October 9, 2017

Clara Balsano
Editor-in-Chief
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Dear Dr. Clara Balsano,

We appreciate your kind consideration of our article entitled "Liver atrophy after percutaneous transhepatic portal embolization occurs in two histological phases: hepatocellular atrophy followed by apoptosis (Manuscript NO.: 35743)." We are also grateful to the reviewer for valuable comments. We have revised the manuscript on the basis of the comments and are now resubmitting it.

Reviewer #1: The manuscript `Liver atrophy after percutaneous transhepatic portal embolization occurs in two histological phases: hepatocellular atrophy followed by apoptosis` by Iwao Y et al. is a very interesting contribution. The study also potentially impacts on understanding ischemia-related pathophysiology in liver relevant for development of many diseases. Comparison of human and pig records, yet does not seem finally reliable, is a good example of sophisticated merging data to assure translation.

Some minor revisions might improve the overall contribution:

1. What was the clinical background in human specimens, discuss their gender, age, diagnoses, interventions, and the differences of data accordingly, which should crucially matter.

Author response

We appreciate your suggestion. The clinical backgrounds of the patients who provided the human specimens are summarized in newly added Table 1. To evaluate the histological changes due to PTPE alone, we excluded patients with viral hepatitis and steatosis from our study. We added the sentence "To facilitate the histological evaluation of liver lobules, 11 patients were excluded because of steatosis." in Page 9 line 14.

Table 1. Clinical backgrounds of the patients who provided the human specimens.

Patient	Gender	Age (years)	Primary tumor site	Surgery
1	F	74	Rectum	Ex Rt
2	F	57	Rectum	Ex Rt
3	M	76	Cecum	Ex Rt
4	M	43	S/C	Ex Rt
5	M	56	Rectum	Ex Rt
6	M	53	Rectum	Ex Rt + nonAnat S3
7	F	47	S/C	Ex Rt
8	F	61	Rectum	Ex Rt
9	F	64	S/C	Ex Rt
10	F	63	Rectum	Ex Rt + nonAnat S3

All patients underwent right-sided percutaneous transhepatic portal embolization.
F, female; M, male; S/C, sigmoid colon; Ex Rt, extended right hemilobectomy; nonAnat S3, non-anatomical liver resection of segment 3.

2. Was the regeneration capacity of the liver considered by Authors ?

Author response

As we described in the Materials and Methods subsection "Animal specimens", all pigs underwent segmental PTPE in a limited area, and formalin-fixed paraffin-embedded specimens were produced from samples resected from the

embolized segment and from a nonembolized lobe (control) far from the lobe containing the embolized segment. This methodology allowed us to evaluate pure histological changes in the embolized area compared to those of the nonembolized lobe (control) without histological regenerative reactions. Therefore, we not only considered the regeneration capacity of the nonembolized area based on our study design but also that of specimens in the control area.

We added the sentence "To evaluate the pure histological changes of the embolized area compared with those of the nonembolized area without histological regenerative reactions," in page 9 line 3.

3. Design is not clearly understood during reading article, I would suggest to provide a scheme and present the human/pig data in parallel where appropriate (mostly imaging) to let the reader assess results comparatively.

Author response

We completely agree with your comment. We added a flow chart describing this study in the newly added figure 1 and summarized the human/pig data in table 1.

Figure 1. Flow chart of the pig specimens and human cases examined in this study

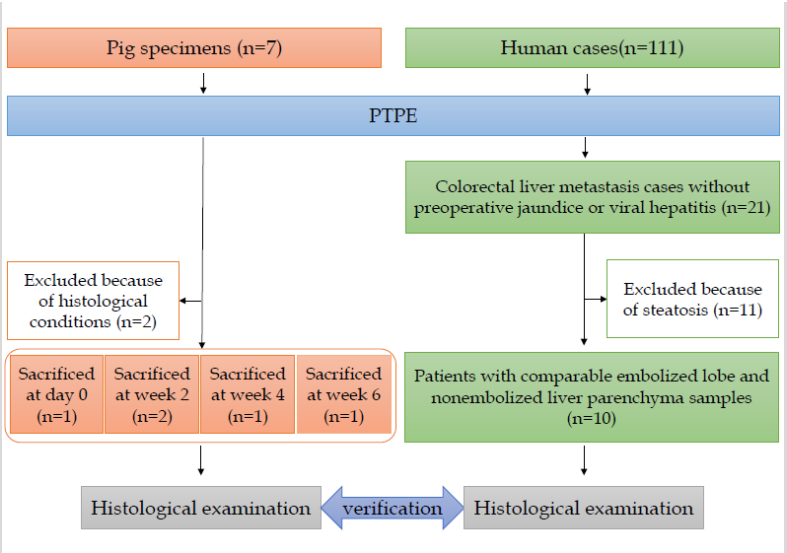

Table 2. Comparison of the results for pigs and humans.

	Pig liver specimens at week 4	Human liver specimens resected around week 4
PV-CV distance	EMB < Cont	EMB < nonEMB
Hepatocyte density	EMB > Cont	EMB > nonEMB
TUNEL-positive cells	EMB > Cont	EMB > nonEMB
LC3 Intensity	N.S.D.	N.S.D.
GS zonation	EMB narrower than Cont	EMB narrower than nonEMB
CYP2E1 zonation	EMB narrower than Cont	EMB narrower than nonEMB

PV-CV, portal vein to central vein; EMB, embolized lobe; Cont, control lobe; nonEMB, nonembolized lobe; N.S.D., no significant difference.

4. In discussion can be included: what therapeutic options could be recommended in each of two phases and chronic ischemia-related liver diseases if relevant.

Author response

This is a very valuable suggestion for us. According to our hypothesis, the first phase occurs in the first 2 weeks after portal venous obstruction and may be a reversible histological change. However, the second phase, which occurs between 2 and 4 weeks after portal venous obstruction, likely involves irreversible change because apoptotic processes have already begun. Therefore, if acute portal obstruction-related liver dysfunction or disease (such as portal thrombus) occurs within about 2 weeks, we consider that we can choose a therapeutic option, for example, aggressive removal of portal thrombus from the vein using internal medicine, interventional radiology, or surgical methods. On the other hand, for conditions such as chronic ischemia-related liver diseases with liver atrophy that arise in the second phase or long after portal

venous obstruction, there are very few therapeutic options, for example, liver transplantation.

Unfortunately, we don't have enough data to prove our hypothesis, especially regarding reversible and irreversible histological changes. Therefore it would be taking speculation too far to describe concrete therapeutic strategies as suggested above. However, our results provide a theoretical basis for planning treatment strategies for acute portal obstruction-related liver dysfunction or disease and chronic ischemic-related liver diseases with liver atrophy.

We added the following sentences to Page 17, line 15 in the limitation paragraph.

Future research will hopefully provide a sound theoretical basis for planning treatment strategies for acute portal obstruction-related liver dysfunction or disease and chronic ischemic-related liver diseases with liver atrophy.

5. Figures: the scales need to be added on the electronic microcopy and on histology. Some imaging data – e.g., punctures with ultrasonographic guidance could be added.

Author response

Thank you for your comment. We added appropriate scales to all figures.
Unfortunately, images giving a clear picture of the ultrasonographic guidance are not available. Furthermore, in this study, we analyzed the pig and human liver specimens but did not perform PTPE.

6. Spelling needs some correction (e.g., plenty of intervals missed, etc.), as well English language improvement.

Author response

A further English check was performed by a native English speaker.

I hope that the revised manuscript is now acceptable for publication.

Yours sincerely,

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