

Extrahepatic toxicity of acetaminophen: critical evaluation of the evidence and proposed mechanisms

Stefanie Kennon-McGill, Mitchell R. McGill

Corresponding author:

Mitchel R. McGill

*Dept. of Environmental and Occupational Health, Fay W. Boozman College of Public Health,
University of Arkansas for Medical Sciences, Little Rock, AR, USA*

Handling editor:

Hartmut Jaeschke

Department of Pharmacology, Toxicology & Therapeutics, Kansas University Medical Center,
University of Kansas, Kansas City, KA, United States

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**EXTRAHEPATIC TOXICITY OF ACETAMINOPHEN: CRITICAL EVALUATION OF THE
EVIDENCE AND PROPOSED MECHANISMS**

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 Nov 24, 2017.

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,

Hartmut Jaeschke, PhD
Associate Editor
Journal of Clinical and Translational Research

Reviewers' comments:

Reviewer #1: This review on the extrahepatic effects of an acetaminophen overdose addresses a relevant issue and is well written and generally comprehensive. However, some additional focus on the nephrotoxicity of acetaminophen, especially in the context of metabolism through the cytochrome P450 system in the kidneys could be included. In this context, a few relevant reviews on the aspect of nephrotoxicity seem to have been omitted (1-4) and these could also be discussed to provide a comprehensive overview of this section, which has been the most studied among the extra-hepatic tissues affected by acetaminophen.

An additional third sub heading on the biological relevance of the proposed mechanisms and their importance in vivo could be provided for each tissue, in addition to the mention in the final conclusion.

References

1. Blantz RC. Acetaminophen: acute and chronic effects on renal function. *Am J Kidney Dis.* 1996 Jul;28(1 Suppl 1):S3-6.
2. Perneger TV, Whelton PK, Klag MJ. Risk of kidney failure associated with the use of acetaminophen, aspirin, and nonsteroidal antiinflammatory drugs. *N Engl J Med.* 1994 Dec 22;331(25):1675-9.
3. Ściskalska M, Śliwińska-Mossoń M, Podawacz M, Sajewicz W, Milnerowicz H. Mechanisms of interaction of the N-acetyl-p-aminophenol metabolites in terms of nephrotoxicity. *Drug Chem Toxicol.* 2015 Apr;38(2):121-5.
4. Bessems JG, Vermeulen NP. Paracetamol (acetaminophen)-induced toxicity: molecular and biochemical mechanisms, analogues and protective approaches. *Crit Rev Toxicol.* 2001 Jan;31(1):55-138.

Reviewer #2: In the present review, the authors critically discuss emerging extra-hepatic adverse effects of acetaminophen (APAP). While nephrotoxicity seems to be proven, hints for other side effects were mainly obtained from epidemiological human studies and molecular mechanisms are largely unknown. However, the general wording in this manuscript is that the absence of mechanistical data argues for absence of an observed extrahepatic side effects. Instead, I would favor a general wording in this article which encourages scientists to carry out mechanistical studies in humans and reliable animal models. For example, any hint for adverse effects of APAP affecting infants' health should be followed.

Regarding liver damage, the authors have nicely reviewed and discussed molecular mechanisms of hepatocellular toxicity of APAP like NAPQI formation, generation of ROS, mitochondrial dysfunction and MAP kinase activation. What is missing is the release of TLR ligands by necrotic hepatocytes and therefore induction of inflammasome activation. This component of APAP hepatotoxicity, called sterile inflammation, has been intensively studies by Kaplowitz and

Mehal and seems to amplify the initial hepatotoxic insult and to increase overall tissue injury. This topic has been nicely reviewed recently by Woolbright and Jaeschke.

Fig. 1: To my knowledge APAP does not induce apoptosis but rather necrosis of hepatocytes and therefore DNA fragmentation is not a principle damage parameter or mechanism of cell death. Rather it seems that mitochondrial DNA is released acting as TLR ligand.

*****Authors response*****

Mitchell R. McGill, PhD
Assistant Professor of Public Health
University of Arkansas for Medical Sciences
4301 W. Markham St., Slot #820
Little Rock, AR 72205
P. 501-526-6696
E. mmcgill@uams.edu

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Re: JCTRes-D17-00017

Dr. Jaeschke,

Thank you for the opportunity to revise our submission entitled "Extrahepatic toxicity of acetaminophen: critical evaluation of the evidence and proposed mechanisms." The reviewer suggestions have helped us to significantly improve the quality of the manuscript.

We have addressed the reviewer comments point-by-point on the following pages, and we have highlighted changes in the manuscript using bold letters.

We hope that you will find our responses to be satisfactory.

Thank you,

Mitch McGill

REVIEWER COMMENTS

Reviewer 1

Comment 1: [Some] additional focus on the nephrotoxicity of acetaminophen, especially in the context of metabolism through the cytochrome P450 system in the kidneys could be included. In this context, a few relevant reviews on the aspect of nephrotoxicity seem to have been omitted (1-4) and these could also be discussed to provide a comprehensive overview of this section, which has been the most studied among the extra-hepatic tissues affected by acetaminophen."

Response: We thank the reviewer for this suggestion. After reading the publications listed by the reviewer, we have determined that the critical points are: 1) There is evidence for increased prevalence of renal disease among frequent, low-dose APAP users; 2) There is evidence for non-P450-mediated bioactivation of APAP in the kidney, particularly via a deacetylase and prostaglandin synthases; 3) There is evidence for differing importance of P450-mediated metabolism in the liver and kidney. **We have heavily modified the section on nephrotoxicity to include discussion of these points and to reference the listed publications.** Please see the revised manuscript for details (pages 8-10).

Comment 2: An additional third sub heading on the biological relevance of the proposed mechanisms and their importance in vivo could be provided for each tissue, in addition to the mention in the final conclusion.

Response: **We have modified the manuscript to include a third sub-heading for each tissue/system, as suggested.** To address this and the suggestion of another reviewer simultaneously, we have titled the new sub-heading "**Biological relevance and future studies**," and we have used the space to summarize what is known concerning the human biological relevance of the relevant studies to date and to recommend future experiments. We appreciate this suggestion; we feel that it has improved readability of the manuscript. Please see the revised manuscript for details.

Reviewer 2

Comment 1: [The] general wording in this manuscript is that the absence of mechanistical data argues for absence of an observed extrahepatic side effects. Instead, I would favor a general wording in this article which encourages scientists to carry out mechanistical studies in humans and reliable animal models. For example, any hint for adverse effects of APAP affecting infants' health should be followed.

Response: We agree with the reviewer that a lack of mechanistic data does not invalidate extra-hepatic adverse effects that have been observed. **We have modified the language throughout the manuscript** to have a more positive tone and to encourage further investigation of the research areas discussed. Furthermore, to address both this comment and comment 2 of reviewer 1, **we have added a third sub-heading entitled "Biological relevance and future studies" to each section.** All changes to the text have been highlighted in bold in the revised version. We have also added the following text to the end of the Introduction section: **"In many cases, there is a paucity of mechanistic data, or the available mechanistic studies suffer from poor design. However, that does not necessarily invalidate empirical observations of adverse effects. We strongly recommend that future investigations use only reliable *in vivo* models and doses that are relevant for the human context."** (Page 4)

Comment 2: What is missing is the release of TLR ligands by necrotic hepatocytes and therefore induction of inflammasome activation. This component of APAP hepatotoxicity, called sterile inflammation, has been intensively studied by Kaplowitz and Mehal and seems to amplify the initial hepatotoxic insult and to increase overall tissue injury. This topic has been nicely reviewed recently by Woolbright and Jaeschke.

Response: We agree that this is an important point to mention. **We have added a brief discussion of the role of inflammation, inflammatory cells, the inflammasome, and DAMPs to the section on APAP-induced liver injury, including a mention of the controversy regarding those issues and references to important recent reviews. (Page 6, paragraph 3, to page 7, paragraph 1)**

Comment 3: Fig. 1: To my knowledge APAP does not induce apoptosis but rather necrosis of hepatocytes and therefore DNA fragmentation is not a principle damage parameter or mechanism of cell death. Rather it seems that mitochondrial DNA is released acting as TLR ligand.

Response: We fully agree that apoptosis is not the mode of cell death in APAP hepatotoxicity. However, nuclear DNA fragmentation is known to occur in both mice and humans after APAP overdose. This has been demonstrated in studies by Corcoran (Ray et al. Toxicol Appl Pharmacol. 1990;106:346-51), Jaeschke (Cover et al. J Pharmacol Exp Ther. 2005;315:879-87; Bajt et al. Toxicol Sci. 2006;94:217-25; McGill et al. J Clin Invest. 2012;122:1574-83), and others (Napirei et al. Hepatology. 2006;43:297-305). The difference between the DNA fragmentation that occurs in APAP-induced necrosis compared to apoptosis is that it is mediated by non-caspase-activated DNases, including Endonuclease G. Although the mechanism of nuclear DNA fragmentation is already explained in the text, we have added a statement in the section on APAP hepatotoxicity to make it more clear: **“Although nuclear DNA fragmentation is widely considered a hallmark of apoptosis, oncotic necrosis is actually the major mode of cell death in the liver after APAP overdose. Studies in both humans and mice demonstrate that apoptosis has, at most, a minor role [70-73].”** (page 6, paragraph 2)

2nd editorial decision
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Dear Authors,

Thank you thoroughly addressing the comments of the reviewers and revising the manuscript accordingly. 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.

Journal of Clinical and Translational Research
Peer review process file 03.201703.005

Thank you for submitting your work to JCTR.

Kindest regards,

Hartmut Jaeschke, PhD
Associate Editor
Journal of Clinical and Translational Research

Comments from the editors and reviewers:
