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Outcome of a rabbit model for late irradiation effects in mandibular oral mucosa and bone: a pilot study

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Dear Ms Helmers,

Reviewers have now commented on your paper. You will see that they are advising that you revise your manuscript. If you are prepared to undertake the work required, I would be pleased to reconsider my decision.

For your guidance, reviewers' comments are appended below.

If you decide to revise the work, please submit a list of changes or a rebuttal against each point which is being raised when you submit the revised manuscript.Also, please ensure that the track changes function is switched on when implementing the revisions. This enables the reviewers to rapidly verify all changes made.

Your revision is due by Jul 14, 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

Ammar Musawi, BDS, MDS
Guest Editor
Journal of Clinical and Translational Research

Reviewers' comments:

Reviewer #1: Lines 68-84: Firstly, SDFI is fairly well established to assess microvascular damage in the oral cavity in humans and animals, which substantially weakens the need to publish a pilot study today. Doing a pilot study to become familiar with a technique that is new to you would not generally be considered publishable, but rather as a typical means to develop expertise. A published "note" at best if something significant was found or if the literature is thin on the topic. Examples of its use in other animals are PMCID: PMC5764044 and PMCID: PMC4409307. In rabbits, Milstein has published several papers such as DOI: 10.1007/s00280-009-1082-x and DOI: 10.1016/j.archoralbio.2010.03.007. When it comes to humans, there many more studies.

Your aim: "The aim of this study was to develop a pilot translational IR model in which microvascular alterations associated with late IR injury could be measured in rabbit oral mucosa and mandibular bone using SDFI and histological analysis." When read in conjunction with most of your introduction suggest little familiarity with the literature and is misleading to readers who may be unfamiliar. Then when you get to your discussion, it seems you must be familiar with at least some of the research (lines 331-332).
To be fair, a study looking specifically at RT and specifically in rabbits may add more supportive information to the literature and thus have some value. I would suggest rewriting the introduction to clearly reflect that numerous studies have increasingly used SDFI in place of invasive techniques over the last decade and that your aim was to specifically look at RT in the commonly used rabbit model using this technique to provide supporting evidence of its use for this application.

Another major weakness is that you stated your goal was to understand lasting effects /late IR injury in an animal model similar as already done in humans (your reference #24) but you only took measurements for 11 weeks. Your argument was based on a hypothesis stated in lines 369-374, but lines 374-385 did clearly point out these weaknesses. Other IR studies in rabbits have gone much longer (e.g., 30 weeks in DOI: 10.1177/019459980112400614). This admission does not resolve the issue of your stated goal and study design conflicting, especially considering there is precedence in the literature of late IR rabbit study design. Along with this, the number of animals led to mixed outcomes, highlighted by group III data measurements using SDFI. Since the aim was the use of this tool, there was notable absence in the discussion specifically and clearly addressing this. It seems the histology data was more valuable. This manuscript would benefit from clearly stating the drawbacks of SDFI, especially when assessing RI during these time points.

Authors' response

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3 July, 2020

Ammar Musawi, BDS, MDS (Associate Professor)
Guest Editor, *Journal of Clinical and Translational Research*

Dear Mr. Musawi,

The authors want to thank the reviewers for their time in carefully reviewing our manuscript. Please find enclosed our revised manuscript titled, "**Outcome of a rabbit model for late irradiation effects in mandibular oral mucosa and bone: a pilot study**".

Below is a point-by-point list of answers raised by the reviewers and modifications recommended by the reviewers. Changes were applied in the text of the manuscript using track changes.

Reviewer #1: Lines 68-84: Firstly, SDFI is fairly well established to assess microvascular damage in the oral cavity in humans and animals, which substantially weakens the need to publish a pilot study today. Doing a pilot study to become familiar with a technique that is new to you would not generally be considered publishable, but rather as a typical means to develop expertise. A published "note" at best if something significant was found or if the literature is thin on the topic. Examples of its use in other animals are PMCID: PMC5764044 and PMCID: PMC4409307. In rabbits, Milstein has published several papers such as DOI: 10.1007/s00280-009-1082-x and DOI: 10.1016/j.archoralbio.2010.03.007. When it comes to humans, there many more studies.

The authors agree that SDFI is well established in the assessment of microvascular pathophysiology in both humans and animals. (lines 89-93). This pilot study did not aim to prove the feasibility of this specific technique. However, the technique was used with the aim to develop an experimental model for the detection of the onset of late irradiation microvascular injury.

Previous experimental rabbit models do not offer consensus regarding follow-up period, number of fractions and total dose that would induce late IR injury (lines 363-366). Therefore, a pilot study was performed with different, cumulative dosages of RT to explore microvascular pathophysiology and with the aim of detecting the onset of late IR microvascular injury. SDFI, a known technique able to detect microvascular pathophysiology and feasible for noninvasive prospective applications was used. Furthermore, as the histological findings of late IR injury are extensively described in previous studies, histology was used to assess the extent of IR injury in each group. To our knowledge, the onset of late IR microvascular injury has never been described in vivo in a rabbit model. This pilot study contributes to the existing literature in revealing the dynamic in

vivo microvascular reaction after exposure to cumulative RT dosages with the goal of attaining a suiting protocol that could represent initiation of late IR injury.

Your aim: "The aim of this study was to develop a pilot translational IR model in which microvascular alterations associated with late IR injury could be measured in rabbit oral mucosa and mandibular bone using SDFI and histological analysis." When read in conjunction with most of your introduction suggest little familiarity with the literature and is misleading to readers who may be unfamiliar. Then when you get to your discussion, it seems you must be familiar with at least some of the research (lines 331-332).

We thank the reviewer for this recommendation. The aim was adjusted and reads as follows, “The aim of this study was to develop a pilot translational IR model in which microvascular alterations associated with *the onset of* late IR injury could be measured in rabbit oral mucosa and mandibular bone.

To be fair, a study looking specifically at RT and specifically in rabbits may add more supportive information to the literature and thus have some value. I would suggest rewriting the introduction to clearly reflect that numerous studies have increasingly used SDFI in place of invasive techniques over the last decade and that your aim was to specifically look at RT in the commonly used rabbit model using this technique to provide supporting evidence of its use for this application.

We appreciate the reviewer recommendation and feel that we should elaborate on this recommendation. As stated above, unfortunately the literature does not provide a “commonly used” late IR rabbit model. The introduction was written focused on the existing RT science. The extensive research that has been done to prove feasibility of SDFI was referred to in the introduction and two similar references (23 and 25) to the ones proposed by the reviewer are already present. The aim of this study was to look at the effects of RT on in vivo microvascular alteration in a rabbit model using a previously proven feasible technique (SDFI). Furthermore, increasing dosages were used with the goal of attaining a suiting protocol that could represent initiation of late IR injury.

Another major weakness is that you stated your goal was to understand lasting effects /late IR injury in an animal model similar as already done in humans (your reference #24) but you only took measurements for 11 weeks. Your argument was based on a hypothesis stated in lines 369-374, but lines 374-385 did clearly point out these weaknesses. Other IR studies in rabbits have gone much longer (e.g., 30 weeks in DOI: 10.1177/019459980112400614). This admission does not resolve the issue of your stated goal and study design conflicting, especially considering there is precedence in the literature of late IR rabbit study design.

The precedence in literature that is mentioned (e.g., 30 weeks in DOI: 10.1177/019459980112400614) poorly describes the methods of irradiation and demarcation of irradiated region. There is no scientific evidence given to support the chosen study-design and time frame. As the results describe clinical

symptoms and histological observations, no information can be deducted about the onset of RT induced microvascular alterations.

Although a longer follow up period might have exposed an additional microvascular effect (lines 374377), no scientific evidence from former experimental rabbit studies appoint where the onset of late IR damage may have started. Therefore, the suitable time frame for the study design was unknown. SDFI offers an opportunity to detect possible changes in microcirculation and microvascular structure over time. The histological observations of group II-IV in this study correspond to previously described RT induced tissue changes, such as loss in vasculature, fibrosis of soft tissue and bone and bone necrosis. This indicates the time frame may have been suitable to detect the onset of late IR injury.

Along with this, the number of animals led to mixed outcomes, highlighted by group III data measurements using SDFI. Since the aim was the use of this tool, there was notable absence in the discussion specifically and clearly addressing this. It seems the histology data was more valuable. This manuscript would benefit from clearly stating the drawbacks of SDFI, especially when assessing RI during these time points.

In hindsight, the authors agree that the histology data of this pilot study seems more valuable in indicating effects produced by RT injury. However, the authors emphasize that there is substantial value in the finding of the presence of histological RT injury in combination with no significant lasting effect of RT on the microcirculation parameters in the overlaying soft tissue.

The observed alterations in the microcirculation were driven by the model and not due to drawbacks or limitations of the technique. Only the mucosa was targeted to be measured by SDFI, not bone. One drawback associated with SDFI in the scope of this study is that it captures an area no greater than 1mm^2, which is added in the discussion.

I hope you would like to reconsider the revised manuscript in its current form for publication.

Thank you for your time and attention.

Yours sincerely,

Renée Helmers, on behalf of the co-authors

2nd Editorial decision
04-Oct-2020

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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,

Ammar Musawi, BDS, MDS
Guest Editor
Journal of Clinical and Translational Research

Comments from the editors and reviewers: