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Editors
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Dear Editors,

We kindly thank you for the comments and suggestions on our manuscript and video. We went carefully through every point to adjust the changes indicated from the Editorial Board and to respond to the questions raised by the reviewers.

Regarding Editorial and Production comments, I confirm that the manuscript was carefully proofread to correct the spelling and grammatical errors and the references were edited to the correct format. Regarding the video, we edited the jump-cuts and the parts you suggested, and also tried to merge better the procedure with the written manuscript. We also reduced the volume by 3 dB, as asked.

About the reviewer's comments, we will address this question by question.

Reviewer #1:

We kindly thank for your comments on the manuscript and the video. We appreciate your enthusiasm with our work.

Major Concerns:

1. How did the authors arrive at this dosage of cisplatin?
We have added a better explanation in the introduction, as the reviewer suggested. In our lab, we decided the best cisplatin doses on studies of cisplatin-induced AKI of murine models, in which the dose was around 10 mg/Kg (equivalent to 10 µg/g). However, this dose wasn't sufficient to induce kidney damage, so we increased the dose to the ones shown in this study.

2. How is cisplatin stock stored?
In this study, we used cisplatin available in drug stores in our country (C-PLATIN, Blau Farmacêutica). This type of cisplatin is used to treat cancer patients and comes in a dark glass bottle in a dilution of 1 mg/mL in a mixture of sodium chloride, hydrochloric acid, sodium hydroxide, and water (concentrations not specified). The fabricant recommended storing the bottle at room temperature (15-30 °C), protected from light. We added this specification at the beginning of the protocol.

3. It is preferable to euthanize adult zebrafish with an overdose of MS-222. This is well established in the zebrafish field and a veterinary requirement observed by AAALAC/and research university IACUCs.

We appreciate the concern on this field, however, we based our protocols of euthanasia on the Normative Resolution N°37 of the Brazilian Government and the Guide for the Care and Use of Laboratory Animals: Eighth Edition (2011) ("The Guide"), both allows to induce euthanasia by hypothermal shock in tropical species of fishes. This protocol is also recommended and approved by the University of Oregon, where we were trained, and is based on the AVMA Guidelines on Euthanasia and the US Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training (IRAC). Further, we confirmed that the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC), mentioned by the reviewer, is based on "The Guide" that reference the same publications that have recommended hypothermal shock as euthanasia method over MS-222 in zebrafish because is less stressful, fast and consistent that the use of MS-222 (Wilson et al. 2009, *Journal of the American Association for Laboratory Animal Science*; Matthews & Varga 2012, *Institute for Laboratory Animal Research*). Because of this, we maintained this part of the protocol as we previously wrote it, although we acknowledge that the approved methods for euthanasia could be different in each country.

4. Rewrite 'with basic functional unit, genetic and developmental similar to mammals'. Suggest: 'and its basic functional unit, the nephron, is conserved with mammals'.
This was corrected in the text.

5. In the video, the researcher is shown monitoring a group of fish after the cisplatin injection. Since the survival rate is high (~30%), it is advisable for the protocol to be edited to suggest that fishes should be singly housed after the injection. This way, fishes are not in tanks with other dead fishes overnight. Furthermore, the protocol should specify how frequently the fishes are monitored (Step 1.9) in the following days. Checking 2 times a day, it would be best practice from a veterinary care perspective (e.g. in the morning and at night, approximately every 12 hours).
We thank for your comments, actually, the fishes were constantly checked the by someone in our team inside the facility where they stayed. The fishes were housed together after the cisplatin injection, fed and monitored for sings of pain or distress, or dead. If any of this happened, the technician would immediately inform to the owner of the experiment. However, we usually did not see dead fishes the first hours after injection and we effectively observed dead fishes only the next day. Nonetheless, we edited this in the video and the text to "monitor the animals twice a day" instead of "next day", as the reviewer indicated.

Minor Concerns: All the minor concerns were related to in-text corrections. We changed all suggestions as the reviewer indicated.

Reviewer #2:

We appreciate your comments and suggestions. This reviewer mentioned a minor concern about the position of the kidney in Figure 1A. We adjusted the position of the kidney and recreated the swim bladder to better understanding.

Reviewer #3:

We kindly thank your comments and suggestions.

Major Concerns:

1. The reviewer suggested a brief introduction of ways we used to assess AKI under stress of any nephrotoxic agent.
We appreciated your comments. We added some information on nephrotoxicity by chemical agents in the introduction to complement this issue.

2. The reviewer also suggests a minor revision of the introduction and the addition of flowcharts to the protocol.
Both suggestions were accepted and added to the manuscript. The flowchart was added as Figure 4.

Minor Concerns:

1. There can be many animals sharing similarities with mammals, why you have specifically selected the zebrafish?
One of the scopes of our lab is to study the regeneration properties of the zebrafish kidneys, for this, we treat them with different probiotics and microbiota-related products and analyze the kidney damage conditions. The available models in zebrafish, at the time that we started this project, were based mainly on the use of gentamycin as a nephrotoxic agent that is also an antibiotic, and this would not be compatible with our treatment (a probiotic bacteria), so we decided to establish a new model using another common nephrotoxic agent, the cisplatin. We added some of this explanation in the text.

2. What are the common routes of exposure of zebrafish to cisplatin under normal conditions?
If we understood well this question, the zebrafish is not exposed to cisplatin in "normal conditions" like in nature. Cisplatin is a controlled antineoplastic chemical that is not available in nature, so zebrafish will not be exposed normally except in a human-controlled manner.

3. Is osmoregulation in this fish common to mammals?
Freshwater fishes like the zebrafish use the kidney like the mammals to regulate osmolarity and water excretion, but also the gills, and in embryonic stages, the skin has also osmoregulatory cells to control ion transportation. It was added a brief explanation in the text.

Thus, we confirm that the manuscript has been read and corrected as the editors and reviewers suggested, as well as we checked that no plagiarism has occurred in the manuscript. We hope you find our manuscript suitable for publication and look forward to hearing from you in due course.

Sincerely,

Camila Morales Fénero
Corresponding Author