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Acetaminophen-induced apoptosis: facts versus fiction

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1st editorial response
7-Jul-2020

Ref.: Ms. No. JCTRes-D-20-00051
Acetaminophen-induced Apoptosis: Facts versus Fiction
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 Aug 06, 2020.

To submit a revision, go to https://www.editorialmanager.com/jctres/ 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:

Reviewer #1: This sweeping review presents an extensive and consistent body of peer-reviewed results forming unequivocal evidence that the mode of cell death caused by acetaminophen (APAP) is oncotic necrosis in humans, animals, and relevant in vitro models. Although apoptosis is increasingly observed with the absence of necrotic death when immortalized cells die following exposure to high concentrations of APAP, the relevance of such finding to in vivo and human exposures is nominal due to the absence of such key cellular components as CYPs and transporters. Biochemical and morphological tools used by investigators to establish mode of cell death are skillfully assessed against a backdrop of events and signaling steps that are common to or can reliably distinguish between necrosis and apoptosis. Results from these tools are placed in the context of recurring flaws in experimental design particularly involving controls, and inappropriate conclusions of cause and effect relationships solely from correlation analysis. A newly revealed molecular switch capable of governing which signaling pathway and mode of cell death occurs, and redundancy in cell death options, add valuable balance for those navigating this important but challenging research field.

The authors present a logical discussion and feasible conclusions that remain with the scope to the presented observations and are highly mechanistic. This reviewer is unaware of hidden flaws in the presented conclusions. The authors are asked to address the following.

Major Points:

1. P23, L31: It is recommended that the authors consider tempering the comment "it also continues to perpetuate poor science and misleads others."

2. P23, L56: It is recommended that the authors consider tempering the comment … "just to bulk up the paper not because they truly believe that apoptosis and not necrosis is the dominant mode …" It is not appropriate for the review authors to impute what other authors in the field believe.

3. P25, L4-5 and P25, L11-12: "The majority of papers …" and "… rarely different from earlier rejected versions …" To retain these comments, the authors of this review are asked to provide data to substantiate these accusations. While the authors are likely 100% correct given the experiences of journal editors and others, inserting it in this review without support is questionable. Without the support of data, the remarks are more well suited to an opinion piece.

Minor Points:

P6, L-48: Correction … the outer mitochondrial …
P9, L6: Grammar … which inhibit … and result ….
P9, L21: Correction … However, the probable lack …

Reviewer #2: This is an excellent review which critically discusses issue of mode of cell death during APAP-induced hepatotoxicity. It is a very important topic as increasing number of studies are being published which do not use proper study design and proper parameters to assess apoptosis and make misleading conclusions/interpretation. It is alarming considering years of careful research by experts on this topic has shown that apoptosis is not a relevant mechanism of cell death in this context. Authors have described these issues in detail with some suggestions for resolving these issues. This will be very helpful for future studies to consider for conducting sound studies on this topic. The manuscript is clearly written and easy to read. The figures and tables are also well presented for clear understanding. The manuscript appears acceptable to me in the current form and I have no further suggestions.

Author's response

POINT-BY-POINT-RESPONSE TO REVIEWERS' COMMENTS

We thank the reviewers for their positive and helpful comments, which helped to improve the manuscript. The following changes were made (highlighted in bold and underlined) in the revised manuscript.

Reviewer 1:
General Comments: This sweeping review presents an extensive and consistent body of peer-reviewed results forming unequivocal evidence that the mode of cell death caused by acetaminophen (APAP) is oncotic necrosis in humans, animals, and relevant in vitro models. Although apoptosis is increasingly observed with the absence of necrotic death when immortalized cells die following exposure to high concentrations of APAP, the relevance of such finding to in vivo and human exposures is nominal due to the absence of such key cellular components as CYPs and transporters. Biochemical and morphological tools used by investigators to establish mode of cell death are skillfully assessed against a backdrop of events and signaling steps that are common to or can reliably distinguish between necrosis and apoptosis. Results from these tools are placed in the context of recurring flaws in experimental design particularly involving controls, and inappropriate conclusions of cause and effect relationships solely from correlation analysis. A newly revealed molecular switch capable of governing which signaling pathway and mode of cell death occurs, and redundancy in cell death options, add valuable balance for those navigating this important but challenging research field.

The authors present a logical discussion and feasible conclusions that remain with the scope to the presented observations and are highly mechanistic. This reviewer is unaware of hidden flaws in the presented conclusions. The authors are asked to address the following.
Response: We thank the reviewer for the positive assessment of our review.

Specific Comments:
Comment 1: P23, L31: It is recommended that the authors consider tempering the comment "it also continues to perpetuate poor science and misleads others."
Response: As suggested we modified the sentence to: "Thus, measuring parameters such Bax and Bcl-2 mRNA or protein expression or using the TUNEL assay and concluding there is apoptosis is not only scientifically unsubstantiated and incorrect, **it also bears the risk that**

others pick up this questionable reasoning and thus perpetuate these wrong mechanistic conclusions." (p. 20)

Comment 2: P23, L56: It is recommended that the authors consider tempering the comment … "just to bulk up the paper not because they truly believe that apoptosis and not necrosis is the dominant mode …" It is not appropriate for the review authors to impute what other authors in the field believe.
Response: As suggested we modified the sentence to: "**most authors seem to include these parameters and others just to add more data without a clear and justifiable rationale. Most importantly, there is rarely if ever a discussion regarding the relative importance of apoptotic versus necrotic cell death despite the fact that contradictory evidence for both forms of cell death are presented."** (p. 21)

Comment 3: P25, L4-5 and P25, L11-12: "The majority of papers …" and "… rarely different from earlier rejected versions …" To retain these comments, the authors of this review are asked to provide data to substantiate these accusations. While the authors are likely 100% correct given the experiences of journal editors and others, inserting it in this review without support is questionable. The remarks are more well suited to an opinion piece.
Response: As suggested we modified these comments as follows: " **Based on our own experience with reviewing a combined number of more than 200 of these types of manuscripts per year, some of them multiple times from different journals, we can conclude that many papers that claim apoptotic cell death in APAP toxicity have been rejected at least once or even multiple times before they found a home. This means that the authors received comments and suggestions for improvement from multiple reviewers. Again, based on our own observations comparing the ultimately published manuscript with earlier submitted versions, which are rarely substantially different, many authors seem to ignore the comments, no matter how valid, and shop around until they find reviewers that accept their paper**." (p. 21)

Minor Points:

P6, L-48: Correction … the outer mitochondrial …
P9, L6: Grammar … which inhibit … and result ….
P9, L21: Correction … However, the probable lack …

Response: the suggestions were addressed as mentioned by the reviewer (highlighted on p. 6 and p. 37).

Reviewer 2:
General Comment: This is an excellent review which critically discusses issue of mode of cell death during APAP-induced hepatotoxicity. It is a very important topic as increasing number of studies are being published which do not use proper study design and proper parameters to assess apoptosis and make misleading conclusions/interpretation. It is alarming considering years of careful research by experts on this topic has shown that apoptosis is not a relevant mechanism of cell death in this context. Authors have described these issues in detail with some suggestions for resolving these issues. This will be very helpful for future studies to consider for conducting sound studies on this topic. The manuscript is clearly written and easy to read. The figures and tables are also well presented for clear understanding. The manuscript appears acceptable to me in the current form and I have no further suggestions.
Response: We thank the reviewer for the positive assessment of our review.

2nd editorial response
8-Jul-2020

Ref.: Ms. No. JCTRes-D-20-00051R1
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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,

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