

Tissue damage with different surgical techniques in a porcine model of liver resection: implications for living-donor liver transplantation?

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Abstract

Background/purpose For living-donor liver transplantation (LDLT) it is of paramount importance to preserve as much viable liver tissue as possible to avoid postoperative complications in the donor and recipient. The depth of tissue damage caused by common surgical techniques for liver resection has not been studied so far.

Methods Here we compared the depth of tissue damage and the immunohistochemical expression of heat shock protein (HSP) 70, a marker for tissue damage, in a porcine model of liver resection, to assess the effect of different surgical techniques, i.e., blunt dissection (BD), and dissection with an ultrasound aspirator (UA), an ultrasound scalpel (US), or a water-jet (WJ).

Results Analysis with linear mixed effects models (LME) showed significantly less tissue damage with BD and UA than with US and WJ (joint p value <0.001). Damage also increased within 6 h after surgery (p value = 0.004). Semiquantitative evaluation of HSP 70 showed increased expression after resection with US compared to all other resection methods (p value <0.001), indicating increased tissue damage with this method.

Conclusion We suggest that in cases of liver resection for LDLT surgeons should reevaluate using US and WJ because of possible excessive tissue damage compared to

BD and UA. Overall we advocate the use of BD as it requires no special equipment and, hence, has considerably higher cost-effectiveness without compromising tissue preservation and clinical outcome and is readily available even in low-tech environments.

Keywords Living-donor liver transplantation · Liver resection · HSP70 · Tissue damage

Introduction

Living-donor liver transplantation (LDLT) is now an accepted treatment for patients suffering from end-stage liver disease [1]. Preserving as much viable liver tissue as possible is paramount for reducing postoperative complications, such as small-for-size syndrome (SFSS), in both the donor and recipient [2].

Concerning liver resection in general it is known that blood loss during liver resection is one of the main factors affecting the perioperative outcomes of patients [3–5]. The following conventional or low-tech methods for parenchymal dissection, which do not require special instruments, have been proposed to reduce blood loss during liver resection: the finger fracture technique or digitoclasis [6, 7], the crush clamp method [8], or simply blunt dissection [9]. Several new techniques, such as dissection with an ultrasound surgical aspirator [10–13], water-jet dissection (also known as hydrojet) [14–18], or dissection by ultrasound scalpel [19–21] have also been established. In many observational studies or prospective trials [22–26], and one meta-analysis [27], the clinical parameters of these methods, such as mortality, length of surgery, blood loss, and perioperative morbidity have been compared but the results are equivocal [27].

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Overall it seems that there is no difference in perioperative morbidity and mortality and that surgeons may choose whichever resection method they favor. Then again, in cases of liver resection for LDLT it is also important to preserve as much viable liver tissue as possible. But the degree of tissue damage caused by these resection methods has never been compared. Here we evaluate these methods in a model of porcine liver resection and compare tissue damage by means of histology and the immunohistochemical expression of heat shock protein (HSP) 70, a marker for liver damage and regeneration [28].

Methods

Four dissection methods, i.e., water-jet dissection (WJ), blunt dissection (BD), and dissection with an ultrasound scalpel (US) and an ultrasound aspirator (UA) were evaluated in a porcine model of liver resection. It was planned to perform seven experiments for each group and to acquire tissue samples directly after and at 3 and 6 h after the surgery. The project and study design were approved by the appropriate German government authorities and the institutional review board.

Animal preparation

Because one pig died during surgery and was excluded from the analysis, a total of twenty-nine healthy female German landrace pigs were prepared for standardized liver resection. Median weight was 29.6 kg (range 22–35 kg) and they were 8–10 weeks old. The animals were treated in accordance with the National Institutes of Health guidelines.

The animals were fasted from midnight the day before the surgery, with free access to water ad libitum. The animals received sedation with intramuscular injection of 8 mg/kg body weight (BW) azaperone (Stresnil; Janssen-Cilag, Neuss, Germany) and 0.025 mg atropine sulfate (Atropinsulfat Braun; B. Braun, Melsungen, Germany). Then they were placed in the supine position and a peripheral ear vein was cannulated for further induction and maintenance of anesthesia and for fluid administration. For induction of general anesthesia, the pigs were injected with 0.005 mg/kg BW fentanyl dihydrogen citrate (Fentanyl-Janssen; Janssen-Cilag) and 12.5 mg/kg BW thiopental sodium (Trapanal; Byk Gulden, Konstanz, Germany). The animals were intubated with a 6.5-mm cuffed endotracheal tube (Portex Blue Line; Smiths Medical Deutschland GmbH, Grasbrunn, Germany) and mechanically ventilated (Respirator Sulla 19 and Pulmonat 19 K1; Dräger, Lübeck, Germany) with 30% oxygen and ambient air. Anesthesia was maintained with continuous intravenous infusion of

2.5 mg/kg BW/h ketamine (Ketanest; Parke-Davis/Pfizer, Karlsruhe, Germany) and 0.1125 mg/kg BW/h piritramide (Dipidolor; Janssen-Cilag). A central venous line was inserted into the left internal jugular vein and an arterial line was placed into the carotid artery for blood pressure measurements and blood sampling. Ventilation was adjusted every 30 min to achieve arterial blood gases of pO_2 120–170 mmHg and pCO_2 35–40 mmHg. A standard electrocardiogram, heart rate, and blood pressure were monitored continuously (Marquette; Hellige Systems, Freiburg, Germany). After obtaining tissue samples at 6 h animals were sacrificed by injection of potassium chloride.

Surgery

After preparation of the animals, baseline values were obtained and the pigs were randomized into one of the four groups. Two surgeons with extensive experience in liver surgery performed the operations. After median laparotomy, the hepatoduodenal ligament and hepatic artery were dissected. A resection line was marked with a monopolar knife between the right and left middle lobe of the porcine liver, roughly corresponding to a hemihepatectomy in humans. The liver was then dissected without occlusion of portal vein or hepatic artery. In addition to the described methods of dissection, hemostasis was performed with titanium clips and 4.0 Prolene sutures (both, Tyco Healthcare Germany, Tonisvorst, Germany).

The technique of hepatectomy with WJ has been described in more detail by Papachristou and Barters [14] and Rau et al. [18]. For this study a WJ system from Saphir Medical was used (HD1-Jet-P Saphir; Saphir Medical, Lyon, France) with 0.15 mm nozzle diameter and a pressure of 14 bar.

Blunt dissection for living-donor liver resection has been described by Obed et al. [9]. In short, Metzenbaum scissors were used for blunt preparation; branches were closed and carefully pressed into the hepatic parenchyma. Tissue was dissected in a millimeter-wise fashion; vessels and biliary structures were exposed and clipped or ligated to both sides.

For a detailed description of the use of US see [19] and [21]. We used an UltraCision Harmonic Scalpel (Ethicon Endo-Surgery; Cincinnati, OH, USA) with a longitudinal blade vibration of 55.5 kHz and a blade movement range of 50–100 μ m.

Surgical resection of the liver with UA has been described in more detail by Hodgson and Aufses [29] and by Fasulo et al. [13]. For this study we used a Cavitron ultrasonic surgical aspirator (CUSA Excel; Valleylab, Boulder, CO, USA) with a straight 23 kHz handpiece.

Perioperatively we also obtained clinical data such as intraoperative blood loss, duration of surgery, hemodynamic

instability, etc. But since this study was not designed to assess the clinical outcome we did not obtain data concerning liver function tests and complications such as bile leaks or infections.

Histopathology

Tissue samples from the resection margin were collected directly after surgery (0 h) and at 3 and 6 h. Samples were fixed in neutral buffered formalin 4% and dehydrated and embedded into paraffin. Thereafter slices of 3–5 μm were prepared for analysis and stained with hemalaun–eosin. Microscopic measurements of tissue damage depth were obtained at 20-fold amplification (Olympus BX 40; Olympus Optical, Hamburg, Germany) and images were transferred via a digital camera (TK-C13811EG; JVC, Friedberg, Germany).

Immunohistochemical staining was carried out with anti-HSP70 antibody as follows: paraffin was removed by embedding the slices in xylol for 20 min. Then the slices were hydrated with ethanol, cleaned with deionized water, and put into TBS-buffer (Tris-buffered saline; Paesel+Lorei, Hanau, Germany) for 5 s. Unspecific protein binding was blocked with bovine serum albumin (BSA) 5% (Paesel+Lorei) and mouse-anti-HSP70 monoclonal antibody (SPA-810; StressGen Biotechnologies, Victoria, Canada) was added for 60 min. After rinsing with TBS-buffer a biotinylated anti-mouse secondary antibody (AB2; DAKO ChemMate, Detection Kit K 5005; Dako, Hamburg, Germany) was applied, the slides were flushed with TBS-buffer for 20 min and then stained with Fast Red (DAKO ChemMate, Detection Kit K 5005; Dako). The staining was stopped by flushing with deionized water. Finally, nuclei were stained with hemalaun. Figure 1 shows a typical example of HSP 70 immunohistochemistry.

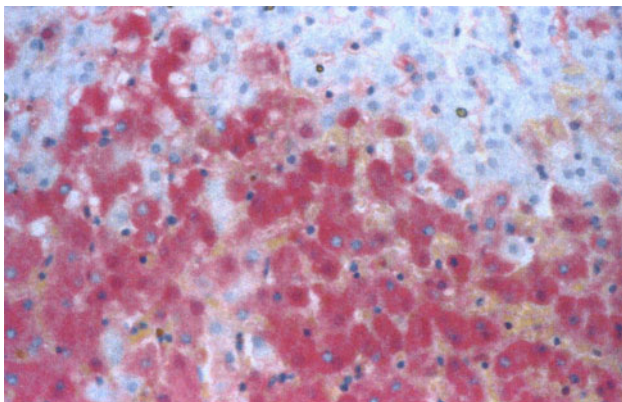


Fig. 1 Immunohistochemistry of heat shock protein (HSP) 70 in the degenerative zone of damaged liver tissue. Red staining indicates expression of HSP70. Cell nuclei have dark blue staining

According to our own preliminary histologic examination of the specimen and from previous publications [10, 11, 15, 25, 30–32] the vertical spread of tissue damage adjacent to the resection area has been divided into two subregions with distinct morphological features, i.e., the exudative zone and the degenerative zone. The exudative zone was characterized by a complete loss of structural integrity with deposition of erythrocytes, fibrin, granulocytes, and viable as well as necrotic hepatocytes. The degenerative zone was characterized by preserved structural integrity with patchy hemorrhages. Hepatocytes were edematous with pale cytoplasm and hyperchromasia or even karyopyknosis of the cell nucleus. The degenerative zone could be delineated from intact liver tissue by an alternating dense infiltration of granulocytes (Fig. 2). We measured the depth of the exudative and degenerative zones in 10 predefined spots per section with 2–4 sections per time point and animal.

As expected we did not observe proper staining of HSP70 in the exudative zone. Thus we evaluated the expression of HSP70 only in the degenerative zone. We defined three sublayers within the degenerative zone which could be evaluated separately if necessary. The maximum intensity of staining was graded semiquantitatively as “low”, “moderate”, or “high”.

Statistical analysis

All statistical calculations were carried out in R (version 2.11.0) [33] with type I error fixed at 0.05. For the main analysis the sum of the depth of the exudative and degenerative zones, measured in μm , was chosen as the response variable. Sensitivity analysis was also carried out with separate depth measurements. Because of non-normal

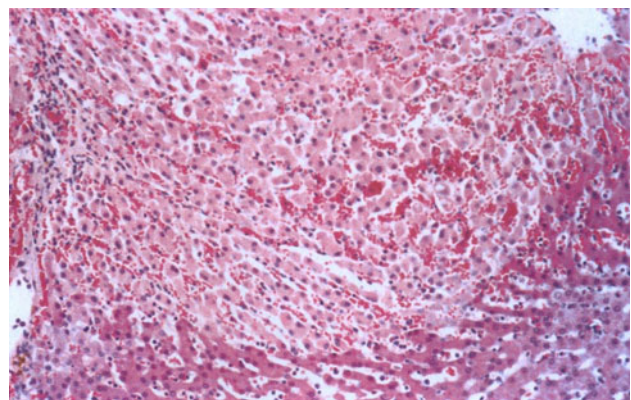


Fig. 2 Hemalaun–eosin staining of liver tissue. In the upper part of the image the degenerative zone can be identified. Edematous hepatocytes with pale cytoplasm and karyopyknosis can be seen in parenchyma with preserved structural integrity and intraparenchymal hemorrhages. In the lower part normal parenchyma is seen with granulocyte infiltrates

distribution the response variable was log-transformed for parametric analysis.

Because of the within-subject correlation of repeated and longitudinal measurements, analysis of variance and ordinary least squares was actually not a valid approach to analyze the data. To account for this correlation we fitted linear mixed effects models (LME) [34]. In LME variance components from the observational units, referred to as random effects, are separated from fixed effects, which are factors with a fixed and reproducible set of possible levels. In our model, the observational unit is the unique and random combination of pig, experimental setting, and surgeon. Hence the observational unit is included in the model as random effect. Covariates with a fixed and reproducible set of possible levels, such as time point and resection method, enter the model as fixed effects.

We used the package “lme4” (version 0.999375-33) for the implementation of LME. First we fitted a “naked model” incorporating only the random effects and a fixed intercept and then added the fixed factors “method” and “time”, and their interaction sequentially. At each step improvement of the new model was checked by likelihood ratio test. Since meaningful p values may not be extracted from the provided t -statistics in the package “lme4”, we used the package “multcomp” (version 1.1-7) to obtain family-wise confidence intervals (CIs) for the final model preserving the overall type I error for multiple comparisons [35].

Counted data from the intensity score measurement were compared with non-parametric tests. First, all groups were compared by the Kruskal–Wallis rank sum test [36] and if there was a significant difference between groups we performed pairwise comparisons with the Mann–Whitney U -test [36] with p values adjusted for multiple comparisons by the Holm procedure [37].

Results

During surgery one pig died due to lung embolism and was therefore excluded from the analysis. To obtain equal group sizes one more pig was allocated, resulting in a total of 29 animals that were used in this study. Surgery in all other pigs was uneventful and there were no significant differences concerning blood loss and operating time. Seven animals were analyzed per resection group and for each animal measurements could be obtained at the time points 0, 3, and 6 h after surgery. For each time point and animal a median of 34 measurements were taken for each zone (range 17–51). Figure 3 shows the median and upper and lower quartiles of overall tissue damage depth, i.e., the sum of the exudative and degenerative zones for the four different resection methods. All analyses were primarily

performed with overall tissue damage depth, i.e., the sum of the exudative and degenerative zones, but we also performed all analyses separately for both zones. These additional sensitivity analyses did not yield significantly different results for the effect of method or time after surgery (not shown).

We then analyzed depth of tissue damage by LME. First we fitted a “naked” model, only including the random effect. Sequential inclusion of the fixed effects “time” and “method” improved the model (p values = 0.004 and <0.001, respectively, compared by likelihood ratio test) but not their interaction (p value = 0.64). It follows that the best LME model that explains variation in the data is a model that includes method and time as fixed effects.

Because of log-transformation we had to interpret the back-transformed estimators for effects as multiplicative rather than additive (Table 1). An exception to this was the estimator for the intercept (402 μm) which can be interpreted as the approximate tissue damage depth in the reference group, i.e., BD at 0 h. Comparison of dissection with UA to BD showed a non-significant decrease to 89% [CI 58–136] of the reference. After resection with WJ there was a barely significant increase of tissue damage depth to 155% [CI 1.01–238]. But the most damaging surgical technique seemed to be US, which led to an increase of tissue damage depth to 238% [CI 155–364] even after correction for experimental effects or other fixed effects such as time after surgery. Concerning the time course of tissue damage depth, the model showed an increase of tissue damage depth from 402 μm directly after resection to 140% [CI 103–190] at 3 h and 153% [CI 113–207] at 6 h (Fig. 4).

Furthermore we measured the expression of HSP70 by immunohistochemistry. Maximum intensity of staining was evaluated for seven experiments for each method at the predefined time points. Frequency counts stratified by method are shown in Fig. 5. In groups BD, WJ, and UA low intensity was counted more frequently than in group US. We could see markedly higher intensity of HSP70 immunohistochemistry after resection with US and a tendency of lower intensity after BD and UA.

Statistical inference of maximum intensity with non-parametric Kruskal–Wallis rank sum test showed a significant difference between the groups (Kruskal–Wallis χ^2 statistic = 24.73 and p value <0.001). Pairwise comparison showed significantly higher scores after resection with US compared to BD (p value <0.001), UA (p value <0.001), and WJ (p value = 0.020), but no difference between any other pair (Table 2). Similar results would have been obtained if the mean intensity instead of the maximum intensity had been compared with the Kruskal–Wallis test (p value <0.001) or pairwise Mann–Whitney U -test

Table 1 Linear mixed effects model estimates for fixed effects

Fixed effect	Log. coefficient	t Statistic	Coefficient	Confidence interval
(Intercept)	6.00	42.29	(402)	NA
Ultrasound aspirator (UA)	-0.12	-0.72	0.89	[0.58, 1.36]
Ultrasound scalpel (US)	0.87	5.15	2.38	[1.55, 3.64]
Water-jet (WJ)	0.44	2.62	1.55	[1.01, 2.38]
Time 3 h	0.34	2.81	1.40	[1.03, 1.90]
Time 6 h	0.43	3.6	1.53	[1.13, 2.07]

The intercept corresponds to the effect of blunt dissection (BD), which is the comparator for all effects. Coefficients in these models are multiplicative rather than additive, e.g., a back-transformed coefficient of 0.89 for ultrasound aspirator corresponds to an 11% decrease compared to blunt dissection, which, in turn, corresponds to an estimated depth of 356 μm with a confidence interval of 232–545. It follows that if a confidence interval of a back-transformed coefficient includes 1 (corresponding to no effect) the effect is non-significant

NA Not applicable

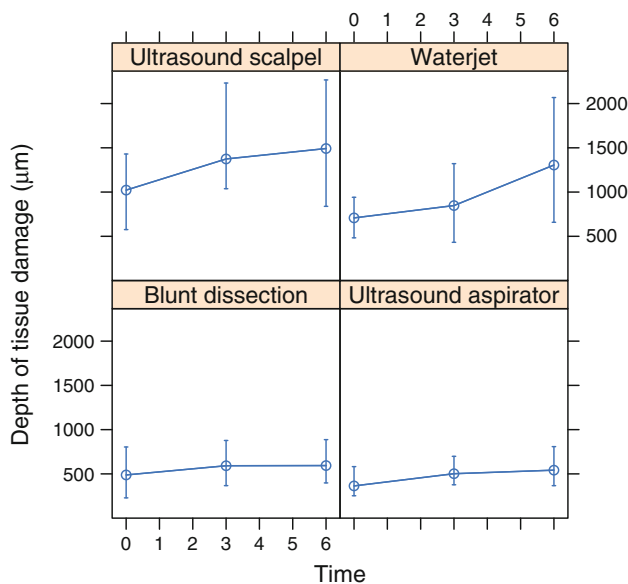


Fig. 3 Observed depth of tissue damage over time stratified for resection method. Shown are median (open circles) and upper and lower quartiles (whiskers) of tissue damage depth

(UA vs. US p value <0.001, BD vs. US p value = 0.001; all other comparisons were non-significant).

Discussion

For LDLT it is of paramount importance to preserve as much viable liver tissue as possible to avoid postoperative complications in the donor and recipient. This is the first experimental study to specifically compare the microscopic tissue damage of BD, UA, US, and WJ in a porcine model of liver resection.

The tissue alterations seen after liver resection in this study were generally in accordance with previous descriptions [10, 11, 15, 25, 30–32]; unfortunately, there is no uniform method to measure depth of tissue damage and

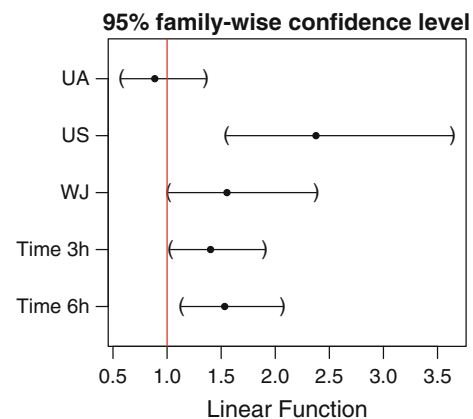


Fig. 4 Family-wise 95% confidence intervals for fixed effects on tissue damage depth according to linear mixed effects models (LME). The estimators are multiplicative and the reference for comparisons is tissue depth after blunt dissection at 0 h. If the confidence interval for a factor does not include 1, statistical significance is implied. UA Ultrasound aspirator, US ultrasound scalpel, WJ water-jet

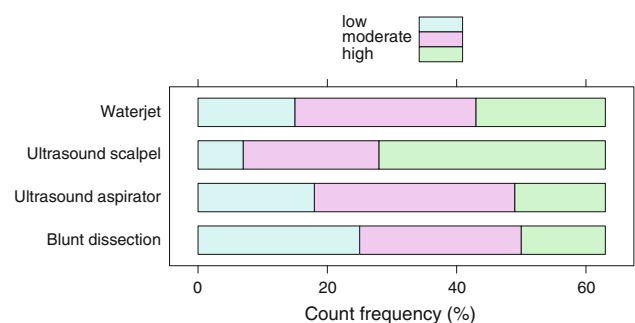


Fig. 5 Frequency of “low”, “moderate”, and “high” maximum intensity scores of HSP70 immunohistochemistry stratified by method of resection

therefore measurements given in the cited studies are generally not comparable. In this study we differentiated two layers of damaged liver tissue according to their morphology: the exudative zone and the degenerative zone.

Table 2 *p* Values of pairwise comparison by non-parametric Mann–Whitney *U*-test of maximum intensity of heat shock protein (HSP) 70 immunohistochemistry

	BD	UA	US
UA	0.547	–	–
US	<0.001	<0.001	–
WJ	0.138	0.547	0.020

Both zones together probably correspond to what most authors describe as “damaged tissue” and therefore we performed our primary analysis with the overall tissue damage. But statistical analyses with depth of only the exudative or degenerative zone did not yield different results and have thus been omitted.

We could show increased tissue damage in plain histology and by the immunohistochemical expression of HSP70 with US compared to the other methods. Although this finding is discordant with the macroscopic evaluation of many clinical investigations [19–21], it has also been noted by Kadesky et al. [38] that there is a major discrepancy between macroscopic and microscopic tissue damage with US. Also, Amaral and Chrostek [39] described a linear increase of tissue damage with the activation time of US, and their estimated depth of tissue damage at an activation time of 3 s, i.e., 1 mm, is in accordance with our estimate of 957 μm .

We did not observe significant difference of tissue damage between BD and UA; and the estimated depth of tissue damage (402 μm for BD and 358 μm for UA) is also in accordance with previous descriptions [11].

There are quite heterogeneous data concerning the amount of tissue damage caused by dissection with the WJ, probably due to differences in equipment and the pressure applied to the WJ [40, 41]. Our findings are in accordance with those of Rau and colleagues [41] concerning the depth of tissue damage, i.e., 623 μm [CI 406–957] in the present study and 800 μm in [41], and concerning comparison with UA (greater damage with WJ in [41] and this study).

Concerning the clinical data we obtained we did not observe any difference in intraoperative blood loss and surgery time, a finding which is in accordance with surgical trials in humans [27]. We did not measure liver function tests because it was not expected to see differences in this model of hemihepatectomy within the short time frame of the experiment. This experiment was also not designed as a small-for-size model, because to assess the effect of the different resection methods on the incidence of SFSS one would need such a large sample size that it would not be practicable for experimental research and probably would not have been approved by the ethics committee.

To sum up, we showed significantly less tissue damage with BD and UA compared to WJ and especially US, as

shown by plain histology and the expression of HSP70. We did not observe any differences between BD and UA. We did not see overt clinical differences between any of the methods in this model of porcine liver resection, a finding which is in accordance with surgical trials in humans [27].

We therefore suggest that in cases of liver resection for LDLT surgeons should re-evaluate using the US because of possible increase of tissue damage. Concerning the other methods, we advocate the use of BD because it does not require any special equipment and, hence, has considerably higher cost-effectiveness without compromising tissue preservation and is readily available even in low-tech environments. Whether the method of tissue dissection influences the incidence and severity of complications and especially SFSS after liver resection for LDLT remains unclear and should be evaluated in randomized controlled trials.

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