Answering Reviewers

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Title: Morphological alterations and redox changes associated with hepatic warm ischemia-reperfusion injury.

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Reviewer Comment: *The patients with a history of cancer and receiving chemotherapy would be expected to have important differences in liver integrity, function and redox state than "healthy/normal" livers. This would be a major confounder. Could this account for the negative results on redox?*

Response: This is indeed a very pertinent question. The patients included in the study had "normal" livers as examined histologically and did not differ from each other. One of the inclusion criteria was that there were no underlying liver diseases apart from the presented diagnosis. Biopsies for further analyses were only acquired from healthy tissues. We understand that depending on the choice of drugs, chemotherapy may modulate the redox balance in the tissue and thus, making it a plausible factor for the negative results on redox. It would have been interesting to compare samples from the same patients before and after chemotherapeutic regimens. However, ethical consideration would not allow us performing such manipulations and thus we could not investigate this further.

Reviewer Comment: *Are the methods used to quantify microscopy findings validated in other publications?*

Response: The choice of morphometric analysis was developed specifically during the course of this study. We believe that the developed method is a pertinent approach to address the observed changes in LSECs.

Reviewer Comment: *The authors state that the short ischemia time is a limitation. Maybe discussing pre-clinical studies that have described changes at precise time-intervals (5, 10, 15, 20, 30, 60 min etc.).*

Response: Many of the clinical studies performed with Portal Triad clamping lack ultrastructural information. Primary endpoints in human studies have revolved around post-operative clinical parameters such as liver function test values, morbidity and mortality, and hospital stay. In our controlled experimental setting we were also able to compare between the ischemic and the reperfused state of the tissue as opposed to before and after surgery. An interesting report suggested by the reviewer by Man et al. (Hepatobiliary & Pancreatic Diseases International 2002; Vol 1, 2:249-257) showed that the hepatic ultrastructure was well preserved after Portal triad clamping in patients even when the accumulated ischemia time reached 120 minutes. Results from our study revealed a better maintenance after reperfusion, given enough time the tissue could recover fully from the I/R injury.

Reviewer Comment: *Interestingly, while the alteration of LSECs were reversible with reperfusion, this did not always occur for crystalline inclusions in hepatocytes, which however were scarcely affected in their morphology. There is a point that is reported in the results, but that is not sufficiently emphasized in the discussion, is the short time taken by the appearance and disappearance of the cell changes in ischemia and reperfusion.*

Response: The crystalline inclusions were observed in hepatocyte mitochondria. We have not discussed it in detail in view of our limited understandings of the inclusions, specifically in association with I/R injury. In an earlier report by Kashiwagi et al. (Yonago Acta medica 1999; 42:135–140), mitochondrial crystalline inclusions have been described as a reversible pathological condition associated with non-specific reactions to cellular injury in liver tissue. The turnover time of mitochondria with crystalline inclusions in the process of mitophagy is not well known from previously published results. Findings from our study showed the presence of these inclusions post perfusion, indicating that the plausible reversal process may require longer time period unless the extent of injury leads to mitophagy.

Previous studies have shown that LSECs are much more susceptible to dynamic changes in the physical and biochemical microenvironment. Their adaptive response including shrinking in cell size can be postulated as a protective mechanism based on their reappearance following post perfusion. It should be noted that blood-flow associated sheer stress is one of the important factors contributing to the phenotypic changes in the endothelial lining (Davies, PF, Nat Clin Pract Cardiovasc Med. 2009 Jan; 6(1): 16–26). Hence the observed changes could be attributed to both the I/R-associated cellular injuries and changes in sheer stress associated with manipulation of blood flow during surgical manipulations.

In view of the uncertainties, we have not speculated our understandings in the discussion section.

Reviewer Comment: *Shorter introduction*

Response: The introduction in our revised version is shortened to keep the information relevant.

Reviewer Comment: *Revision of method section*

Response: We have thoroughly revised the method section. A more detailed explanation is provided in the revised version of the manuscript, specifically in the sections “Transmission and electron microscopy” and “Immunoelectron microscopy”.