Author’s response to reviews

Title: Short-term treatment with taurolidine is associated with liver injury

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Author’s response to reviews:

Reviewers’ comments:

Reviewer #1:

The paper by Fahrner et al provides evidences for liver toxicity by Taurolidine. Results are clear, but what it is not is whether the concentration they consider toxic is the same used in the clinics. In other words, did the authors investigated a dose commonly used for therapy? This is a missing important point that authors should absolutely discuss, otherwise there’s no reason to talk about toxicity. In the pharmacokinetics of Taurolidine, what is the range of concentrations they used? Is it still far from the toxic dose-response curve?

Thank you for this valuable comment which is not easy to answer. As already mentioned in the manuscript, taurolidine has been postulated to have no severe side effects mainly in oncological animal studies, except of one animal study showing liver toxicity after long-term systemic use of taurolidine (Arlt et al 2012). Most of the clinical treatments are/were locally (peritoneal and pleural cavity, catheter-lock therapy) without measurements of systemic drug levels. There are only few clinical reports with intravenous injection of Taurolidine: e.g. in two patients taurolidine was used in case of glioblastoma by intravenous injection over weeks (cumulative dose 840 g), without any clinical relevant side effects (Stendel et al. 2004), furthermore one
patient with gastric cancer was treated with taurolidine, without reported drug toxicity, but elevated liver enzymes (Braumann et al. 2006). All case reports have in common, that there was no systematic investigation of drug toxicity, and the patients number was very small. Therefore an exact comparison regarding drug toxicity between humans, rodents and cell culture experiments is not possible.

We clarified this fact in the text (introduction (p. 4, 3rd paragraph, and discussion (p. 14, first paragraph)).

Minor comments:

English language must be revised by a native speaker.

The manuscript has been extensively revised through a native speaker (see Acknowledgement section).

Page 14, add “alpha” to TNF

We revised the text appropriately.

Fig. 2E: in the graph, please indicate the concentration of Taurolidine.

A revised graph and figure legend was included.

Reviewer #2:

The manuscript brings experimental data which may be interesting for medicinal scientific community. In addition, the presented data might be applicable to determine safety of a clinically important compound. A complex of relevant in vitro and in vivo methods was employed in the study. The manuscript is divided in standard sections and meets Journal’s requirements. However, several shortcomings can be found through the submitted manuscript.

Major comments:

1. Why did the authors use the geometric mean as statistical parameter (p.10, l.9)? The adequate method of evaluation for the performed tests should employ arithmetic means. The reviewer recommends to recalculate experimental data and use arithmetic means for
ANOVA test should be utilized for statistical evaluation of differences between the experimental groups.

Statistical analysis was recalculated as suggested. One-way-ANOVA testing was used to analyze statistical significance and the method specified in the “Method” section.

2. The results in Fig. 1, part A and part B are presented as associated continuing data. However, these data are independent, not continuing, obtained paralelly using different concentrations of taurolidine. These parts of Fig. 1 have to be changed in column graphs similarly as data presented in parts C and D of the figure

We changed the figure appropriately.

Minor comments:

1. The HepaRG cells are better to seed at a density 2.6 x 10^4 cells/cm^2. But the used amount 2.7 x 10^4 cells/cm^2 (p.6, l.61) can be acceptable even a higher number of the seeded cells can lead to stopping of their differentiation.

The given number reflects the number of cells during the initial growth phase where no differentiation is initiated. Cell differentiation is induced subsequently by addition of 2% DMSO. This is now specified in the “Methods” section.

2. The hydrocortisone-hemisuccinate concentration employed for HepaRG cell culturing (p. 7, l. 5) should be 50 μM instead of 50 mM.

Thank you for this important note. The typing error was corrected and changed to 50 μM.

3. Information on weight and age of the used rats should be added to the part "Animal preparation and experimental setting" (p. 6).

We included information for age and weight on page 6, paragraph 2.

4. Was the liver removed from anesthetized but living animals (p.6, l.34)? The presented formulation is not clear. There is no information on how and when the animals were euthanized.
We included specifications in regard to tissue collection on page 6 in the second paragraph.

5. The authors use systematically the term "Taurolidine". However, it is clear from the context that they mean compound called taurolidine. Standard generic name should be written using low initial letter. Such unclear designation can be confusing. For example, the doses of taurolidine in Methods (p.6, lines 24-28) expressed as mg/kg probably mean amount of taurolidine but not a formulation called Taurolidine. It has to be clearly explained and corrected.

Thank you for this comment. We changed the text appropriately, as “taurolidine” is a commercially available compound/drug. We included a short explanation on this topic within the “Methods” section on page 6 in the second paragraph.

6. The sentence "As previously described, cell differentiation was induced and cells used up to four weeks.” (p.7, l.14-17) is not clear. How long did the differentiation take place? When were the cells taken for experiments? These details should be clarified.

We specified the passage on page 7 in paragraph 1.

“In a humidified cell incubator at 5% CO2 and 37°C cells were cultured to grow for 14 days with renewal of the medium every three to four days. As described previously, cell differentiation was induced by addition of 2% DMSO for 14 days. Differentiated cells were used for up to four weeks.”

Reviewer #3:
The manuscript is well written, and the information is useful. I would like this paper can be published in BMC PT.

Please provide the detail exposure protocol about HepaRG cells with Tauroline treatment for 24 hours.

We included a paragraph in the “materials and methods” section (p. 8, first paragraph).

Moreover, could you get 24h LC50/LD50 of Taurolidine for HepaRG cells, which will be an useful basic toxicological data for this medication.

We performed additional experiments to answer this question and included a new supplementary Figure 1 (page 11, first paragraph) showing the LC50 of taurolidine for HepaRG cells.