile salts and their role in liver regeneration. It is in some areas very detailed, contains a wealth of information and is broadly referenced.

Comments
Major:
1. This review lacks illustrations. There should be a least a figure each on the "interplay of organs" (Abstract), relevant transporters in hepatocytes and intestine and the network of transcription factors regulating bile salts in hepatocytes and ileocytes. This would make the review much more comprehensible for the non-experts.

2. I suggest a table summarizing the (physiologic) effects of the different transcription factors

covered in this overview, in particular in the context of bile salts during liver regeneration.

3. The nomenclature for genes and proteins is not as recommended by the respective committees. E.g. protein names, regardless of the species are given in all caps: e.g. NTCP, rNTCP, mNTCP. Please consult the corresponding websites.

4. According to the abstract, the role of bile salts in the interplay between different organs is covered. This is not true: The authors focus (rightly) on the gut-liver axis. They should either adapt the abstract or include additional relevant organs, e.g. kidney, adrenals…

5. The authors have to make it very clear (and I suggest to arrange the overview such) when they talk about rodent species (mice and rats differ) and when they talk about humans. Currently, the species are rather mixed, even in individual paragraphs. It is also of great importance that the authors keep in mind that bile salt metabolism (phase I and II reactions) is species dependent: e.g. sulfation versus glucuronidation. This should become more clear.

6. The authors should describe the bile salt pool and its distribution in the body, which is different between different species. Alan Hofmann has published several excellent reviews where the pertinent information can be found. It should be kept in mind that hepatectomy removes part of the bile salt pool and as such diminishes the bile salt load to the remaining liver.

7. This journal is focusing on translational research. I miss a paragraph to the human relevance of the animal information presented here. Currently, there are lots of interesting concepts and novel procedures being developed aimed at improving liver regeneration in patients. This is of outmost importance in cases of liver resections due to various indications.

minor:
1. Page 4: The myth of Prometheus is nice, but I wonder whether the ancient Greek really knew about the capacity of the liver to regenerate. I suggest skipping ref. 2.

2. Ref. 34 is incomplete

3. Page 13: LCA, DCA and the others mentioned are not conjugated and hence did not undergo phase II reactions.

4. Page 14: MRP3 is not expressed at the canalicular membrane: Ref 230 uses a nonspecific antibody, ref. 231 shows no data on subcellular localization of MRP3.

5. Page 18: The role of MRP3 for intestinal bile salt efflux is not as clear as stated here. MRP3 is highly expressed in the colon. Please define eFXR.

6. Page 27: MRP2 is most likely not relevant for canalicular export of sulfated bile salts as demonstrated by the group of Sugiyama and others.

7. Page 32: OATP1A2 is not expressed in hepatocytes.

8. Page 33: MRP2 and MDR1 are not bile salt transporters in a strict sense. In fact, patients

with mutations in the gene coding for BSEP have normal MRP2 and MDR1, but nevertheless develop very early in childhood sever liver disease, which can develop into liver failure.

Authors' rebuttal

We would like to thank the reviewers for taking the time to critically appraise our manuscript. Their comments (and praise) are very much appreciated. Included below are the reviewer's comments with our point-by-point reply in red text.

It took us quite some time to implement everything to the fullest possible extent. We ask the reviewers' understanding for including hand-drawn figures in the revised version. The figures are currently being prepared by a professional illustrator who has worked with us in the past (we cannot provide examples at this point to maintain anonymity). We did this to save time in case our paper gets accepted.

Furthermore, please note that we have included 2 versions in the resubmission; one clean file and a subsequent file with tracked changes. Due to the extensive modifications, we wanted to include a clearly legible version as well as an annotated version for the reviewers to cross-check our implemented changes.

REVIEWER 1

Comments to Author:

Authors present a review article describing the role of bile acids in liver regeneration. Authors include underlying mechanisms triggered by different cell types responsible for liver growth and how bile acid homeostasis impact on such complicated mechanisms. In addition, authors link each chapter to clinical implications in this field. Though this is an extended review and very well written, some issues should be addressed:

1) Authors describe underlying mechanisms extensively. Unfortunately, some very important things may get lost though the style and structure of the manuscript. Therefore, I would suggest including some graphic illustrations on important mechanisms, cells, receptors, etc.

Authors' response: In order to make the review more comprehensible for non-experts and to create better overview, the following figures were added to the review:

Figure 1 – Changes in hepatic hemodynamics that lead to liver regeneration
Figure 2 – Intercellular signals that initiate liver regeneration
Figure 3 – Hepatocellular bile acid transporters
Figure 4 – Chronological flowchart of mitogenic signaling by bile acids Figure 5 – Hepatocyte-enterocyte interplay after PHx

Moreover, Table 1 was inserted to summarize the targets of bile acids, regulatory directionality, their downstream effectors, and their biological function.

2) The well written text would gain more reputation, if authors include bullet sentences or provide short summaries.

Authors' response: Excellent point. We have summarized key regulatory pathways in bullet point style.

Selection and to highlight references of special interest, could also help the reader to focusing on important findings and publications.

Authors' response: This goes against the copyediting style of the journal. However, because we agree with you, we have indirectly underscored the more important findings by associating the author's last name with the key finding(s).

Page 24 Huang, Ma [28], [222], and [336]
Page 29 Uriarte et al. [22], Kong et al. [257], and Padrissa-Altés et al. [23]
Page 30 [402]
Page 32 [347] and [411]
Page 33 Dai et al. [349]
Page 33 [296]
Page 34 Huang et al [29] and [350]
Page 35 [401]
Page 42 Lo Sasso et al. [293]
Page 43 Kim et al. [496]

3) Authors connect basic mechanisms in each chapter to the human situation. For the reader it would become easier, if authors could summarize the main translational aspects in a separate chapter at the end. Furthermore, the reader would appreciate a summary or table including the most recent and important studies completed or recruiting patients or even planned in this field.

Authors' response: In chapter 3 and 4, paragraphs were added that summarize the human relevance for the information presented in the corresponding sections. In these chapters, findings in rodents are connected to the human situation. The main translational aspects are also summarized in Table 1.

4) A separate short chapter including future implications at the end of the review could also be of interest.

Authors' response: At the end of the review, a paragraph was added that summarizes future implications described earlier in the review.

REVIEWER 2
Comments to Author:
This overview deals with the physiology of bile salts and their role in liver regeneration. It is in some areas very detailed, contains a wealth of information and is broadly referenced.

Comment
s Major:

1. This review lacks illustrations. There should be a least a figure each on the "interplay of organs" (Abstract), relevant transporters in hepatocytes and intestine and the network of transcription factors regulating bile salts in hepatocytes and ileocytes. This would make the review much more comprehensible for the non-experts.

Authors' response: In order to make the review more comprehensible for non-experts and to create better overview, the following figures were added to the review:

Figure 1 – Changes in hepatic hemodynamics that lead to liver regeneration
Figure 2 – Intercellular signals that initiate liver regeneration
Figure 3 – Hepatocellular bile acid transporters
Figure 4 – Chronological flowchart of mitogenic signaling by bile
acids Figure 5 – Hepatocyte-enterocyte interplay after PHx

Moreover, Table 1 was inserted to summarize the targets of bile acids, regulatory directionality, their downstream effectors, and their biological function.

2. I suggest a table summarizing the (physiologic) effects of the different transcription factors covered in this overview, in particular in the context of bile salts during liver regeneration.

Authors' response: Table 1 was added to the review and summarizes the different transcriptional factors that are discussed in the review.

3. The nomenclature for genes and proteins is not as recommended by the respective committees. E.g. protein names, regardless of the species are given in all caps: e.g. NTCP, rNTCP, mNTCP. Please consult the corresponding websites.

Authors' response: The nomenclature for genes and proteins has been updated. All protein names are now given in capitals. Gene names are written cursively and are given in capitals when it concerns the human gene and in lowercase letters for rodent gene names.

4. According to the abstract, the role of bile salts in the interplay between different organs is covered. This is not true: The authors focus (rightly) on the gut-liver axis. They should either adapt the abstract or include additional relevant organs, e.g. kidney, adrenals.

Authors' response: We agree with the reviewer that our article primarily focuses on the gut-liver axis. Although the abstract starts with the fact that liver regeneration involves multiple organs, the abstract finishes with the fact that FXR agonist modulate liver regeneration through te gutliver axis, which may benefit patients. Since our main reason for writing this article is gaining more knowledge in order to help patients that have undergone a PHx, our review focuses on the gut-liver signaling axis. The final sentence of the abstract announces that the gut-liver signaling axis will be highlighted. In order to

prevent further miscommunication towards readers, the last sentence now reads: "… in the context of bile acid homeostasis **in the liver** and gut-liver signaling axis."

5. The authors have to make it very clear (and I suggest to arrange the overview such) when they talk about rodent species (mice and rats differ) and when they talk about humans. Currently, the species are rather mixed, even in individual paragraphs. It is also of great importance that the authors keep in mind that bile salt metabolism (phase I and II reactions) is species dependent: e.g. sulfation versus glucuronidation. This should become more clear.

Authors' response: We agree that the species is not always clearly stated throughout the article. Therefore, the species were specified.

6. The authors should describe the bile salt pool and its distribution in the body, which is different between different species. Alan Hofmann has published several excellent reviews where the pertinent information can be found. It should be kept in mind that hepatectomy removes part of the bile salt pool and as such diminishes the bile salt load to the remaining liver.

Authors' response: Chapter 3 now contains a comprehensive description of the bile acid pool composition and distribution throughout the human and rodent body. Chapter 3.1.1. describes the mechanism by which bile acids are synthesized in the liver. Chapter 3.1.2. follows up with a description of the transport of bile acids in the enterohepatic circulation, including an overview of the main bile acid transporters on enterocytes and hepatocytes. Chapter 3.1.3. provides background information on the signaling molecules, including nuclear receptors, which are addressed in the subsequent sections.

7. This journal is focusing on translational research. I miss a paragraph to the human relevance of the animal information presented here. Currently, there are lots of interesting concepts and novel procedures being developed aimed at improving liver regeneration in patients. This is of outmost importance in cases of liver resections due to various indications.

Authors' response: In chapter 3 and 4, paragraphs were added that summarize the human relevance for the information presented in the corresponding sections. In these chapters, findings in rodents are connected to the human situation.

minor:

1. Page 4: The myth of Prometheus is nice, but I wonder whether the ancient Greek really knew about the capacity of the liver to regenerate. I suggest skipping ref. 2.

Authors' response: Reference 2 was skipped. We have rephrased the sentence on page 5 (former page 4) to:

'Factors that control the liver-to-body weight ratio, or the 'hepatostat,' are only partially understood [2].'

2. Ref. 34 is incomplete

Authors' response: The sentence: **"Secondly, the surgical trauma after PHx causes damaged and dying cells to leak their intracellular content"** (page 7) is now substantiated with refs 11 and 12.

3. Page 13: LCA, DCA and the others mentioned are not conjugated and hence did not undergo phase II reactions.

Authors' response: '**and phase II (conjugation) metabolism**.' has been deleted. The metabolic phases are further explained at the beginning of chapter 3

4. Page 14: MRP3 is not expressed at the canalicular membrane: Ref 230 uses a nonspecific antibody, ref. 231 shows no data on subcellular localization of MRP3.

Authors' response: Thank you for pointing out this mistake. MRP3 is expressed on the basolateral membrane of hepatocytes and not on the canalicular membrane.

5. Page 18: The role of MRP3 for intestinal bile salt efflux is not as clear as stated here. MRP3 is highly expressed in the colon. Please define eFXR.

Authors' response:

Chapter 3.2 has been rewritten in order to improve the overall structure of the article and to align this paragraph with previous paragraphs. The statements concerning MRP3 have been revised. In the newly added paragraph 3.1.3, eFXR and hFXR are described in the following sentences: " **The best studied nuclear receptor, farnesoid X receptor (FXR), is primarily expressed in the liver and intestines and, as shown in Figures 4 and 5, distinct pathways are initiated by hepatocellular FXR (hFXR) [252] and enterocytic FXR (eFXR) [253] that are involved in post-PHx liver regeneration and bile acid homeostasis [28, 254]."**

6. Page 27: MRP2 is most likely not relevant for canalicular export of sulfated bile salts as demonstrated by the group of Sugiyama and others.

Authors' response: P40 (former page 27) has been corrected to: **"… and MRP2 (canalicular export of glucuronidated bile acids [467]) …"**

7. Page 32: OATP1A2 is not expressed in hepatocytes.

Authors' response: P35 (former page 33) now reads; **"As described before, the extraction of bile acids from the portal circulation by hepatocytes is facilitated by NTCP (encoded by *SLC10A1/Slc10a1*), and OATP isoforms OATP1B1 (*SLCO1B1*), OATP1B3 (*SLCO1B3*), and OATP2B1 (*SLCO2B1*) [507-510], all located at the basolateral membrane of hepatocytes [238, 509, 510]."**

8. Page 33: MRP2 and MDR1 are not bile salt transporters in a strict sense. In fact, patients with mutations in the gene coding for BSEP have normal MRP2 and MDR1, but nevertheless develop very early in childhood sever liver disease, which can develop into liver failure.

Authors' response: The function of MRP2 and MDR3 in liver regeneration is now addressed in paragraph 3.1.2. This paragraph explains that, besides bile acids, MRP2 also mediates canalicular transport of bilirubin conjugates, glutathione and drugs. This paragraph also describes that MDR3 secretes phosphatidylcholine, the major phospholipid in bile, into the bile.

2nd Editorial decision

Date: 25-Jan-2018

Ref.: Ms. No. JCTRes-D-16-00007R1
Post-hepatectomy liver regeneration in the context of bile acid homeostasis and the gut-liver signaling axis
Journal of Clinical and Translational Research

Dear author(s),

Reviewers have submitted their critical appraisal of your paper. The reviewers' comments are appended below. Based on their comments and evaluation by the editorial board, your work was FOUND SUITABLE FOR PUBLICATION AFTER MINOR REVISION.

If you decide to revise the work, please itemize the reviewers' comments and provide a point-by-point response to every comment. An exemplary rebuttal letter can be found on at http://www.jctres.com/en/author-guidelines/ under "Manuscript preparation." Also, please use the track changes function in the original document so that the reviewers can easily verify your responses.

Your revision is due by Feb 24, 2018.

To submit a revision, go to https://jctres.editorialmanager.com/ and log in as an Author. You will see a menu item call Submission Needing Revision. You will find your submission record there.

Yours sincerely,

Yao Liu
Editorial Board Member
Journal of Clinical and Translational Research

Reviewers' comments:

Reviewer #2: The authors have adequately dealt with my comments and I have nothing to add with one exception:

The graphical abstract and figures one to three and five are hand-made. I suggest that the authors either use power point or another graphic software to provide these figures in a proper way or seek professional help. Having graphically nice figures will very much improve the impact of this review. Parenthetically, figure 5 is labelled as figure 4 in the draft.

Authors' rebuttal

The Authors' did not provide a rebuttal with their revised manuscript.

3rd Editorial decision

Date: 25-Jan-2018

Ref.: Ms. No. JCTRes-D-16-00007R2
Post-hepatectomy liver regeneration in the context of bile acid homeostasis and the gut-liver signaling axis
Journal of Clinical and Translational Research

Dear authors,

I am pleased to inform you that your manuscript has been accepted for publication in the Journal of Clinical and Translational Research.

You will receive the proofs of your article shortly, which we kindly ask you to thoroughly review for any errors.

Thank you for submitting your work to JCTR.

Kindest regards,

Yao Liu
Editorial Board Member
Journal of Clinical and Translational Research

Comments from the editors and reviewers: