を伴わない肉芽腫形成性の胆管炎が特徴的であり，胆管周囲にリンパ球の集簇を認めることもある．鑑別診断としては，急性拒絶反応，慢性拒絶反応，ウイルス性肝炎(HCV, CMV)などである．

4 PBC再発の頻度と危険因子

Gautamらのメタ解析によると，16の原著論文に報告された1,241例の移植患者の内204例(16%)が移植後のPBC再発と診断されていた[5]．いくつかの報告について表1にまとめる．観察が長期になればより多くの再発症例が診断される[15]．Liermann Garciaらによるとプロトコール肝生検によるPBCの再発率は4年で18%，10年で30%と報告されている[4]．また同報告では，ドナーと患者の年齢，タクロリムス使用，温阻血時間が再発の危険因子と報告した．しかし，その後の報告で一定した危険因子の報告はない．免疫抑制剤としてタクロリムスを投与した患者の方がサイクロスポリンAを投与した患者よりもPBCの再発を多く認めるとする報告は複数みられる[19~23]．生体肝移植症例の検討において，MoriokaらはHLA-A, HLA-B, HLA-DRミスマッチが少ないほうが，PBC再発の頻度が高いと報告している[24]．しかしながらHashimotoらは同様の結果を得ていない[25]．Moriokaら，Hashimotoらいづれのグループもプロトコール肝生検に基づいた診断ではなく，肝胆道系酵素の上昇を認めて実施した肝生検所見に基づいてPBCの再発を診断している．そのためHLAとプロトコール肝生検によるPBC再発との関連については不明であり検討が必要である．

5 PBC再発の治療と予後

再発を診断した場合，ウルソデオキシコール酸(UDCA; ursodeoxycholic acid)を投与することが多い．Charatcharoenwitthayaらは，36カ月以上にわたるUDCA投与によりALPとALTが正常化したが，UDCAを投与しない群ではALPとALTの正常化は22%の症例にとどまったと報告した[19]．ただしこの報告では，UDCA投与と患者およびグラフト生存率との関連は認めなかった．

PBC再発そのものが肝移植後の患者およびグラフト生存に関与するという証拠は限られる．Hytiroglouらの報告[26]では，PBCの再発をきたした7例中1例が再移植を受けた後敗血症で死亡している．しかし，他の報告では，PBCの再発は再移植や死亡へ有意な影響を及ぼさないとされる[21,23]．

6 オーバーラップ

PBCの症例のうちAIHの特徴をあわせ持つ，オーバーラップ症候群は9%～19%に認めるとされる[27]．しかしながらオーバーラップ症候群について正確に診断することは容易ではない．Silveiraらは，PBCと診断した症例のうち，International Autoimmune Hepatitis Group (IAHG)の診断基準に基きAIHを厳密に診断したオーバーラップ症候群について，治療効果と予後について報告している．それによると，オーバーラップ症候群では，PBCのみの患者に比較し食道静脈瘤，上部消化管出血，腹水の合併頻度，および肝移植または死亡のリスクが高い[28]．オーバーラップ症候群に対する肝移植のみを取り扱った報告はないが治療としてUDCAおよびコルチコステロイドが有効なことから[27]，肝移植の際にもそれらの使用について考慮する必要がある．また，Hytiroglouらの報告[26]では，再移植を受けた症例を含め3症例で自己免疫性肝炎(AIH; autoimmune hepatitis)に合致す

る病理所見を認めており，肝移植後のPBCとAIHのオーバーラップが示唆されている．

7 結 語

原発性胆汁性肝硬変に対する肝移植の成績，再発について述べた．末期PBCに対する肝移植の成績は良好である．肝移植後の組織学的評価において，約20％～30％の症例にPBCの再発を認める．しかし，これまでPBCの再発により再肝移植を要したとの報告はあるものの，患者およびグラフト生存率への有意な影響については報告されていない．今後より長期の観察とともに，PBC再発が予後に与えるインパクトは増すだろう．

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Hepatology Research

Hepatology Research 2012; 42: 166–170 doi: 10.1111/j.1872-034X.2011.00908.x

Original Article

Predictive factors for cholangiocarcinoma associated with hepatolithiasis determined on the basis of Japanese Multicenter study

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Aim: The aim of this study was to delineate predictive factors for cholangiocarcinoma in patients with hepatolithiasis, and to establish optimal management for hepatolithiasis from the viewpoint of carcinogenesis on the basis of a Japanese nationwide survey for hepatolithiasis.

Methods: The Hepatolithiasis Research Group was organized in 2006 by the Ministry of Health, Labour and Welfare of Japan, and conducted a nationwide survey. The research group collected data on 336 cases of hepatolithiasis in 2006, in a cross-sectional survey involving 2592 institutions in Japan. Predictive factors for cholangiocarcinoma associated with hepatolithiasis were analyzed by univariate and multivariate analyses of clinicopathological and therapeutic factors.

Results: Twenty-three patients had cholangiocarcinoma. Histories of choledocoenterostomy and liver atrophy were found to be significantly predictive factors by multivariate analysis. In 87.5% of cases of cholangiocarcinoma with liver atrophy, cholangiocarcinoma was located in the atrophic lobes. The method of reconstruction did not affect the incidence of cholangiocarcinoma (choledochojejunostomy vs. choledochoduodenostomy; side-to-end vs. side-to-side anastomosis).

Conclusions: Choledocoenterostomy and liver atrophy may increase the risk of developing cholangiocarcinoma. Choledocoenterostomy is thus contraindicated in patients with hepatolithiasis. An aggressive resection strategy is recommended for an atrophic segment.

Key words: cholangiocarcinoma, hepatolithiasis, management

INTRODUCTION

HEPATOLITHIASIS IS CHARACTERIZED by its intractable nature and frequent recurrence, requiring multiple operative interventions. In addition to frequent occurrences of cholangitis and chronic sepsis, it is widely known that long-standing hepatolithiasis leads to cholangiocarcinoma.[1] Development of cholangiocarcinoma is a major problem, and highly indicates a worse prognosis and treatment outcomes. Diagnosis of cholangiocarcinoma associated with hepatolithiasis by imaging studies is difficult, because of stone artifacts in the intrahepatic bile duct.

In 2006, the Hepatolithiasis Research Group was organized by the Ministry of Health, Labour and Welfare of Japan to investigate the epidemiology, pathogenesis, carcinogenicity, and therapeutic options of hepatolithiasis. The Hepatolithiasis Research Group conducted a nationwide, multi-institutional, cross-sectional survey of hepatolithiasis cases, and analyzed the clinicopathologic features.

The aims of this study were to delineate predictive factors for cholangiocarcinoma in patients with hepatolithiasis, and to establish an optimal management strategy for hepatolithiasis from the viewpoint of carcinogenesis.

METHOD

IN 2006, THE research group performed a nationwide, cross-sectioned survey by sending a questionnaire to 2592 institutions in Japan regarding hepatolithiasis patients who have a history of hospital visits and treatment history in 2006, and received replies from 319 institutions including 336 patients. Collected data included gender, age, period from onset to treatment, other malignant neoplasms, history of biliary tract diseases, history of choledocoenterostomy, complications, symptoms, location of stones, stone classification, bile duct stenosis, bile duct dilatation, liver atrophy,

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Received 15 July 2011; revision 31 August 2011; accepted 2 September 2011.

administration of ursodeoxycholic acid (UDCA), residual stones after treatment, symptoms after treatment and stone recurrence. Atrophy was defined as a >50% reduction in liver volume as determined by imaging studies.[2] Patients with unclear demography were excluded. A total of 325 patients were included and analyzed in this study. Patients consisted of 168 male and 157 female patients with a median age of 65 (range, 2–98 years). Collected data were subjected to further analysis to determine predictive factors for cholangiocarcinoma associated with hepatolithiasis. Risk factors for cholangiocarcinoma were determined by univariate analysis using the χ^2 test or Mann–Whitney analysis. Factors with a *P*-value <0.25 in the univariate analysis were then included in a forward stepwise multiple logistic regression model. Statistical analysis was carried out using SAPW Statistics ver.18 (SPSS Japan Inc., Tokyo, Japan). In multivariate analysis, a *P*-value of <0.05 was considered statistically significant.

RESULTS

TWENTY-THREE PATIENTS had cholangiocarcinoma. Among 36 potential risk factors shown in Table 1, six showed $P < 0.25$ in univariate analysis, namely, other malignant neoplasms, history of choledocoenterostomy, bile duct dilatation, liver atrophy, UDCA administration, and symptoms after treatment (Table 1). Among these six factors, two (history of choledocoenterostomy and liver atrophy) remained significant in the multivariate analysis (Table 2).

Eight cases of hepatolithiasis with liver atrophy were complicated by cholangiocarcinoma. In seven of eight cases (87.5%), cholangiocarcinoma was located in the atrophic segments of the liver (Table 3). The relationship between the method of reconstruction and development of cholangiocarcinoma was not clear. There was no significant difference in the incidence of cholangiocarcinoma between choledochoduodenostomy and choledochojejunostomy (Table 4). Furthermore, the incidence was not significantly different between end-to-side and side-to-side anastomosis (Table 5). In cases without liver atrophy, history of choledocoenterostomy was a significant predictive factor for cholangiocarcinoma associated with hepatolithiasis (Table 6).

DISCUSSION

THE PRESENT STUDY showed that liver atrophy (odds ratio: 4.424) and history of choledocoenterostomy (odds ratio: 3.718) were found to be predictive factors for cholangiocarcinoma associated with hepatolithiasis in multivariate analysis. Previous reports showed that bilirubin stones,[3] irregular ductal stricture or obstruction, liver atrophy,[4] portal obstruction in portograms,[5] being over 40 years old, having a long history of hepatolithiasis with weight loss, a high level of serum alkaline phosphatase, a low level of serum albumin, a high level of the serum carcinoembryonic antigen, and/or hepatolithiasis in the right or both lobes[6] were predictive factors for cholangiocarcinoma associated with hepatolithiasis. On the other hand, Chijiiwa *et al.* described that the incidence of cholangiocarcinoma associated with hepatolithiasis was significantly higher in patients with cholesterol stones than in those with brown pigment stones.[7] However, age, symptoms, stone classification, and stone location were not significantly predictive in the present series.

In this study, liver atrophy was a predictive factor for cholangiocarcinoma associated with hepatolithiasis. Ohta *et al.* reported the presence of fibrosis periportal tissue, portal vein narrowing, and mild atrophy of the liver.[8] In 87.5% of cases of cholangiocarcinoma with liver atrophy, cholangiocarcinoma was located in the atrophic lobes. Hepatectomy is recommended for hepatolithiasis with liver atrophy. Regarding the outcomes of hepatectomy for hepatolithiasis, many authors reported about the usefulness and safety of hepatectomy. Uenishi described that hepatectomy might thus offer another advantage in eliminating the risk of new development of cholangiocarcinoma.[9] In patients with hepatectomy, the incidences of stone recurrence, postoperative cholangitis, cholangiocarcinoma, and mortality were lower than in those without hepatectomy.[10–12]

Choledocoenterostomy was a predictive factor for cholangiocarcinoma associated with hepatolithiasis in this study. The method of reconstruction did not affect the incidence of cholangiocarcinoma (choledochojejunostomy vs. choledochoduodenostomy; side-to-end anastomosis vs. side-to-side anastomosis). Xiao-Feng *et al.* described that the incidence of severe cholangitis after choledochoduodenostomy was significantly higher than that in the preoperative state.[13] Furthermore, Li *et al.* reported that 24% of patients who underwent choledochojejunostomy showed postoperative cholangitis.[14] Kusano *et al.* reported that the incidence of cholangitis was significantly higher in patients who underwent choledocojejunostomy than in those who underwent noncholedocoenterostomy.[15] By histopathologic analysis, Ohta *et al.* observed hyperplasia of varying degrees in the epithelium of large bile ducts in

Table 1 Univariate analysis between patients with and without cholangiocarcinoma

	With cholangiocarcinoma $n = 23$ (%)	Without cholangiocarcinoma $n = 302$ (%)	*P*-value
Gender			
Male	11 (47.8)	157 (52.0)	0.829
Female	12 (52.2)	145 (48.0)	
Age (median) (range)	67 (37–81)	65 (2–98)	0.724
Period from onset to treatment (month, median) (range)	6 (0–480)	3 (0–564)	0.799
Other malignant neoplasms	0 (0)	26 (8.6)	0.236
History of biliary tract diseases	16 (69.6)	189 (62.6)	0.655
History of choledocoenterostomy	10 (43.5)	70 (23.2)	0.042
Complications			
Viral hepatitis	0 (0)	13 (4.3)	0.610
Liver cirrhosis	0 (0)	12 (4.0)	1.000
Diabetes mellitus	3 (13.0)	30 (9.9)	0.716
APBJ	2 (8.7)	19 (6.3)	0.652
Heart disease	2 (8.7)	30 (9.9)	1.000
Hyper tension	0 (0)	22 (7.3)	0.384
Obesity	2 (8.7)	14 (4.6)	0.315
Total	14 (60.9)	173 (57.3)	0.829
Symptoms			
Abdominal pain	13 (56.5)	170 (56.3)	1.000
Fever	11 (47.8)	131 (43.4)	0.671
Jaundice	5 (21.7)	52 (17.2)	0.572
Nausea	1 (4.3)	9 (3.0)	0.525
Total	19 (82.6)	236 (78.1)	0.794
Location of stones			
Intrahepatic duct only	14 (60.9)	165 (54.6)	0.666
Both intra- and extrahepatic ducts	9 (39.1)	137 (45.4)	
Right lobe	6 (26.1)	66 (21.9)	0.608
Left lobe	9 (39.1)	150 (49.7)	0.390
Both lobes	8 (34.8)	86 (28.5)	0.486
Stone classification			
Bilirubin stones	12 (52.2)	133 (44.0)	0.517
Cholesterol stones	1 (4.3)	20 (6.6)	1.000
Black pigment stones	4 (17.4)	32 (10.6)	0.302
Hybrid stone	0 (0)	14 (4.6)	0.610
Mixture stones	0 (0)	3 (1.0)	1.000
Bile duct stenosis	5 (21.7)	69 (22.8)	1.000
Bile duct dilatation	9 (39.1)	82 (27.2)	0.232
Liver atrophy	8 (34.8)	46 (15.2)	0.035
UDCA administration	15 (65.2)	152 (50.3)	0.198
Residual stones after treatment	3 (13.0)	38 (12.6)	1.000
Symptoms after treatment	6 (26.1)	40 (13.2)	0.114
Stone recurrence	2 (8.7)	26 (8.6)	1.000

APBJ, Anomalous pancreatobiliary junction; UDCA, ursodeoxycholic acid.

association with areas of chronic proliferative cholangitis in the vicinity of the impacted stones, and mucosal dysplasia may be a precursor to the cholangiocarcinoma.[16] In this study, abdominal pain, fever, and jaundice were found not to be significant factors. We suggest that choledocoenterostomy induces inflammatory changes of the bile duct even without symptoms, and continuous inflammation may lead to the risk of

Table 2 Predictive factors for cholangiocarcinoma associated with hepatolithiasis determined by logistic regression analysis

	P-value	Odd ratio	95% confidence interval
History of choledocoenterostomy	0.007	3.718	1.431–9.659
Liver atrophy	0.004	4.424	1.618–12.095

Table 3 Relationship of location between liver atrophy and cholangiocarcinoma

Case	Location	
	Liver atrophy	Cholangiocarcinoma
1	S2, 3	B2, 3
2	S5, 8	B5-8
3	S2, 3	B2, 3
4	S6	B6
5	S2, 3	Hilar, extrahepatic duct
6	S2, 3	B3
7	S2, 3	B2, 3
8	S2, 3	B3

cholangiocarcinoma. In cases without liver atrophy, history of choledocoenterostomy was a significantly predictive factor for cholangiocarcinoma associated with hepatolithiasis. Choledocoenterostomy should be avoided as much as possible for radical treatment of hepatolithiasis with or without liver atrophy. In patients without liver atrophy, other procedures, such as percutaneous transhepatic cholangioscopic lithotomy (PTCSL) or endoscopy, are recommended.

In conclusion, choledocoenterostomy may increase the risk of developing cholangiocarcinoma and should be avoided as much as possible in patients with hepatolithiasis. An aggressive resection strategy is recommended for an atrophic segment.

ACKNOWLEDGMENTS

THE AUTHORS WISH to thank all the doctors who participated in this multicenter survey. This study was supported by the Research Program of Intractable Disease provided by the Ministry of Health, Labor, and Welfare of Japan.

Table 4 Reconstructive techniques for cholangiocarcinoma: choledochoduodenostomy vs. choledocojejunostomy

	With cholangiocarcinoma $n = 12$ (%)	Without cholangiocarcinoma $n = 68$ (%)	*P*-value
Choledochoduodenostomy	3 (25.0)	9 (13.2)	0.376
Choledocojejunostomy	9 (75.0)	59 (86.8)	

Table 5 Reconstructive techniques for cholangiocarcinoma: end-to-side anastomosis vs. side-to-side anastomosis

	With cholangiocarcinoma $n = 8$ (%)	Without cholangiocarcinoma $n = 53$ (%)	*P*-value
End-to-side anastomosis	6 (75.0)	45 (84.9)	0.607
Side-to-side anastomosis	2 (25.0)	8 (15.1)	

19 cases: unclear.

Table 6 History of choledocoenterostomy and cholangiocarcinoma in patients without liver atrophy

	With cholangiocarcinoma $n = 15$ (%)	Without cholangiocarcinoma $n = 256$ (%)	*P*-value
With choledocoenterostomy	8 (53.3)	68 (26.6)	0.036
Without choledocoenterostomy	7 (46.7)	188 (73.4)	

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Ann Surg Oncol (2011) 18:3624–3631
DOI 10.1245/s10434-011-1800-6

Annals of
SURGICAL ONCOLOGY
OFFICIAL JOURNAL OF THE SOCIETY OF SURGICAL ONCOLOGY

ORIGINAL ARTICLE – HEPATOBILIARY TUMORS

Adjuvant Chemolipiodolization Reduces Early Recurrence Derived from Intrahepatic Metastasis of Hepatocellular Carcinoma After Hepatectomy

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ABSTRACT

Background. The recurrence of hepatocellular carcinoma is still high even after surgery. Two general recurrence patterns occur: intrahepatic metastasis (IM) and multicentric carcinogenesis (MC). The aim of this study was to investigate the effectiveness of adjuvant chemolipiodolization for reducing IM or MC recurrences after surgery.

Methods. A retrospective case-control study was carried out. From April 2005, adjuvant chemolipiodolization was performed in 63 initial hepatocellular carcinoma patients 3 months after surgery. Sixty-four patients who underwent surgery between April 2001 and March 2005 were analyzed as the control group. Recurrence-free and overall survival as well as prognostic factors were analyzed univariately and multivariately.

Results. The 2-year recurrence-free survival was 57% in the chemolipiodolization group and 37% in the control group ($P = 0.02$). However, there was no significant difference at 5 years after surgery ($P = 0.09$). The 5-year overall survival rates in the chemolipiodolization and the control groups were 82.4 and 55.7%, respectively ($P = 0.04$). Cox proportional multivariate analysis revealed that adjuvant chemolipiodolization was an independent favorable prognostic factor for 2-year recurrence-free survival, and the odds ratio [95% confidential interval] was 0.55 [0.34–0.90] ($P = 0.02$). However, adjuvant chemolipiodolization was not an independent favorable prognostic factor for 5-year overall survival.

Conclusions. Adjuvant chemolipiodolization can reduce the risk of early recurrences, which would be mainly IM derived. However, chemolipiodolization did not reduce late phase recurrences after surgery, which would be mainly MC derived. To prevent late phase recurrences, another novel strategy would be needed.

Hepatocellular carcinoma (HCC) is the fifth most common cancer in the world. Although surgical resection is considered to be a potentially curative treatment, the recurrence rates of HCC are still high. The recurrence rates are about 50–60% at 2 years and 80% at 5 years.[1–3]

In HCC, the recurrence pattern is different from other cancers, and two major origins are thought to underlie the pattern of recurrence: microscopic intrahepatic metastases from the primary tumor (IM), and other newly developed HCCs induced by multicentric carcinogenesis (MC).[4,5] As a result of these unique recurrence patterns of HCC, there are two peaks in the incidence of recurrences after surgery.[1] Previous studies have revealed that 2 years after surgery was the inflection point for the disease-free survival curve.[6,7] Early recurrences that occurred within 2 years of surgery were mainly thought to be IM recurrences and were thought to have a worse prognosis. On the other hand, the late recurrences that occurred more than 2 years after surgery were mainly thought to be MC recurrences and were thought to be the result of viral hepatitis or cirrhosis of the remnant liver.

Previous studies identified various risk factors for IM or MC recurrences. Adverse tumor factors such as tumor size, number of tumor, vessel involvement, and serum alfa-fetoprotein (AFP) levels were found to be risk factors.[3,8] Underlying liver status such as serum albumin level, prothrombin time, serum bilirubin level that would be summarized in Child-Pugh status were also found to be risk

First Received: 12 January 2011;
Published Online: 26 May 2011

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factors.[8,9] Others reported that operative blood loss or blood transfusion and anatomical resection would affect the prognosis after surgery.[10–12]

On the basis of the unique recurrence pattern of HCC and identified risk factors for recurrence, previous studies have reported favorable results for various adjuvant therapies such as systemic chemotherapy, locoregional chemotherapy, and adoptive immunotherapy.[13–18] However, most of these articles had small sample sizes or a short duration of observation and did not clearly discuss their preventive effect by distinguishing IM from MC. Moreover, meta-analyses did not show any recommendation for adjuvant anticancer therapy against HCC.[19,20] However, randomized control trials have demonstrated that there are some promising treatments, such as lipiodol injection.[14,17,18]

The benefits of 131-iodine-labeled lipiodol (^{131}I-lipiodol) injection into the hepatic artery for reducing intrahepatic recurrences have been reported in various countries.[14,21,22] However, in Japan and other countries where ^{131}I-lipiodol was not used, injecting an emulsion of lipiodol and chemotherapeutic agents into the hepatic artery (chemolipiodolization) was proposed.[23,24] Considering the results of the previous studies, our institution has decided to propose the use of adjuvant chemolipiodolization as a new protocol for patients undergoing surgical resection for HCC.

In this retrospective case-control study, we examined the validity of adjuvant chemolipiodolization by analyzing a retrospective series of matched patients treated in our institution before and after this treatment was administered, and we estimated whether chemolipiodolization could reduce IM and/or MC.

METHODS

Patients

This study was designed as a retrospective case-control study and was conducted in accordance with the Declaration of Helsinki and the ethical guidelines for clinical studies from the Ministry of Health, Labor and Welfare in Japan.

Because previous adjuvant lipiodolization studies demonstrated about 20% improvement of recurrence, we calculated that we would need a sample size of about 120 patients to detect 20% difference in 2-year recurrence-free survival at 5% type I error and 80% power for a one-tailed log-rank test.[14,21]

From April 2005 to March 2008, 64 patients who underwent curative resection with no macroscopic residual tumor for initial HCC at Wakayama Medical University Hospital were intended to receive adjuvant chemolipiodolization 3 months after surgery. Any patient who experienced recurrence within 3 months after surgery would be administered transarterial chemoembolization (TACE) instead of adjuvant chemolipiodolization and they were enrolled in this study in an intention-to-treat analysis fashion. The patients with a Child-Pugh score of C were excluded from this treatment because of insufficient liver function. All of the patients were given information about the adjuvant chemolipiodolization and its probable benefits and risks. During this period, one patient was determined to have a Child-Pugh score of C at 3 months after surgery. Therefore, 63 patients were defined as an adjuvant treatment group and reviewed in this study. Of these patients, 58 patients were R0 resection and 5 were R1 resection. Among them, 6 patients experienced recurrence within 3 months after surgery and underwent TACE instead of adjuvant chemolipiodolization.

The patients who underwent surgery from April 2001 to March 2005 with matched criteria were enrolled as the control group. A total of 64 patients underwent curative resection with no macroscopic residual tumor for initial HCC during that period. There was no patient with a Child-Pugh score of C by 3 months after surgery. Of these patients, 60 patients were R0 resection and 4 were R1 resection. Therefore, 64 patients were defined as the control group in this study. Among them, 5 patients were diagnosed with a recurrence within 3 months after surgery.

Adjuvant Chemolipiodolization

At 3 months after surgery, the patients were examined by computed tomography (CT) during arteriography, and CT during arterial portography to examine the remnant liver. As previously described, the patients diagnosed with recurrences by this examination were administered TACE. Otherwise, adjuvant chemolipiodolization was performed as follows: 40 mg of epirubicin and 6 mg of mitomycin C were dissolved in 5 ml of water-soluble contrast medium (Omnipaque, Daiichisankyo, Japan). The solution was then mixed with 10 ml of lipid contrast medium (Lipiodol Ultra-Fluid, Terumo, Japan) and the prepared mixture emulsions were injected into the proper hepatic artery through a catheter. The dose of the emulsions was 0.1 ml/kg body weight in this study. Adjuvant chemolipiodolization was administered only one time in this study.

Surveillance

All of the patients were followed up at Wakayama Medical University Hospital every 2–3 months for more than 2 years. Blood examinations were performed every 2–3 months. Ultrasonography and abdominal contrast enhanced dynamic

CT was also alternately performed every 2–3 months as the imaging follow-up. If a recurrence was suspected by ultrasonography, contrast enhancement dynamic CT was performed at that time to reconfirm the lesion.

The image findings were reported by blinded radiologists and the presence of an intrahepatic recurrence was determined by the existence of a hypervascular nodule in early phase with a perfusion defect in the portal phase under contrast enhancement dynamic CT. If an extrahepatic recurrence was suspected, lung CT or bone scintigraphy was performed. An extrahepatic recurrence was determined by the existence of a tumor. After detecting any recurrence, appropriate therapeutic modalities were administered, and the same surveillance was performed.

The primary outcome in this study was defined as the recurrence-free survival especially within 2 years after hepatectomy and the secondary outcome in this study was defined as the overall survival.

Data Collection

The following 16 variables were collected for each patient as potent risk factors for recurrence and survival: age, gender, etiology of underlying liver disease (hepatitis C virus [HCV], hepatitis B virus [HBV], and daily alcohol intake), Child-Pugh score (A or B), indocyanine green retention rate at 15 min (ICG R_{15}), The Cancer of the Liver Italian Program (CLIP) score (0, 1, 2, or 3), primary tumor size, number of tumors (single or multiple), vascular involvement (negative or positive), serum AFP levels, liver resection procedure (major or minor), resection margin (R0 or R1), tumor differentiation (well, moderate, or poor) and blood loss during the operation.

Tumor size, number and vascular involvement were measured by the resection specimen. Resection margin and tumor differentiation were pathologically defined. More than sectionectomy and lobectomy were defined as major liver resection and the others were defined as minor liver resection.

Additionally continuous variables of age, ICG R_{15}, main tumor size, serum AFP levels, blood loss were also categorized by cutoff values of 69 years old, 15%, 5 cm, 400 IU/ml, and 880 ml, respectively.

Statistical Analyses

Continuous variables were expressed as the medians with interquartile ranges and compared by the Mann–Whitney *U*-test. Dichotomous variables were compared by the Chi-square test or Fisher's exact test. Recurrence-free survival and the overall survival rate were analyzed by the Kaplan–Meier method and log rank test.

In order to adjust for confounding variables, chemolipiodolization and 16 collected variables were analyzed univariately by a Cox proportional hazard model for recurrence-free survival and overall survival and then potent significant variables were entered into multivariate analyses. *P* values of <0.05 were considered statistically significant.

RESULTS

The patient backgrounds between those treated with and without adjuvant chemolipiodolization therapy are

TABLE 1 Patients backgrounds between those treated with or without chemolipiodolization

Variable	Chemolipiodolization		*P* value
	(−) (n = 64)	(+) (n = 63)	
Age (year)	67 (63, 73)	70 (65, 76)	0.08
Gender (male/female)	44/20	53/10	0.04
HCV antibody (+/−)	41/23	31/32	0.09
HBs antigen (+/−)	8/56	7/56	0.81
Daily alcohol intake (+/−)	19/45	25/38	0.24
Child-Pugh score (A/B)	58/6	60/3	0.31
ICG R_{15} (%)	9.9 (7.6, 14.4)	15.0 (10.0, 20.0)	<0.001
CLIP score (0/1/2/3)	30/20/12/2	30/19/13/1	0.94
Primary tumor size (cm)	4.1 (2.8, 6.6)	4.5 (2.9, 6.4)	0.96
No. of tumors (single/multiple)	48/16	46/17	0.79
Vascular involvement (+/−)	11/53	12/51	0.78
Serum AFP levels (ng/ml)	28.6 (6.0, 136.0)	14.3 (6.1, 162.8)	0.75
Liver resection (major/minor)	36/28	32/31	0.54
Resection margin (R1/R0)	4/60	5/58	0.74
Tumor differentiation (well/moderate/poor)	13/48/3	14/38/11	0.06
Blood loss (ml)	978 (513, 1850)	740 (420, 1335)	0.09

Continuous variables are expressed as the medians (25th, 75th percentile)

HBs hepatitis B surface, *CLIP* cancer of the liver Italian program

summarized in Table 1. The distributions of gender and ICG R_{15} were different between the two groups. However, the Child-Pugh score was similar between the two groups. The tumor status, such as tumor size, number of tumors, presence of vascular involvement, serum AFP levels, and tumor pathological differentiation were not statistically significantly different between the two groups. Therefore, the distributions of the CLIP scores were similar between the two groups. The surgical parameters such as operation procedure, resection margin, and blood loss were not different between the two groups.

There were no complications higher than grade 3 in Common Terminology Criteria for Adverse Event among the patients who received adjuvant chemolipiodolization.

The recurrence-free survival curves after surgery for each group are shown in Fig. 1. At 2 years after surgery, recurrences were observed in 40 patients (63%) without chemolipiodolization. On the other hand, 27 patients (43%) with chemolipiodolization experienced recurrence ($P = 0.02$). However, at 5 years after hepatectomy, these curves were no longer significantly different ($P = 0.09$).

The overall survival curves after hepatectomy for each group are shown in Fig. 2. Although the duration of observation was different between the two groups (the median follow-up period was 35 months in the chemolipiodolization-positive group and 53 months in the chemolipiodolization-negative group), the overall survival rates of the chemolipiodolization-positive and -negative groups were 92.1 and 82.8% at 2 years after surgery ($P = 0.12$) and were 82.4 and 55.7% at 5 years after surgery ($P = 0.04$).

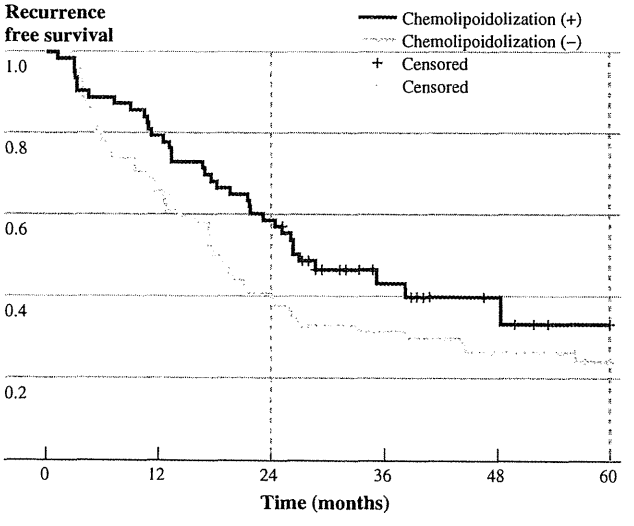

FIG. 1 Kaplan–Meier recurrence-free survival curves of patients with adjuvant chemolipiodolization and those without. The log rank test was performed at 2 and 5 years after the surgery ($P = 0.02$ and 0.09, respectively)

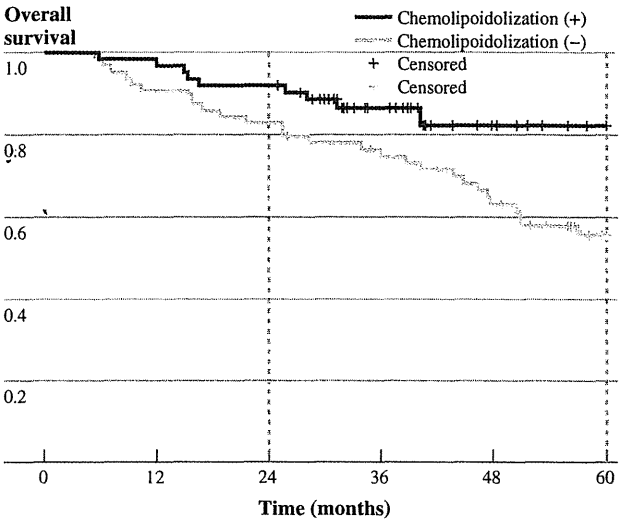

FIG. 2 Kaplan–Meier overall survival curves of patients with adjuvant chemolipiodolization and those without. The log rank test was performed at 2 and 5 years after the surgery ($P = 0.12$ and 0.04, respectively)

In order to evaluate the independent prognostic factors for 2-year recurrence-free survival after hepatectomy, a Cox proportional hazard model was used (Table 2). In univariate analysis, the number of tumors, blood loss and chemolipiodolization were recognized as potent prognostic factors. The odds ratios [95% confidential interval (CI)] were 2.16 [1.31–3.57], 1.67 [1.03–2.71] and 0.51 [0.29–0.87], respectively. To adjust for confounding factors, these three variables were entered into multivariate analysis. The number of tumors and chemolipiodolization were recognized to be independent factors, and the odds ratios [95% CI] were 1.57 [1.35–3.73] and 0.55 [0.34–0.90], respectively.

A Cox proportional hazard model was also used in order to evaluate the independent prognostic factors for overall survival after hepatectomy (Table 3). In univariate analysis, gender, primary tumor size, number of tumors, poor differentiation, blood loss and chemolipiodolization were recognized as potent prognostic factors. The odds ratios [95% CI] for these factors were 2.22 [1.12–4.40], 2.67 [1.35–5.26], 2.65 [1.31–5.17], 4.14 [1.11–15.5], 2.15 [1.07–4.32] and 0.46 [0.21–0.98], respectively. In this study, there were 35 survival events. In multivariate analysis there need to be 10–15 events per variable; therefore, the top three variables (primary tumor size, number of tumors and blood loss) were entered into multivariate analysis. Only the number of tumors was found to be an independent factor for overall survival, with an odds ratio [95% CI] of 2.18 [1.08–4.37].

The patient outcome in terms of recurrence data at 2 years after surgery is shown in Table 4. The recurrence

TABLE 2 Univariate and multivariate analyses of prognostic factors for recurrence within 2 years after hepatectomy

Variable	*N*	Univariate analysis			Multivariate analysis		
		Odds ratio	95% CI	*P* value	Odds ratio	95% CI	*P* value
Age							
<69 (year)	64	1					
≥69 (year)	63	0.88	0.55–1.42	0.61			
Gender							
Male	97	1					
Female	30	1.49	0.87–2.56	0.15			
HCV antibody							
(−)	55	1					
(+)	72	1.61	0.98–2.65	0.06			
HBs antigen							
(−)	112	1					
(+)	15	0.54	0.22–1.35	0.19			
Daily alcohol intake							
(−)	83	1					
(+)	44	1.03	0.63–1.69	0.90			
Child-Pugh score							
A	118	1					
B	9	1.06	0.39–2.93	0.90			
ICG R_{15}							
<15%	78	1					
≥15%	49	0.93	0.57–1.53	0.78			
Primary tumor size							
≤5 cm	75	1					
>5 cm	52	1.43	0.89–2.32	0.14			
Number of tumors							
Single	94	1			1		
Multiple	33	2.16	1.31–3.57	0.003	1.57	1.35–3.73	0.002
Vascular involvement							
(−)	104	1					
(+)	23	1.37	0.76–2.48	0.29			
Serum AFP levels							
<400 ng/ml	105	1					
≥400 ng/ml	22	1.60	0.89–2.88	0.12			
Liver resection							
Minor	59	1					
Major	68	1.13	0.70–1.83	0.62			
Resection margin							
R0	118	1					
R1	9	1.10	0.45–2.43	0.91			
Tumor differentiation							
Well	27	1					
Moderate	86	1.26	0.68–2.32	0.46			
Poor	14	1.35	0.54–3.38	0.52			
Blood loss							
<880 ml	65	1			1		
≥880 ml	62	1.67	1.03–2.71	0.04	1.57	0.96–2.56	0.07
Chemolipiodolization							
(−)	64	1			1		
(+)	63	0.51	0.29–0.87	0.01	0.55	0.34–0.90	0.02

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TABLE 3 Univariate and multivariate analyses of prognostic factors for overall survival

Variable	*N*	Univariate analysis			Multivariate analysis		
		Odds ratio	95% CI	*P* value	Odds ratio	95% CI	*P* value
Age							
<69 (year)	64	1					
≥69 (year)	63	1.37	0.70–2.67	0.36			
Gender							
Male	97	1					
Female	30	2.22	1.12–4.40	0.02			
HCV antibody							
(−)	55	1					
(+)	72	1.86	0.91–3.80	0.09			
HBs antigen							
(−)	112	1					
(+)	15	0.37	0.09–1.55	0.17			
Daily alcohol intake							
(−)	83	1					
(+)	44	0.64	0.30–1.37	0.25			
Child-Pugh score							
A	118	1					
B	9	1.27	0.39–4.15	0.70			
ICG R_{15}							
<15%	78	1					
≥15%	49	0.74	0.35–1.54	0.42			
Primary tumor size							
≤5 cm	75	1			1		
>5 cm	52	2.67	1.35–5.26	0.005	2.00	0.98–4.01	0.06
Number of tumors							
Single	94	1			1		
Multiple	33	2.65	1.31–5.17	0.006	2.18	1.08–4.37	0.03
Vascular involvement							
(−)	104	1					
(+)	23	1.96	0.94–4.10	0.07			
Serum AFP levels							
<400 ng/ml	105	1					
≥400 ng/ml	22	1.66	0.76–3.67	0.21			
Liver resection							
Minor	59	1					
Major	68	1.21	0.62–2.37	0.57			
Resection margin							
R0	118	1					
R1	9	1.13	0.35–3.69	0.84			
Tumor differentiation							
Well	27	1					
Moderate	86	2.42	0.84–6.95	0.10			
Poor	14	4.14	1.11–15.5	0.04			
Blood loss							
<880 ml	65	1			1		
≥880 ml	62	2.15	1.07–4.32	0.03	1.72	0.84–3.54	0.14
Chemolipiodolization							
(−)	64	1					
(+)	63	0.46	0.21–0.98	0.04			

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TABLE 4 Patterns of recurrence at 2 years after hepatectomy

Pattern of recurrence	Chemolipiodolization	
	(−)	(+)
Intrahepatic metastasis		
≤3 nodules	21	14
>3 nodules	17	9
Extrahepatic metastasis	2	4

patterns were not statistically significantly different between the two groups.

DISCUSSION

This study revealed that adjuvant chemolipiodolization could reduce recurrences at 2 years after surgery, which implies that it can prevent intrahepatic metastasis (IM). Moreover, a multivariate Cox proportional hazard model revealed that adjuvant chemolipiodolization was an independent favorable factor for reducing recurrences at 2 years after hepatectomy. However, this effect disappeared at 5 years after surgery, which suggests that the technique does not effectively prevent MC.

Concerning the survival outcome, chemolipiodolization appeared to improve the overall survival at 5 years after surgery. The patients with earlier recurrence tend to have a less favorable outcome.[3,6,25] Therefore, reducing IM recurrences by adjuvant chemolipiodolization would affect the overall survival at 5 years after surgery. However, adjuvant chemolipiodolization was not an independent favorable prognostic factor for overall survival in this study. This might be because the duration of follow-up observation in the chemolipiodolization-positive group was relatively short, and did not provide sufficient statistical power for the multivariate Cox proportional hazard model.

Previously ^{131}I-lipiodol was reported in various countries as an adjuvant therapy after curative liver resection for HCC that provided a favorable survival benefit.[14,21,22,26] In studies performed in China and France, ^{131}I-lipiodol provided prolonged survival benefit on recurrence-free and overall survival, and the effect was sustained for 5–7 or 8 years after surgery.[26,27] However, in contrast to these studies, a study from Italy which evaluated the prognostic impact of ^{131}I-lipiodol on HCV-associated HCC revealed that the improvement of disease-free survival rate was only over the short term, up to 15 months, and that the advantage had faded at 36 months after surgery.[22] In the previous Chinese study, more than 80% of the patient population had HBV infection. In the French study, although the prevalence of viral hepatitis was not described in the report, alcoholic hepatitis was the leading cause of HCC and was responsible for 60% of all HCC cases in France.[28] Contrary to the previous studies, the underlying liver disease in the present study was HCV hepatitis in 56.7% of patients (72 of 127), HBV hepatitis in 11.8% of patients (15 of 127) and other nonviral etiology in 33.1% of patients (42 of 127). As Japanese HCC was primarily the result of HCV hepatitis, the beneficial effect of adjuvant lipiodolization would be predicted to be similar to the Italian study, because it appears that the efficacy of chemolipiodolization is influenced by the underlying liver disease.

Adjuvant interferon (IFN) therapy is also a candidate for preventing HCC recurrences. Although IFN was reported to have direct antitumor effects, most previous studies administered IFN expecting an additional effect on hepatitis C, and reported favorable results.[29–31] From one randomized, controlled study for HCV-associated HCC, adjuvant IFN therapy could not prevent recurrences within 2 years from surgery, but could greatly reduce late recurrences that occurred more than 2 years after surgery.[32] This would indicate that treatment of the underlying viral hepatitis is more important for reducing late or MC recurrences. Therefore, a combination of therapeutic strategies targeted for reducing IM recurrences by using our approach and for MC recurrences by using IFN should be examined in order to determine whether the incidence of intrahepatic recurrences can be further reduced.

This study has some limitations. The most problematic limitation is that this study is not a randomized prospective study. Therefore, some confounders might affect our results. However, multivariate analysis revealed that adjuvant chemolipiodolization was an independent favorable factor for reducing early recurrences. Second, the patient occurred recurrence within 3 months after surgery was administered TACE instead of adjuvant chemolipiodolization in this study and which might bias our results. However, excluding these patients from analysis would bring another selection bias. Therefore, this study was performed by intention-to-treat fashion. Third, in survival analysis, the duration of follow-up observation was not very long in the chemolipiodolization group. Therefore, further follow-up will be needed for a more definitive survival analysis and a prospective study also will be needed to verify our retrospective result.

In conclusion, adjuvant chemolipiodolization could reduce the incidence of recurrences for 2 years after curative resection, and resulted in a favorable overall survival at 5 years after the surgery. However, this effect was limited to reducing IM (early) recurrences. In order to further prevent recurrences, a strategy for reducing MC (late) recurrences will be needed. A combination of chemolipiodolization with antiviral therapy could provide additional survival benefit in cases where virus-associated HCC is dominant.

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Langenbecks Arch Surg (2011) 396:1101–1107
DOI 10.1007/s00423-011-0778-7

ORIGINAL ARTICLE

Combined intraoperative use of contrast-enhanced ultrasonography imaging using a sonazoid and fluorescence navigation system with indocyanine green during anatomical hepatectomy

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Received: 15 August 2010 / Accepted: 8 March 2011 / Published online: 29 March 2011

Abstract
Purpose The clear demarcation line is ideal for real-time surgical navigation imaging during hepatectomy.
Methods The study population was comprised of 22 patients with moderate liver cirrhosis scheduled to undergo an anatomical liver resection for the treatment of hepatocellular carcinoma. This study set out to assess the clinical value of the concomitant intra-operative use of contrast-enhanced intra-operative ultrasound using Sonazoid™, and a fluorescence navigation system (PDE) with ICG, as a novel tool for patients undergoing an anatomical liver resection.
Results Following portal pedicle ligation for anatomical resection, 2 min after injection of ICG, the segments to be resected were detected as a negative-brightness area using PDE fluorescence. Sonazoid™ administration provides a parenchymal transectional line, as the margin of a loss of blood flow shows a hypo-enhanced image, and the resectional line of the parenchyma can be confirmed by CE-IOUS. Although the demarcation line of the liver surface after the portal pedicle ligation was apparent in 17 patients, the resection line using PDE was clearly detected in all 22 patients ($p<0.018$).
Conclusions The combined use of these methods is therefore considered to be useful and safe for surgeons, as an additional tool for performing a liver resection.

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Keywords Fluorescence navigation system (Photo Dynamic Eye: PDE) · Contrast-enhanced intra-operative ultrasound (CE-IOUS) · Sonazoid™ · Anatomical hepatic resection · Kupffer-phase image

Introduction

Intra-operative fluorescent imaging techniques using indocyanine green (ICG) (Diagnogreen Inj., Daiichi Sankyo, Tokyo, Japan) have been used to assess the detection of sentinel lymph nodes metastasis in gastric and breast cancer [1, 2]. Recently, some studies reported that liver tumors such as hepatocellular carcinoma (HCC) and metastatic carcinoma exhibited strong fluorescence in patients who had been administered ICG several days prior to surgery during a routine preoperative liver function test [3]. Previous studies of hepatobiliary surgeries using ICG-fluorescent imaging to identify liver cancers, biliary congested areas, and the biliary tracts during cholecystectomy have been reported [4, 5]. These techniques are based on the finding that ICG binds to plasma proteins, and protein-bound ICG emits light with a peak wavelength of around 830 nm when excited with near-infrared light [4]. In our department, anatomic hepatic resection, defined as the complete removal of at least one Couinaud segment containing the tumor, is the standard treatment for patients with HCC [6]. Two minutes after intravenous ICG injection following portal pedicle ligation via a posterior intrahepatic approach, we found that the demarcation line on the liver surface was clearly detectable using Photo Dynamic Eye (PDE).

Intra-operative ultrasonography (IOUS) has been shown to yield significant new information not identified on preopera-

tive imaging, which either determines the resectability or changes the operative plan; it is considered to be the gold standard, thereby achieving universal usage. Recently, to find occult metastases during a hepatectomy in patients with HCC and liver metastases, contrast-enhanced IOUS (CE-IOUS) was performed using Sonazoid™ (GE Healthcare, Oslo, Norway), a new second-generation ultrasound perflubutane microbubbles agent with a median diameter of 2 to 3 nm that provides a parenchyma-specific contrast image based on its accumulation in the Kupffer cells in the liver [7]. Sonazoid is present on the late Kupffer-phase image with a long duration (approximately 2 h after injection) following vascular- and sinusoidal-phase images. SonoVue (Bracco Spa, Milan, Italy) has already been used as a microbubble agent in CE-IOUS, but the duration of the contrast enhancement was shorter, with a mean duration of 4 to 5 min [8]. After portal pedicle ligation for anatomical resection, intravascularly administered Sonazoid™ provides a parenchyma-specific contrast image based on its accumulation in the Kupffer cells, and we were able to identify the parenchymal transactional line as the margin with loss of blood flow shown as a hypo-enhanced image for over 1 h. Therefore, we were able to confirm the resectional line of the parenchyma with IOUS at any time. The aim of the present study was to assess the clinical value of the combined use of fluorescence navigation system (PDE) using indocyanine green and CE-IOUS with Sonazoid, as a novel tool in patients undergoing anatomical liver resection.

Patients and method

Patients' backgrounds

From January 2008 to December 2009, 22 consecutive patients (mean age, 73.5 years; SD, 9.8 years; range, 43–82 years of age; 14 males and eight females) were enrolled to undergo an anatomical liver resection for hepatocellular carcinoma (HCC) at Wakayama Medical University Hospital (WMUH). The anatomical resection procedures included a hemihepatectomy (removal of right lobe in four patients, the removal of a left section in four patients), a sectionectomy (removal of the anterior section in three patients, removal of posterior section in five patients), and a segmentectomy (removal of 8 in four patients, segment 6 in two patients) based on Couinaud's classification. The operative procedures conducted in the anatomic resections are shown in Table 1. All patients were fully informed of the study design according to the Ethical Committee on Clinical Investigation of Wakayama Medical University Hospital and consented in writing to participate in this study.

In all patients, multi-detective CT scanning (MDCT) and magnet resonance imaging (MRI) were performed in a standardized preoperative examination within 2 weeks before the hepatic resection. The CT diagnoses were based on a triphasic contrast-enhanced protocol using a 64-row multi-detective CT scanner (Aquilion 64, Toshiba Medical, Japan), and MR imaging was performed using the 1.5 T MRI system (Philips, Achieva, Netherlands) with an 8-channel body phased-array coil. The use of MDCT allows for a three-dimensional image construction of the liver and a preoperative estimation of the resected liver weight using the CT-volumetry software program Virtual Place Advance (AZE Inc., Tokyo, Japan) [9]. We evaluate the consistency of the shape and/or weight of the resected specimen with those estimated based on preoperative 3D-CT using this software program. Preoperative 3D simulation images taken during the resection of segment 8 arranging from the MDCT are shown in Fig. 1.

Table 1 Operative producers of an anatomic resection using combined PDE and CE-IOUS

Hepatectomy procedure	No.	No. of cases in which the demarcation line was detected	
		Only ligated in the portal pedicle	Adding use of PDE
Right liver (S5 S6 S7 S8)	4	4	4
Left liver (S2 S3 S4)	4	3	4
Posterior sector (S6 S7)	5	4	5
Anterior sector (S5 S8)	3	2	3
S8 segment	4	2	4
S6 segment	2	2	2
Total	22	17	22*

*$p<0.018$ vs. only ligated in the portal pedicle

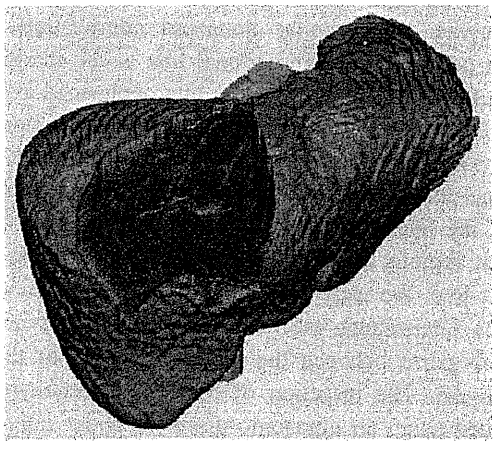

Fig. 1 Preoperative 3D simulation image during segment 8 resection arranging from the MDCT using the Virtual Place Advance software program. Virtual landmark veins were found in the cutting surface: the right hepatic vein, the middle hepatic vein, and the stump of the Glisson of segment 8

Equipment of PDE and CE-IOUS system

Intra-operative fluorescent techniques using the commercially available near-infrared (NR) fluorescence imaging system (PDE; Hamamatsu Photonics K.K. Hamamatsu, Japan) activates ICG with emitted light at a wavelength of 760 nm and filters out light with wavelengths below 820 nm. The light source was a light-emitting diode (LED), and the detector was a charge-coupled device (CCD) camera. The fluorescence signals were sent to a digital video processor for display on a TV monitor. When ICG binds to human plasma, ICG is detectable as a fluorescent signal using the PDE infrared observation camera system. A contrast-enhanced (CE-IOUS) system with Sonazoid™ was performed on an Aloka ProSound α10 system (Aloka Ltd., Tokyo, Japan) equipped with a micro-convex 3.5 MHz frequency probe. None of the authors or Wakayama Medical University Hospital received any financial benefits by advertising these equipments, and there are no conflicts of interest to declare.

Surgical technique

Following the portal pedicle ligation via the posterior intrahepatic approach for anatomical hepatectomy, 1 min after ICG was injected in the cephalic vein at a dose of 0.5 mg/kg body weight, the negative-brightness area of the liver area without blood flow was clearly detected on the liver surface using the PDE system. At the same time, the contrast agent Sonazoid™ was administered at a dose of 0.0075 ml/kg by a manual bolus injection following a flush with 3.0 ml of normal saline. This contrast agent is characterized by prolonged maintenance of its contrast attributes and allows continuous vascular and postvascular imaging. Microbubbles of the perflubutane-based contrast agent are phagocytosed by reticuloendothelial cells in the liver 10–30 min after injection, enhancing the liver parenchyma. Intravascular Sonazoid administration provides a parenchymal transactional line at the margin with a loss of blood flow, shown on a hypo-enhanced image for a long duration approximately 1 h, and the resectional line of the parenchyma could be confirmed with CE-IOUS at any time during hepatectomy. Following contrast administration, the liver parenchyma enhances uniformly, and the ischemic regions were easily identified appearing as a dark contrast free. We were able to recognize the resection line with the intermittent use of CE-IOUS by sandwiching a piece of gauze between the cutting surfaces of the liver parenchyma. The resection of the liver parenchyma was performed using an ultrasonic dissector under intermittent clamping by means of occlusion of blood inflow, both pedicular or selective, 20 min and then release for 5 min using a rubber tape with a tourniquet.

As for the statistical analyses, the chi-square test was used to evaluate the differences of the number of patients by Stat View program (version 5 Hulinks, Tokyo, Japan). Statistical significance was defined as a P value of <0.05.

Results

The surgical procedures used during the anatomic resections are shown in Table 1. Although the demarcation line of the liver surface after the portal pedicle ligation was apparent in 17 patients, the resection line using PDE was clearly detected in all 22 patients ($p<0.018$). The median blood loss during hepatectomy was 480 ml (range 130 to 1,250 ml) and the median duration of surgery was 280 min (range 140 to 380 min). There were no problems with the PDE and CE-IOUS procedure. No postoperative complications, such as bile leakage, developed as a result of either type of liver resection in this study. The median hospitalization period was 11 days (range 8 to 15 days). The surgical procedures conducted in the anatomic resections are shown in Table 1. After the ligation of the portal pedicle prior to the parenchymal dissection for anatomic resection, 17 of 22 cases (77%) had the demarcation line appear on the liver surface without using ICG-fluorescent imaging, but in all cases, the discolored area indicating the ischemic area of the liver was detected following the use of the PDE system. The demarcation line of the liver segment was visible with the PDE camera system, even 10 s after ICG injection. In our cases, intravenous ICG has not been associated with any adverse effects apart from occasional allergic reactions. For example, Fig. 2 shows the time course of the appearance of the negative-brightness area of segment 8.

Immediately after the administration of Sonazoid, the portal veins, hepatic veins, and the normal liver parenchyma, but not the segment with the loss of blood flow, were uniformly enhanced following portal pedicle ligation. For example, segment 8 was due to be removed and was identified as a filling defect during hepatectomy (Fig. 3). Approximately 60 min after the injection, the remnant liver was identified by contrast enhancement. Our standard views are shown after the anatomic resection of segment 8 (Fig. 4). After performing the liver resection, we confirmed that the positive-brightness area on the remnant liver was clearly detected on the raw liver surface using the ICG-fluorescent images. With regard to the benefit of using both methods after clamping of the pedicle, clear discoloration marks on the liver surface could be confirmed with the PDE system, and the resectional line of the parenchyma could be confirmed with CE-IOUS at any time during hepatectomy.

If the metastatic lesion was in the remnant liver, the filling defects were clearly confirmed with CE-IOUS at any time

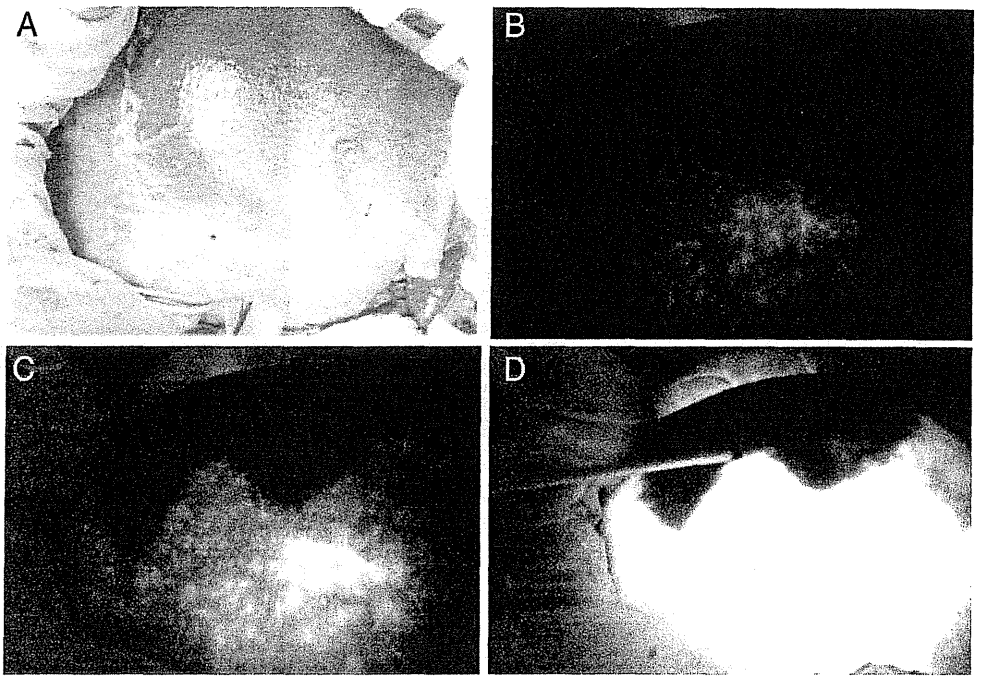

Fig. 2 The time course of the appearance of the negative-brightness area in segment 8 with the intra-operative PDE image after intravenous ICG injection. **a** Normal view of pre-injection. **b** One minute after injection. **c** Two minutes after injection. **d** Ten minutes after injection

within 30 min after the intravenous injection of Sonazoid. Figure 5 shows IOUS and CE-IOUS images of an HCC metastasis with liver cirrhosis at segment 7. The metastatic lesion was unclearly detected as a slightly hypoechoic mass, which could not be differentiated from fibroid indurations based on the liver cirrhosis with IOUS; however, the CE-IOUS view of the same lesion revealed the metastatic lesion to be a clear hypoechoic mass at the late Kupffer phase.

Two hours after venous ICG injection, its excretion into the bile was monitored by PDE, and when used with bile duct imaging was a better choice for detecting the cutting portion of bile duct (Fig. 6). The PDE system for imaging of the extrahepatic biliary duct can detect the cutting portion of the bile duct to permit real-time monitoring of the resection of liver parenchyma.

We evaluated the correlation of the weight of the resected specimen with that estimated based on preoperative 3D-CT using this software program. Figure 7 shows the correlation diagram between the estimated volume of the resected specimen and the true weight of the removed specimen. The correlation coefficient showed a satisfactory result for the estimate (R=0.982).

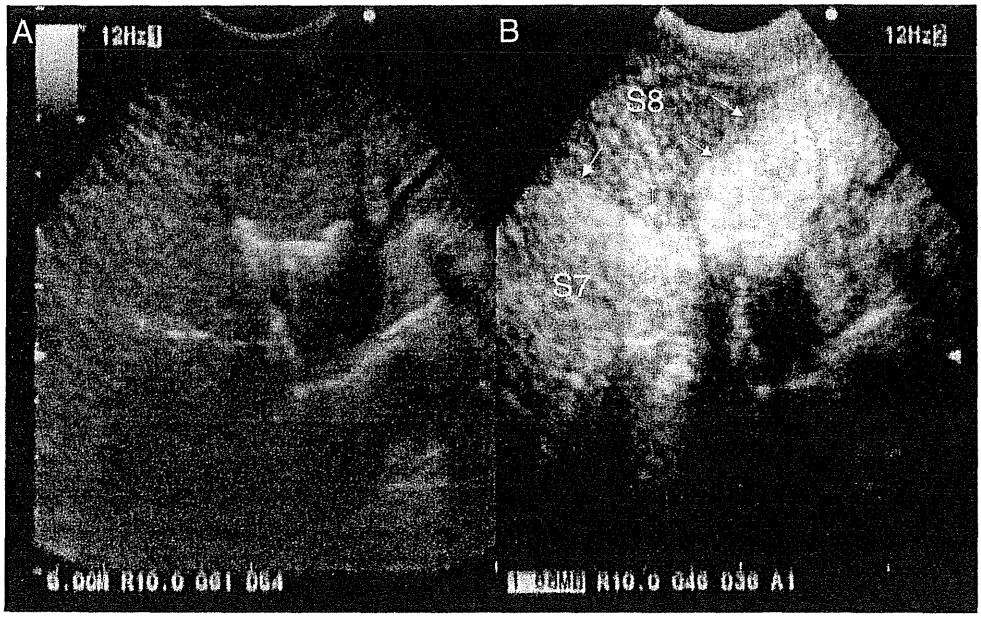

Fig. 3 IOUS image and CE-IOUS image of the Kupffer phase using Sonazoid after the ligation of the segment 8 pedicle. **a** Non-enhanced IOUS cannot provide a parenchymal transactional line. **b** Sonazoid administration provides a clear parenchymal transactional line as the margin with loss of blood flow, as shown on the hypo-enhanced image