Figure Legends

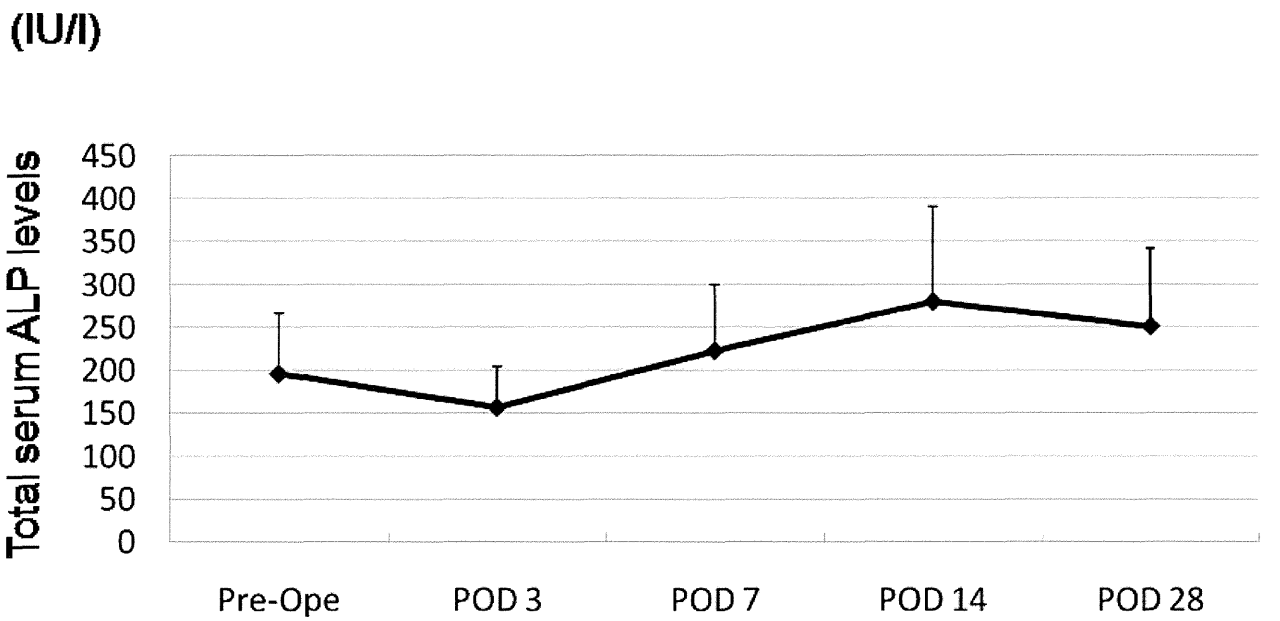

Figure 1. Total ALP levels after hepatectomy in donors of living donor liver transplantation. Abbreviations : ALP, Alkaline phosphatase; POD, Postoperative day.

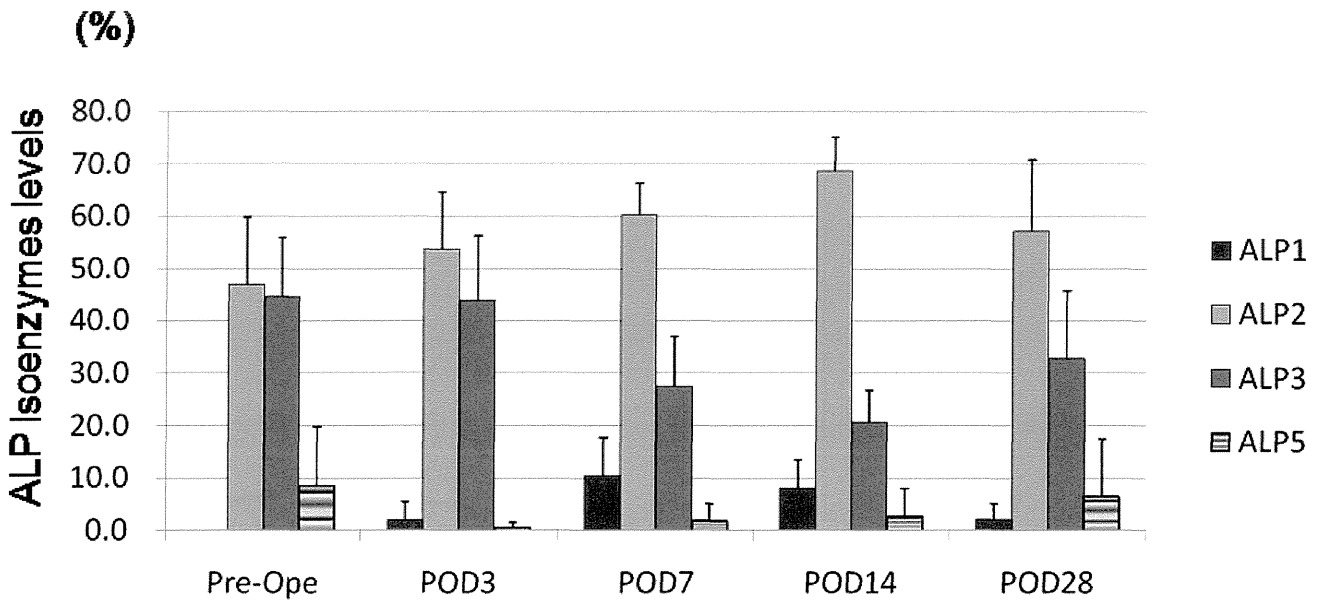

Figure 2. Postoperative transition of the ratio of ALP isoenzymes in donors of living donor liver transplantation. Abbreviations : ALP, Alkaline phosphatase; POD, Postoperative day.

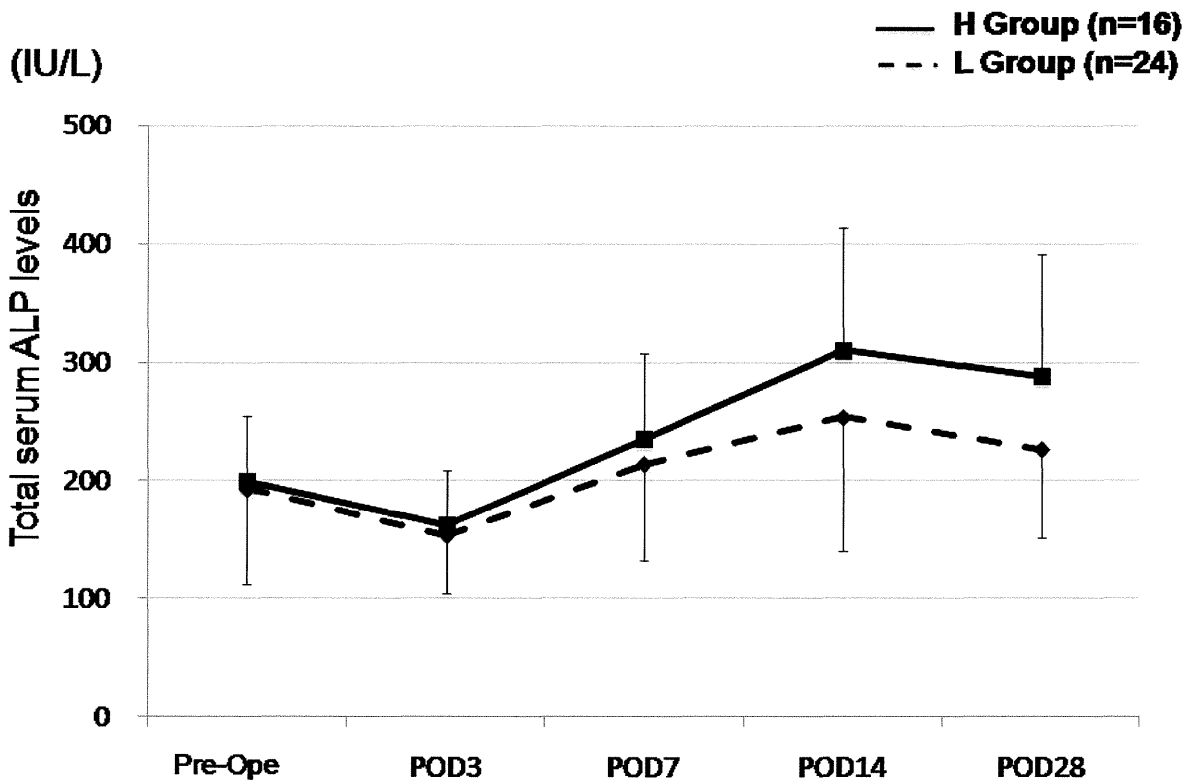

Figure 3. Postoperative total ALP levels in each group according to liver regeneration rates. Abbreviations : ALP, Alkaline phosphatase; H group, High regeneration group; L group, Low regeneration group.

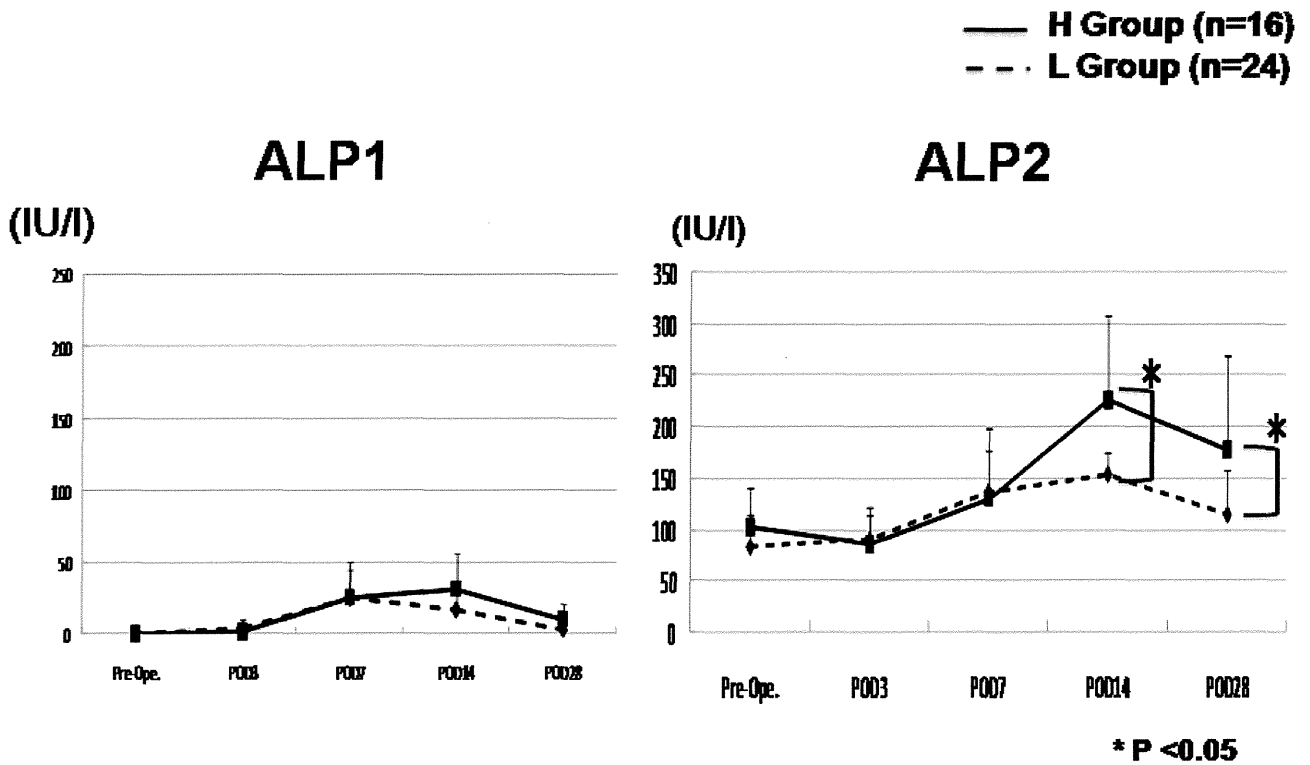

Figure 4. Expression of the ALP isoenzyme levels according to liver regeneration rates. Abbreviations : ALP, Alkaline phosphatase; H group, High regeneration group; L group, Low regeneration group.

TABLE 1

Characteristics of LDLT donors in each group according to liver regeneration rates.

	H group (n=16)	L group (n=24)	p-value
Age	37 (21 - 63)	32 (20 - 55)	N.S.
Resected liver volume (%)	62.0 (37.4 - 70.8)	35.1 (27.8 - 66)	<0.001
Rt lobe vs. Lt. lobe	13 : 3	4 : 20	<0.001

H group: high regeneration rate group

L group: low regeneration rate group

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ORIGINAL ARTICLE

Is a fluorescence navigation system with indocyanine green effective enough to detect liver malignancies?

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Published online: 1 July 2013

Abstract
Background Although several reports have shown the efficacy of a fluorescence navigation system (FNS) with indocyanine green (ICG) to detect liver malignancies during hepatectomy, the real accuracy of this procedure is not yet clear. This study aimed to analyze the actual efficacy of ICG-FNS in cirrhotic and non-cirrhotic livers.
Methods Ten cirrhotic whole livers explanted from liver transplant recipients and 23 non-cirrhotic livers from patients who underwent hepatectomy for various kinds of liver tumors were investigated with ICG-FNS. All surgical specimens were analyzed macroscopically and pathologically.
Results In the patients with a cirrhotic liver, most nodules illuminated by ICG-FNS were diagnosed as regenerative nodules pathologically. The positive predictive value was 5.4%. There was a significant difference in positive predictive value to detect malignant liver tumors between cirrhotic liver and non-cirrhotic liver (5.4% vs 100%, $P < 0.0001$). In the non-cirrhotic livers, 11 of 33 (32.4%) tumors were not recognized by ICG-FNS through the liver surface before resection. There was a significant difference in the depth from the liver surface to tumor between illuminated nodules and non-illuminated nodules (1.5 mm vs 11.6 mm, $P < 0.01$).
Conclusions It is necessary to know the limitation of ICG-FNS when detecting liver malignancies in both cirrhotic and non-cirrhotic livers.

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Keywords Fluorescence navigation system · Indocyanine green · Liver tumor

Introduction

In liver surgery, in order to achieve absolute removal of tumors, it is essential to recognize even small tumors and to ensure the accurate surgical margin intraoperatively. Intraoperative ultrasonography has been the gold standard to detect liver tumors since it was introduced by Makuuchi et al. [1]. Recently, several reports have shown the efficacy of intraoperative navigation surgery using a fluorescence navigation system with indocyanine green (ICG-FNS) [2, 3].

Indocyanine green-fluorescence navigation system has generally been used to detect the sentinel lymph nodes in the breast [4, 5], gastric [6–8], lung [9, 10], and esophageal cancers [11] and to intraoperatively assess graft patency in vascular surgery [12, 13]. The adaption of ICG-FNS was expanded for hepatobiliary surgery [3, 14, 15]. Ishizawa et al. [2] reported that fluorescence intraoperative cholangiography with ICG is a safe and valuable procedure for a road map of biliary tract anatomy and identification of liver tumor through the visualization of the disordered biliary excretion of ICG in real time. However, the actual efficacy of ICG-FNS to detect liver tumors is not clear. For example, false positive detection might occur because regenerative nodules develop as liver damage progresses [16]. Also, small tumors located deep in the liver parenchyma might not be detected by this procedure.

Therefore, the aim of this study was to evaluate the actual efficacy of ICG-FNS in cirrhotic or non-cirrhotic liver separately.

Table 1 Characteristics of patients with a cirrhotic liver and a non-cirrhotic liver

	Cirrhotic liver for LDLT ($n = 10$)	Non-cirrhotic liver for liver resection ($n = 23$)
Gender (male : female)	6:4	16:7
Age[a]	61 (38–72)	67.5 (60–90)
liver disease	HCV:8, HBV:1, alchohol:1	HCV:5, HBV:5, NBNC:4, NL:9
liver tumor	HCC:9	HCC:12, metastases:9, CCC:1, Carcinoid:1
Total-bilirubin (mg/dl)[a]	3.2 (0.5–8.8)	0.8 (0.3–1.4)
AST (IU/l)[a]	52 (30–89)	28 (13–285)
ALT (IU/l)[a]	29 (15–75)	27 (10–100)
Albumin (g/dl)[a]	2.6 (2.1–3.8)	4.2 (2.9–5.2)
Platetlet ($\times 10^4$/mm^3)[a]	6.4 (2.3–13.4)	16.4 (10.8–27.6)
PT (INR)[a]	1.47 (1.07–1.95)	1.00 (0.87–1.13)
ICGR15 (%)[a]	44 (24–70)	11 (2–15)
Child–Pugh score[a]	9 (5–12)	5 (5–7)
MELD score[a]	14 (7–22)	–

ALT alanine aminotransferase, *AST* aspartate aminotransferase, *CCC* cholangiocarcinoma, *HBV* Hepatitis B virus, *HCC* Hepatocellular carcinoma, *HCV* Hepatitis C virus, *ICGR15* indocyanine green retention rate at 15 minutes, *INR* international normalized ratio, *MELD score* model for endstage liver disease score, *NBNC* non-HBV non HCV, *NL* normal liver, *PT* prothorombin time

[a] Median (range)

Materials and methods

Patients

Ten cirrhotic whole livers were obtained from living donor liver transplant (LDLT) recipients. The original diseases included hepatitis C virus (HCV)-cirrhosis in eight, hepatitis B virus (HBV)-cirrhosis in one, and alcoholic cirrhosis in one. Patient characteristics are listed in Table 1. In addition, we evaluated 23 non-cirrhotic liver specimens obtained from patients who underwent hepatectomy for various kind of liver tumors (hepatocellular carcinoma [HCC] in 12, metastasis of colorectal cancer in nine, cholangiocarcinoma in one, and hepatic carcinoid in one) (Table 1).

Examination methods

As a fluorescence source, we used ICG (Diagnogreen, Daiichi Sankyo, Tokyo, Japan), which had been intravenously injected before surgery at a dose of 0.5 mg/kg as part of a routine liver function test. The intervals between the ICG injection and surgery ranged from 2 days. As a fluorescent imaging tool, we used Photo Dynamic Eye-II (PDE-II, Hamamatsu Photonics, Hamamatsu, Japan), which filtered out light with a wavelength below 820 nm, and 36 light-emitting diodes with a wavelength of 760 nm. The camera imaging head was positioned between 20 and 30 cm above the surgical specimen. The marking suture was performed near the site of the illuminated nodules during 10 min while putting the emission of light situation in the video. Any illuminated nodules clearly visualized from the liver surface regardless of signal intensity, size and illuminated pattern were marked. The recognition of the illuminated nodules was clarified by two independent surgeons (TT and IM). Surgical specimens were observed from every angle, and cut to include each tumor's maximum diameter based on gross inspection. After the operation, the number of the illuminated lesions in the video and the number of the marking sites were counted and macroscopically analyzed. In addition, the number of illuminated lesions that were detected through the liver surface and the number of identifiable tumors by pathological examination were analyzed.

Statistical analysis

Results for continuous variables are expressed as the median (range). The positive predictive value was calculated as the number of pathological tumors in illuminated nodules. Data for continuous variables were compared using the Mann–Whitney *U*-test. We set statistical significance at $P < 0.05$.

Results

Efficacy of ICG-FNS to detect HCCs in a cirrhotic liver

The median number of preoperatively detected tumors with various modalities in the 10 patients with cirrhotic liver was 2 (0–4). However, as shown in Figure 1, the median number of illuminated nodules was 20 (7–37), and the median

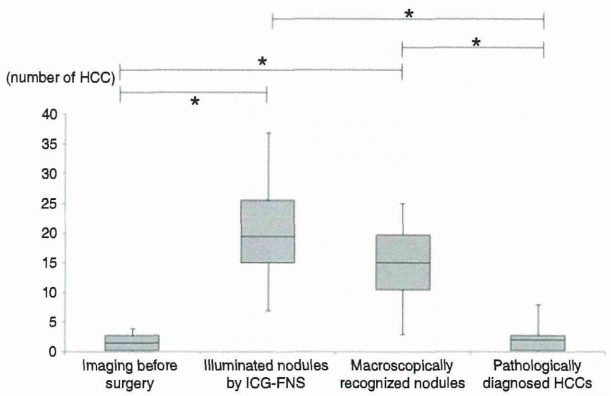

Fig. 1 The number of detected nodules in a cirrhotic liver. The median number of preoperative, illuminated, macroscopic, pathological tumors with cirrhotic liver was, in order, 2 (0–4), 20 (7–37), 16 (3–25), and 2 (0–8). There were significant differences between preoperative tumors and both illuminated nodules and macroscopic tumor ($P < 0.001$). Moreover, there were also significant differences between pathological tumor and both illuminated nodules and macroscopic tumor ($P < 0.001$). *$P < 0.05$

number of nodules to be macroscopically considered as tumors was 16 (3–25). The number of pathologically diagnosed as tumors was 2 (0–8), so the positive predictive value was only 5.4% (0–24.2%). The number of false negatives was 14 (4–17). There were significant differences between the numbers of preoperatively detected tumors and the number of both illuminated nodules and those macroscopically recognized as tumors, as shown in Figure 1 ($P < 0.001$). Moreover, there were also significant differences between the numbers of nodules pathologically diagnosed as tumors and the number of both illuminated nodules and nodules macroscopically recognized as tumors ($P < 0.001$). HCC on the liver surface with ICG-FNS revealed fluorescence in Figure 2a. The area that was actually illuminated was also macroscopically observed as HCC in Figure 2b. The same part was pathologically diagnosed as HCC in Figure 2c. However, the other areas that were illuminated and observed as nodules were diagnosed as regenerative nodules (Fig. 2d–f).

Efficacy of ICG-FNS to detect malignant tumors in a non-cirrhotic liver

The median number of preoperatively detected tumors with various modalities in 23 patients with non-cirrhotic liver was 1 (1–4). The median number of illuminated nodules and nodules macroscopically recognized as tumors was 1 (0–3) and 1 (1–4), respectively. Finally, the median number of nodules pathologically diagnosed as liver tumors was 1 (1–4), so that the positive predictive value to detect malignant tumors was 100%. There were no significant differences between the groups (Supporting information Fig. S1). There was a significant difference in positive predictive value to detect HCC or malignant tumors between the cirrhotic livers and the non-cirrhotic livers (5.4% vs 100%; $P < 0.0001$). Although some tumors could be detected from the liver surface (Fig. 3a,b) and positive predictive value to detect malignant tumors in the non-cirrhotic livers was 100%, other tumors could not be detected from the liver surface (11/34, 32.4%). These nodules were finally recognized after the liver was cut, including tumors (Fig. 3c,d) and defined as non-illuminated nodules. There was a significant difference in the median depth from the liver surface between illuminated nodules ($n = 23$) and non-illuminated nodules ($n = 11$) (1.5 mm vs 11.6 mm, $P < 0.01$) (Fig. 4a). Although there was no significant difference, non-illuminated nodules tended to be smaller than illuminated nodules (illuminated nodules, 32.4 mm vs non-illuminated nodules, 20.7 mm; $P = 0.058$) (Fig. 4b). Furthermore, the tumor size of almost all non-illuminated nodules located within 10 mm depth tended to be smaller than 10 mm (Fig. 4c).

Discussion

Indocyanine green is usually used to directly measure the actual functional state of the liver [17]. ICG is a water-soluble, inert compound that is injected intravenously. It mainly binds to plasma proteins, is taken up by hepatocytes, and is excreted unchanged into the bile. Moreover, ICG is known to absorb infrared rays [18]. Lights, and specially near-infrared light (NIR) in the biological window (700–900 nm), can be exploited for intraoperative imaging guidance. Tanaka et al. demonstrated that (1) the appearance of the surgical field is not altered, (2) it is safe, (3) wavelengths in the 800 nm range penetrate relatively deeply into living tissue, and (4) there has been a tremendous recent effort in developing general-purpose NIR fluorophores that can be conjugated to targeting or other molecules, thus creating "contrast agents" matched to any desired surgical application [19]. Therefore, ICG injection combined with FNS is widely used to detect sentinel lymph nodes [4–11], because this technique is convenient and safe for assessing lymph node status in the oncological field. Recently, adaptation of ICG-FNS was expanded further to hepatobiliary surgery and there have been some reports of its capability in detecting tumors [2, 3]. Furthermore, several reports also showed that ICG-FNS was effective to check the bile leakage and the surgical margin [2, 3, 14, 15].

Our team sectioned whole livers with HCC removed from recipients who underwent living donor liver transplantation and fully investigated them to detect small HCCs [20]. Generally, hepatocytes have the ability to reproduce by

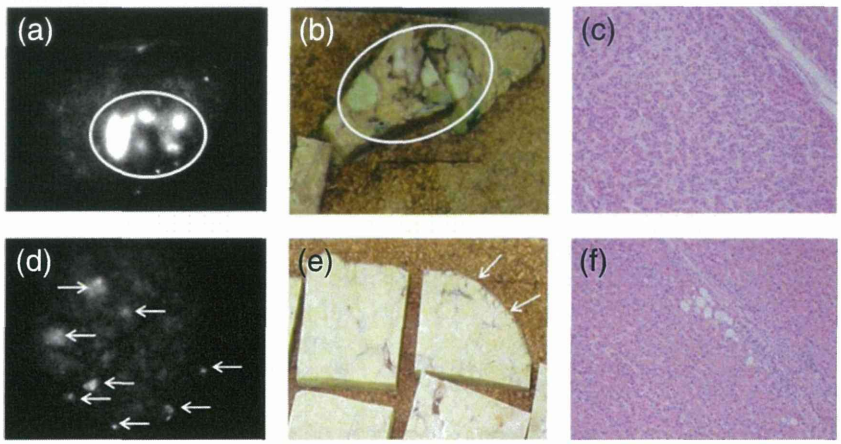

Fig. 2 Indocyanine green-fluorescence navigation system (ICG-FNS) for patients with cirrhotic liver and pathological results. (**a**) Hepatocellular carcinomas (HCCs) on the liver surface with ICG-FNS revealed the illuminated nodules. (**b**) The area that actually emitted light was also macroscopically observed as liver tumor. (**c**) The same area was pathologically diagnosed as HCC. (**d**) The other areas on the liver surface in the same case also revealed the illuminated nodules. (**e**) The areas that emitted light were macroscopically observed as nodules. (**f**) Finally, these areas were diagnosed as regenerative nodules

themselves even if the hepatocytes are exposed to disorders. However, when hepatocytes are exposed to inflammation for a long time and repeatedly, hyperplasia of the collagen fiber deposits occurs strongly and many benign nodules, such as regenerative nodules, in a cirrhotic liver appear surrounded by collagen fiber in the extracellular matrix. In addition, the structure of the hepatic lobule is disturbed and the function of hepatocytes is affected [21]. On the other hand, as described above, ICG binds to plasma proteins, one of which is ligandin. Ligandin is the binding protein of ICG and is uniformly distributed over hepatocytes in a normal liver. The expression of ligandin deviates with liver damage, and it is not expressed in areas of necrosis, fibrosis, or severe inflammation, so that the expression of ligandin becomes relatively rich in the regenerative area [22]. Finally, the coloration of ICG is thought to accumulate in the regenerative nodules [16]. In fact, in our series of cirrhotic livers, many false positive nodules were illuminated, possibly because of the severe liver function disorder and the biliary excretion disorders [2, 16, 21, 22]. However, Ishizawa et al. demonstrated that the signal intensity of the noncancerous liver parenchyma was higher in patients with an unfavorable ICG retention rate and in patients who had received the ICG injection within 24 h before surgery [2]. Although the interval longer than 2 days might be better to obtain a good lesion-to-liver contrast, especially in patients with advanced cirrhosis, we routinely perform the ICG test when the patients were hospitalized generally 2 days before the operation. Also, the optimal interval between ICG injection and surgery to detect tumors remains controversial.

Second, we attempted to evaluate liver resection cases with non-cirrhotic livers. The aim of this study was to determine whether the fluorescence navigation system was effective or not to detect tumors. Therefore, we did not

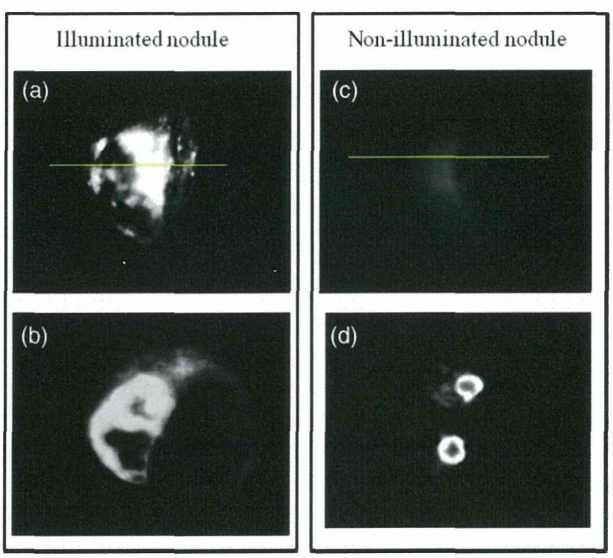

Fig. 3 Indocyanine green-fluorescence navigation system (ICG-FNS) for patients with liver resection with non-cirrhotic liver. Liver tumors in illuminated nodules could be observed as illuminated nodules on the liver surface (**a–b**); liver tumors in non-illuminated nodules could not be observed as illuminated lesions on the surface, and finally recognized after the liver was cut including the tumor under ultrasound (US) guidance (**c–d**)

evaluate the illuminated pattern of tumors. However, we got the same results as Ishizawa's report concerned with illuminated pattern of liver tumors (data not shown) [2]. It was clear that although false positive nodules were not observed in the non-cirrhotic livers, smaller tumors and tumors that were located deeper than 10 mm from the liver surface to tumor were difficult to recognize as illuminated nodules. Kim et al. have already described that near-infrared light penetrates human tissues to a depth of

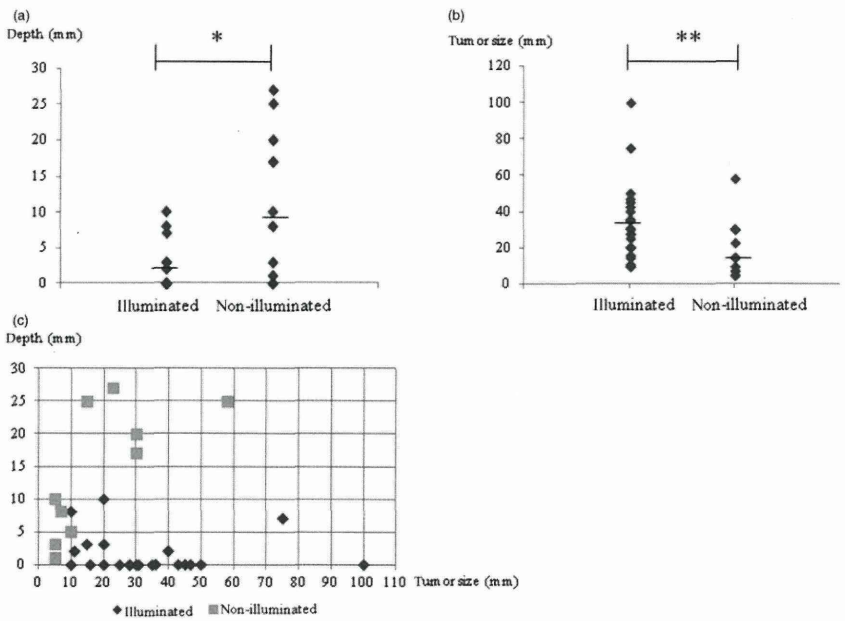

Fig. 4 Comparison between illuminated nodules and non-illuminated nodules on the liver surface in a non-cirrhotic liver. (**a**) There was a significant difference in median depth from the liver surface between illuminated nodules and non-illuminated nodules (1.5 mm vs 11.6 mm, $P < 0.01$). (**b**) Non-illuminated nodules tended to be smaller than illuminated nodules (illuminated nodules, 32.4 mm vs non-illuminated nodules, 20.7 mm; $P = 0.058$). (**c**) Correlation diagram of the depth and tumor size presented. The tumor size of almost all non-illuminated nodules located within 10 mm depth tended to be smaller than 10 mm

about 5–10 mm and Ishizawa et al. have also described that cancer detectability using the fluorescent imaging technique seems mainly to depend on the depth of the tumors from the liver surface because of the limited tissue penetration of near-infrared light [2, 23]. Therefore, in our study, ICG-FNS was not able to detect tumors located deeper than 10 mm. Possibly, 10 mm is the maximum limit of depth for ICG-FNS to detect tumors. Based on these results, we suggested that the capability of detecting tumors with ICG-FNS alone was not sufficient in both cirrhotic and non-cirrhotic livers.

We conclude that although ICG-FNS have the capability of detecting tumors and checking the bile leakage and surgical margin, it is necessary to know the limitation of ICG-FNS when searching liver malignancies in cirrhotic livers or small and deep liver malignancies in non-cirrhotic livers. Additional modalities including ultrasound should be adapted for use with this procedure to detect small liver tumors.

Author contribution Study design: Takayuki Tanaka. Acquisition of data: Takayuki Tanaka, Takanobu Hara, and Izumi Muraoka. Analysis and interpretation: Takayuki Tanaka, Mitsuhisa Takatsuki, Masaaki Hidaka, and Akihiko Soyama. Manuscript drafted by: Takayuki Tanaka and Mitsuhisa Takatsuki. Revision: Takayuki Tanaka and Susumu Eguchi. Statistical Advice: Takayuki Tanaka, Tomohiko Adachi and Tamotsu Kuroki

Conflict of interest None declared.

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Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Fig. S1 Number of detected nodules in a non-cirrhotic liver. The median number of preoperative, illuminated, macroscopic, and pathological tumors with non-cirrhotic liver was, in order, 1 (1–4), 1 (0–3), 1 (1–4), and 1 (1–4). There were no significant differences in each group.

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Original Paper

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Evaluation of immune function under conversion from Prograf to Advagraf in living donor liver transplantation

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Authors' Contribution:
A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
G Funds Collection

Summary

Background: Although some reports have shown the safety and efficacy of conversion from Prograf to Advagraf in liver transplantation, there have been no reports showing the change of immune function after conversion. The aim of this study is not only to analyze the safety and efficacy of conversion from Prograf to Advagraf, but also to evaluate the immune function using the ImmuKnow assay.

Material/Methods: Of the 168 living donor liver transplantation (LDLT) patients, 21 recipients whose liver function was stable after discharge in outpatient clinic and who agreed to conversion from Prograf to Advagraf were enrolled in this study. Liver, renal, and immune functions were retrospectively reviewed.

Results: There were no significant differences in liver and renal function after conversion from Prograf to Advagraf. The intracellular adenosine triphosphate levels before and after conversion were 263±157 and 256±133 ng/ml, respectively, and there was also no significant difference in immune function. None of the recipients showed adverse effects, rejection, or severe infection during the study. It should be further noted that none of the recipients had to increase the dose of Advagraf, while five of 21 recipients (24%) were able to reduce the dose of Advagraf during this study.

Conclusions: Conversion from Prograf to Advagraf in LDLT can be performed safely and effectively without affecting liver, renal, and immune function.

Key words: **Advagraf • tacrolimus • ImmuKnow • LDLT**

Full-text PDF: http://www.annalsoftransplantation.com/download/index/idArt/883953

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Tables: 1
Figures: 4
References: 21

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BACKGROUND

Immunosuppressive therapy is essential to preserve graft function in solid organ transplant recipients [1]. Prograf (Astellas Pharma, Inc.), which is a calcineurin inhibitor developed as an oral twice-daily medicine containing tacrolimus, has been the standard therapeutic regimen all over the world [2]. However, the oral twice-daily regimen has led to non-compliance, and non-compliance causes life-threatening rejection and late graft dysfunction [3,4]. To prevent this, Advagraf (Astellas Pharma, Inc.), a modified tacrolimus formation, was developed as an oral once-daily medicine. At present, conversion to Advagraf therapy has been accepted in various stable organ transplant recipients [5–11].

However, there have been no reports that show the actual changes of immune function after conversion. The ImmuKnow assay (Cylex™ ImmuKnow®-the Cylex Immune Cell Function Assay, Cylex, Inc., USA), which was approved by the Food and Drug Administration in 2002, has been shown to be capable of directly measuring the global immune response, especially T-cell-mediated immunity in transplant recipients. This assay has been shown to reliably distinguish between immune profiles of overimmunosuppression and underimmunosuppression and has been reported to be a convenient, non-invasive, *in vitro* assay, and to be effective as an immune monitoring tool for organ transplant recipients [12,13]. The aim of this study is to analyze the safety and efficacy of conversion from Prograf to Advagraf using not only liver and renal function but also immune function using the ImmuKnow assay.

MATERIAL AND METHODS

Patients

A total of 168 recipients underwent living donor liver transplantation (LDLT) from August 1997 to September 2011 at Nagasaki University Hospital. Of these recipients, 21 who underwent conversion from Prograf to Advagraf were enrolled in this study. They included 13 men and 8 women, with a median age at transplantation of 59 (range, 2–73). Original diagnoses included 3 hepatitis C virus (HCV) cirrhosis, 7 hepatitis B virus (HBV) cirrhosis, 5 alcoholic liver cirrhosis, and 6 others. Of these patients, 8 had hepatocellular carcinoma. The characteristics of the patients are shown in Table 1.

Table 1. The characteristic of the recipients.

Variable	Recipients (n=21)
Gender (male: female)	13: 8
Age	59 (2–73)
Original diagnosis*	HBV-LC: 2 HBV-LC/ HCC: 5 HCV-LC/ HCC: 3 Alcoholic LC: 5 BA: 4 FHF: 2
Duration between LDLT and conversion (months)	33 (7–171)
Duration after conversion (months)	8 (3–29)
Dose of Advagraf at conversion (mg/day)	2 (1–4)

* HBV – hepatitis B virus; HCV – hepatitis C virus; LC – liver cirrhosis; HCC – hepatocellular carcinoma; BA – biliary atresia; FHF – fulminant hepatic failure.

Protocol of immunosuppressant

The baseline protocol of immunosuppressants consisted of Prograf and steroids. The steroids were discontinued three to six months after staged reduction, as long as the liver function was stable without rejection. Prograf was initiated at the dose of 1 mg twice a day after transplantation, and regulated to adjust the desired tacrolimus trough level, 10–15 ng/ml within one month after transplantation and 5–10 ng/ml thereafter. In the outpatient clinic, Prograf was gradually reduced as long as the liver function was stable, and maintained at a minimal dose to prevent both adverse effects and rejection. The indications of the conversion were that liver functions had been stable for at least the three previous months in the outpatient clinic before conversion and that the recipient's fully informed consent to conversion was given. The initial dose after conversion to Advagraf started with the dose equivalent to the dose of Prograf at conversion.

Laboratory evaluation

Tacrolimus trough (Tac), total bilirubin (T-Bil), alanine aminotransferase (ALT), estimated Glomerular Filtration Rate (eGFR), serum creatinine (Cr), and fasting blood sugar (FBS) levels were recorded just before conversion and at the last follow-up and evaluated retrospectively.

The ImmuKnow assay

The immune function was evaluated using Cylex™ ImmuKnow®-the Cylex Immune Cell Function Assay (Cylex, Inc. USA). This assay

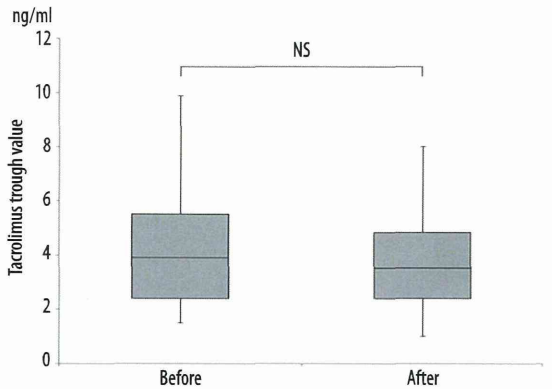

Figure 1. The change of the tacrolimus trough level before and after conversion. Tac levels before and after conversion were 3.9±2.4 and 3.5±2.1 ng/ml, respectively and there was no significant difference in Tac.

was performed according to the manufacturer's protocol [14]. A whole blood sample was collected from each recipient just before conversion and at the last follow-up. The blood sample was collected into an 8-ml sodium heparin vascutainer tube and tested within 10 hours. The whole blood was diluted with a sample diluent, added to a microtiter plate well, and incubated with phytohemagglutinin for 15 to 18 hours in a 37°C, 5% CO_2 incubator. The following day, CD4+ cells were positively selected within the microwells with magnetic particles coated with anti-human CD4 monoclonal antibody (Dynabeads, Dynal, Oslo, Norway) and a strong magnet (model 1050 magnet tray, Cylex, Inc., Columbia, MD) and washed to remove residual cells. A lysing reagent was added to release intracellular adenosine triphosphate (ATP). A luciferin/luciferase mixture was then added to the cell lysate. Within 10 minutes after the addition of the enzyme, released ATP was measured with a GloRunner™ Microplate Luminometer (Turner Biosystems CA).

Statistical analysis

Results for continuous variables were expressed as the median (range). Data for continuous variables were compared using the Mann-Whitney U test. We set statistical significance at $p<.05$.

Results

Change in Tac level and liver functions after conversion.

As shown in Figure 1, the Tac levels before and after conversion were 3.9±2.4 and 3.5±2.1 ng/ml, respectively, and there was no significant difference in Tac. Figure 2 shows liver function. The serum ALT levels before and after conversion were 25±13 and 25±19 IU/l, respectively, and the serum T-Bil levels were 0.9±0.5 and 30.9±0.5 mg/dl, respectively. There was no significant difference in liver function.

Change in renal functions and FBS levels after conversion

Figure 3 shows renal function and FBS level. The serum eGFR levels before and after conversion were 66.8±29.0 and 64.1±27.8 ml/min/1.73 m^2, the serum Cr levels were 0.87±0.23 and 0.82±0.27 mg/dl, and the serum FBS levels were 92±32 and 93±35 mg/dl, respectively. There was no significant difference in renal function or FBS level.

Change in ATP levels after conversion

Figure 4 shows the immune function. The ATP levels before and after conversion were 263±157 and 256±133 ng/ml, respectively. There was also no significant difference in immune function. In addition to these results, none of the recipients showed adverse effects, rejection, or severe

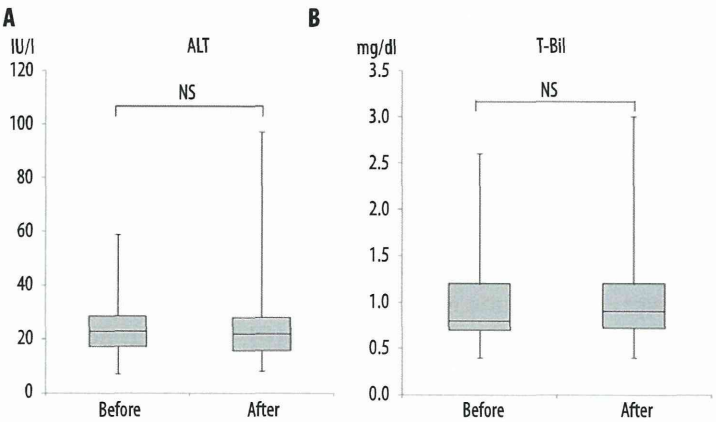

Figure 2. The change of liver functions before and after conversion. (**A**) Serum ALT levels before and after conversion were 25±13 and 25±19 IU/l, respectively. (**B**) Serum T-Bil levels were 0.9±0.5 and 30.9±0.5 mg/dl, respectively. There was no significant difference in liver function.

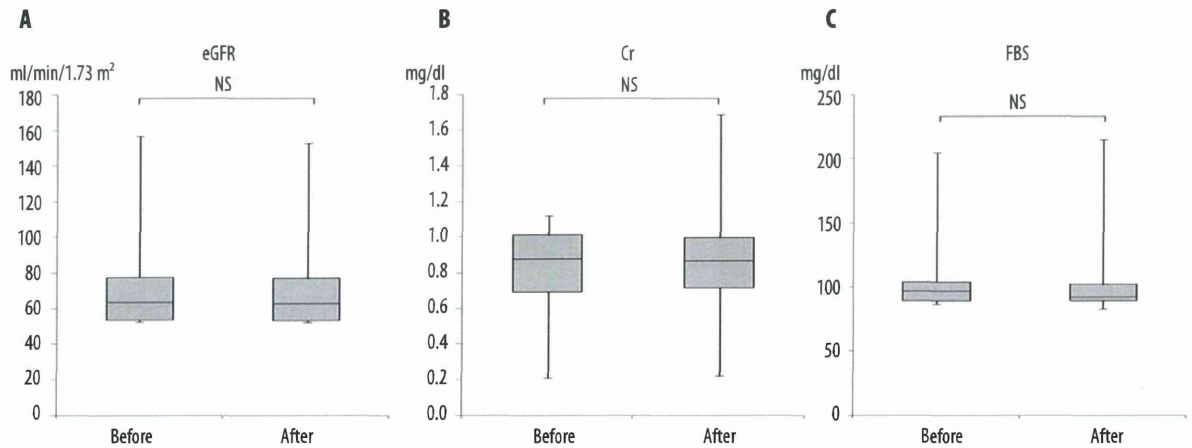

Figure 3. The change of renal functions and FBS before and after conversion. (**A**) Serum eGFR levels before and after conversion were 66.8±29.0 and 64.1±27.8 ml/min/1.73 m², respectively. (**B**) Serum Cr levels were 0.87±0.23 and 0.82±0.27 mg/dl, respectively. (**C**) Serum FBS levels were 92±32 and 93±35 mg/dl, respectively. There was no significant difference in renal functions or FBS level.

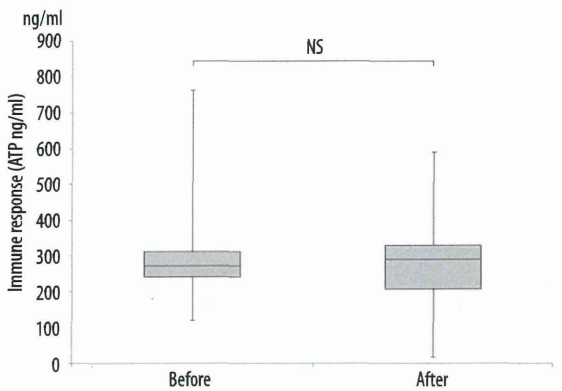

Figure 4. The change of immune function before and after conversion. ATP levels before and after conversion were 263±157 and 256±133 ng/ml, respectively. There was also no significant difference in immune function.

infection during the study. It should also be noted that none of the recipients had to increase the dose of Advagraf, and five of the recipients (24%) could reduce the dose of Advagraf without rejection during this study.

Discussion

Although some reports have shown the safety and efficacy of conversion from Prograf to Advagraf with regard to liver and renal function [8–11], the actual immune function has not yet been clarified. Liver transplantation has been the standard therapeutic option for end-stage liver diseases and reduces the mortality and morbidity of end-stage liver diseases as reflected in the 1- and 5-year survival rates [15–17]. This is mainly the result of improved immunosuppression due to the introduction of a calcineurin inhibitor. Prograf was the immediate-release form of tacrolimus and the oral twice-daily medicine used to prevent various complications in solid organ transplantations and has been accepted as the standard therapeutic regimen all over the world [2,18,19]. However, the estimated rates of nonadherence to immunosuppressive regimens in solid organ transplant recipients range from 15 to 55% [15–17]. Nonadherence has been identified as a leading cause of preventable graft loss [3,4]. It has been proposed that simpler dosing regimens, such as an oral once-daily regimen, may help to improve adherence in transplant recipients [20]. In fact, the prolonged-release form of tacrolimus (Advagraf) was developed as an oral once-daily medicine, and some data have shown that an oral once-daily regimen was associated with an increased likelihood of patient adherence compared with an oral twice-daily regimen [21]. Some reports have evaluated liver and renal function before and after conversion and have shown that the conversion can be applied to liver transplant recipients [8–11]. This study was also able to suggest that conversion does not affect liver and renal function, which is consistent with previous reports.

Additionally, we adapted the ImmuKnow assay to evaluate of the actual immune function. This assay was approved by the US Food and Drug Administration in 2002 for measuring CD4+ T cell immunity [5]. A meta-analysis by Kowaski et al. reported that this assay was useful in monitoring the immune response and assessing the relative risk of infection and rejection [6]. However, no reports have evaluated the safety and efficacy of conversion from Prograf to Advagraf with regard to immune function using this assay. As a result, there

was no significant difference in immune function before and after conversion; this result suggested that conversion also did not affect immune function. In addition, it was important that none of the recipients showed adverse effects, rejection, or severe infection and none had to increase the dose of Advagraf, while five of 21 recipients (24%) were even able to reduce the dose of Advagraf during this study. In our policy of immunosuppression, especially in long-term cases, we reduce and maintain the dose of immunosuppressant as long as possible, keeping the lowest level of tacrolimus needed to prevent rejection. According to the results of this study, Advagraf might be a feasible treatment for avoiding an overdose of tacrolimus.

Conclusions

This study suggested that the conversion of Advagraf can be safely and effectively applied to stable LDLT recipients without affecting liver, renal, and immune function.

Disclosure

The authors have no conflicts of interest or funding to disclose.

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A hybrid method of laparoscopic-assisted open liver resection through a short upper midline laparotomy can be applied for all types of hepatectomies

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Abstract
Background Although hepatectomy procedures should be designed to provide both curability and safety, minimal invasiveness also should be pursued.
Methods We analyzed the data related to our method for laparoscopy-assisted open resections (hybrid method) through a short upper midline incision for various types of hepatectomies. Of 215 hepatectomies performed at Nagasaki University Hospital between November 2009 and June 2012, 102 hepatectomies were performed using hybrid methods.
Results A hybrid method was applicable for right trisectionectomy in 1, right hemihepatectomy in 32, left hemihepatectomy in 29, right posterior sectionectomy in 7, right anterior sectionectomy in 1, left lateral sectionectomy in 2, and segmentectomy in 7 patients, and for a minor liver resection in 35 patients (12 combined resections). The median duration of surgery was 366.5 min (range 149–709) min, and the median duration of the laparoscopic procedure was 32 min (range 18–77) min. The median blood loss was 645 g (range 50–5,370) g. Twelve patients (12 %) developed postoperative complications, including bile leakage in three patients, wound infections in two patients, ileus in two patients, and portal venous thrombus, persistent hyperbilirubinemia, incisional hernia, local liver infarction each in one patient. There were no perioperative deaths.
Conclusions Our method of hybrid hepatectomy through a short upper midline incision is considered to be applicable for all types of hepatectomy and is a reasonable approach with no abdominal muscle disruption, which provides safe management of the hepatic vein and parenchymal resection even for patients with bilobular disease.

Keywords Hepatectomy · Minimally invasive liver resection · Hybrid method · Living-donor hepatectomy · Midline incision

Liver resection is one of the most challenging fields of minimally invasive surgery. In 2007, Koffron et al. [1] reported 300 minimally invasive liver resections (MILR) for hepatic lesions. In their report, they employed three different methods of liver resection: pure laparoscopic liver resection, hand-assisted laparoscopic resection, and laparoscopy-assisted open resection (hybrid) as the MILR. Compared with open hepatic resection, all of their MILR procedures were less invasive and were associated with a shorter operation time, lower blood loss, and shorter hospital stay, with the same rates of local recurrence and complications.

In a worldwide review of laparoscopic liver resection performed in 2009, pure laparoscopic resections were performed in 75 % of cases, hand-assisted laparoscopic resections in 17 %, and a hybrid procedure was done in only 2 % of cases [2]. However, according to the review, the resected area of the liver was a wedge resection in 45 % and left lateral section in 20 %, revealing that only 23 % of procedures were performed for anatomical resections larger than a sectionectomy.

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We have employed hybrid liver resection with hand-assisted laparoscopic liver mobilization and subsequent liver resection with the hanging maneuver [3] and a two-surgeon technique through a short upper midline incision. Our initial data on hybrid liver resection were herein analyzed to clarify the parameters related to their use for various types of hepatectomies.

Patients and methods

Of 215 hepatectomies performed between November 2009 and May 2013 at Nagasaki University Hospital, we employed laparoscopy-assisted open resections (hybrid method) for 102 patients (47 %).

The contraindications for the hybrid procedure were as follows: (1) cases with a previous history of upper abdominal laparotomy; (2) tumor involvement of the diaphragm or a tumor large enough to require an anterior liver resection; and (3) cases with portal or hepatic venous tumor thrombus. The resections for small tumors located in the anterocaudal side of the liver and left lateral sectionectomy were performed under pure laparoscopic liver resection. As a standardized anatomical resection of the normal liver, we chose living-donor left hemihepatectomy for comparison of the surgical outcomes of the hybrid technique and the open procedure.

Among the analyzed patients who underwent hybrid resection, the median age (62 males, 40 females) was 59 years (range 21–85) years (Table 1). The patients' median height was 161 cm (range 145–181) cm, and the median weight was 60 kg (range 37–88) kg. The body mass index (BMI) was 22.8 (range 16.5–31.6).

Table 1 Patient characteristics

Age (years)	59 (range, 21–85)
Sex (M:F)	62:40
Height (cm)	161 (145–181)
Weight (kg)	60 (37–88)
BMI	22.8 (16.5–31.6)
Indications	**Number**
Hepatocellular carcinoma	32
Metastatic liver tumor	14
Hilar cholangioma	3
Intrahepatic cholangioma	5
Epithelial hemangioendothelioma	1
Hepatic carcinoid	1
Cystoadenoma	1
Caroli's disease	1
Living donor	44

All but one patient had Child-Pugh grade A status; one patient was considered to have Child-Pugh grade B disease. The liver functional reserves were as follows: the indocyanine retention rate at 15 min (ICGR15) had a median of 11 % (range 1–30 %), and the median 99mTc-GSA scintigraphy receptor index [ratio of the liver to heart-plus-liver radioactivity at 15 min (LHL15)] was 0.922 (range 0.826–0.975). The liver functional reserve evaluations, including the ICG retention test and 99mTc-GSA scintigraphy, were not performed for living liver donors.

The primary reasons for the operations were hepatocellular carcinoma in 32, metastatic liver cancer in 14, hilar cholangiocarcinoma in 3, intrahepatic cholangiocellular carcinoma in 5, hepatic epithelial hemangioendothelioma in 1, hepatic carcinoid in 1, cystadenoma in 1, Caroli's disease in 1, and living liver donor in 44 (Table 1). The surgical methods employed were a right trisectionectomy in 1, right hemihepatectomy in 32, left hemihepatectomy in 29, right posterior sectionectomy in 7, left-lateral sectionectomy in 2, and segmentectomy (S5, 6, 7) in 7 patients, and a minor liver resection was performed in 35 patients (combined in 12; Table 2). We evaluated surgical outcomes in the patients who underwent the hybrid procedure. We also compared the surgical outcomes of the hybrid procedure and open procedure for living-donor hemihepatectomy.

The Mann–Whitney U test was applied to compare the groups. $P < 0.05$ was considered to be statistically significant.

Surgical techniques

Patients were placed in the supine position with their arms adducted, and a urinary catheter and arterial and central venous lines were inserted. An 8-cm upper midline laparotomy was made, followed by a 5-mm umbilical incision for the laparoscope. The round, falciform, and coronary ligaments were divided, and a wound retractor was installed. Before starting the laparoscopic procedure, a surgical towel was inserted through the upper midline incision to displace the small intestine and colon away

Table 2 Types of hepatectomies

Types of hepatectomies	Number
Right trisectionectomy	1
Right hemihepatectomy	32
Left hemihepatectomy	29
Anterior sectionectomy	1
Posterior sectionectomy	7
Left lateral sectionectomy	2
Segmentectomy	7
Minor liver resection	35 (combined in 12)

from the surgical site. A GelPort (Applied Medical, CA, USA) was attached to the wound retractor at the 8-cm incision, and a 5-mm trocar was placed in the right lateral upper abdomen under pneumoperitoneum (CO_2 at 8 mmHg; Fig. 1A). This configuration enabled the first assistant surgeon, who stood on the left side of the patient, to use the hand port for liver manipulation. The primary surgeon stood on the right side and used the right lateral 5-mm port for dissection. Using laparoscopic electrocautery and a hand assist, the right lobe of the liver was mobilized until the inferior vena cava (IVC) was recognized for all types of hepatectomies. The IVC does not need to be exposed fully at this stage to avoid incidental massive bleeding.

For patients indicated for left-side hepatectomy, the left triangle ligament also was dissected through the 5-mm port placed through the GelPort (Fig. 1B). After these mobilizations, the midline incision was extended to 10 cm for left-side anatomical resection and 12 cm for right-side anatomical resection, and a wound retractor was applied. For minor partial resections, even for multiple lesions, the 8-cm incision was still used. The wound was retracted and opened with the Omnitract retractor. For a right-side hepatectomy, the short hepatic veins were divided under direct view, and the right hepatic vein was encircled and a 6-mm Penrose drain was placed for a subsequent liver hanging maneuver through a midline incision (Fig. 2A). For an extended left hemihepatectomy, the common trunk of the middle and left hepatic veins was carefully encircled. The left hepatic vein was isolated and encircled in advance of parenchymal resection, when it could be performed safely. A Penrose drain also was placed between the hepatic veins for the liver hanging maneuver for left hemihepatectomies.

When cholecystectomy was necessary, we performed it by an open procedure. Hilar dissection was conducted through the midline incision under direct vision (Fig. 2B).

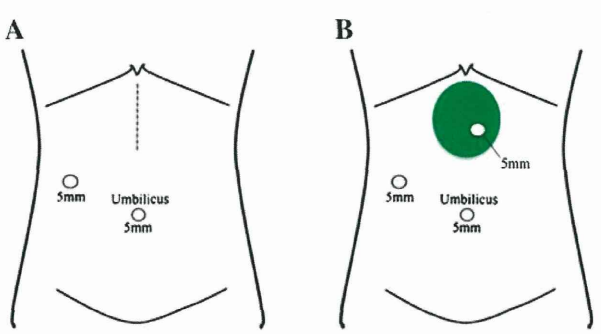

Fig. 1 The trocar placement. **A** *Dotted line* shows the upper midline incision. A 5-mm camera port is inserted from the umbilicus. Another 5-mm trocar is used for dividing the ligaments for mobilization of the right lobe. **B** When mobilizing the left lobe of the liver is necessary, a 5-mm trocar is inserted through a GelPort handport device

By placing surgical towels in the right subphrenic space, the liver can be stabilized in an ideal position by setting the intended transection line in the middle of the incision (Fig. 3).

The 4-0 polypropylene stay sutures were placed at the anterocaudal edge of the liver along the plane of the intended transection. The chief surgeon dissected the hepatic parenchyma from the patient's right side using a Cavitron ultrasonic surgical aspirator (CUSA) system (Integra Life Sciences, Plainsboro, NJ, USA), whereas the assistant surgeon used a saline-linked cautery device (Dissecting Sealer DS 3.5; Salient Surgical Technologies, Portsmouth, NH, USA) from the patient's left side. The occlusion of the hepatic arterial and portal inflow was not performed in any of the cases. The liver parenchyma was dissected with the CUSA, and the intraparenchymal vascular anatomy was defined so that a decision on the hemostatic technique could be made based on the vessel size. The saline-linked cautery device was used to coagulate and divide the dissected vessels that were 3 mm or smaller in diameter. Vessels larger than 3 mm in diameter were ligated with 3-0 or 4-0 synthetic polyester ties and were sharply divided. The few larger vessels were ultrasonically dissected and controlled with 4-0 absorbable monofilament transfixing sutures and were then sharply divided. The traction on the stay sutures was used to separate and to expose the deepening transection plane. During the parenchymal dissection, the upward traction on the tape (hanging maneuver) allowed the surgeon to follow a direct plane and facilitated the exposure and hemostasis of the deeper parenchymal plane in front of the IVC [5, 13]. A closed suction drain was inserted at the conclusion of each procedure.

Preparations for an open hepatectomy were always executed as a backup plan before surgery.

Results

The median length of the operation was 366.5 min (range 149–709) min. The median duration of the laparoscopic procedure was 32 min (range 18–77) min. The median blood loss was 645 g (range 50–5,370) g. There were no macroscopic or microscopic-positive margins seen in any of the patients. No cases were converted to conventional open hepatectomy with subcostal incision. The postoperative complications included surgical site infections in two patients, bile leakage in three patients, ileus in two patients, local liver infarction, portal venous thrombus, incisional hernia, and postoperative hyperbilirubinemia each in one patient. According to the Clavien–Dindo classification [4], the patient with portal venous thrombus and the patient with ileus was a grade III complication, whereas the others