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Abstract

To diagnose hepatocellular carcinoma (HCC) functionally and immediately, we examined the usefulness of indocyanine green (ICG) injection during ultrasound-guided liver biopsy. Liver specimens were obtained after intravenous ICG injection by ultrasound-guided biopsy from 251 space-occupying lesions (SOL) in 136 patients. The tissues were immediately examined for ICG uptake using an infrared Vidicon camera and were also subjected to histopathological examinations. Of the 112 ICG-negative biopsy specimens, 105 were histologically diagnosed as HCC, 6 as dysplastic nodules (DN) and 1 as a regenerative nodule (RN). Of the 139 ICG-positive specimens, 18 were diagnosed as HCC, 1 as DN and 120 as RN. The sensitivity of the absence of ICG uptake (SEAIU), the specificity of the absence of ICG uptake (SPAIU), and the positive predictive value of the absence of ICG uptake (PPAIU) for the diagnosis of HCC were 85.3%, 94.5% and 93.8%, respectively. Of the 251 SOLs, 184 were less than 2 cm. SEAIU, SPAIU and PPAIU for the diagnosis of these small HCC were 85.3%, 94.5% and 91.4%, respectively. These results support the reliability of ICG injection during ultrasound-guided liver biopsy to diagnose even small HCC.

KEYWORDS: indocyanine green, ultrasound, liver biopsy, diagnosis, hepatocellular carcinoma

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Usefulness of Indocyanine Green Injection during Ultrasound-Guided Liver Biopsy for the Diagnosis of Small Hepatocellular Carcinoma

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To diagnose hepatocellular carcinoma (HCC) functionally and immediately, we examined the usefulness of indocyanine green (ICG) injection during ultrasound-guided liver biopsy. Liver specimens were obtained after intravenous ICG injection by ultrasound-guided biopsy from 251 space-occupying lesions (SOL) in 136 patients. The tissues were immediately examined for ICG uptake using an infrared Vidicon camera and were also subjected to histopathological examinations. Of the 112 ICG-negative biopsy specimens, 105 were histologically diagnosed as HCC, 6 as dysplastic nodules (DN) and 1 as a regenerative nodule (RN). Of the 139 ICG-positive specimens, 18 were diagnosed as HCC, 1 as DN and 120 as RN. The sensitivity of the absence of ICG uptake (SEAIU), the specificity of the absence of ICG uptake (SPAIU), and the positive predictive value of the absence of ICG uptake (PPAIU) for the diagnosis of HCC were 85.3%, 94.5% and 93.8%, respectively. Of the 251 SOLs, 184 were less than 2 cm. SEAIU, SPAIU and PPAIU for the diagnosis of these small HCC were 85.3%, 94.5% and 91.4%, respectively. These results support the reliability of ICG injection during ultrasound-guided liver biopsy to diagnose even small HCC.

Key words: indocyanine green, ultrasound, liver biopsy, diagnosis, hepatocellular carcinoma

In recent years, our ability to detect small hepatocellular carcinomas (HCC) has increased due to advances in ultrasound technology (1) and the establishment of a follow-up system for patients with chronic liver

diseases, especially liver cirrhosis. It is sometimes difficult to accurately diagnose small space-occupying lesions (SOL) of the liver detected at ultrasound (US) examination by other imaging modalities, including routine angiography, computed tomography (CT) and magnetic resonance imaging (MRI) (2). In addition, alpha-fetoprotein (AFP) and des- λ -carboxy prothrombin levels are not always elevated in patients with small HCC (3, 4). Hence, ultrasound-guided liver biopsy (USGB) is generally carried out for the diagnosis of small HCC (5). However, a high percentage of small HCC are well-differentiated (WD) (6) and are sometimes difficult to distinguish from dysplastic nodules (DN) (7, 8) by histological examination. Although characteristic findings for small HCC involving increased cellularity, increased nucleus/cytoplasm (N/C) ratio, and pseudoglandular formation were reported (9), the difference between HCC and DN in the degree of each of these 3 findings have not been defined. Therefore, it is very important to develop a method to support present diagnostic systems.

Itoshima *et al.*, reported that the uptake of indocyanine green (ICG) was not visible in malignant liver tumors and suggested that ICG injection might be useful for identifying malignant tumors on the liver surface at peritoneoscopic examination (10). In the present study, we applied this method during USGB of small SOL of the liver and evaluated its usefulness as a quick and an easy functional method of diagnosis of HCC.

Materials and Methods

USGB after ICG injection was carried out for 251 SOL (range = 5 × 4 mm to 55 × 52 mm) in 136 cirrhotic

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patients (range = 49 to 69 years; 40 women, 96 men) who were admitted to the 1st Department of Internal Medicine, Okayama University Medical School, between February 1988 and July 1992. Of the 251 SOL examined by US (115 hyperechoic, 136 hypoechoic), 60 were less than 1 cm, 124 were between 1 cm and 2 cm, and the remaining 67 were over 2 cm in diameter. HBsAg was positive in 17 of 136 patients. AFP (range = 7.6 to 5139 ng/ml) and PIVKA-II (range = 0 to 4.7 AU/ml) were examined as tumor markers. Informed consent was obtained from each patient before USGB.

For each kg of body weight, 2 or 5 mg ICG (Daiichi Pharmaceutical Co. Ltd., Japan) was dissolved in saline (10 ml), mixed with 25 % albumin solution (20 ml), and the mixture was injected intravenously. About 20–40 min after the injection, liver tumor tissue was obtained by USGB using a 21-gauge needle (Majima needle, Top, Japan). The ICG uptake of the specimens was examined immediately after USGB with a visible light camera and an infrared Vidicon camera with 805 nm wavelength (Hamamatsu; C-1000, Hamamatsu Kougyou Co. Ltd., Japan), which is the peak absorbance of infrared light by ICG (11). In a preliminary study, we injected 5 mg of ICG per kg of body weight, because this dose has previously been used in peritoneoscopic examination for the evaluation of liver function in our department (12). However, we later decreased the dose to 2 mg per kg of body weight, since this dose was sufficient to reveal the difference between ICG-positive and -negative tissues when examined with the infrared Vidicon camera.

The ICG uptake was compared with the histopathological findings of the sections stained with Hematoxylin-Eosin and silver impregnation. The histological grade of HCC was determined based on the criteria outlined by the International Working Party (8). Two investigators diagnosed each sections separately. When their diagnoses were different, the investigators checked the section together and a final diagnosis was obtained.

Results

The ICG uptake by the biopsy specimens could not be readily evaluated using a visible light camera (Fig. 1A) but was easily detected with an infrared Vidicon camera (Fig. 1B). With the infrared camera, the ICG-stained tissue was black in contrast to the unstained tissue which appeared lucid or slightly dark. The stained specimen showed non-malignant histological findings (Fig. 1C), whereas the unstained specimen appeared as HCC (Fig. 1D).

Of 139 ICG uptake-positive and of 112 ICG uptake-negative SOLs, 79 (56.8 %) and 58 (51.8 %) showed a hyperechoic pattern, respectively. Fig. 2 is an example of ICG-positive non-malignant tissue and ICG-negative HCC containing almost the same amount of fat deposits.

Of the 139 ICG-positive specimens, 18 were histologically diagnosed as HCC (12 WD, 6 moderately-differentiated), 1 as DN with slightly increased cellularity, and the remaining 120 specimens were regenerative nodules (RN) (Table 1). Of the 112 specimens in which

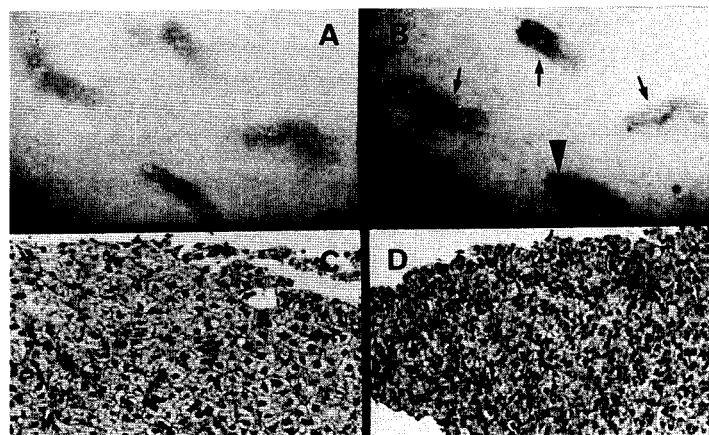


Fig. 1 Indocyanine green (ICG) uptake of a biopsy specimen observed with a visible light camera (A) and an infrared Vidicon camera (B). The latter clearly revealed ICG-positive area as black (arrowhead) and ICG-negative area as lucid or slightly dark (arrows). The ICG-positive tissue yielded non-malignant histological findings (C), while the negative tissue showed the features of moderately-differentiated hepatocellular carcinoma (D).

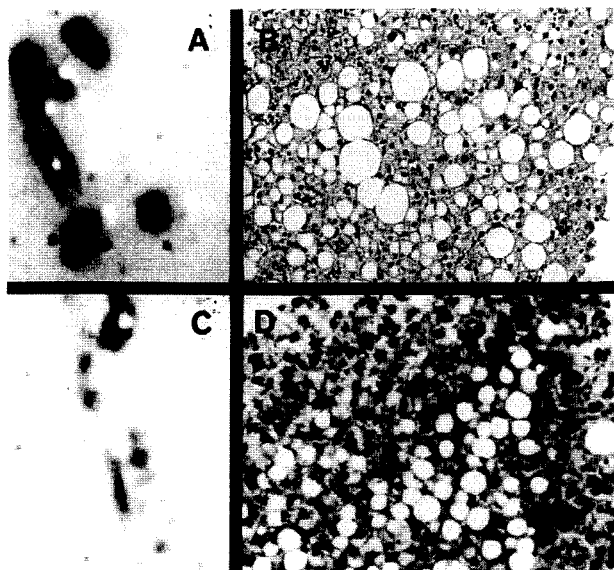


Fig. 2 ICG-positive non-malignant tissue (A, B) and ICG-negative hepatocellular carcinoma (C, D) both with moderate fat infiltration. Note that ICG-positive regenerating tissue was demonstrated simultaneously on panel C. A, C: Observation with infrared Vidicon camera; B, D: HE staining. ICG: See legend to Fig. 1.

Table 1 Indocyanine green (ICG) uptake and histological gradings of HCC

ICG uptake	RN	DN	WD	MD	PD	Total
Positive	120	1	12	6	0	139
Negative	1 (0.8%)	6 (85.7%)	31 (72.1%)	44 (88.8%)	30 (100%)	112 (44.6%)
Total	121	7	43	50	30	251

(): ICG-negative space occupying lesion (SOL)/Total SOL. RN: regenerative nodule; DN: dysplastic nodule; WD: well-differentiated hepatocellular carcinoma; MD: moderately-differentiated hepatocellular carcinoma; PD: poorly-differentiated hepatocellular carcinoma (HCC).

Table 2 ICG uptake and histological gradings of small HCC

ICG uptake	RN	DN	WD	MD	PD	Total
Positive	102	1	10	1	0	114
Negative	0 (0%)	6 (85.7%)	23 (69.7%)	23 (95.8%)	18 (100%)	70 (38.0%)
Total	102	7	33	24	18	184

(): ICG-negative SOL/Total SOL. Abbreviations: See Table 1.

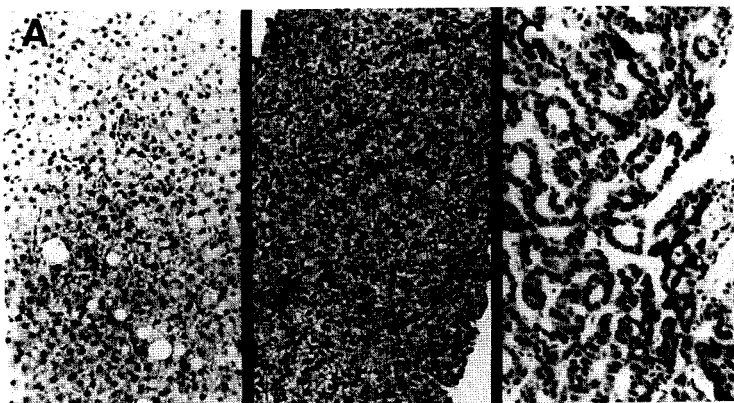


Fig. 3 Histological appearance of three hepatocellular carcinoma (HCC) specimens in which ICG uptake was not recognized. Well-differentiated (A), moderately-differentiated (B), and pseudoglandular (C) HCCs are shown. The absence of ICG uptake was observed even in well-differentiated HCC. ICG: See legend to Fig. 1.

ICG uptake was not detected, 105 (93.8%) were histologically diagnosed as HCC. These consisted of 31 WD, 44 moderately-differentiated (MD) and 30 poorly-differentiated (PD) HCC. The histological diagnoses in 7 other specimens were DN (6 cases) and RN (1 case). The sensitivity of the absence of ICG uptake (SEAIU) were 0.8, 85.7, 72.1, 88.8 and 100.0 in RN, DN, WD-HCC, MD- and PD-HCC, respectively. SEAIU, the

specificity of the absence of ICG uptake (SPAIU) and the positive predictive value of the absence of ICG uptake (PPAIU) for the diagnosis of HCC were 85.3%, 94.5% and 93.8%, respectively. HCC developed in 3 out of 5 patients with ICG-negative DN, but not in patients with ICG-positive DN that were evaluated over at least an 18-month follow-up period (data not shown). Of 251 specimens, 184 were less than 2 cm (Table 2). SEAIU,

SPAIU and PPAIU for the diagnosis of small HCC were 85.3 %, 94.5 % and 91.3 %, respectively. The histological appearance of ICG-negative HCC specimens is shown in Fig. 3. There was no significant difference of ICG uptake between WD-HCC, MD- and PD-HCC ($P = 0.5695$).

Discussion

Tumors less than 2 cm are generally very difficult to diagnose by other imaging methods. The high SEAIU, SPAIU and PPAIU in small HCC show the reliability of this method for diagnosing small HCC. This SEAIU is higher than that of immunohistochemical staining of AFP (33–60 %) and PIVKA-II (55–72 %) (13–15).

The term DN is now generally used to describe SOL in which the cellularity of the hepatocytes is slightly increased relative to the surrounding tissue (8). Because HCC developed frequently in DN (9, 16, 17), DN is also considered to be a premalignant stage of HCC. However, it is not clear whether the phenotypic character of DN is malignant or whether malignant cells developed within the DN. In this experiment, DN showed very similar SEAIU as did HCC (85.7 % and 85.3 %, respectively). In addition, HCC developed in patients with ICG-negative DN, but not in patients with ICG-positive DN. Thus, it appears likely that ICG-negative DN is malignant, although sampling errors or misreading of histological examinations due to the limited size of the samples were possible. We previously examined the relationship between ICG uptake and the DNA ploidy pattern of histologically similar borderline lesions (BL) in the rat hepatocarcinogenesis model. Over 70 % of ICG-negative BL showed aneuploidy or multiploidy, whereas all ICG-positive BL showed diploidy pattern (18). These findings support the hypothesis that ICG-negative DN is malignant.

Since ICG-loaded peritoneoscopic examination revealed decreased ICG uptake in fatty liver tissue (19), we examined whether the absence of ICG uptake in HCC was due to fatty infiltration or phenotypical change of HCC by evaluating the relationship between ICG uptake and echogenic findings (20). The fact that the incidence of the hyperechoic SOL, which usually consist of fat deposition (20–22), is very similar among ICG-positive and ICG-negative SOL (56.8 % and 51.8 %, respectively), indicates that the absence of ICG uptake in HCC nodules is not simply due to the fat infiltration. The examples of

ICG-positive non-malignant tissue and ICG-negative HCC containing almost the same amount of fat deposition also support this conclusion. Most of the ICG-negative biopsy specimens diagnosed as HCC and SEAIU in WD-HCC, MD-, PD-HCC are similar. In light of this, the absence of ICG uptake may be a common phenomenon in malignant hepatocytes, regardless of the differentiation grade of HCC. This data also suggests that the effect of a tumor-induced desmoplastic reaction to the ICG uptake is low. The existence of ICG-positive cases, especially in WD-HCC, suggests that this phenotypic change is not the cause but the result of malignant transformation.

Although the mechanism by which ICG uptake is absent in malignant cells remains obscure, it is possible that either the decrease in a ligand of ICG or the disappearance of the ICG binding protein on plasma membrane may be involved. With regard to the former mechanism, we recently reported that rat liver nodules induced by 2-acetylaminofluorene showed diminished ICG uptake during the process of hepatocarcinogenesis. This loss was partly due to decrease in intracellular glutathione-S-transferase (GST), a ligand of ICG (23). However, the decrease of GST could not totally account for the absence of ICG uptake and the participation of an ICG receptor such as an organic anion binding protein was hypothesized (24).

Tissues obtained by USGB usually contain both cirrhotic and cancerous portions and it is usually impossible to selectively obtain cancerous portions only before histological examination. Very high SEAIU, SPAIU and PPAIU for the diagnosis of malignant SOL make the immediate assessment of malignancy of the liver SOL functionally after USGB possible. The method described herein is unique and very useful for selecting cancerous tissues quickly and easily. This method has the further advantage that the biopsy material can be reliably used for subsequent diagnostic tests, thus minimizing the number of biopsies needed and decreasing the patient's risk of biopsy complications.

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