Journal of Clinical and Translational Research
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Mitochondrial dysfunction as a mechanism of drug-induced hepatotoxicity: current understanding and future perspectives

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1st Editorial decision

Date: 24-Mar-2018

Ref.: Ms. No. JCTRes-D-18-00002
Mitochondrial dysfunction as a mechanism of drug-induced toxicity: current understanding and future therapies
Journal of Clinical and Translational Research

Dear authors,

Reviewers have now commented on your paper. Two reviewers have advised a reject and one reviewer, who has more than 45 years of experience in the field, is advising minor revision. I would therefore like to give you the opportunity to resubmit your work, granted that you pay particular attention to the comments of reviewers 2 and 3. 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 and one reviewer report is attached separately to this email.

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 23, 2018.

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

Do not hesitate to call on me if you require assistance.

Yours sincerely,

Michal Heger
Editor-in-Chief
Journal of Clinical and Translational Research

Reviewers' comments:

Reviewer #2: There is no doubt that mitochondrial dysfunction as a mechanism of drug-induced toxicity is well-accepted as a topic of importance and worthy of review. In this review the authors have focused on drug-induced mitochondrial dysfunction in the onset of hepatotoxicity viewed from several view-points: its role as a causative and/or signal transduction mechanism; the prospect of mechanism-based biomarkers of hepatotoxicity and future work. The authors have selected several examples of drugs/classes of drugs on which to provide an in-depth commentary of the role of mitochondrial dysfunction. However, it is this reviewer's opinion that these examples are not well selected from the field and not representative of the many mechanisms by which drugs can interact with, or induce dysfunction of, the mitochondria, or even those most associated with mitochondria dysfunction in hepatotoxicity. For example, interaction with mtDNA, fatty acid oxidation or mt protein synthesis. In addition, in places, important details on the compound-specific mitochondrial mechanism of action are missed. Please refer to more detailed comments below.

Title: The title is not representative of the content of the review. In particular it does not clearly state that the review is specifically focused upon hepatotoxicity. In addition "future therapies" is not clearly defined. Does this mean future therapies which may induce dysfunction or could be used to mitigate dysfunction? In either case this reviewer could not find an area of the text which can be matched to this section of the title.
Acetaminophen: This section is very well reviewed and covers in great detail the different pathways induce by APAP that impact on the mitochondria. This is a topic that has not been reviewed at this level elsewhere and may be more suited to a separate review, especially when combined with the biomarker section. However, throughout this section the authors do not clearly differentiate whether the presented evidence has originated from in vitro, in vivo or human studies which is important as a reader to assess the whole picture in terms of species differences or model limitations (an example of this can be found on p6, lines 7 - 26)
Diclofenac, RFD/IND, AED, Herbal: These sections appear rather brief and less detailed in comparison to the APAP section. Furthermore, much of these sections are given to describing the hepatotoxicity of the compound rather than mitochondrial mechanisms. A section of text in the diclofenac section appears speculative (pg 14 lines 41 - 58, with no references).
AED: This is a very important topic and deserving of inclusion. However, again this section is light on mitochondrial mechanistic details. For example, important several important mitochondrial targets and pathways of valproic acid have not been included (carnitine, CPT, inhibition, β-oxidation, urea cycle, mitochondrial GSH, lipid peroxidation). In addition the detail of the clear links between hepatotoxicity and mitochondrial disease (e.g Alpers disease)

are only briefly mentioned and not clearly explained.

Reviewer #4: This is a robust piece of work covering a significant part of litterature with > 200 refs. The narrative is detailed but good to follow.
Although the review is OK as it is but would perhaps even be better if one would include (all easy to do):
1. provide a table with all relevant drugs that cause hepatotoxicity due to mitochondrial injury;
2. give some info on the importance of drug-drug interaction including alcohol;
3. the importance of genetic susceptibilty;
4. mention interaction of drugs with nuclear hormone receptors (e.g. rifampicine and PXR; role of CAR) and its relevance for mitotoxicity

There is additional documentation related to this decision letter. To access the file(s), please click the link below. You may also login to the system and click the 'View Attachments' link in the Action column.

Author's rebuttal

POINT-BY-POINT RESPONSE TO REVIEWERS' COMMENTS AND SUGGESTIONS

We thank the reviewers for their comments and suggestions. We strongly feel that their efforts have helped us to improve the manuscript.

Reviewer 2:

1) Title: The title is not representative of the content of the review. In particular it does not clearly state that the review is specifically focused upon hepatotoxicity. In addition "future therapies" is not clearly defined. Does this mean future therapies which may induce dysfunction or could be used to mitigate dysfunction? In either case this reviewer could not find an area of the text which can be matched to this section of the title.

Response: *We have modified the title to read "Mitochondrial dysfunction as a mechanism of drug-induced hepatotoxicity: current understanding and future perspectives", as per the reviewer's suggestions.*

2) Acetaminophen: This section is very well reviewed and covers in great detail the different pathways induced by APAP that impact on the mitochondria. This is a topic that has not been reviewed at this level elsewhere and may be more suited to a separate review, especially when combined with the biomarker section. However, throughout this section the authors do not clearly differentiate whether the presented evidence has originated from in vitro, in vivo or human studies which is important as a reader to assess the whole picture in terms of species differences or model limitations (an example of this can be found on p6, lines 7 - 26)

Response: *Additional text differentiating in vitro or in vivo experiments has been added in the APAP section where appropriate.*

3) Diclofenac, RFD/IND, AED, Herbal: These sections appear rather brief and less detailed in comparison to the APAP section. Furthermore, much of

these sections are given to describing the hepatotoxicity of the compound rather than mitochondrial mechanisms. A section of text in the diclofenac section appears speculative (pg 14 lines 41 - 58, with no references).

Response: *The section on APAP is more detailed since more mechanistic details are known about APAP-induced hepatotoxicity when compared to the other drugs. Nevertheless, the sections on Dicolofenac, RFD/IND and AED have been extensively re-written with a focus on the mitochondrial mechanisms (Diclofenac-pages 13-16, RMP/INH-pages 18-21, AED-pages 26,27).*

4) AED: This is a very important topic and deserving of inclusion. However, again this section is light on mitochondrial mechanistic details. For example, important several important mitochondrial targets and pathways of valproic acid have not been included (carnitine, CPT, inhibition, β-oxidation, urea cycle, mitochondrial GSH, lipid peroxidation). In addition the detail of the clear links between hepatotoxicity and mitochondrial disease (e.g Alpers disease) are only briefly mentioned and not clearly explained.

Response: *The AED section has been extensively re-written with a focus on the mitochondrial mechanisms and the pathways mentioned by the reviewer have been included (pages 26,27).*

Reviewer 3:

1) The basis is of the selection of the described drugs/herbs should be motivated.

Response: *The basis for selection of the described herbs has been now included with modification of the last paragraph of the introduction to the section on herbs as below: "As it is difficult to identify specific compounds in herbal preparations that cause liver damage and some of the damage is idiosyncratic, relatively few studies focus on mechanisms of liver damage caused by herbal supplements, and fewer still examine the effect of specific ingredients on organelles such as mitochondria to decipher the cause of liver injury. However, mitochondriamediated apoptosis is a major pathway implicated in liver injury caused by herbal supplements in general, and information is available on the mitotoxicity of specific constituents such as pyrrolizidine alkaloids and neo-clerodane diterpenes. Hence, we take these two types of herbal constituents as examples to dissect their role in the mitochondria-mediated apoptotic pathway"(page 33).*

2) Also other hepatotoxic drugs may elicit mitochondrial damage, as amiodarone.

Response: *A new section on amiodarone has now been added after the section on AED (pages 28, 29).*

3) The overall structure needs to be drastically improved, particularly in the Discussion section; each drug, and its effect, is described separately, but a comprehensive and summarizing overview, including a description of common mechanisms of mitochondrial stress is completely missing. See for instance Table 1 in the review of Stephan Krähenbühl in 2001.

Response: *The summary paragraph has now been better delineated and modified to include a summary of the mitochondrial effects depicted as a new Table 1 (page 40).*

4) For the description of the acetaminophen toxicity it is important to note that sensitivity to acetaminophen is species-specific, therefore studies in mice cannot be easily translated to humans.

Response: *While it is true that sensitivity to acetaminophen overdose is species specific in rodents, with rats being resistant (1), it was shown extensively that mechanisms of APAPinduced liver injury in most mouse strains and humans are very similar (2,3).*

5) Although indicated in the title, future therapies targeting the effects of drug-induced mitochondrial dysfunction in liver are hardly/not addressed.

Response: *The title has been modified as follows "Mitochondrial dysfunction as a mechanism of drug-induced hepatotoxicity: current understanding and future perspectives"*

The manuscript contains many language errors, and the punctuation is often incorrect. For example: the second sentence in the abstract: "Their central role in cellular metabolism as well as abundant numbers in hepatocyte makes them important targets for drug-induced hepatotoxicity". This should be rewritten to: "Their central role in energy metabolism, as well as their high abundance in hepatocytes, make them important targets for drug-induced hepatotoxicity.

Response: *The abstract has been corrected as suggested.*

6) The usage of abbreviations is inconsistent (e.g. for APAP vs acetaminophen).

Response: *Acetaminophen has now been consistently abbreviated as APAP.*

References

1) McGill MR, et al. Acetaminophen-induced liver injury in rats and mice: comparison of protein adducts, mitochondrial dysfunction, and oxidative stress in the mechanism of toxicity. Toxicol Appl Pharmacol. 2012 Nov 1;264(3):387-94.

2) McGill MR, et al. The mechanism underlying acetaminophen-induced hepatotoxicity in humans and mice involves mitochondrial damage and nuclear DNA fragmentation. J Clin Invest. 2012 Apr;122(4):1574-83.

3) Xie Y, et al. Mechanisms of acetaminophen-induced cell death in primary human hepatocytes. Toxicol Appl Pharmacol. 2014 Sep 15;279(3):266-74.

Reviewer 4:

1) Provide a table with all relevant drugs that cause hepatotoxicity due to mitochondrial injury;

Response: *A new table 1 has been included describing mitochondrial effects of the discussed drugs.*

2) Give some info on the importance of drug-drug interaction including alcohol;

Response: *A new section on additional factors influencing the effects of drugs on mitochondrial function has been added to address this (page 30).*

3) The importance of genetic susceptibility;

Response: *A new section on additional factors influencing the effects of drugs on mitochondrial function has been added to address this (page 30).*

4. Mention interaction of drugs with nuclear hormone receptors (e.g. rifampicine and PXR; role of CAR) and its relevance for mitotoxicity

Response: *A new section on the role of nuclear hormone receptors has been added in the rifampicin section as below:*

"***Role of nuclear hormone receptors:*** *RMP is a well-known ligand for nuclear receptors such as the pregnane X receptor (PXR), and high dose RMP has been shown to stimulate nuclear translocation of mPXR in the liver of mice by indirect activation, resulting in the transactivation of Cyp3a11 and other PXR-target genes [141]. In addition, hepatic PXR was rapidly activated in rifampiin treated mice, with transcriptional upregulation of the PXR target, PPARγ, ultimately resulting in increased uptake of fatty acids from circulation into the liver [142]. However, while RMP activation of PXR and its effect on cytochrome P450, glucuronosyltransferases and pglycoprotein activities to modulate metabolism of other drugs is well known [143], mitochondrial targets subsequent to PXR activation in the context of*

RMP hepatotoxicity have not been extensively studied. Enzymatic analysis of CYP3As and CYP2B in mitochondria from pig liver however, showed upregulation in response to rifampicin [144] (page 20).

2nd Editorial decision

Date: 18-May-2018

Ref.: Ms. No. JCTRes-D-18-00002R1
Mitochondrial dysfunction as a mechanism of drug-induced hepatotoxicity: current understanding and future perspectives
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 Jun 17, 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,

Michal Heger
Editor-in-Chief
Journal of Clinical and Translational Research

Reviewers' comments: see attached

There is additional documentation related to this decision letter. To access the file(s), please click the link below. You may also login to the system and click the 'View Attachments' link in the Action column.

Author's Rebuttal

RESPONSE TO REVIEWERS' COMMENTS AND SUGGESTIONS

We thank the reviewer for his suggestions, which have helped us to improve the manuscript.

Reviewer 3: The second sentence in the abstract: "Their central role in cellular metabolism as well as abundant numbers in hepatocyte makes them important targets for drug-induced hepatotoxicity". This should be rewritten to: "Their central role in energy metabolism, as well as their high abundance in hepatocytes, make them important targets for drug-induced hepatotoxicity.

The sentence was corrected in the revision into: "Their central role in energy metabolism as well as their high abundance in hepatocytes makes them important targets for drug-induced hepatotoxicity".

The usage of abbreviations is also still inconsistent, I gave previously the example of APAP, but it also accounts to other abbreviations (e.g. NAC).

Response: *The sentence in the abstract has been corrected as suggested. N-acetylcysteine has been abbreviated (NAC) as mentioned (pages 24, 29 & 34)*

3rd Editorial decision

Date: 18-May-2018

Ref.: Ms. No. JCTRes-D-18-00002R2
Mitochondrial dysfunction as a mechanism of drug-induced hepatotoxicity: current understanding and future perspectives
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.

In the meantime, could you please provide a list of abbreviations for your manuscript? We would like to include this list in the final version.

Thank you for submitting your work to JCTR.

Kindest regards,

Michal Heger
Editor-in-Chief
Journal of Clinical and Translational Research

Comments from the editors and reviewers: