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ORIGINAL ARTICLE

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

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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

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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

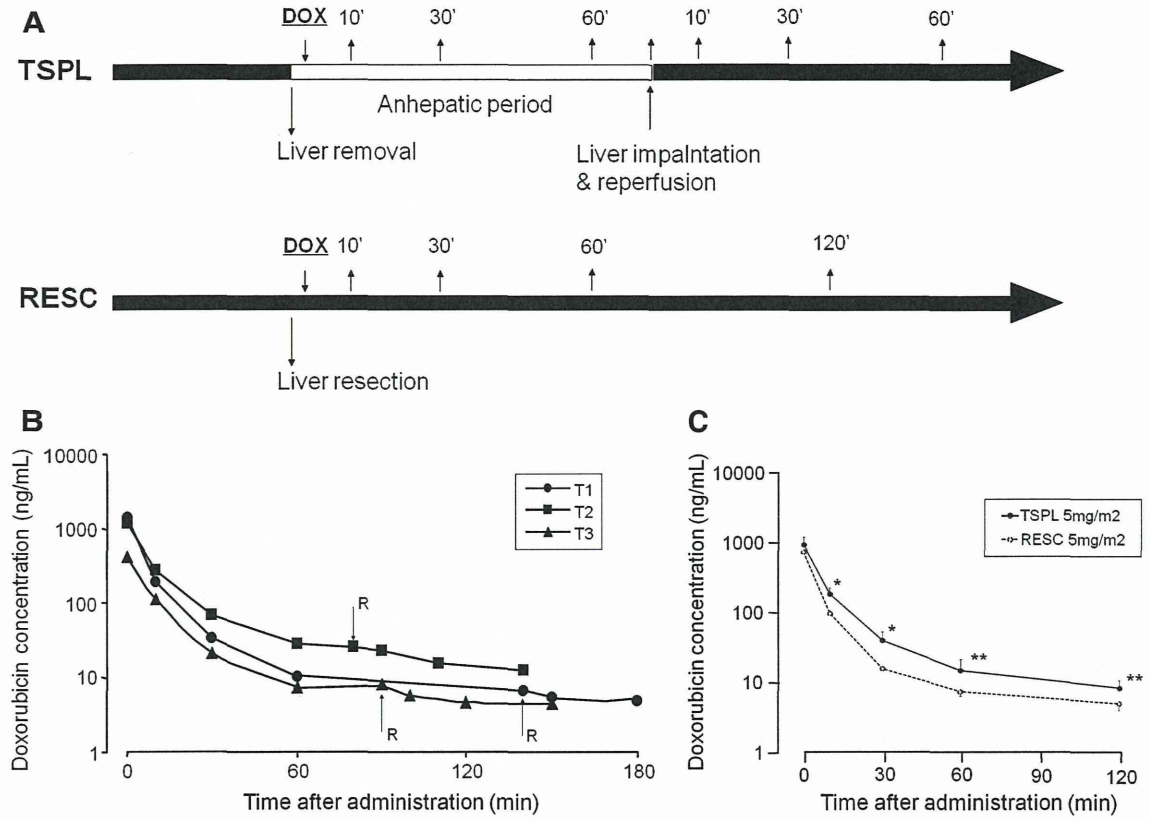

Fig. 1 Perioperative administration of doxorubicin in patients undergoing liver transplant or resection. **a** A schematic depicting doxorubicin (DOX) administration and sample collection. DOX was administered 5 min after the removal of the cirrhotic liver (TSPL) or liver resection with portal vein tumor 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 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.

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

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.

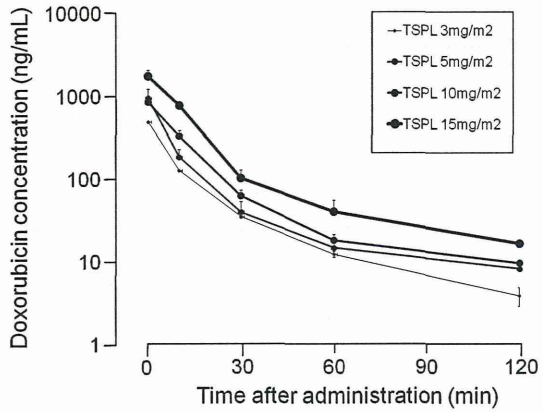

Fig. 2 The mean change in serum doxorubicin concentration in liver transplant patients (TSPL) treated perioperatively with 3, 5, 10, or 15 mg/m^2 doxorubicin. Each patient was treated with the indicated dose of doxorubicin ($n = 3$ per dose level). Data are expressed as mean ± standard error

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.

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Hepatic Artery Reconstruction in Living Donor Liver Transplantation: Risk Factor Analysis of Complication and a Role of MDCT Scan for Detecting Anastomotic Stricture

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Abstract
Background In partial liver transplantation, reconstruction of the hepatic artery is technically highly demanding and the incidence of arterial complications is high. We attempted to identify the risk factors for anastomotic complications after hepatic artery reconstruction and examined the role of multidetector-row computed tomography (MDCT) in the evaluation of the reconstructed hepatic artery in liver transplant recipients.
Methods A total of 109 adult-to-adult living donor liver transplantations (LDLT) were performed at our institute between 1999 and July 2011. Hepatic artery reconstruction was performed under a surgical microscope (MS group, $n = 84$), until we began to adopt surgical loupes (4.5×) for arterial reconstructions in all cases after January 2009 (SL group, $n = 25$). A dynamic MDCT study was prospectively carried out on postoperative days 7, 14, and 28, and at postoperative month 3, 6, and 12 after April 2005 ($n = 60$).
Results There were no cases of hepatic artery thrombosis and six cases (5.5 %) of interventional radiology-confirmed hepatic artery stenosis (HAS). Risk factor analysis for HAS showed that ABO-incompatible LDLT was associated with HAS. Use of surgical loupes provided superior results as compared to anastomosis under a surgical microscope, and it also provided the advantage of reduced operative time. The MDCT procedure was useful for detecting HAS; however, the false positive rate was relatively high until 3 months after the LDLT (100 % sensitivity and 72.8 % specificity at 3 months).
Conclusions Hepatic arterial anastomosis using surgical loupes tended to be time-saving and to yield similar or better results than traditional microscope-anastomosis. The use of MDCT aided the diagnosis of HAS, although the substantial false positive rate should be borne in mind in clinical practice.

Abbreviations

DUS	Doppler ultrasonography
HAS	Heoatic artery stenosis
IVR	Interventional radiology
LDLT	Living donor liver transplantation
MELD score	Model for end-stage liver disease score
MDCT	Multidetector-row CT
POD	Postoperative day
POM	Postoperative month
RI	Resistive index
SMA	Superior mesenteric artery

Introduction

Hepatic artery reconstruction is the most important surgical procedure for liver transplantation, and complications associated with this vascular reconstruction, such as hepatic artery thrombosis or stenosis, may have a significant influence on the recipients' prognosis. In partial liver transplantation, where the hepatic arterial system should be reconstructed using a branch of the hepatic artery, such as the right hepatic artery in right liver grafting and the left and middle hepatic arteries in left liver grafting, reconstruction of the hepatic artery is technically highly demanding and the incidence of arterial complications is

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