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Dear Editor-in-Chief: Masatoshi Kudo, MD, PhD, Professor,

We would like to thank you and your reviewer for considering our manuscript (ESPS Manuscript NO: 2443) for publication in the *World Journal of Hepatology*. We are honored that both you and your reviewers feel that there is some importance to this study. Below we have addressed, on a point by point basis, all of the comments made by the reviewers. All changes have been made in our updated submission. Changes to the original manuscript appear in red. We eagerly look forward to your further comments.

World Journal of Hepatology

ESPS Manuscript NO: 2443

In vivo assessment of intratumoral aspirin injection to treat hepatic tumors

Author ID: 02462242

We are available for any clarification that may still be necessary.

Thank you very much for your attention and consideration of our manuscript.

Sincerely yours,

Rafael Denadai, MD (**Author ID: 02462242**)

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REPLY TO REVIEWERS

Reviewer 01557553:

1. *“The “intervention” group was exposed to intralesional 10% acetylsalicylic acid solution. In studies like this important chemical parameters of the solution should be listed – pH, osmolarity, solvent. Any of the above could be able to have effects on viable tissues. It is impossible to discriminate in this study whether the necrosis of the tumor was related to chemical characteristics of the solution or other actions of the ASA.”*
2. *“In order to clarify the above in studies like this the third control group should be added, and the latter usually is exposed to a solution with similar chemical characteristics - pH, osmolarity etc.”*

Reply: We appreciate all of the feedback given by Reviewer 01557553.

1. Chemical parameters of the solution were included in the methods section, as requested. In studies conducted by our group, these effects were tested, as well as use other substances alone. The acetylsalicylic acid is a substance greatly known and studied from the point of view biochemical and pharmacological; there are several studies demonstrating the various effects. Therefore, the aim of this study was not to analyze the biochemical and pharmacological aspects of the solution; the purpose was to answer the initial question: this solution causes (or not) cytolysis and localized tissue necrosis and whether the solution can stimulate apoptosis, as observed in all previous studies by our group where such use have been proposed. Another important aspect is that several clinical and experimental studies have been conducted to find new uses for aspirin. All these aspects were included in detail at the end of the discussion section.
2. As already explained, the option not to use other control groups based on results obtained in previous studies we based the propose and study these effects with aspirin. Our goal was and is being used this solution in several experimental

models (*in vitro* and *in vivo*) with the ultimate goal of base new treatment proposals. All these aspects were included in detail at the end of the discussion section.

Reviewer 00181269:

"The aim of this study is well defined – evaluation the effects of 10% aspirin on VX-2 hepatic tumors in rabbits as a method for treating hepatic metastases. Therapy of liver metastases in whole seems to have good potential to improve patients' survival. Methodology and sample group – Authors compare four randomly divided groups of rabbits, the total size of sample seems to be sufficient for proving the hypothesis. Qualitative and quantitative studies of all liver were performed, selected followed parameters are without any comments. Results – show very good effect of 10% aspirin in the target group, statistical analyses is clear, without any doubt. The conclusion is well defined in connection with presented results of submitted study."

Reply: Thank you for your comments. The language of the manuscript was completely revised by a specialized company.

Reviewer 02441744:

"I think this is a really interesting study but unfortunately it lacks in some details

which could be really relevant at the time to interpret the results. It is important to explain better the process of preparation of each solution giving data about the pH, osmolarity... which could influence the results. In the discussion be careful with guessing the causes of some findings, i.e. the cause of high levels of ALP in group 4. Try to explain the results according to the pathological results. Be a little more critic with your results: explain which have been the weak points and why you think all the results are not due to the chemical differences between the two solutions.”

Reply: We appreciate all of the feedback given by Reviewer 02441744.

1. In studies conducted by our group, these effects were tested, as well as use other substances alone. The acetylsalicylic acid is a substance greatly known and studied from the point of view biochemical and pharmacological; there are several studies demonstrating the various effects. Therefore, the aim of this study was not to analyze the biochemical and pharmacological aspects of the solution; the purpose was to answer the initial question: this solution causes (or not) cytolysis and localized tissue necrosis and whether the solution can stimulate apoptosis, as observed in all previous studies by our group where such use have been proposed. All these aspects were included in detail at the end of the discussion section.

2. The elevation of ALP in group 4 was a consequence of tumor growth, as was observed in the control group, was not observed in the treated groups. This aspect was included in detail in the discussion section.

Reviewer 00070537:

"The research is well done and treats an important field for medical practice in hepatology. However, a correct title will be "Direct Aspirin Treatment of VX-2 Liver Tumors - An Experimental Study in Rabbits" as the research worked with VX-2 carcinomas and not with metastases. Further researches have to insist on the way of administrating aspirin within the tumor (maybe including repetitive transcutaneous administration of aspirin under ultrasound guidance) as to obtain complete destroying of the tumor. Longer time follow up of treated rats on larger lots will be also of great importance before clinical trials."

Reply: We appreciate all of the feedback given by Reviewer 00070537.

1. The manuscript was edited entirely by a specialized company, as requested by the Editorial Board of the WJH. Thus, the term "metastasis" was removed from the title as requested.
2. Longer time follow up of treated rats will be subject to further studies. Probably new applications may be needed, as is done in treatment of metastasis in humans (alcohol and radiofrequency). All these aspects were included in detail at the end of the discussion section.

REPLY TO EDITORIAL SUGGESTIONS

Additionally, follow our answers about the changes made to meet the Editorial suggestions:

1. A language certificate by professional English language editing companies (the edit has achieved Grade A: priority publishing; no language polishing required after editing) was attached as a supplement to the manuscript as requested.
2. The number of words in the abstract has been modified as requested.
3. Tables and Legends for Figures are at the end of the manuscript as requested.
4. PubMed citation numbers for the reference list (PMID and DOI) were included as requested.

We are available for any clarification that may still be necessary.

Thank you very much for your attention and consideration of our manuscript.

Sincerely yours,

The authors

(Author ID: 02462242)
