before LDLT. We assess the patency and flow direction of the main portal vein (PV) and SPV and detect the size and PVF of the SR shunts. A large SR shunt is defined as a shunt with diameter of >10 mm and PVF of >400 ml/min.

The recipient operation is performed in piggyback fashion. After complete graft revascularization, we manage a large SR shunt according to preoperative portal venous hemodynamics and intraoperative PVF of the graft. In patients showing partial steal of SMV blood flow by the SR shunt preoperatively and in whom PVF of the graft is measured as <1,000 ml/min, we dissect the shunt at the site of inflow to the LRV, with or without splenectomy. In patients showing complete steal of SMV blood flow by the SR shunt preoperatively, we divert SMV and SPV blood flow by ligation at the SPV root for prophylactic management of the large SR shunt as follows. The main PV is dissected towards the upper edge of the SPV root (Fig. 1a). After tunneling of the SMV, tape is introduced above the main PV and SMV (Fig. 1b). When the lower edge of the SPV root can be exposed safely by pulling the tape caudally, the SPV root is encircled and tied from the upper side using nonabsorbable sutures (Fig. 1c). In patients for whom the above-mentioned approach to the SPV root cannot be carried out safely, ligation is performed as follows. After the dorsal side of the SPV root is dissected from the retroperitoneal tissue, a nonabsorbable suture is introduced behind the SPV root (Fig. 2a). After tunneling of the SMV, another suture is introduced above the main PV and SMV (Fig. 2b). After both sutures are tied at the lower side, the suture above the main PV and SMV is pulled up over the pancreas (Fig. 2c). Using this method, the SPV root can be safely encircled and tied by the suture (Fig. 2d). The SR shunt is not dissected, and blood from the spleen can flow into the LRV via the SR shunt. In this method, the liver graft is supplied with PVF consisting of SMV blood flow alone. Graft PVF is measured by Doppler US before and after the diversion of SMV and SPV blood flows.

Statistical Analysis

Values are expressed as the mean ± standard error of the mean. Statistical analysis was performed using Student's t test and the Mann–Whitney test. The level of significance was defined as $p<0.05$.

Results

Between August 1996 and December 2011, a total of 280 LDLTs were performed at Okayama University Hospital. Based on preoperative evaluations using 3D-CT and Doppler US, we identified 25 patients with a spontaneous large SR shunt. In ten of these 25 patients, preoperative direction of blood flow in the SPV was hepatopetal, and SMV blood flow was not stolen by the SR shunt. In the remaining 15 patients, the direction of preoperative blood flow in the SPV was hepatofugal. Of these 15 patients, SMV blood flow showed partial steal by the SR shunt in eight patients and complete steal by the shunt in the other seven patients. The above-mentioned surgical techniques were applied in five of these seven patients.

Two of these seven patients, who were treated before the introduction of diversion of SMV and SPV blood flow at our institution, developed postoperative steal of the graft PVF by a large SR shunt. In one of these patients in whom the SR shunt was not occluded during the transplant procedure in the early stage of our LDLT program, graft PVF was completely stolen by the preserved SR shunt on postoperative day (POD) 9 due to steroid-resistant acute rejection. Although we dissected the SR shunt at the site of inflow to the LRV on POD 9 while commencing treatment for rejection, liver graft function deteriorated rapidly, and the patient died on POD 39. In the other patient, who underwent ligation of the SR shunt at the site of inflow to the LRV during the transplant procedure, graft PVF was completely stolen by the residual SR shunt on POD 2 due to severe ischemic graft injury. We diverted SMV and SPV blood flow by ligation at the SPV root on POD 2, leading to the recovery of liver graft function.

The remaining five of the seven patients with preoperative complete steal of SMV blood flow by the SR shunt underwent diversion of SMV and SPV blood flow during the transplant procedure. Mean graft weight was 641±47 g (range, 482–767 g), and mean graft-to-recipient body weight ratio was 0.84±0.06 % (range, 0.70–1.05 %). Mean blood loss was 2,495±908 ml (range, 800–5,100 ml), and mean operative time was 543±28 min (range, 490–599 min). The mean PVF of grafts in these five patients before SR shunt management was low (582±67 ml/min), but it increased significantly to 1,361±124 ml/min after diversion of SMV and SPV blood flow.

In 11 patients, the SR shunt was dissected at the site of inflow to the LRV. Among these 11 patients, graft PVF was stolen by the residual SR shunt in two patients, and portal vein thrombosis derived from the SR shunt developed in another two patients. In contrast, in the five patients who underwent diversion of SMV and SPV blood flow, there was no posttransplant morbidity related to the SR shunt and no portal vein complications. No mortality was encountered, and the mean length of postoperative hospital stay was 50±3.8 days (range, 41–60 days).

Discussion

The optimal management for a spontaneous large SR shunt remains controversial in liver transplantation. In adult

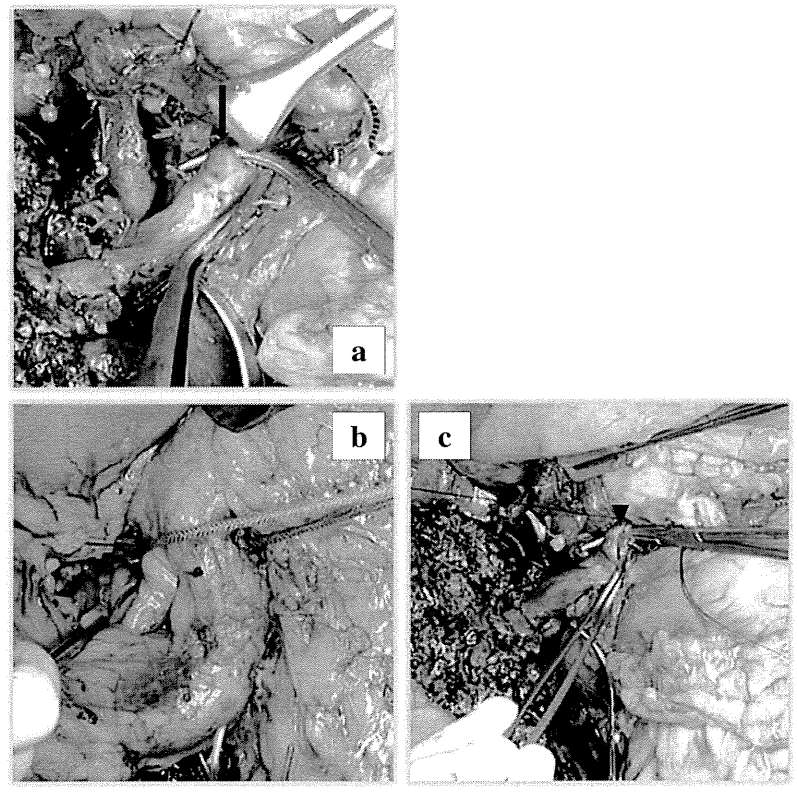

Figure 1 **a** The main PV is dissected towards the upper edge of the SPV root. In this case, the left coronary vein (*arrow*) is transected. **b** Tunneling of the SMV is performed, and the tape is introduced above the main PV and SMV. **c** The SPV root (*arrowhead*) is encircled from the upper side and is tied using a nonabsorbable suture.

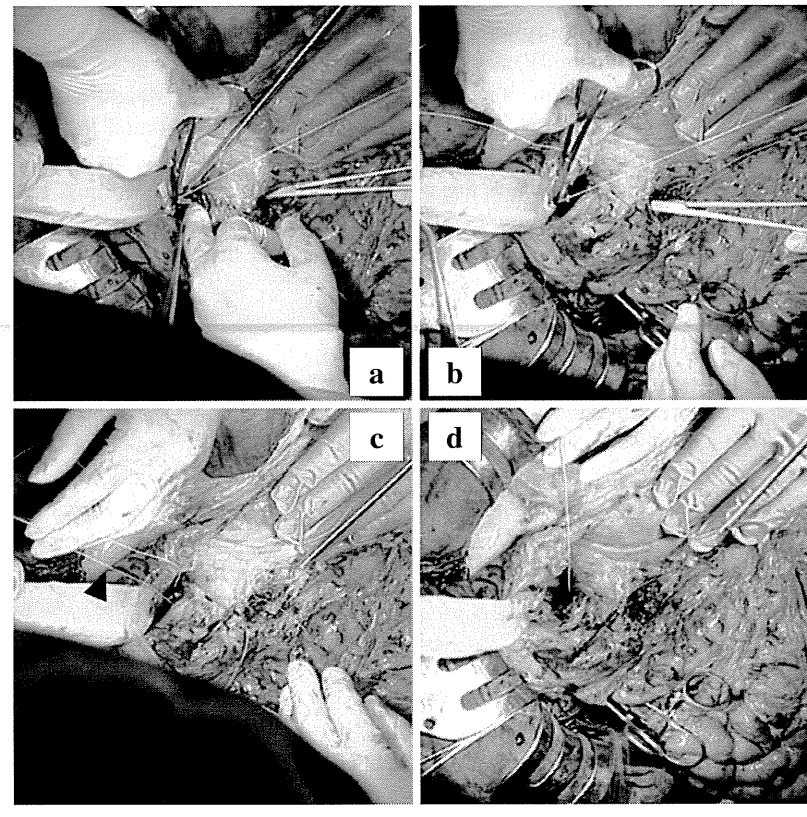

Figure 2 **a** After the dorsal side of the SPV root is dissected from the retroperitoneal tissue, a nonabsorbable suture is introduced behind the SPV root. **b** After tunneling of the SMV, the other suture is introduced above the main PV and SMV. **c** After both sutures are tied at the lower side, the suture above the main PV and SMV (*arrowhead*) is pulled up over the pancreas. **d** The SPV root can be safely and promptly encircled and tied by the suture.

LDLT, as sufficient restoration of the liver vascular bed cannot be achieved in the early postoperative period; posttransplant portal hypertension caused by acute rejection or severe ischemic damage is more severe than in DDLT. The steal of graft PVF by the preserved large SR shunt might thus be more likely in adult LDLT than in DDLT. Based on these issues, several authors have reported that prophylactic management of large SR shunts is necessary to achieve good patient and graft survival, particularly in association with adult LDLT.[1,2,8]

We have introduced a method of diverting SMV and SPV blood flow for the prophylactic management of a large SR shunt in LDLT. Indications for this method were decided according to the preoperative assessment of portal venous hemodynamics, including direction of blood flow in the main PV and the degree of steal of SMV blood flow by the SR shunt. We have applied this method for five patients in whom SMV blood flow had been completely stolen by the SR shunt preoperatively, resulting in excellent postoperative course without morbidity related to the shunt or portal vein complications.

Large SR shunts that have resulted in complete steal of SMV blood flow via the SPV before liver transplantation can often cause the steal of graft PVF immediately after liver transplantation or in various posttransplant conditions causing increased intrahepatic vascular resistance, such as acute rejection or severe ischemic damage.[1–3,8,9] In addition, such SR shunts provoke phlebosclerosis and narrowing of the main PV, intensifying the steal of graft PVF and requiring replacement of the main PV using an interposed vein graft. Several approaches to large SR shunts have thus been applied in liver transplantation.

Ligation of the LRV is a simple, safe procedure, but has a potential risk of detrimental effects on renal function.[1,8,9] Direct division of the SR shunt with splenectomy is technically difficult and is associated with an increased incidence of portal vein complications.[6,7] Ligation of the SR shunt at the site of inflow to the LRV is an effective method but carries the risk of postoperative steal of graft PVF by the development of other residual SR shunts, leading to graft dysfunction.[1,8] On the other hand, the diversion of SMV and SPV blood flow by ligation at the SPV root is a reliable method to ensure prevention of steal from the graft PVF by the SR shunt and to decrease the incidence of portal vein complications.

This method of diverting SMV and SPV blood flows poses issues in terms of the complexity of procedures and the risk of bleeding. However, in our experience, ligation of the SPV root can be performed safely and without bleeding, since collateral vessels around the head of the pancreas are rare because of the large SR shunt. Furthermore, when the SPV root cannot be safely approached from the upper side, we perform the ligation using the procedures described in Fig. 2.

Adequate PVF is essential for postoperative regeneration of the liver after partial liver transplantation.[4,5] Conversely, excessive PVF causes tissue injury in the liver graft and inhibits postoperative liver regeneration. Several groups have thus tried to maintain graft PVF and/or portal venous pressure at an optimal level by selecting occlusion or preservation of the existing portosystemic shunt.[4,10,11] In the present study, diversion of SMV and SPV blood flows by ligation at the SPV root increased graft PVF to optimal levels, as suggested in previous reports.[4,10] Although intraoperative assessment of portal venous pressure was not undertaken in our study, SMV blood flow alone, without SPV blood flow, might be adequate to achieve postoperative liver regeneration of partial liver grafts.

Conclusion

We have applied a diversion method of SMV and SPV blood flow by ligation at the SPV root for prophylactic management of large SR shunts, which had stolen SMV blood flow completely before LDLT. This new surgical approach to large SR shunts can be performed safely and completely prevents morbidity related to such shunts after LDLT.

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World J Surg (2013) 37:2202–2210
DOI 10.1007/s00268-013-2117-3

World Journal of Surgery

Laparoscopy-Assisted Hybrid Left-Side Donor Hepatectomy

Shigeru Marubashi · Hiroshi Wada · Koichi Kawamoto · Shogo Kobayashi · Hidetoshi Eguchi · Yuichiro Doki · Masaki Mori · Hiroaki Nagano

Published online: 5 June 2013

Abstract
Background Laparoscopic liver resection developed for live liver donors has the advantage of reducing the physical and mental stress in donors. However, its safety and efficacy still remain to be established. We aimed to evaluate the feasibility, safety and efficacy of laparoscopy-assisted hybrid donor hepatectomy (LADH) to obtain left side grafts.
Patients and methods A total of 31 consecutive live liver donors of left side liver grafts underwent LADH, including left lateral segmentectomy ($n = 17$) and left liver resection with or without the caudate lobe ($n = 14$) (LADH group). We compared the clinical data between the LADH group and the group of donors in whom traditional open donor hepatectomy was performed to procure the liver graft (open donor hepatectomy [ODH] group, $n = 79$).
Results Laparoscopy-assisted hybrid donor hepatectomy was feasible in all patients, and there was no mortality over a follow-up period of 13.9 ± 9.8 months. The operative time to procure a left-lobe graft was significantly longer in the LADH group (510 ± 90 min) than in the ODH group ($P < 0.001$). A large right lobe on CT (RPv distance) was identified as a significant risk factor for prolonged operative time ($P = 0.007$). Evaluation using the SF36-v2 questionnaire revealed faster recovery of the physical component summary score and bodily pain score in the LADH group than in the ODH group.
Conclusions Laparoscopy-assisted hybrid donor hepatectomy for procuring left side grafts was safe and effective up to the left liver with the caudate lobe. Left-lobe LADH in donors with a large right lobe should be carefully planned in view of the potential surgical difficulty.

S. Marubashi (✉) · H. Wada · K. Kawamoto · S. Kobayashi · H. Eguchi · Y. Doki · M. Mori · H. Nagano
Department of Surgery, Osaka University Graduate School of Medicine, 2-2 Yamadaoka, Suita, Osaka 565-0871, Japan
e-mail: smarubashi@gesurg.med.osaka-u.ac.jp

Introduction

In spite of the growing number of liver transplantations from brain-dead donors around the world, donor shortage still remains a significant problem. As a result, living donor liver transplantation (LDLT) is still necessary in Japan as well as other Asian and Western countries. Needless to say, the most important issue in LDLT is donor safety, and several reported donor deaths emphasize the great importance of this factor, and even minor morbidities should be minimized with the surgery conducted by an experienced surgeon [1, 2]. Donor surgery in live donors substantially affects quality of life, with the patients often developing wound infection, pain, and deformity [3–5]. A recent report of donor morbidities in Japan showed that the incidence of donor surgery-related morbidities was 8.4 % in total, and the leading morbidity was bile leak (2.6 %), followed by wound infection (1.2 %) [5].

Laparoscopy-assisted hybrid hepatectomy or laparoscopic liver resection has been developed for live liver graft donors, as well as for the treatment of benign or malignant tumors [3–6]. Several studies have shown its advantage over traditional open surgery in reducing the physical and emotional stress experienced by patients [7–13]. However, its safety and efficacy remain to be established.

Among the surgeries on live donors to procure liver grafts, that for obtaining a "left lobe including the caudate" graft is technically the most difficult, and very limited studies have reported the use of laparoscopic procedures to harvest left liver plus caudate lobe grafts [9]. Left liver plus

caudate lobe grafts have been used to obtain the maximum graft volume from the left side of the donor liver in adult-to-adult LDLT [14, 15]. It is important to adopt this principle, regardless of whether a laparoscopy-assisted procedure or traditional open surgery is employed.

We have experience in performing more than 140 live donor hepatectomies, as previously reported [16]. We also have sufficient experience in performing laparoscopy-assisted hepatectomy for the treatment of liver tumors. Based on this considerable experience, we began to perform laparoscopy-assisted hybrid donor hepatectomy, initially to obtain left lateral section grafts, and then, with accumulating experience, first, left liver without the caudate grafts, and finally left liver plus caudate lobe grafts.

The aim of the present study was to evaluate the safety and efficacy of laparoscopy-assisted hybrid donor hepatectomy (LADH) to procure left-side grafts, including left lateral section, left liver without the caudate, and left liver plus caudate lobe grafts.

Patients and methods

The study protocol was approved by the Human Ethics Review Committee of Osaka University Graduate School of Medicine (No. 750). A signed consent was obtained from each donor prior to operation. The study protocol was registered in the UMIN clinical trial registry (ID: UMIN000003886).

Study design

The study was a non-randomized prospective cohort study. The primary endpoint was mortality and morbidity of laparoscopy-assisted hybrid donor hepatectomy, and the secondary endpoint was the postoperative quality of life (QOL) of the living donors as evaluated in terms of analgesic requirement and the SF36v2 questionnaire for postoperative QOL.

Donor evaluation

Donor evaluation was based on the criteria approved by the ethics review committee of Osaka University. All living liver donors were adults between 20 and 65 years of age. Donor candidates with systemic diseases such as hypertension, diabetes mellitus, or psychiatric disease, and those receiving medications for any systemic disease were strictly excluded. Preoperative evaluation consisted of a complete history and physical examination, and laboratory tests (complete blood count, blood chemistry, coagulation profile, hepatitis B or C virus markers, and serological profiles for other infectious diseases). Donors also underwent chest and abdominal radiography, four-phase multidetector computed tomography (MD-CT) and drip-infusion cholangiography computed tomography (DIC-CT) with three-dimensional reconstruction. Liver volumetric analysis was conducted routinely with the Virtual Place software ver. 2.0 (AZE, Tokyo, Japan) and/or the Synapse Vincent 3D image analysis system (Fujifilm Corporation, Tokyo, Japan).

Graft selection

The criteria for donor selection have been described previously [16]. Briefly, the graft type was determined by the results of the volumetric study with MD-CT. The requirements for living donation were (1) an estimated volume of the remnant liver of more than 35 % of the whole liver volume of the donor, and (2) an estimated donor graft liver volume of more than 40 % of the recipient's standard liver volume (SLV).

Donor surgery

Open donor surgery

The methods employed for donor hepatectomies have been described previously [16]. All donors received a midline incision with bilateral subcostal incisions (Mercedes incision). Big incisions were an essential part of open donor surgery to secure the best possible field and assure donor safety during the operation. The bilateral costal incision was shorter in left lateral sectionectomy than in left lobectomy. Standard total length of incision was 25 cm in left lateral sectionectomy and 40 cm in left lobectomy. Surgery has been performed under general anesthesia without epidural anesthesia since July 2009. Basic techniques for donor hepatectomy were based on the strategy of no metal clips, no inflow occlusion, and minimal dissection of the liver hilum, as described previously [16].

Laparoscopy-assisted hybrid donor surgery (LADH)

A midline incision about 7 cm long was first made, and later extended an additional 1 cm or more, as needed. The round ligament and falciform ligament were divided. Liver wedge biopsy was obtained from segment 3 of the liver and sent for histopathological evaluation. A Gelport was placed and a 12 mm trocar was inserted through the Gelport, followed by establishment of pneumoperitoneum at 10 cm H_2O. A flexible 10 mm scope was used for the laparoscopic procedure. A 12 mm trocar was inserted at the umbilicus, and then the scope was reinserted from this second trocar, after which 5 mm trocars were placed as shown in Fig. 1, two for left lateral sectionectomy or three for left lobectomy. The left triangular ligament was

dissected up to the left hepatic vein under either full laparoscopic guidance or as a hand-assisted maneuver. For obtaining a left with caudate lobe graft, a 12 mm trocar was placed through the Gelport, and the caudate was mobilized from the inferior vena cava (IVC) under laparoscopic view (Fig. 1b). The short hepatic vein from the caudate was preserved if it measured more than 5 mm in diameter. The Arantius duct was transected, and the left and middle hepatic veins were mobilized from the IVC as far as possible. For left lobectomy with or without the caudate lobe, the right triangular ligament was dissected and the right lobe was mobilized with the hand-assisted laparoscopic surgery (HALS) technique. Dissection between the right adrenal gland and the liver was not necessary. Under the hybrid procedure, dissection around the right hepatic vein and pericaval region was carefully performed until the right lobe was fully mobilized. Pneumoperitoneum was ended after checking hemostasis. For left lobectomy, the incision was extended to 10–12 cm, then a retractor was placed. Dissection around the right hepatic vein was performed under direct vision at this point [17]. Cholecystectomy, hilar dissection, the liver hanging maneuver, and liver parenchymal dissection were performed under direct vision through the small midline incision in LADH. We applied the same procedure as that in the open technique in terms of not using any metal clips or inflow occlusion, with minimal dissection of the liver hilum.

Postoperative management and care

A drain was placed at the end of the operation, and was removed on postoperative day 2–3. Postoperative pain control was initiated immediately after operation with intravenous continuous fentanyl infusion at 0.5 μ/kg per hour for 40 h. Donors could receive bolus doses of fentanyl at 0.5 μ/kg per bolus every hour, as needed, up to 40 h after the operation, and flurbiprofen 50 mg or loxoprofen 50 mg thereafter.

Enhanced MDCT was performed on postoperative days (POD) 7, 14, and 28, and at 3, 6, and 12 months after operation. Doppler ultrasonography was performed on POD 1 to rule out the presence of a thrombus in the hepatic artery or portal vein. Donors were considered to be ready for discharge from the hospital on treatment with an oral proton pump inhibitor when the liver function tests were normal or improving satisfactorily, and they were capable of eating sufficient oral intake (more than 80 % of normal adult food).

Postoperative morbidities and evaluation of the health-related QOL after donor surgery

Postoperative morbidities were evaluated based on the Clavien–Dindo classification [18, 19]. Health-related QOL was evaluated preoperatively and at 1, 3, 6, and 12 months after the surgery with the Short Form-36, version 2 (SF36-v2) questionnaire [20].

Assessment of potential difficulty in left-lobe laparoscopy-assisted hybrid donor hepatectomy

Laparoscopy-assisted hybrid donor hepatectomy (LADH) could be more difficult to perform in obese or big male donors. Preoperatively, we calculated the distance between the abdominal wall and the front of the spine at the level of

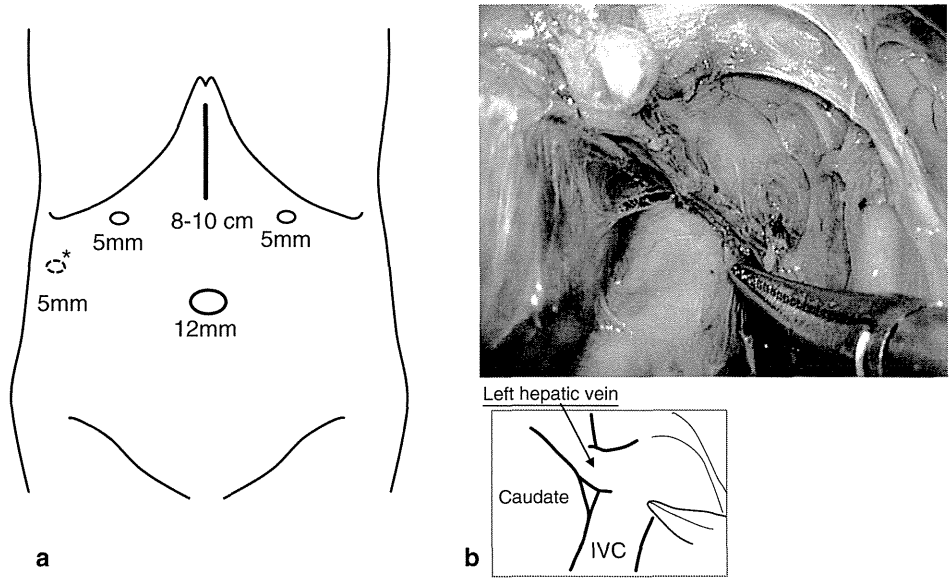

Fig. 1 Laparoscopy-assisted hybrid donor surgery. **a** Skin incision and trocar sites. An upper abdominal midline incision was made over a length of 7–8 cm for left lateral sectionectomy and a length of 10–12 cm for left lobectomy. A 12 mm trocar was placed through the umbilicus, and 2 trocars (5 mm) were placed in the hypochondriac region of either side. A third trocar (5 mm, *) was placed in the right flank for left lobectomy. **b** Mobilization of the left liver plus caudate. The Spiegel lobe of the caudate was completely mobilized under laparoscopic guidance

the portal bifurcation (WS distance), and the maximal distance between the surface of the right lobe and the portal vein bifurcation (RPv distance) on donor CT scans (Fig. 2a, b).

Evaluation of the feasibility and safety of laparoscopy-assisted hybrid donor liver surgery

Living donors who underwent LADH were divided into groups: those who underwent left lateral sectionectomy (LADH-lateral group) and those who underwent left lobectomy with or without the caudate lobe (LADH-left group). Living donors who underwent open donor hepatectomy were also divided into groups: those who underwent left lateral sectionectomy (open donor hepatectomy [ODH]-lateral group) and those who underwent left lobectomy with or without the caudate lobe (ODH-left group).

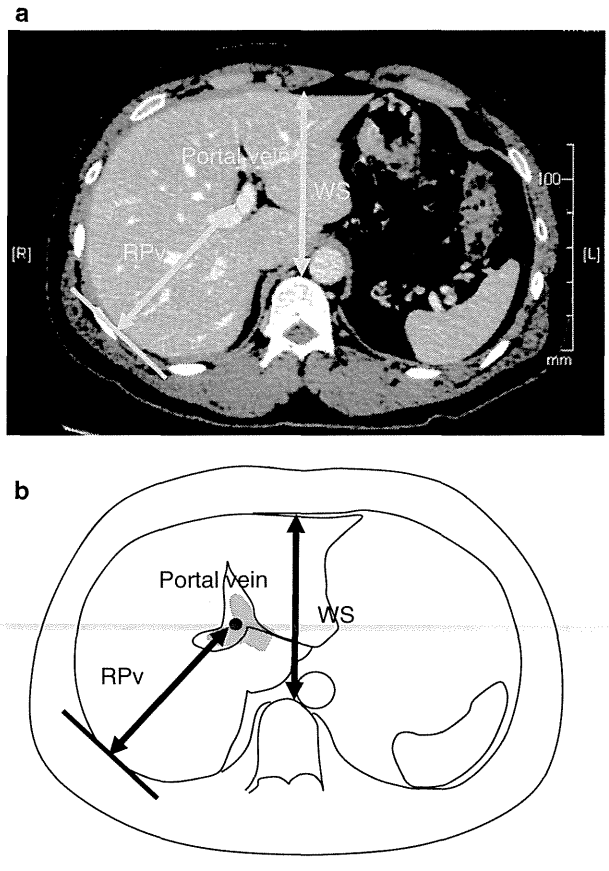

Fig. 2 Distance between the abdominal wall and the front of the spine at the level of the portal bifurcation (*WS distance*) and RPv distance. WS distance was defined as the distance between the abdominal wall and the front of the spine at the level of the portal bifurcation, and the maximal distance between the surface of the right lobe and the portal vein bifurcation (*RPv distance*) was defined as the maximal distance between the surface of the right lobe and the portal vein bifurcation on preoperative CT scans. **a** CT scan image. **b** Schematic view

The demographic characteristics, operative parameters, postoperative morbidities, results of the SF36-v2 questionnaire evaluation, analgesic requirement, and serum C-reactive protein levels measured preoperatively and on POD 1, 3, 7, and 14 were compared between the ODH and LADH groups.

The analgesic requirement was compared between the LADH ($n = 31$) and recent open donor groups (after July 2009 [$n = 21$]), when we stopped using epidural anesthesia and started to use intravenous fentanyl for 40 h after surgery in July 2009.

Statistical analysis

Results are expressed as mean ± standard deviation. Statistical examination of the correlations was based on the Pearson product-moment correlation. Clinical data of the donors were compared with Student's t test. P values <0.05 were considered to indicate statistical significance.

Results

A total of 31 consecutive live liver donors of left-side liver grafts underwent LADH between April 2009 and March 2012; of these, 17 donors underwent left lateral sectionectomy (LADH-lateral group), including one case of in situ S3 monosegmentectomy, and 14 donors underwent left lobe resection with or without the caudate lobe (LADH-left group). We compared the clinical outcomes between the LADH group ($n = 31$) and donors who had undergone open donor hepatectomy (ODH group; $n = 79$) prior to this period in our hospital, which were either open left lateral sectionectomy (ODH-lateral group; $n = 32$), including one case of reduced-left lateral sectionectomy or open left lobe resection with or without the caudate lobe (ODH-left group; $n = 47$).

There was no perioperative or postoperative mortality in any of the donor groups, and all the donors were healthy without any sustained physical or mental problems at 13.9 ± 9.8 months after the donor hepatectomy.

The demographic characteristics of the donors were similar between the LADH group and the ODH group (Table 1). The length of the midline incision was 7.5 ± 0.7 cm in the LADH-lateral group and 10.5 ± 1.4 cm in the LADH-left group. The operative time was 375 ± 65 min in the LADH-lateral group and 508 ± 94 min in the LADH-left group; the operative time was significantly longer in the LADH-left group than in the ODH-left group ($P < 0.001$). The volume of blood loss was similar between the LADH and ODH groups. The

postoperative length of hospital stay was 9.0 ± 2.3 days in the LADH-lateral group and 11.5 ± 3.6 days in the LADH-left group, which were significantly shorter than those for the donors who had undergone open surgery ($P = 0.019$).

The operative time was similar between the donors who underwent left lobe resection with the caudate ($n = 6$) or without the caudate lobe ($n = 8$), and it was not associated with the body mass index (BMI) or the WS distance. Of note, the operative time increased as the RPv distance increased ($P = 0.014$, $r = 0.637$) (Fig. 3a, b). The operative time was significantly longer in the donors with an RPv distance equal to >10 cm ($n = 6$) as compared with donors with an RPv distance of less than 10 cm ($n = 8$) ($P = 0.007$) (Fig. 3c). No significant correlation was observed between the volume of blood loss and the RPv distance or WS distance.

Laparoscopy-assisted hybrid donor hepatectomy was feasible, without any need for conversion to open surgery, in all patients in the LADH group. During the laparoscopic procedure, two incidental injuries (one to the diaphragm and one to the right hepatic vein) occurring during mobilization of the right lobe were successfully managed by finger compression under the HALS technique and subsequent suturing under direct vision through the midline incision. In one of the patients, however, elongation of the midline incision to 15 cm was necessitated; in the other, the procedure was completed through the planned 12 cm midline incision.

Table 1 Characteristics of the laparoscopy-assisted hybrid donor hepatectomy (LADH) group and the open donor hepatectomy (ODH) group

	LADH ($n = 31$)	ODH ($n = 79$)	P value
Age, years	35.8 ± 8.4	37.8 ± 10.1	0.369
Gender (male)	13 (41.9 %)	54 (68.4 %)	0.011
Body mass index (BMI), kg/m^2	21.3 ± 3.6	22.6 ± 3.1	0.075
Type of resection			
Left lateral section (LLS)	16	31	0.174
Reduced left lateral section (rLLS)	1	1	(Left vs. LLS)
Left lobe without caudate (left)	8	10	
Left lobe with caudate (left-C)	6	37	
Operative time, min	435 ± 103	383 ± 73	0.005
Estimated blood loss, ml	353 ± 396	456 ± 347	0.197
Length of hospital stay after surgery, days	10.3 ± 3.3	18.3 ± 16.7	0.019
Complication (Clavien–Dindo grade)			
1	1 (3.2 %)	7 (8.9 %)	0.653
2	0	1 (1.3 %)	
3a	2 (6.5 %)	8 (10.1 %)	
3b	0	1 (1.3 %)	
4/5	0	0	

After the donor surgery the amount of pain medication needed up until the seventh POD after 40 h of systemic fentanyl infusion was compared between the LADH group ($n = 31$) and the recent ODH group ($n = 21$), and was found to be similar between the two groups (Fig. 4a). Likewise, the serum C-reactive protein (CRP) levels after surgery were similar between the LADH and recent ODH group (Fig. 4b).

Postoperative morbidity, defined with the Clavien–Dindo classification [18], was established as grade ≥2 in two donors (6.7 %) with delayed gastric emptying which required fiberoptic endoscopy ($n = 2$) for correcting rotation of the stomach, and both recovered within 2 weeks after the donor surgery. No bile leak or other morbidity was observed.

There was no mortality related to the LADH procedure among the graft recipients. The graft survival rate of the 17 pediatric recipients who received the left lateral section grafts from the LADH-lateral group was similar to that of the 32 pediatric recipients who received the left lateral section grafts from the ODH-lateral group ($P = 0.877$, log rank test) (Fig. 5a). The graft survival rate of the 14 recipients (9 adults and 5 children) who received the left lobe grafts in the LADH-left group was slightly better but statistically similar to that of the 47 recipients (32 adults and 15 children) who received the left lobe grafts in the ODH-left group ($P = 0.237$, log rank test) (Fig. 5b).

A total of 29 donors from the LADH group could be evaluated by the SF36-v2 questionnaire. Comparison with the preoperative test results revealed that the scores for all six components decreased significantly at 1 month after the surgery; thereafter, the physical functioning (PF) score, general health perception (GH) score, vitality (VT) score, social functioning (SF) score, and mental health (MH) score recovered by 3 months, while the role physical (RP) score, bodily pain (BP) score, and role emotional (RE) score recovered by 6 months after the surgery. The PCS score, which was decreased at 1 month after the surgery, recovered by 6 months, and the mental component summary (MCS) score, which was decreased at 1 month after the surgery, recovered by 3 months (Fig. 6).

Discussion

Despite close attention being paid to preventing donor mortality and morbidity in living donor hepatectomies, it is inevitable to encounter them at a certain incidence.

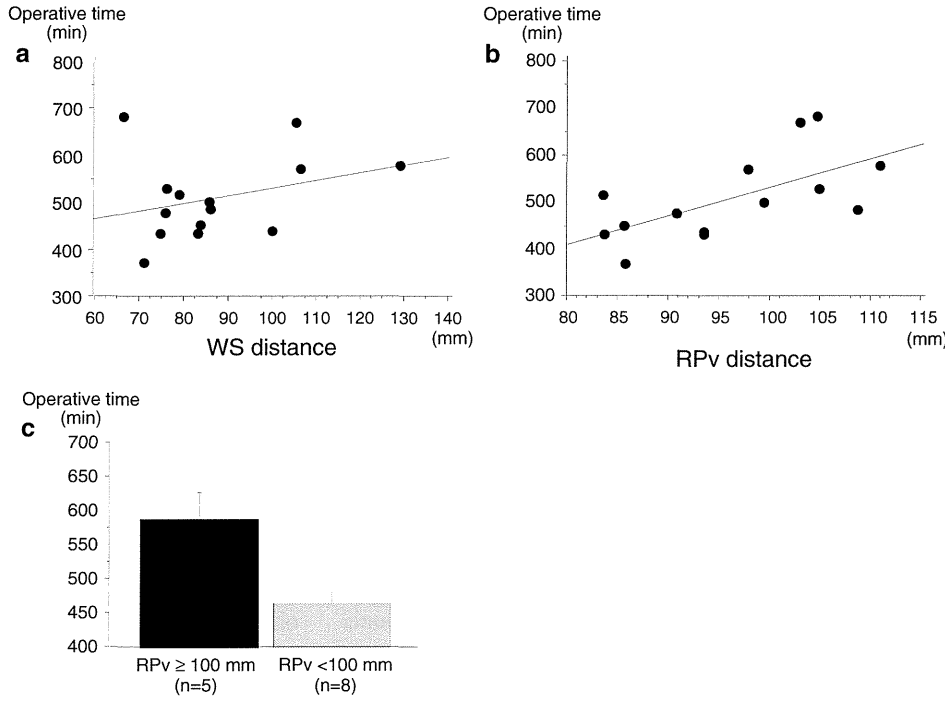

Fig. 3 Relationship between the WS distance and RPv distance and the operative time in the LADH-left group ($n = 14$). **a** WS distance and operative time. There was no significant correlation between these two parameters. ($r = 0.239$). **b** RPv distance and operative time. There was a significant correlation between the RPv distance and the operative time; operative time (min) $= -76.4 + 6.10 \times$ RPv (mm); $P = 0.014$, $r = 0.637$. **c** The operative time was significantly longer in donors with an RPv distance equal to or >10 cm ($n = 6$) than in those with an RPv distance of <10 cm ($n = 8$) ($P = 0.007$)

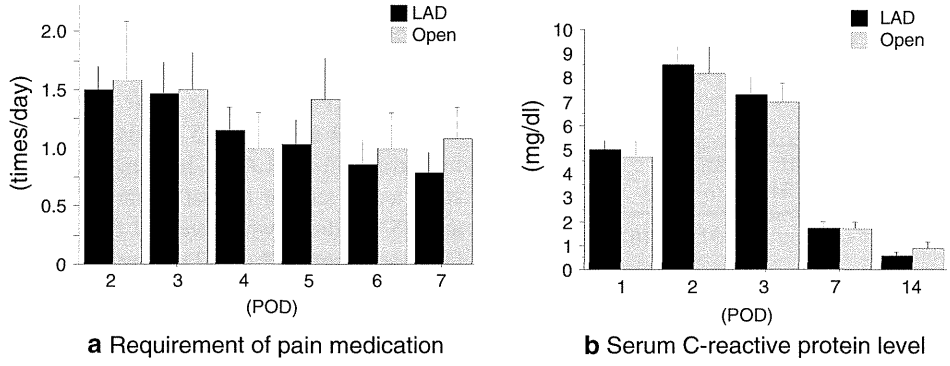

Fig. 4 Analgesic agent requirement and serum C-reactive protein level. (LADH: $n = 31$, ODH group: $n = 21$). **a** Analgesic agent requirement from postoperative day (POD) 2 to POD 7. While the requirement was higher in the ODH group after POD 5, there was no significant difference between the LADH and ODH groups. **b** The serum CRP level peaked on POD 2 in both groups, with no significant difference in the level change between the LADH and ODH groups

Therefore many surgeons consider that the traditional open donor hepatectomy with a big incision is appropriate, merely for reasons of safety. In addition, donor protection is very important in terms of reduction of physical and mental stresses, and also provision of support for recovery from the surgery to a healthy daily life as before the operation. Laparoscopic surgery was introduced in the field of donor hepatectomy, first from left lateral sectionectomy [6] and on to right lobectomy [17], and these techniques have been rapidly spread worldwide. However, parenchymal dissection in laparoscopic view is not always a familiar technique to most hepatobiliary surgeons who are experts in open donor hepatectomies. LADH has been developed based on its advantageous characteristics of less invasiveness for living liver donors and the familiarity of direct parenchymal dissection to hepatobiliary surgeons. One of the other important features of this procedure is the safety we have observed during laparoscopic surgery because of the advantages of hand-assisted surgery.

In our series there were two significant complications during right lobe mobilization: a right diaphragmatic injury and an injury to the right hepatic vein. In each case the surgeon was able to make a successful recovery, initially using fingers in the hand-assisted technique, without any

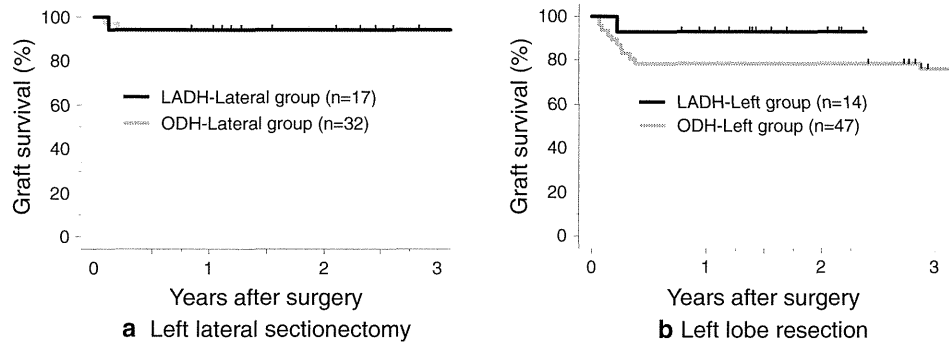

Fig. 5 The graft survival rates after liver transplantation. **a** Left lateral sectionectomy. The graft survival rates were similar between the LADH and the ODH groups ($P = 0.877$, log rank test). **b** Left lobe resection with caudate or without caudate. The graft survival rate in the LADH group was slightly better than that of the ODH group, although there was no significant difference between the LADH and the ODH groups ($P = 0.237$, log rank test)

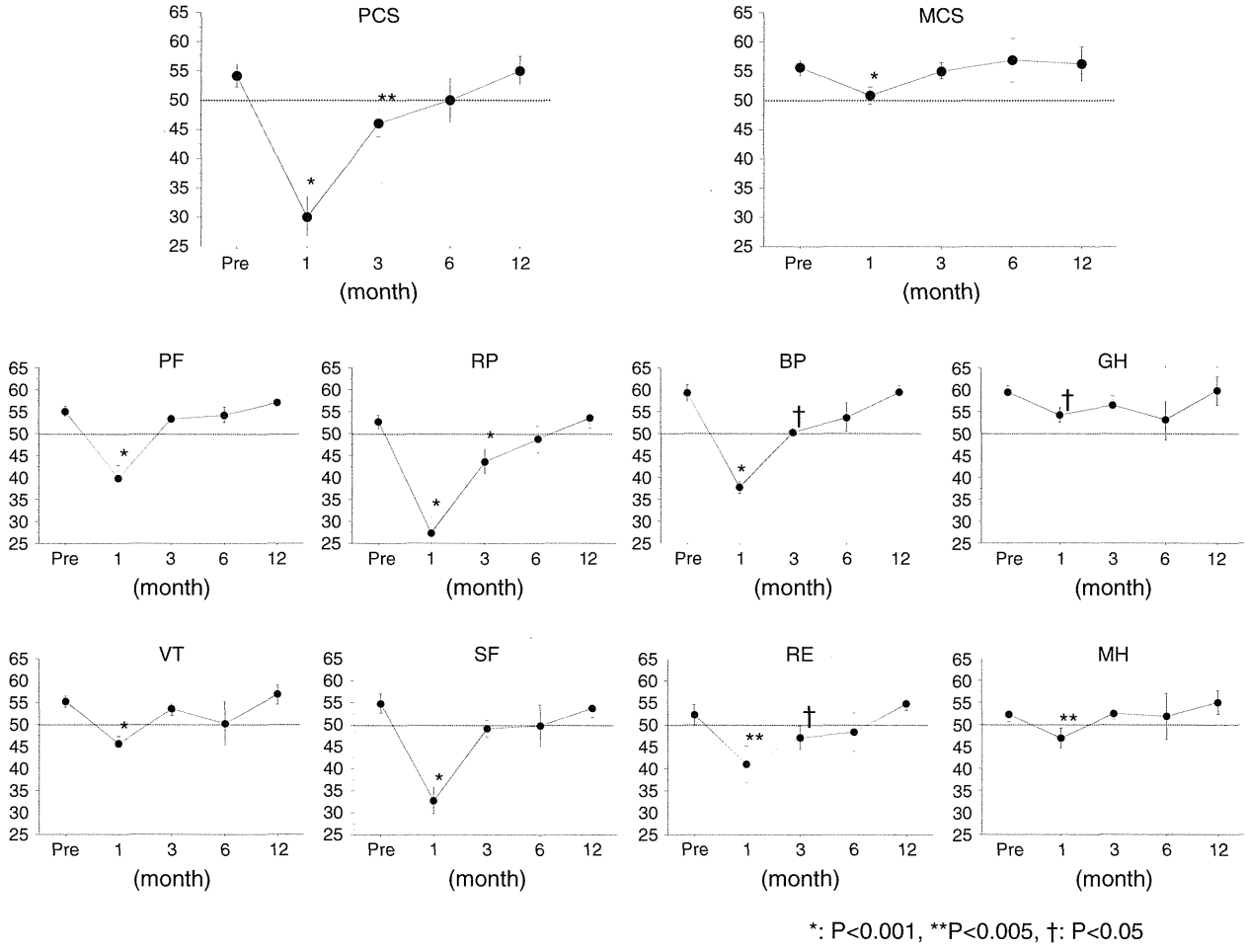

Fig. 6 Evaluation by the Short Form-36, version 2 (SF36-v2) questionnaire. *PCS* physical component summary, *MCS* mental component summary, *PF* physical functioning, *RP* role physical, *BP* bodily pain, *GH* general health perceptions, *VT* vitality, *SF* social functioning, *RE* role emotional, *MH* mental health. * $P < 0.001$; ** $P < 0.005$; † $P < 0.05$

problem under HALS technique. Nevertheless, the safety and efficacy of LADH has not been established, and few feasibility studies are reported [7, 9–11]. Therefore, the purpose of the present study was to investigate the safety and efficacy of the laparoscopic procedure for procuring left liver grafts.

The technique of LADH is quite demanding, and adequate experience with both open donor hepatectomy and laparoscopic mobilization of the left and right hemi-liver is required. Thus, it is important to ensure that LADH is performed by surgeons with adequate experience in both donor hepatectomy and laparoscopic liver mobilization, under the assumption that "experienced" surgeons in donor hepatectomy would be able to perform donor left lobectomy with the caudate by themselves without any supervision.

We have reported the adequacy of our open donor hepatectomy previously, and have also performed laparoscopic hepatectomies very actively. Having established these two bases, we started to perform LADH in a stepwise manner, from LADH-lateral to LADH-left surgery; we believe that this stepwise approach was fundamental from the point of view of preserving the donor safety. We conducted research to determine the best sites for ports, the number of ports, the method for dissecting the liver hilum and hepatic veins in 10 cases of laparoscopy-assisted hybrid left lateral sectionectomy, and then proceeded to left lobe surgery with or without the caudate.

The target length of the midline incision was 7–8 cm for LADH-lateral in our series. This was sufficient to perform hilar dissection and dissection of the liver parenchyma for lateral segmentectomy. For left lobectomy, the incision was extended to 10 cm or longer to ensure an adequate view of the liver parenchyma for dissection. Thus, the mean length of the midline incision was 7.5 ± 0.7 cm for left lateral sectionectomy and 10.5 ± 1.4 cm for left lobectomy. It is noteworthy that the length of the skin incision was uniform in spite of differences in body constitution or BMI in LADH, which could not be expected in open donor hepatectomy.

In our series blood loss was similar between the LADH and ODH groups. The operative time for left lateral sectionectomy was similar between the LADH and ODH groups, but that for left lobectomy was much longer in the LADH group than in the ODH group ($P < 0.001$). No improvement was seen even with case experience (data not shown), suggesting that the longer operative time for left lobectomy was needed because of the small incision in the LADH group.

The operative time in the LADH-left group was associated with the RPv distance, but not with the WS distance. An RPv distance of over 10 cm was identified as a significant risk factor for a prolonged operative time. At first, in fact, we hypothesized that the WS distance might influence the difficulty level of left-lobe LADH. However, no correlation was noted between the WS distance and the duration of operation. We then calculated the RPv distance, because we thought that the difficult cases tended to have a larger right lobe. During the left-lobe LADH procedure, the right lobe is mobilized and rotated toward the midline incision to allow performance of hybrid surgery through the small midline incision. Our results showed that the longer the RPv distance, the longer the duration of left-lobe LADH, suggesting that the volume of the right lobe of the liver had a greater impact on this procedure than the depth of the abdomen. Because left-lobe LADH is expected to be more difficult and to take a longer time in donors with an RPv distance >10 cm in left-lobe LADH, the operation type and explanations to the donors should be carefully conducted preoperatively.

Again in our series, two incidental events occurred during LADH that may have been avoided by a surgeon with greater experience in laparoscopic right lobe mobilization. However, both incidental injuries were easily treated with the help of a hand inserted into the abdomen, which is the one of the advantages of the HALS technique. In case of unexpected incidents such as these, the HALS technique is quite useful and safer than pure laparoscopic surgery, which is one of the reasons why we adopted HALS. It is fundamental in donor surgery not to expose the donor to any avoidable danger.

Postoperative morbidity was rather rare in the LADH group, and the length of hospital stay after surgery was shorter in the LADH group than that in the ODH group ($P = 0.028$), indicating that the safety of the procedure was comparable to that of the well-established open procedure. Serum CRP level is one of the markers of acute-phase reactions to surgery; however, in the present series it failed to reflect any advantage of the laparoscopic procedure, with the smaller skin incision, over the open procedure. In studies comparing open and laparoscopic colorectal surgery, no significant differences in the serum levels of interleukin (IL)-1, IL-6, IL-8, or interferon γ (IFN-γ), all of which are known to be acute-phase cytokines, were found between the laparoscopic surgery and open surgery groups [21]. These results showed that the invasiveness of the surgery was not different between the open and laparoscopic techniques, at least as evaluated by measurements of the serum cytokine levels, even though the patients in the LADH group recovered more rapidly after surgery and discharge than those of the ODH group.

The length of hospital stay after surgery was significantly reduced in the LADH group. Although the length of hospital stay was much longer as compared with that reported from the West in both the open and LADH groups [7], it is our policy to keep the donors in the hospital until the absence of any influence of the surgery in the daily lives of the patients, except for requirement of a minimal amount of pain medications.

In the short-term evaluation, the analgesic requirement during the first week after surgery was similar between the LADH and ODH groups. However, in the longer-term evaluation, the QOL after surgery as evaluated using the SF36-v2

questionnaire showed recovery of both the PCS summary score and the BP pain score by 6 months after operation. Considering the previous report of evaluation of living liver donors by the SF36-v2 questionnaire [20], recovery from bodily pain and physical disturbance after surgery was quicker in the LADH group than in the ODH group. These results showed that LADH may be less invasive and have a positive impact on the postoperative QOL in the donors.

The graft survival rates in the recipient patients, which were fundamental and important in evaluating the outcome of donor hepatectomy, were similar between the LADH group and the ODH group either in left lateral sectionectomy or left lobe resection. The slight difference in the graft survival rates between the LADH-left and the ODH-left groups in left lobe resection was considered to have resulted in part because of the different time periods in which the surgeries had been performed. These results could also strengthen the positive evaluation of the LADH procedure from the standpoint not only of the donors but also the recipients.

This study was not a randomized or high-volume study. Therefore, the results should be interpreted cautiously. Nonetheless, the results suggesting that LADH was safe and feasible, and provided a better QOL after surgery in our series, may justify continuation of LADH for procuring left liver grafts.

One of the problems in our series was that the operative time for procuring a left liver graft with LDAH was significantly longer than that of open surgery. The operative time for left-lobe LADH depends on the duration of open procedures, suggesting that more experience in hilar dissection and parenchymal transection under the hybrid procedure would be important for reducing the operative time. Another approach could be increasing the length of the incision to more than 10 cm, especially in donors with an RPv distance of more than 10 cm.

In conclusion, LADH was safe and feasible for harvesting left liver grafts in the hands of surgeons with experience in both open donor surgery and laparoscopic surgery, and use of the procedure had a positive impact on the postoperative QOL in the donors, although the prolonged duration of the procedure in the LADH-left group needs to be improved with further experience and improvements in the technique of LADH. Left-lobe LADH should be carefully planned in donors with an RPv distance of more than 10 cm, in view of the potential surgical difficulty.

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Cancer Chemother Pharmacol (2013) 72:1325–1333
DOI 10.1007/s00280-013-2311-x

ORIGINAL ARTICLE

Evaluation of safety parameters and changes in serum concentration in liver transplant recipients treated with doxorubicin during the anhepatic period

Shogo Kobayashi · Hiroshi Wada · Naoki Hama · Hirofumi Akita · Koichi Kawamoto · Hidetoshi Eguchi · Koji Umeshita · Yuichiro Doki · Masaki Mori · Hiroaki Nagano

Received: 8 May 2013 / Accepted: 25 September 2013 / Published online: 13 October 2013

Abstract
Purpose Because of the recurrence of hepatocellular carcinoma (HCC) at the graft after liver transplantation, circulating HCC cells may be present during the anhepatic period. Intravenous doxorubicin (DOX) is used during the anhepatic period to combat these cells; however, pharmacokinetics data have been poorly analyzed. This study aims to investigate DOX administration during the anhepatic period.
Patients and methods We administered 5 mg/m^2 DOX immediately after liver removal and compared serum DOX concentrations at several intervals during the anhepatic period in patients who underwent liver transplantation because of liver cirrhosis and HCC ($n = 3$) and patients who underwent liver resection owing to HCC with portal vein tumor thrombi ($n = 5$). We also measured serum DOX concentrations and pharmacokinetic parameters in transplant patients that received 3–15 mg/m^2 DOX ($n = 3$ per dose level). We evaluated transplant patients' adverse drug reactions and survival.
Results At 10 and 30 min after DOX administration, serum DOX concentrations were elevated two- to threefold in transplant patients versus resection patients. Dose escalation in transplant patients exhibited a prolonged $T_{1/2}$ in the one-compartment model and $T_{1/2}$ β in the two-compartment model, as well as a dose-dependent elevation of the area under the curve. No obvious adverse drug reactions were noted at 3–15 mg/m^2 DOX. In transplant patients, 5-year recurrence-free survival was 68.8 %; overall survival was 100.0 %.
Conclusion During the anhepatic period, serum DOX concentrations were elevated two- to threefold, $T_{1/2}$ was prolonged dose dependently, and up to 15 mg/m^2 DOX could be safely administered.

Keywords Liver transplantation · Hepatocellular carcinoma · Doxorubicin · Pharmacokinetics · Anhepatic period

S. Kobayashi · H. Wada · N. Hama · H. Akita · K. Kawamoto · H. Eguchi · K. Umeshita · Y. Doki · M. Mori · H. Nagano (✉)
Department of Surgery, Osaka University, Suita, Osaka 565-0871, Japan
e-mail: hnagano@gesurg.med.osaka-u.ac.jp

Introduction

Viral hepatitis and cirrhotic liver are major risk factors associated with hepatocellular carcinoma (HCC) [1, 2]. Because these are chronic conditions that also affect liver function, and some cases of HCC are contraindicated for surgical resection because of poor liver function. In these cases, liver transplantation is becoming an alternative strategy to combat this tumor, even in patients with Child-Pugh C liver function [3, 4]. Although Milan and other criteria [4–6] have proposed indications for liver transplantation due to HCC with cirrhotic liver, the prognosis in patients with HCC exceeding these criteria is quite poor [5–8]. Accordingly, several authors have tried neo-adjuvant therapy for down-staging, as well as intra-operative and post-operative adjuvant chemotherapy [4, 9, 10]. Because of HCC recurrences at the liver graft after transplantation, some authors have suggested that circulating HCC cells may be present during the anhepatic period [11–14]. Adjuvant chemotherapies have been tried against these small clusters of HCC cells [15].

Doxorubicin (DOX) is one of the major drugs employed against HCC in several situations, both for unresectable HCC and in an adjuvant setting. For example, several clinicians have performed adjuvant chemotherapy with DOX

after the resection of HCC with portal vein tumor thrombus (PVTT) [16, 17]. In liver transplantation, several clinicians have tried chemotherapy during the anhepatic period [18, 19] or adjuvant chemotherapy with DOX [9, 20] in patients with HCC exceeding Milan criteria.

However, pharmacological analysis of DOX during the anhepatic period and after reperfusion during liver transplantation is rarely investigated. This drug is mainly metabolized in the liver, and the serum concentration would reportedly remain high in patients with liver dysfunction [21–24]. In dogs, serum DOX concentration was measured during the anhepatic period and exhibited only a 50 % reduction in total body clearance [25, 26]. In the present study, we measured serum DOX concentration during the anhepatic period in the transplant recipients. We also compared these results to serum DOX concentrations in patients who underwent liver resection. Furthermore, we evaluated safety by performing a detailed investigation of the adverse events and adverse drug reactions in these series.

Patients and methods

Patients

Between 2003 and 2011, we measured serum DOX concentration in 12 patients who underwent liver transplantation because of liver cirrhosis and HCC (TSPL group). We also measured serum DOX concentration in five patients who underwent liver resection and PVTT removal owing to HCC with PVTT (RESC group). The first three patients in the TSPL group were treated with 5 mg/m^2 DOX, and we compared pharmacokinetic data from the TSPL group with data from the RESC group. Previous data [25, 26] indicated that DOX clearance would be reduced by 50 %; therefore, for safety reasons, we administered 5 mg/m^2 DOX (the common dose for systemic administration in the context of HCC is 45–75 mg/m^2 [27–29]) and compared the pharmacokinetic data of the TSPL and RESC groups. After pharmacokinetic data were confirmed in the TSPL group at 5 mg/m^2 DOX, we administered DOX at several dose levels (3, 10, and 15 mg/m^2), calculated pharmacokinetic data, and evaluated adverse events at each dose level. Patients' characteristics were prospectively collected. All patients underwent surgery at our institution. The protocol was approved by the institutional review board at our hospital, and written informed consent was obtained from each patient.

DOX administration, sample collection, and measurement DOX concentration

The time course of DOX administration and sample collection is depicted in Fig. 1a. In the TSPL group, patients underwent liver transplantation because of liver cirrhosis with HCC. At 5 min after explantation of the cirrhotic liver, 3–15 mg/m^2 of DOX were administered intravenously. Five milliliter peripheral blood samples were obtained at 0, 10, 30, 60, and 120 min after DOX administration until reperfusion. We also collected blood samples at 0, 10, 30, and 60 min post-reperfusion. The RESC group underwent liver resection with the removal of PVTT. We administered 5 mg/m^2 DOX to each RESC patient 5 min after the liver resection was completed. Blood samples were obtained at 0, 10, 30, 60, and 120 min after DOX administration.

All blood samples were stored at 4 °C, centrifuged at 3,000 rpm for 10 min, and frozen at −80 °C before the DOX concentrations were measured. Serum DOX concentrations were measured by high-pressure liquid chromatography at Kyowa Hakko Kogyo Co., Ltd., Japan. The serum concentration curves, pharmacokinetic parameters, and area under the DOX concentration curve from 0 to 120 min (AUC_{120}) were determined for each patient. Various parameters were calculated using the one- or two-compartment infusion model ($C(t) = Ae - \alpha t$ for the one-compartment model and $C(t) = Ae - \alpha t + Be - \beta t$ for the two-compartment model) and LAB Fit Curve Fitting Software 7.2.41 (Wilton and Cleide Pereira da Silva, Brazil). AUC_{120} was calculated using the trapezoidal model.

Evaluation of adverse events and adverse drug reactions

We evaluated adverse events and adverse drug reactions according to CTACE version 4.0, retrospectively, during the first 7 days after the surgery. For adverse drug reactions, we considered events that were unrelated to liver transplantation and the use of immunosuppressant medications.

Statistical analysis

Data were expressed as mean ± standard error. Differences between groups were tested using Student's t test and the chi-squared test, and differences were considered statistically significant at $p < 0.05$. All statistical analyses were performed using StatView J-5.0 software (SAS, Cary, NC).

Results

Comparison of pharmacokinetic parameters between TSPL and RESC groups at 5 mg/m^2 DOX

We summarized these patients' characteristics in Table 1. Major characteristics (e.g., age, sex, body height and weight, and ratio of hepatitis) were similar between the groups. Characteristics specific to liver function were expected to be worse in the TSPL group than in the RESC

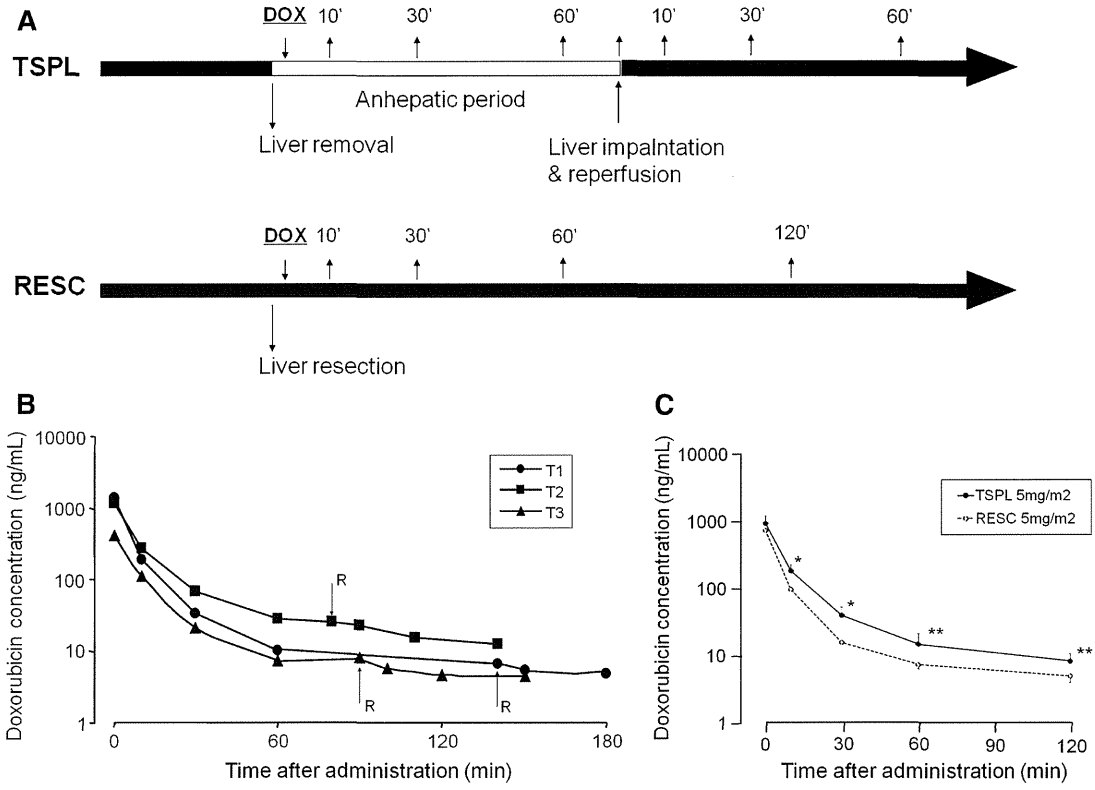

Fig. 1 Perioperative administration of doxorubicin in patients undergoing liver transplant or resection. **a** A schematic depicting doxorubicin (DOX) administration and sample collection. DOX was administered 5 min after the removal of the cirrhotic liver (TSPL) or liver resection with portal vein tumor thrombi (RESC). Peripheral blood samples were obtained at 0, 10, 30, 60, 120, and 180 min, as indicated. Blood samples were also obtained at the same intervals after reperfusion in the TSPL group. **b** Change in serum doxorubicin concentration in TSPL patients after DOX administration (5 mg/m^2). Each line indicates the serum DOX concentration in an individual TSPL patient (T1, T2, and T3). R, reperfusion. **c** The mean change in serum DOX concentration in the TSPL ($n = 3$) and RESC ($n = 5$) groups after DOX administration (5 mg/m^2). Data are expressed as mean ± standard error. $^*p < 0.05$, $^{**}p < 0.1$

group; however, only albumin and Child-Pugh classification were worse among TSPL patients. Renal function (serum creatinine level) did not differ between the groups ($p = 0.3118$).

The mean duration of the anhepatic period (from clamp of the portal vein of the recipient to reperfusion) was 101 min in the TSPL group. We compared serum DOX concentrations at 0, 10, 30, and 60 min after DOX administration; data at 120 min served as a reference. Reperfusion seemed to have almost no influence on the serum DOX concentration (Fig. 1b). The concentration from 0 min to 120 min (C0–C120) is depicted in Table 2 and Fig. 1c. At 10 and 30 min after DOX administration, serum DOX concentrations were significantly higher in TSPL patients than in RESC patients. Although the levels at 60 and 120 min were numerically higher in the TSPL group, the data only trended toward statistical significance. The other pharmacokinetic parameters are also described in Table 2. The area under curve from 0 to 120 min (AUC_{120}) was numerically higher in TSPL patients and approximately 1.5-fold higher than in RESC patients.

We employed both the one-compartment and two-compartment models to evaluate half-life ($T_{1/2}$) because the $T_{1/2}$ of alpha phase (also known as the distribution phase) was longer during the anhepatic period and the $T_{1/2}$ of beta phase (also known as the elimination phase) was unchanged in dogs [25, 26]. These findings indicated that the pharmacokinetic analysis of DOX during the anhepatic period is more important during the alpha phase, and for this reason, we decided to employ the one-compartment model. In our hands, the two-compartment model revealed that the $T_{1/2}$ of alpha phase was longer in the TSPL group than in the RESC group, although the difference did not reach statistical significance, and the $T_{1/2}$ of beta was shorter among TSPL patients than RESC patients. In contrast, the one-compartment model indicated that the $T_{1/2}$ trended longer in the TSPL group than in the RESC group.

Table 1 Characteristics of liver transplant (TSPL) and resection (RESC) patients who were treated with 5 mg/m^2 doxorubicin

Variables	TSPL	RESC	*p* value
v*n*	3	5	
Age	57 ± 5.4	56 ± 2.0	0.6939
Sex	2 (67 %)	4 (80 %)	0.6733
Male (%)			
Body high (cm)	164 ± 4.1	171 ± 2.7	0.2213
Body weight (kg)	66 ± 5.2	70 ± 2.8	0.5739
Body surface area (m^2)	1.67 ± 0.07	1.77 ± 0.04	0.3129
Hepatitis			
HBV (%)	1 (33 %)	3 (60 %)	0.4652
HCV (%)	2 (67 %)	1 (20 %)	0.1869
Preoperative liver function			
Aspartate aminotransferase (IU/L)	82 ± 30	53 ± 14	0.4460
Alanine aminotransferase (IU/L)	69 ± 31	46 ± 11	0.5300
Prothrombin time-INR	1.33 ± 0.15	1.22 ± 0.02	0.5299
Total bilirubin (mg/dL)	4.8 ± 1.31	0.9 ± 0.16	0.0918
Albumin (g/dL)	2.7 ± 0.20	3.9 ± 0.13	**0.0079**
Creatinine (mg/dL)	0.6 ± 0.10	0.8 ± 0.10	0.3118
Child-pugh score	10.7 ± 1.5	5.0 ± 0.0	0.0599
Child-pugh classification			
A	0	5 (100 %)	**0.0183**
B	1 (33 %)	0	
C	2 (67 %)	0	
MELD score	15.0 ± 2.5	8.2 ± 0.37	0.1109
Anhepatic period (min)	101 ± 22	N/A	
Cold ischemia time (min)	69 ± 18	N/A	
Warm ischemia time (min)	46 ± 12	N/A	
Operation period (min)	703 ± 41	541 ± 95	0.1767
Estimated blood loss (min)	4,307 ± 1,699	3,984 ± 2,226	0.9120
Graft or remnant liver lobe			
Left (%)	1 (33 %)	3 (60 %)	0.4652
Right (%)	2 (67 %)	2 (40 %)	
GW/SLV	0.54 ± 0.06	N/A	
Dose of doxorubicin (mg/m^2)	5	5	

Bold values indicate statistical significance at $p < 0.05$

MELD score, model for end stage liver disease score; RESC, patients who underwent liver resection and portal vein tumor thrombi removal due to hepatocellular carcinoma; TSPL, patients who underwent liver transplantation due to liver cirrhosis and hepatocellular carcinoma; and *GW/SLV,* graft weight/standard liver volume

Change in serum DOX concentration in TSPL patients at 3, 5, 10, and 15 mg/m^2 DOX

We summarized TSPL patients' characteristics in Table 3. Ninety percent of the patient population was male, and the mean body surface area was 1.78 m^2. The mean

Table 2 Pharmacokinetic parameters in liver transplant (TSPL) and resection (RESC) patients after administration of 5 mg/m2 doxorubicin

	TSPL	RESC	*p* value
n	3	5	
Dose of doxorubicin (mg/m^2)	5	5	
Plasma concentration (ng/mL)			
C0	975 ± 165	760 ± 171	0.2575
C10	189 ± 46	99 ± 12	**0.0233**
C30	40 ± 13.8	16 ± 2.9	**0.0315**
C60	15 ± 6.6	7.4 ± 1.1	0.0903
C120	8.3 ± 2.4	5.0 ± 1.0	0.0940
AUC_{120} (ng min/mL)	9,642 ± 2,519	6,162 ± 877	0.0808
One-compartment model			
A	974 ± 173	760 ± 171	0.2583
α	0.156 ± 0.20	0.197 ± 0.019	0.1056
$T_{1/2}$(min)	4.6 ± 0.54	3.6 ± 0.32	0.0774
Two-compartment model			
A	902 ± 267	739 ± 173	0.3040
B	73 ± 27	22 ± 5	**0.0235**
α	0.183 ± 0.020	0.218 ± 0.018	0.1301
β	0.023 ± 0.004	0.014 ± 0.002	**0.0359**
$T_{1/2}$ α(min)	3.9 ± 0.5	3.3 ± 0.3	0.1276
$T_{1/2}$ β(min)	31.7 ± 4.9	55.4 ± 10.9	0.0822

Bold values indicate statistical significance at $p < 0.05$

AUC_{120}, area under concentration curve from 0 to 120 min; RESC, patients who underwent liver resection and portal vein tumor thrombi removal due to hepatocellular carcinoma; and TSPL, patients who underwent liver transplantation due to liver cirrhosis and hepatocellular carcinoma

MELD score was 16.0. Because one patient was received a transplanted liver from a deceased donor, the mean cold ischemia time was 137 min and one graft liver was whole liver. However, the anhepatic period was 118 ± 11 min, and there appeared to be no large difference among the patients. We observed changes in serum DOX concentration at 3, 5, 10, and 15 mg/m^2 (Fig. 2). Pharmacokinetic parameters are summarized in Table 4. AUC_{120} increased in a dose-dependent manner, with the exception that AUC_{120} at 10 mg/m^2 was slightly lower. The $T_{1/2}$ of serum DOX concentrations was prolonged in alpha phase of the one-compartment model and in beta phase of the two-compartment model, according to dose escalation of DOX. Maximum serum concentration was 2,440 ng/mL at 15 mg/m^2 DOX administration.

Adverse events in TSPL patients at 5, 10, and 15 mg/m^2 DOX

We evaluated adverse events in TSPL patients using CTCAE version 4.0 during the first 7 days after liver

Table 3 Characteristics of transplant (TSPL) patients

Variables	TSPL
n	12
Age	53 ± 7.1
Sex	
Male (%)	11 (92 %)
Body high (cm)	168 ± 1.7
Body weight (kg)	71 ± 3.4
Body surface area (m^2)	1.78 ± 0.05
Hepatitis	
HBV (%)	3 (25 %)
HCV (%)	7 (58 %)
Preoperative liver function	
Aspartate aminotransferase (IU/L)	58 ± 11
Alanine aminotransferase (IU/L)	47 ± 12
Prothrombin time-INR	1.68 ± 0.18
Total bilirubin (mg/dL)	5.5 ± 1.51
Albumin (g/dL)	2.9 ± 0.15
Creatinine (mg/dL)	0.8 ± 0.11
Child-pugh score	5.0 ± 0.0
Child-pugh classification	
B	4 (33 %)
C	8 (67 %)
MELD score	16.0 ± 1.43
Anhepatic period (min)	118 ± 11
Cold ischemia time (min)	137 ± 46
Warm ischemia time (min)	44 ± 4
Operation period (min)	811 ± 36
Estimated blood loss (min)	7,975 ± 1,769
Graft liver lobe	
Left (%)	2 (17 %)
Right (%)	9 (75 %)
Whole (%)	1 (8 %)
GW/SLV	0.54 ± 0.06
Dose of doxorubicin (mg/m^2)	3–15

GW/SLV, graft weight/standard liver volume; MELD score, model for end stage liver disease score; and TSPL, patients who underwent liver transplantation due to liver cirrhosis and hepatocellular carcinoma

transplantation (Table 5). Because of liver transplantation, Grade 3–4 decreased platelet count and hyperbilirubinemia was noted in almost all patients. Grade 1 diarrhea at 5 mg/m^2 was noted owing to elementary diet. Two patients at 10 mg/m^2 presented with Grade 1 abnormal echocardiogram (sinus tachycardia). Other Grade 1–2 adverse events were compatible with the regular postoperative course after liver transplantation. Regarding DOX-related adverse drug reactions, both symptoms and laboratory data were unremarkable.

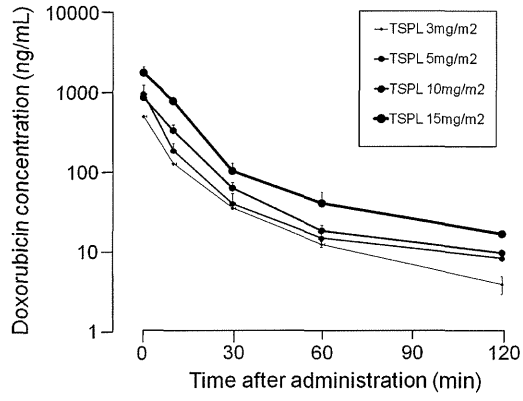

Fig. 2 The mean change in serum doxorubicin concentration in liver transplant patients (TSPL) treated perioperatively with 3, 5, 10, or 15 mg/m^2 doxorubicin. Each patient was treated with the indicated dose of doxorubicin ($n = 3$ per dose level). Data are expressed as mean ± standard error

Tumor factors and survival in TSPL patients

As preliminary data, we investigated the recurrence-free survival and overall survival in TSPL patients. Tumor characteristics are summarized in Table 6. Briefly, this patient population featured 58 % multiple HCCs, 50 % exceeding Milan criteria, and no portal vein tumor thrombus. One patient underwent intra-portal 5-fluorouracil infusion. With a median observation period of 4.1 years (range, 1.7–9.9 years), the 5-year recurrence-free survival was 68.8 %, and the overall survival was 100.0 %. Two patients with HCC exceeding Milan criteria experienced HCC recurrence: One patient, who suffered from over 20 HCCs (maximum diameter, 3.3 cm) with microscopic vascular invasion and was treated with 3 mg/m^2 DOX, experienced liver metastasis at 1.0 year post-transplantation. Another patient, who suffered from 4 HCCs (maximum diameter, 1.5 cm) and was treated with 5 mg/m^2 DOX, experienced lymph node metastasis at 5.0 years post-transplantation. The former patient who was treated with 3 mg/m^2 DOX died of HCC at 6.2 years post-transplantation.

Discussion

In the current study, we demonstrated the elevation of serum DOX concentration during the anhepatic period. Briefly, the concentrations at 10 and 30 min after DOX administration (C10 and C30) were elevated two- to three-fold during liver transplantation in comparison with liver resection. $T_{1/2}$ in the one-compartment model tended to be prolonged. In contrast, in the two-compartment model, $T_{1/2}$ α was prolonged, but was not significantly so, and $T_{1/2}$ β

Table 4 Pharmacokinetic data from transplant (TSPL) patients for each dose of doxorubicin

Dose of doxorubicin (mg/m^2)	AUC $_{120}$ (ng min/mL)	One-compartment model			Two-compartment model					
		A	α	$T_{1/2}$ (min)	A	B	α	β	$T_{1/2}$ α (min)	$T_{1/2}$ β (min)
3	6,060	507	0.134	5.52	419	90	0.182	0.0285	3.8	26.6
5	9,642	974	0.156	4.57	902	73	0.183	0.0230	3.9	31.7
10	12,227	880	0.094	7.58	880	25	0.104	0.0083	6.6	83.1
15	25,882	1,804	0.084	8.87	3,038	38	0.139	0.0069	5.0	100.0

AUC_{120}, area under concentration curve from 0 to 120 min; TSPL, patients who underwent liver transplantation due to liver cirrhosis and hepatocellular carcinoma

Table 5 Adverse events during the first 7 days after liver transplantation

Dose of doxorubicin (mg/m^2)	5		10		15	
CTCAE Grade	Grade 1/2	Grade 3/4	Grade 1/2	Grade 3/4	Grade 1/2	Grade 3/4
Symptom						
Diarrhea	1	0	0	0	0	0
Rash	0	0	0	0	0	0
Fever	0	0	0	0	0	0
Biliary tract infection	0	0	0	0	0	0
Other infection	0	0	0	0	0	0
Laboratory data						
Abnormal ECG	0	0	2	0	0	0
Neutropenia	1	0	2	0	1	0
Anemia	3	0	3	0	3	0
Platelet	0	3	0	3	0	3
Creatinine	0	0	2	0	1	0
Aspartate aminotransferase	3	0	3	0	3	0
Alanine aminotransferase	3	0	3	0	3	0
Alkaline phosphatase	0	0	1	0	0	0
Total bilirubin	0	3	0	3	2	1
Prothrombin time	2	0	1	0	0	0
Albumin	1	0	2	1	3	0

CTCAE Common Terminology Criteria for Adverse Events, version 4.0

tended toward being shortened. The AUC was elevated in a dose-dependent manner. No obvious adverse drug reactions were noted at the maximum dose of DOX during the anhepatic period.

Serum concentrations of drugs, including DOX, have rarely been investigated in liver dysfunction during the anhepatic period. The change in serum DOX concentration during the anhepatic period was marked by rapid decline after administration and two- to threefold elevation at C10 and C30. First, the rapid decrease after DOX administration was also noted in the normal liver [21]. This rapid decrease might be a result of distribution to the other organs and blood vessels [30] (e.g., DOX is mainly distributed to the spleen and lung in rats). At C10 and C30, serum DOX concentrations were sustained at two- to threefold; these data are similar to previous studies in dogs, which compared concentrations during the anhepatic period versus in normal whole liver [25, 26]. Using both one- and two-compartment models, we observed that DOX $T_{1/2}$ was prolonged by minutes. In contrast, $T_{1/2}$ β was shortened in the two-compartment model by approximately 10 min. These findings are compatible with previous reports of serum concentration in dogs [25].

An additional discussion point is the effect of the duration of the anhepatic period. The anhepatic period is regularly within 2 h (especially in living donor liver transplantations), and our findings demonstrate that the length of the anhepatic period appears to have limited influence on the serum DOX concentration. In comparisons of normal and cirrhotic liver, the DOX concentration in cirrhotic liver reached levels that were six- to eightfold higher than in normal liver at 48 h after DOX administration [31, 32]. In contrast, serum DOX concentration during the anhepatic period was limited two- to threefold higher

Table 6 Tumor factors in liver transplant (TSPL) patients

Variables	TSPL
n	12
HCC	
Multiple (%)	7 (58 %)
Maximum size (cm)	1.3 ± 0.3
PVTT (%)	0
Exceeding Milan criteria(%)	6 (50 %)
Preoperative treatment	
Transcatheter arterial chemo-embolization (%)	5 (42 %)
Local ablation (radiofrequency, microwave) (%)	5 (42 %)
Complete necrosis (%)	2 (17 %)
AFP (ng/mL)	527 ± 313
Histology	
Early HCC	1 (8 %)
Well differentiated HCC	2 (17 %)
Moderately differentiated HCC	4 (33 %)
Poorly differentiated HCC	3 (25 %)
Micro PVTT (%)	1 (8 %)

AFP, α-fetoprotein; *HCC,* hepatocellular carcinoma; *PVTT,* portal vein tumor thrombi; and *TSPL,* patients who underwent liver transplantation due to liver cirrhosis and hepatocellular carcinoma

in patients undergoing transplant than in those undergoing resection. In other words, the factor of liver function (anhepatic or not) appears to influence serum DOX concentration only between 30 min and 120 min after DOX administration. The elevation of the serum DOX concentration is likely limited because the anhepatic period is so short.

The final discussion points regarding pharmacokinetic analysis are the AUC and peak serum DOX concentrations during the anhepatic period. As the administered dose of DOX escalated, the AUC increased to 25,000 ng min/mL (approximately 400 ng h/mL), the peak DOX level reached 2,500 ng/mL in actual measurements and 3,000 ng/mL in estimates calculated from the one- and two-compartment models. The peak serum DOX concentration reportedly contributes to cardiac toxicity in addition to cumulative dose [33–35]. From our findings during the anhepatic period, the AUC of 15 mg/m^2 (our maximum dose) was much lower than when DOX was administered as a systemic bolus; however, the peak level of 15 mg/m^2 was almost equal to a systemic bolus administration of 150 mg/m^2 DOX in previous studies [36, 37]. When comparing the adverse events between "reported 150 mg/m2 of bolus DOX administration" and "our 15 mg/m2 of DOX during anhepatic period," the reported data showed 50 % of febrile neutropenia and 16.7 % of Grade 3–4 nausea/vomiting [36], our data showed 100 % of Grade 3/4 thrombocytopenia, 33 % of hyper bilirubinemia, and no cardiac toxicities, and our data were compatible with "the regular postoperative course" after living donor liver transplantation. The adverse events differed markedly between the previous reports and our data in the present study and may be associated with different causes (adverse drug reaction in the previous report versus regular postoperative course in the present report). Therefore, these might be non-drug-related adverse events that depend on conditions other than peak DOX level, which would indicate that our series did not reveal any severe adverse drug reactions. There remains the possibility that the differences were caused by AUC. However, there is a persistent possibility of severe adverse drug reactions in future series. It will be necessary to check patients' vital signs, physical status, and examinations carefully during any phase II study, because of high peak serum DOX concentration during the anhepatic period.

Regarding the anticancer effect of DOX, we achieved approximately 70 % 5-year recurrence-free survival and 100 % 5-year overall survival in this series. However, a previous randomized trial revealed that adjuvant chemotherapy is ineffective after transplantation [19]. They administered 15 mg/m^2 of DOX intra-operatively (they did not describe whether or not this was during the anhepatic period). Our maximum dose was 15 mg/m^2 DOX during the anhepatic period; the serum DOX concentration did reach 10–100 ng/mL until 120 min. Our previous evaluation showed that the IC_{50} of DOX in several cultured hepatocellular carcinoma cell lines varied from 10 to 100 ng/mL [30]. Although it is difficult to keep serum DOX concentration similar to in vitro studies, the serum concentration appeared to exceed the IC_{50}s demonstrated in vitro.

The recommended dose for DOX during the anhepatic period should be 15 mg/m^2, with careful monitoring for adverse drug reactions. Additional studies, such as a phase II study, are needed to verify adverse drug reactions and should be paired with monitoring of changes in mAFP-expressing cells during the perioperative period to evaluate efficacy. Several researchers have mentioned the existence of HCC cells and/or a niche in the bone marrow in published work [38–40], and it is necessary to evaluate bone marrow cells during the perioperative period. The main limitation of this study is the difficulty of distinguishing between adverse drug reactions and regular postoperative course, and higher doses of DOX might be necessary.

In conclusion, up to 15 mg/m^2 DOX was safely administered during the anhepatic period. However, further investigation is necessary to estimate treatment efficacy, with careful monitoring of adverse events.

Conflict of interest The authors declare no conflicts of interest.