because the mechanisms of action of TAC-101 and other retinoids are considered cytostatic as opposed to cytotoxic, and the TTP in this study may be comparable to those in studies of sorafenib (2.8–5.5 months).[2,3]

In conclusion, our results suggest that TAC-101 is well tolerated at an oral dose of 20 mg/day (dose level 2). This dose, given once daily after breakfast for 14 consecutive days followed by a 7-day rest period, was determined to be the RD for HCC. Additional studies of TAC-101 as a single agent as well as in combination with molecular-targeted agents such as sorafenib are warranted to further delineate potential clinical benefits and risks.

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Disclosure Statement

The authors have no conflict of interest.

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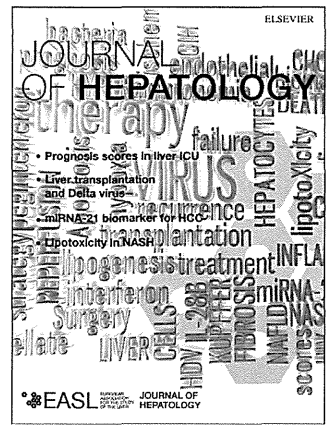

Non-hypervascular Hypointense Nodules Detected by Gd-EOB-DTPA-enhanced MRI is a Risk Factor for Recurrence of HCC after Hepatectomy

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Non-hypervascular Hypointense Nodules Detected by Gd-EOB-DTPA-enhanced MRI is a Risk Factor for Recurrence of HCC after Hepatectomy

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Key words: heparocellular carcinoma, Gd-EOB-DTPA-enhanced MRI, non-hypervascular hypointense nodule, hepatobiliary phase, hepatectomy, recurrence

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List of Abbreviations

Gd-EOB-DTPA, gadolinium-ethoxybenzyl-diethylenetriamine pentaacetic acid; MRI, magnetic resonance imaging; HCC, hepatocellular carcinoma; US, ultrasonography; MDCT, multidetector-row computed tomography; TFE, turbo field echo; CTHA, computed tomography during hepatic arteriography.

Conflict of interest

There is no conflict of interest on this study.

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Abstract

Background & Aims: The gadolinium-ethoxybenzyl-diethylenetriamine pentaacetic acid (Gd-EOB-DTPA)-enhanced magnetic resonance imaging (MRI) often depicts non-hypervascular hypointense hepatic nodules during the hepatobiliary phase in patients with hepatocellular carcinoma (HCC). It is not unclear whether the presence of these nodules is associated with HCC recurrence after hepatectomy. We conducted prospective observational study to investigate the impact of the presence of non-hypervascular hypointense hepatic nodules on the hepatobiliary phase of Gd-EOB-DTPA-enhanced MRI on the recurrence of HCC after hepatectomy. *Methods*: A total of 77 patients who underwent hepatectomy for primary, non-recurrent, hypervascular HCC were prospectively followed up after hepatectomy. Post-operative recurrence rates were compared according to the presence of non-hypervascular hypointense nodules on preoperative Gd-EOB-DTPA-enhanced MRI. *Results*: Recurrence rates after hepatectomy were higher in patients with non-hypervascular hypointense nodules (risk ratio 1.9396 [1.3615-2.7222]) and the presence of non-hypervascular hypointense nodules was an

independent factor associated with postoperative recurrence (risk ratio 2.1767 [1.5089-3.1105]) along with HCC differentiation and portal vein invasion. Whereas no differences were found in the rate of intrahepatic metastasis recurrence based on the preoperative presence of non-hypervascular hypointense hepatic nodules, the rate of multicentric recurrence was significantly higher in patients with preoperative non-hypervascular hypointense hepatic nodules. *Conclusions*: Patients with preoperative non-hypervascular hypointense hepatic nodules detected during the hepatobiliary phase of Gd-EOB-DTPA-enhanced MRI are at higher risk of HCC recurrence after hepatectomy, mainly due to multicentric recurrence.

Introduction

Hepatocellular carcinoma (HCC) is the sixth most common cancer worldwide and the third most common cause of cancer-related death [1,2]. In Japan, HCC is the third and fifth most common causes of death from cancer in men and women, respectively [3]. Tremendous efforts have been made to improve various imaging techniques including ultrasonography (US), multidetector-row computed tomography (MDCT) [4,5], and magnetic resonance imaging (MRI) [6] for the detection of hepatic nodules, including small early-stage HCC tumors in high-risk patients under surveillance.

The liver-specific contrast agent gadolinium-ethoxybenzyl-diethylenetriamine pentaacetic acid (Gd-EOB-DTPA), which is taken up by hepatocytes, has been in clinical use for dynamic MRI studies since February 2008 in Japan. Gd-EOB-DTPA provides both dynamic and liver-specific hepatobiliary MR images [7-10]. In the hepatobiliary phase, hepatic lesions that lack normally functioning hepatocytes are imaged as an absence of hepatocyte-selective enhancement as compared with normal parenchyma

[10,11]. The use of Gd-EOB-DTPA-enhanced MRI increases detection of concurrent non-hypervascular hepatic nodules as hypointense nodules during the hepatobiliary phase in patients with HCC. It is controversial whether the presences of these non-hypervascular hepatic nodules detected in patients with typical hypervascular HCC lesions have an impact on the recurrence of HCC after treatment.

In the present study, we attempted to evaluate the impact of concurrent non-hypervascular hepatic nodules detected as hypointense nodules during the hepatobiliary phase of Gd-EOB-DTPA-enhanced MRI on postoperative recurrence in patients who underwent hepatectomy with curative intent for HCC.

Methods

Patients, Treatment and Follow-up

This prospective study was conducted after the approval by the hospital institutional review board and carried out in compliance with the Helsinki Declaration. Patient enrollment was carried out between February 2008 and December 2011. A total of 102 patients underwent hepatectomy

as a curative treatment for primary, non-recurrent HCC during the study period at Ogaki Municipal Hospital. Gd-EOB-DTPA-enhanced MRI could not be performed prior to hepatectomy in 25 patients, including 11 patients who had been referred from another institution only for hepatectomy and 14 patients who could not receive examination due to metal implants, history of allergy to contrast medium, tattoos, or claustrophobia. The remaining 77 patients who underwent Gd-EOB-DTPA-enhanced MRI within 2 weeks prior to hepatectomy were studied. The initial diagnosis of HCC before treatment was based on appropriate imaging characteristics according to criteria of the guidelines by the American Association for the Study of Liver Diseases (12,13). The final diagnosis of HCC was confirmed by pathologic diagnosis of resected specimens.

Decisions regarding individual treatments were based on Japanese treatment guidelines for HCC [14]. In all patients, HCC tumors were resected with ample margins; enucleation of tumors without margins was not performed.

After hepatectomy, all patients were prospectively followed for 8.5 months to 55.4 months (median follow-up, 34.1 months) until the end of

September 2012 at our institution, with US and either MDCT or MRI every 3 to 6 months. Regular monitoring of serum tumor markers (alpha-fetoprotein, *lens culinaris* agglutinin-reactive alpha-fetoprotein, and des-gamma-carboxy prothrombin) was performed every 3 months. When an elevation in tumor markers was detected, additional imaging (usually MDCT or MRI) was performed to check for HCC recurrence. Recurrence was diagnosed by pathologic examination of resected specimens when patients underwent re-hepatectomy. In the remaining patients, HCC was diagnosed by appropriate imaging characteristics according to criteria of the guidelines by the American Association for the Study of Liver Diseases [12,13]. Recurrent HCC was categorized into two groups prior to the study as intrahepatic metastasis recurrence or multicentric recurrence according to a previous study [15,16]. Intrahepatic metastasis recurrence was defined as recurrent tumors consisting of moderately or poorly differentiated HCC with the same or lower degree of differentiation than the primary tumors on pathologic examination or hypervascular tumor without non-hypervascular peripheral regions in a same hepatic segment on imaging examination. Multicentric recurrence was defined according to previously reported

criteria with some modifications [17,18] as follows: (i) the recurrent tumor consists of well-differentiated HCC occurring in a different hepatic segment, than moderately or poorly differentiated pre-existing HCCs; (ii) both the primary and recurrent tumors are well-differentiated HCCs; and (iii) the recurrent tumor contained regions of dysplastic nodules in peripheral areas based on pathologic examination or contained non-hypervascular regions in peripheral areas of hypervascular tumor on imaging examination.

Preoperative Imaging Examinations of Liver Nodules by Gadolinium-Ethoxybenzyl-Diethylenetriamine Pentaacetic Acid-enhanced MRI and Confirmation of Non-hypervascular Hypointense Hepatic Nodules

All patients underwent Gd-EOB-DTPA-enhanced MRI within 2weeks of hepatectomy. MRI was performed using a 1.5-T whole-body MRI system (Intera Achieva 1.5T NOVA; Philips Medical Systems) with a phased-array body coil as the receiver coil. T1-weighted sequences were acquired with the following parameters: T1-weighted turbo field echo (TFE) in-phase and opposed-phase transverse (TE, opposed-phase 2.3,

in-phase 4.6; flip angle, 12°; matrix size, 256 X 512; scan percentage, 70) with 3.5-mm section thickness, a 0-mm intersection gap, and a 38 cm field of view. After intravenous injection of Gd-EOB-DTPA (Primovist; Bayer Schering Pharma, Osaka, Japan), T1-weighted transverse gradient-echo sequences (high-resolution isotropic volume examination [THRIVE] with spectral presaturation with inversion recovery [SPIR], 4/1.8; flip angle, 12°; matrix size, 256 X 512; scan percentage, 78.54) with 3.5-mm section thickness, a 0-mm intersection gap, and a 38 cm field of view were obtained. Gd-EOB-DTPA was administered intravenously as a bolus at a rate of 2 mL/sec (0.1 mL/kg, maximum dose of 10 mL) through an intravenous cubital line (20–22 gauge), which was flushed with 20 mL of saline using a power injector (Sonic Shot; Nemoto Kyorindo, Tokyo, Japan). The timing for dynamic arterial phase imaging was determined using MR fluoroscopic bolus detection of the descending aorta (Bolus Trak; Philips Medical Systems). The mean delay times (time interval between the start of bolus administration and the start of image acquisition) for the arterial, portal, and delayed phases were 20, 60, and 180 seconds, respectively. Immediately after the dynamic study, a respiration-triggered

single-shot T2-weighted sequence with a reduction factor of 4 (1,200/100; flip angle, 90°; matrix size, 400 X 512) with 7-mm section thickness, a 1-mm intersection gap, and a 38 cm field of view was obtained with SPIR. The 20-min-delayed hepatobiliary phase [19] was obtained with a T1-weighted TFE sequence (TR/TE, 4/1.8; flip angle, 12°; matrix size, 256 X 512) with 3.5-mm section thickness, a 0-mm intersection gap, and a 38 cm field of view. All the sequences were obtained with parallel imaging (SENSE). Hypointense hepatic nodules during the hepatobiliary phase of Gd-EOB-DTPA-enhanced MRI were nodules greater than 3.5 mm with low-intensity.

Prior to hepatectomy, all patients underwent CT during hepatic arteriography (CTHA) [20-22] to evaluate the intranodular blood supply, and to confirm the hypervascularity of HCC lesions and the lack of hypervascularity of non-hypervascular hepatic nodules.

All imaging findings were evaluated by radiologist (Y.S.) and by hepatologist (H.T.) independently, being blind to the clinical data. When the imaging assessment was discordant between two reviewers, consensus was made through the discussion.

Statistical Analyses

Differences in percentages between groups were analyzed using the chi-square test. Differences in mean quantitative values were analyzed by the Mann-Whitney U test. The date of hepatectomy was defined as time zero for calculations of recurrence rates. In the analysis of the overall recurrence rate, patients in whom HCC did not recur were censored, and those in whom HCC recurred were not censored. In the analysis of the intrahepatic metastasis recurrence rate, patients in whom HCC did not recur or patients with multicentric HCC recurrence were censored, and those in whom HCC recurred as intrahepatic metastases were not censored. In the analysis of the multicentric recurrence rate, patients in whom HCC did not recur were censored and patients with multicentric HCC recurrence were not censored, while those in whom HCC recurred as intrahepatic metastases were excluded from the analysis. The Kaplan-Meier method [23] was used to calculate recurrence rates, and the log-rank test [24] was used to analyze differences.

The Cox proportional hazards model [25] was used for univariate

and multivariate analyses of factors related to recurrence. Variables analyzed included patient age and sex, Child-Pugh class (A/B), tumor size, number of tumors (single/multiple), differentiation of resected HCC (well-differentiated/moderately or poorly differentiated), growth pattern of resected HCC (expansive growth/infiltrative growth), portal vein invasion of resected HCC (absent/present), and presence of non-hypervascular hypointense nodules on the hepatobiliary phase of Gd-EOB-DTPA-enhanced MRI (absent/present). Data analyses were performed using JMP statistical software, version 6.0 (Macintosh version; SAS Institute, Cary, NC). All p values were derived from 2-tailed tests, with $p<0.05$ accepted as statistically significant.

Results

Patients Characteristics and Imaging Findings

Patients consisted of 56 males and 21 females with a mean age of 68.3 ± 7.6 years (range, 46-82 years). A total of 40 non-hypervascular hypointense hepatic nodules were identified during the hepatobiliary phase of Gd-EOB-DTPA-enhanced MRI in 28 of 77 patients (36.4%). The size of

non-hypervascular hypointense nodules was 1.17 ± 0.38 cm (range, 0.4-2.1 cm). Two of 40 non-hypervascular hypointense hepatic nodules (5.0%) were identified by T2-weighted sequence as high-intensity nodules. Nodules were not identified both by T1- and T2-weighted sequences in case of the other 38 non-hypervascular hypointense hepatic nodules. Two nodules were located in segment II of the liver, 7 in III, 1 in IV, 10 in V, 6 in VI, 4 in VII, and 10 in VIII, respectively. Among 28 patients with non-htpervascular hypointense nodules, 19 patients had one non-htpervascular hypointense nodule, 6 patients had 2 nodules, and the remaining 3 patients had 3 nodules. Non-hypervascular hypointense nodules were resected along with HCC lesions during hepatectomy in 10 patients because they were included within the intended area of resection. Therefore, we categorized these 10 patients and the 49 patients in whom non-hypervascular hypointense nodules were not detected by preoperative Gd-EOB-DTPA-enhanced MRI as the hypointense nodule (-) group and the remaining 18 patients who had residual hypointense nodules after hepatectomy as the hypointense nodule (+) group. Of 13 hypointense nodules in 10 patients resected along with HCC at hepatectomy, 3 nodules

were diagnosed as well-differentiated HCC and the remaining 10 nodules were diagnosed as dysplastic nodules on pathologic examination.

Table 1 compares the preoperative characteristics of the study patients. No differences were found in patient age and sex, etiology, liver function, and tumor progression as evaluated by preoperative imaging examinations and by post-operative pathologic examinations. Multiple HCC nodules were resected in 6 patients (10.2%) of hypointense nodule (-) group and 3 patients (16.7%) of hypointense nodule (+) group, without the difference in proportions. No difference was observed in the length of follow-up period.

Recurrence Rate after Hepatectomy According to the Presence of Non-hypervascular Hypointense NodulesDetected during Preoperative Gadolinium-Ethoxybenzyl-Diethylenetriamine Pentaacetic Acid-enhanced MRI

We determined the recurrence rate in patients after hepatectomy with curative intent based on the presence of non-hypervascular hypointense hepatic nodules identified during the hepatobiliary phase of

Gd-EOB-DTPA-enhanced MRI (Figure 1). The recurrence rate was significantly higher in patients in the hypointense nodule (+) group than the hypointense nodule (-) group (p<0.0001). In the univariate analysis, HCC differentiation and portal vein invasion were identified as factors associated with the rate of recurrence after hepatectomy along with preoperative non-hypervascular hypointense nodules by Gd-EOB-DTPA-enhanced MRI. In the multivariate analysis, these factors were confirmed to be independently associated with the rate of recurrence (Table 2). Among 18 patients with hypointense nodule (+) group, recurrence was observed in 7 of 11 patients with one non-hypervascular hypointense nodule, whereas recurrence was observed in all 7 patients with multiple non-hypovascular hypointense nodules.

Patterns of Recurrence after Hepatectomy According to the Presence of Non-hypervascular Hypointense Nodules Detected during Preoperative Gadolinium-Ethoxybenzyl-Diethylenetriamine Pentaacetic Acid-enhanced MRI

In 30 patients with HCC recurrence after hepatectomy, 16 patients

(53.3%) had intrahepatic metastasis recurrence and 14 patients (46.7%) had multicentric recurrence. There was no difference in the rate of intrahepatic metastasis recurrence between patients in the hypointense nodule (+) group and the hypointense nodule (-) group (p=0.8852). In contrast, patients in the hypointense nodule (+) group had a significantly higher rate of multicentric recurrence than patients in the hypointense nodule (-) group (p<0.0001, Figure 2). Univariate and multivariate analyses revealed that portal vein invasion was independently associated with intrahepatic metastasis recurrence but not preoperative non-hypervascular hypointense nodules detected by Gd-EOB-DTPA-enhanced MRI (Table 3). The presence of preoperative non-hypervascular hypointense nodules detected by Gd-EOB-DTPA-enhanced MRI was the only factor associated with multicentric recurrence in univariate and multivariate analyses (Table 4). Among 8 HCCs that recurred multicentrically in the hypointense nodule (+) group, 6 nodules (75.0%) had existed as non-hypervascular hypointense hepatic nodules on Gd-EOB-DTPA-enhanced MRI before hepatectomy and progressed to hypervascular HCC tumors (Figure 3), while the other 2 nodules (25.0%) newly occurred as multicentric recurrence after