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Manuscript title: **Oxidative stress and extracellular matrices after hepatectomy and liver transplantation in rat models of shear stress with portal hypertension and/or cold ischemia followed by warm reperfusion**

Responses to Reviewers

1. Comparisons between groups

Thank you for your valuable suggestion.

According to your suggestion, we added the statistical differences between groups and also added the mentions in the revised manuscript and Table 2, as described below.

Statistical differences between groups

As described above, the data in comparisons with the controls were shown. Statistical differences between groups were summarized in Table 2.

2. Poor survivals in 60% hepatectomy group

Thank you for your valuable suggestion.

Though 60% hepatectomy is standard experimental procedure, insufficient liver remnant volume resulted in poor prognosis.

According to your suggestion, we simply added mention, as 'Insufficient liver remnant resulted in poor survivals after 60%-hepatectomy. Especially, 40%-SOLT showed very poor survivals.' in the revised manuscript.

3. Oxidative stress marker

Thank you for your valuable suggestion.

According to your suggestion, we added data of MDA and new Figs 4AB in the revised manuscript, as described below.

Oxidative stress

The western blotting intensities of MDA in each group are shown in Fig. 4A. In comparison with the controls (1.00 ± 0.10), normalized MDA showed significant differences in 60%-hepatectomy (1.64 ± 0.39, P = 0.0074), 100%-OLT (2.12 ± 0.78, P = 0.0133), and 40%-SOLT (2.30 ± 0.26, P <0.0001) (Fig. 4B).

4. Free radicals scavenging system

Thank you for your valuable suggestion.

Preliminary data in our Lab suggested that free radicals scavenging system showed no differences, if surgery models caused considerable liver damage. Mild liver damages (such as 40%-hepatectomy) showed differences in SOD and catalase (data not shown).

According to your suggestion, we added mention, as 'Shear stress with portal hypertension and/or CIWR injury after liver surgeries in this study caused considerable liver damage.', in the revised manuscript.

'Shear stress with portal hypertension and/or CIWR injury after liver surgeries in this study caused considerable liver damage. A possible explanation is that

this scavenging system failed to stimulate some reactive molecules because of considerable damage after liver surgery.'

5. Apoptotic induction in 100%-OLT

Thank you for your valuable suggestion.

The group with only CIWR injury (i.e., 100%-OLT) surely caused OS-induced damage and subsequent apoptotic induction, this group showed differences with control, not in PI3K/Akt, but in ATM/H2AX. Hence, we suggested that CIWR injury induce apoptosis due to OS via the ATM/H2AX pathway.
According to your suggestion, we dded mention in the revised manuscript, as described below.

'Our results with ATM and H2AX clearly showed that OS after liver surgery caused DNA-damage signaling and triggered subsequent DNA repair. In this study, groups with only CIWR injury (i.e., 100%-OLT) caused OS-induced damage and subsequent apoptotic process. However, this group showed differences not in PI3K/Akt, but in ATM/H2AX. This results suggested that CIWR injury induce apoptosis due to OS via the ATM/H2AX pathway.'

6. Conclusion section

Thank you for your valuable suggestion.

Based on the data in 100%-OLT, we suggested that the inhibition of apoptotic

induction due to OS via the ATM/H2AX pathway may be important for strategy against CIWR injury, even in the condition of sufficient liver volume.
On the other hand, under the conditions with insufficient liver remnant (i.e., 60%-hepatectomy and 40%-SOLT), the prevention of apoptotic induction due to OS via the Akt/PI3K pathway may be key to improving postoperative course.
Based on the data in zymography (function), only MMP-9 showed statistical differences in all groups after surgeries, though some factors showed differences in assays by western blotting (expression). Note that MMP-9 showed differences in both western blotting and zymography in the group of CIWR injury with insufficient liver volume (i.e., 40%-SOLT). Therefore, we suggested that MMP-9 may be a reliable therapeutic target, especially in the condition of CIWR injury with insufficient liver volume (40%-SOLT).
According to your suggestion, we added mentions in the revised manuscript, described below.

'The inhibition of apoptotic induction due to OS via the ATM/H2AX pathway may be important for strategy against CIWR injury, even in the condition of sufficient liver volume. Under conditions with insufficient liver remnant, the prevention of apoptotic induction due to OS via the Akt/PI3K pathway may be key to improving postoperative course. Also, MMP-9 may be a reliable therapeutic target, especially in the condition of CIWR injury with insufficient liver volume.'