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Original Research

Optimal Scanning Protocol of Arterial Dominant Phase for Hypervascular Hepatocellular Carcinoma with Gadolinium-Ethoxybenzyl-Diethylenetriamine Pentaacetic Acid-Enhanced MR

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Purpose: To investigate optimal delay time of hepatic arterial phase in Gadoxetate-enhanced MR for detecting hypervascular hepatocellular carcinoma (HCC).

Materials and Methods: Forty-five patients with 85 hypervascular HCCs and 9 patients with 16 hypervascular HCCs underwent Gadoxetate- and Gd-DTPA-enhanced MR at 1.5 Tesla (T) system, respectively. All HCCs were analyzed 10–38 s after injection using a time-resolved dynamic MR sequence with keyhole data sampling. Seven sequential phase images (1 phase = 4 s) were obtained during a single breath hold of 28 s. Time–intensity curves of the abdominal aorta, liver parenchyma, and HCC were obtained, then aortic contrast arrival time, time of peak HCC enhancement, duration time of HCC and aortic enhancement, and time delay from aortic contrast arrival to peak enhancement of HCC were measured.

Results: Aortic contrast arrival time was 15.1 ± 2.9 s, time of peak HCC enhancement 29.9 ± 4.6 s, duration time of HCC enhancement 17.4 ± 6.4 s postinjection of Gadoxetate. Duration of aortic enhancement (23.6 ± 3.5 s) of Gadoxetate-enhanced MR was significantly less than that of Gd-DTPA-enhanced MR (26.3 ± 2.8 s) ($P < 0.0059$).

Conclusion: Peak enhancement time of HCC on Gadoxetate-enhanced MR imaging occurred at 14.6 ± 4.6 s after aortic contrast arrival.

Key Words: MRI; Gadoxetate; hepatocellular carcinoma; bolus tracking; Gd-DTPA

J. Magn. Reson. Imaging 2011;33:864–872.

IN MR EXAMINATION of patients with chronic liver disorders, dynamic MR imaging using conventional extracellular contrast agent, such as gadolinium diethylenetriamine pentaacetic acid (Gd-DTPA), is essential for detection and characterization of focal liver lesions because it enables hemodynamic analysis of the lesion (1–3). Enhanced MR imaging with tissue-specific contrast agent, such as superparamagnetic iron oxide, is also useful for detection and characterization of focal liver lesions because it provides functional evaluation of the reticuloendothelial system of the lesion (4,5). Gadoxetate (Gadolinium ethoxybenzyl diethylenetriamine; Gd-EOB-DTPA), which enables combined dynamic and hepatocyte-specific imaging in the one examination, has recently become clinically available (6,7). Gadoxetate behaves as an extracellular contrast agent in the early phase after intravenous injection and as a hepatocyte-specific agent in the hepatobiliary phase (8).

In detecting hypervascular hepatocellular carcinoma (HCC), the hepatic arterial phase (HAP) in dynamic imaging is important because moderately–poorly differentiated (and a part of well-differentiated) HCCs tend to occur as hypervascular nodules (9,10). Generally, liver MR imaging of HCC requires bolus injection and a bolus-tracking technique (11), although test injection technique and fixed arterial phase are also used. The total amount and concentration of Gadoxetate (0.025 mmol/kg) used clinically is smaller than that of Gadolinium-diethylenetriamine pentaacetic acid (Gd-DTPA) (0.1 mmol/kg); however, to the best of our knowledge, optimal delay time of HAP for Gadoxetate has not been reported, and thus requires investigation.

The purpose of our study was to investigate the enhancement patterns in Gadoxetate-enhanced MR

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Received May 28, 2010; Accepted December 17, 2010.
DOI 10.1002/jmri.22487
View this article online at wileyonlinelibrary.com.

imaging in patients with hypervascular HCC and to determine the optimal scan delay time for HAP.

MATERIALS AND METHODS

Patient Population

This study followed the principles of the Declaration of Helsinki. Informed consent was obtained from all patients who underwent contrast-enhanced MR imaging with Gd-DTPA or Gadoxetate between September 2007 and October 2009 (September 2007 to January 2008; Gd-DTPA, February 2008 to October 2009; Gadoxetate). Institutional Review Board approval was obtained, although MR imaging in this study was acquired during routine clinical examination of liver tumors.

Definition of Hypervascular HCC

All examinations (both Gadoxetate and Gd-DTPA enhanced MR imaging) were reviewed by two radiologists with more than 10 years experience in liver MR imaging. Any interpretation discrepancies were resolved by consensus with the participation of a third radiologist. The reviewers were told that the patients had chronic hepatitis or cirrhosis, and treatment history was available. The 70-s, 5-min, and 20-min post-gadoxetate injections were performed, whereas, 70 s for the portal-venous phase and 5 min for the equilibrium phase were obtained in the patients with Gd-DTPA. Each arterial phase was evaluated for the presence of early enhancing hypervascular HCCs on dynamic MR imaging. Hypervascularity is defined as higher intense than surrounding liver parenchyma at the arterial phase. On Gadoxetate enhanced MR imaging, lower uptake of Gadoxetate than surrounding liver parenchyma at the hepatobiliary phase (20 min after injection) is regarded as HCC. Washout at the equilibrium phase (5 min after injection) is defined as HCC on Gd-DTPA enhanced MR imaging. On Gadoxetate enhanced MR imaging, less than 2-cm HCCs were homogeneously enhanced at arterial phases. Twenty-five of 85 HCCs showed more than 2 cm in size, 10 of 25 HCCs (>2 cm) had homogeneously arterial enhancement, whereas 15 of 25 HCCs had heterogeneous arterial enhancement. Thus, the 15 HCCs with heterogeneous arterial enhancement were measured at the intratumoral hyper-enhancing lesions. All of 16 HCCs were homogeneously enhanced at arterial phase in Gd-DTPA enhanced MR imaging.

Gadoxetate-Enhanced MRI

Of the 52 consecutive patients who underwent Gadoxetate-enhanced MR imaging for detection of liver tumor, 7 were excluded from the study because of marked artifacts on the arterial phase of dynamic MR imaging of the liver due to insufficient breath holding. A final total of 45 patients (33 men, 12 women; mean age, 68 years; age range, 45–82 years) with 85 untreated hypervascular HCCs in liver cirrhosis or chronic hepatitis were included in the study. Eighty-five HCCs were diagnosed using computed tomography (CT) and/or contrast-enhanced ultrasonography (US), based on the radiologic criteria of hypervascularity of HCC, contrast washout at the portal and/or equilibrium phase, response to transarterial chemoembolization (TACE), and interval progression in size. Follow-up CT or US imaging data were used to confirm the diagnosis of HCC if necessary. T2-weighted images were used to differentiate HCC from liver hemangioma. In six cases, a diagnosis of HCC was established histologically by percutaneous liver biopsy. Mean tumor size of hypervascular HCC was 17.2 ± 11.0 mm (range; 6–57 mm).

The treatment histories of our patients were as follows: TACE in 5 patients, radiofrequency ablation (RFA) in 4 patients, both TACE and RFA in 21 patients, hepatic intra-arterial chemotherapy in 1 patient, and hepatic resection in 3 patients; 11 patients had no treatment. These procedures were performed more than 2 months after treatment. As stated above, only untreated HCCs were evaluated (Table 1). TACE was performed super-selectively (and RFA performed focally), and these treatments were performed more than 2 months before the imaging obtained in the present study. The patient population consisted of Child-Pugh grade A in 32 patients and Child-Pugh grade B in 13 patients; no patients were Child-Pugh grade C. Causes of liver dysfunction were hepatitis B (n = 6), hepatitis C (n = 29), both hepatitis B and C (n = 2), neither hepatitis B nor C (n = 8). Mean patient body weight was 63 ± 10.4 kg (range; 44–86 kg).

Gd-DTPA Enhanced MRI

Of the 23 consecutive patients who underwent Gd-DTPA enhanced MR imaging for detection of liver tumor, 4 were excluded from the study because of marked artifacts on the arterial phase of dynamic liver MR imaging due to insufficient breath holding. We studied 19 patients (13 men, 6 women; mean age, 70 years; age range, 58–76 years) with suspected liver tumor in liver cirrhosis or chronic hepatitis who underwent dynamic MR imaging with Gd-DTPA. Nine of 19 patients had 16 hypervascular HCCs. Mean tumor size was 17.3 ± 5.5 mm (12 ± 32 mm).

Patient population, who underwent Gd-DTPA enhanced MRI, consisted of Child-Pugh grade A in 13 patients, Child-Pugh grade B in 4 patients, and 2 patients were Child-Pugh grade C. Causes of liver dysfunction were hepatitis B (n = 4), hepatitis C (n = 13), neither hepatitis B nor C (n = 2). Mean patient body weight was 56 ± 12.5 kg (range; 35–80 kg). Nine of 19 patients had hypervascular HCC. The treatment histories of nine patients with HCC were as follows: TACE in 1 patient, RFA in 1 patient, both TACE and RFA in 4 patients, and hepatic resection in 2 patients; 1 patient had no treatment. These procedures were performed more than 3 months after treatment. As stated above, only untreated HCCs were evaluated in our study (Table 1). TACE was performed super-selectively (and RFA performed focally), and these

Table 1
Data of Patient Population in Our Study

Parameters	Gadoxetate-enhanced MRI	Gd-DTPA-enhanced MRI
No. of patients	45	19
Sex	Male (33 pts) female (12 pts)	Male (13 pts) female (6 pts)
Age	Mean 68 (range 45 - 82) years	Mean 70 (range 58 - 76) years
Weight	63 ± 10.4 (range 44 - 86) kg	56 ± 12.5 (range 35 - 80) kg
Treatment history	TACE (5 pts), RFA (4 pts), TACE and RFA (21 pts) Hepatic intra-arterial chemotherapy (1 pt), Hepatic resection (3 pts), no treatment history (11 pts)	TACE (1 pt), RFA (1 pt), TACE and RFA (4 pts) Hepatic resection (2 pts), no treatment history (1 pt)
Hepatitis	B (6 pts), C (29 pts), B and C (2 pts), neither B nor C (8 pts)	B (4 pts), C (13 pts), neither B nor C (2 pts)
Elevation of tumor maker	PIVKA-II (27 pts), AFP (27 pts) PIVKA-II or AFP (38 pts)	PIVKA-II (6 pts), AFP (7 pts) PIVKA-II or AFP (8 pts)
Child–Pugh grade	A (32 pts), B (13 pts), C (none)	A (13 pts), B (4 pts), C (2 pts)
Differentiation (number)	Poor (1), moderately (5)	Poor (1), moderately (3)
HCC size	Mean 17.2 ± 11.0 (range 6 - 57) mm	Mean 17.3 ± 5.5 (range 12 - 32) mm
HCC location (number)	S1 (1), S2 (12), S3 (5), S4 (15), S5 (12), S6 (14), S7 (8), S8 (18)	S1 (1), S2 (1), S3 (1), S4 (1), S5 (1), S6 (4), S7 (2), S8 (5)

*TACE = transarterial chemo-embolization; RFA = radiofrequency ablation; PIVKA-II = protein induced by Vitamin K absence or antagonists-II; AFP = alpha-fetoprotein; HCC = hepatocellular carcinoma; pts = patients.

treatments were performed more than 3 months before the imaging obtained in the present study.

MR Imaging Technique

All patients underwent time-resolved dynamic MR imaging (both Gadoxetate and Gd-DTPA) of the entire liver using a 1.5 Tesla (T) clinical MR scanner (Gyroscan Intera Nova; Philips Medical Systems, Best, the Netherlands) equipped with a high-performance gradient system; a commercially available four-element phased-array body coil with improved signal detection was used for parallel imaging. For dynamic MR imaging of HAP, we used a 3-dimensional Fourier transformation (3DFT) gradient echo sequence with keyhole data sampling (four-dimensional high-resolution isotropic volume examination [4D THRIVE] sequence with fat saturation) (12,13); we obtained parallel imaging with Sensitivity Encoding (SENSE) and an MR scanner equipped with a gradient system (maximal gradient amplitude = 30 mT, slew rate = 150 T/m/s).

A full reference 3D data set without contrast was obtained initially, during a single breath hold. The patients were then administered intravenous injection of 0.025 mmol/mL/kg of Gadoxetate by power injector via the antecubital vein at a rate of 2 mL/s, followed by normal saline at a rate of 2 mL/s (14). Dynamic MR imaging with 0.1 mmol/mL/kg of Gd-DTPA was performed using the same sequence, parameters. Total injected volume (Gadoxetate + normal saline or Gd-DTPA + normal saline) was 40 mL in our protocol.

Imaging protocol of our study was as follows: Dynamic MR imaging was then performed at seven sequential phases (4 s/phase) from 10 to 38 s after initiation of the injection of Gadoxetate and Gd-DTPA, during a single breathhold. The center time of each imaging phase was used for numerical analysis. Therefore, the first, second, third, fourth, fifth, sixth, and seventh phases were obtained at 12, 16, 20, 24, 28, 32, and 36 s, respectively, after initiation of the injection of Gadoxetate and Gd-DTPA. In addition, for the diagnosis of HCC, portal venous phase and hepatobiliary phase imaging were obtained at approximately 70 s and 20 min, respectively, after initiation of the injection of Gadoxetate. For Gd-DTPA enhanced MRI, portal venous phase and equilibrium phase were used at approximately 70 s and 180 s. The acquisition parameters of 4D THRIVE were as follows: TR = 3.9 ms, TE = 1.9 ms, flip angle = 15°, matrix size = 256 × 256, slice thickness = 2.5 mm, field of view = 340 mm, SENSE factor = 2. Eighty slices were obtained at each arterial phase.

In the keyhole technique, a single acquisition of peripheral *k*-space corresponding to the reference scan is acquired, after which a predefined portion of the central region of ky–kz space is repeatedly acquired. In reconstruction, the central, dynamic part of *k*-space is combined with the static periphery of *k*-space that was acquired during the reference scan. The size of the central region can be configured: the smaller the size, the higher the temporal resolution. By adopting a 40% keyhole rate, dynamic MR images were acquired every 4 s.

Image Interpretation

Analysis by Time–Intensity Curve

The arterial enhancement patterns of HCC were evaluated using time–intensity curve (TIC) analysis. Dynamic MR imaging (both Gadoxetate and Gd-DTPA) sets were sent to a clinical workstation (ViewForum; Philips Medical Systems, Tokyo, Japan). Using a multi-window frame setting on this system, a region of interest (ROI) was placed on the abdominal aorta at the level of celiac artery. After HCC and liver parenchymal signal were measured at the same slice, the

Table 2
Comparison Between Gadoxetate and Gd-DTPA-Enhanced MR for Aortic and HCC Enhancement Analyzed by Time-Intensity Curves

	Gadoxetate	Gd-DTPA	*P* value
1) Aortic contrast arrival time	15.1 ± 2.9 s	17.6 ± 3.0 s	<0.00013
2) Time of peak aortic enhancement	21.0 ± 4.9 s	22.4 ± 5.6 s	NS
3) Duration time of aortic enhancement	23.6 ± 3.5 s	26.3 ± 2.8 s	<0.0059
4) Aortic enhancement (Aorta / muscle ratio)	5.6 ± 1.7	8.1 ± 1.7	<0.000013
5) Beginning time of HCC enhancement	20.8 ± 6.1 s	22.5 ± 4.1 s	NS
6) Duration time of HCC enhancement	17.4 ± 6.4 s	17.5 ± 4.1 s	NS
7) Time of peak HCC enhancement	29.9 ± 4.6 s	31.8 ± 3.7 s	NS
8) Time delay from aortic contrast arrival to peak enhancement of HCC	14.6 ± 4.6 s	14.5 ± 3.8 s	NS

*HCC = hepatocellular carcinoma; NS = not significant; Gadoxetate = Gadolinium ethoxybenzyl diethylenetriamine (Gd-EOB-DTPA); Gd-DTPA = Gadolinium-diethylenetriamine pentaacetic acid; aorta/muscle ratio; signal of aorta/signal of latissimus dorsi muscle ratio.

enhancement values were calculated using the ratio of the signal of HCC and liver parenchyma to the signal of latissimus dorsi muscle. TIC was created using commercially available Excel software (Microsoft Excel 2007). Thus, hemodynamic change of HCC through seven arterial phases was quantitatively analyzed. We calculated the following parameters by evaluating all phases of the dynamic series: (i) Arrival time of contrast agent at the abdominal aorta (aortic contrast arrival time), (ii) Time of peak aortic enhancement, (iii) Duration time of aortic enhancement (Aorta/muscle ratio 1.5), (iv) Aortic enhancement (Aorta/muscle ratio), (v) Beginning time of HCC enhancement (up 10% of HCC enhancement, in comparison to surrounding liver parenchyma), (vi) Duration time of HCC enhancement, (vii) Time of peak HCC enhancement, and (viii) Time delay from aortic contrast arrival to peak enhancement of HCC. The center time of each imaging phase was used for numerical analysis. Aortic enhancement was defined as aorta/muscle ratio. Duration of aortic enhancement was defined as the time with aorta/muscle ratio greater than 1.5 for both contrast agents.

Statistical Analysis

Comparison between Gadoxetate and Gd-DTPA enhanced MRI was made in all parameters analyzed by TIC curve. Mann-Whitney's U test was used for statistical analysis; A *P* value <0.05 was considered significant.

RESULTS

Analysis by Time–Intensity Curve

Aortic Enhancement

The eight analyzed parameters are listed in Table 2. Aortic contrast arrival time and duration time of aortic enhancement of Gadoxetate were significantly shorter than those of Gd-DTPA. Aortic enhancement of Gadoxetate was lower than that of Gd-DTPA.

Aortic contrast arrival time was variable. Eighteen of 45 patients with Gadoxetate-enhanced MR and 3 of 9 patients with Gd-DTPA had already contrast arrival to the aorta at first phase. Others (27 of 45 patients with Gadoxetate-enhanced MR and 6 of 9 patients with Gd-DTPA) showed dynamic inflow of the aorta since second phase.

HCC Enhancement

One case of typical hypervascular HCC is shown in Figures 1 and 2. Time of peak HCC enhancement of Gadoxetate showed a relatively wide range, from the third to seventh phases (29.9 ± 4.6 s; Table 2). 33 of 85 HCCs showed at 24 s (4 of 7 phases) in beginning time of HCC (Fig. 3), and 14, 29, 35, and 7 of 85 HCCs showed enhancement duration of 8, 12, 16, and 20 s in duration time of HCC, respectively. Twenty-nine of 85 HCCs showed peak enhancement of Gadoxetate at the sixth phase (32 s postinjection; Fig. 4).

In 18 HCCs, the enhancement continued to increase at the seventh phase (36 s) and peak HCC enhancement was expected to occur after the seventh phase in Gadoxetate enhanced MRI. Furthermore, in 41 HCCs, persistent enhancement remained evident at the seventh phase (Fig. 5).

In 8 of 16 HCCs in Gd-DTPA enhanced MRI, the HCC enhancement continued to increase at the seventh phase. And peak HCC enhancement was seen at forth phase in Gd-DTPA enhanced MRI.

Of interest, HCC enhancement was not affected according to different volume of contrast agent (Gadoxetate is half the volume of Gd-DTPA).

The time delay from aortic contrast arrival to peak enhancement of HCC was 14.6 ± 4.6 s (Table 2), which enables optimal HCC enhancement to be obtained when using bolus tracking in Gadoxetate-enhanced MRI.

Other parameters, such as aortic contrast arrival time, time of peak aortic enhancement, beginning time of HCC enhancement, HCC enhancement in the arterial phase, time of peak HCC enhancement, were shown as no significant difference between Gadoxetate and Gd-DTPA in Table 2.

Time intensity curve of HCC/muscle ratio was obtained (Fig. 6), and 12 to 24 s from aortic contrast arrival showed higher enhancement of HCC in Gadoxetate-enhanced MRI.

Twenty-six of 45 patients (53 of 85 HCCs) had TACE, and 19 of 45 patients (32 of 85 HCCs) did not have TACE. Time delay from aortic contrast arrival to peak enhancement of HCC with TACE (14.3 ± 4.9 s) is as same as that of HCC without TACE (15.0 ± 3.9 s) (Table 3). Other 5 parameters, such as aortic contrast arrival time, time of peak aortic enhancement, beginning time of HCC enhancement, HCC enhancement in

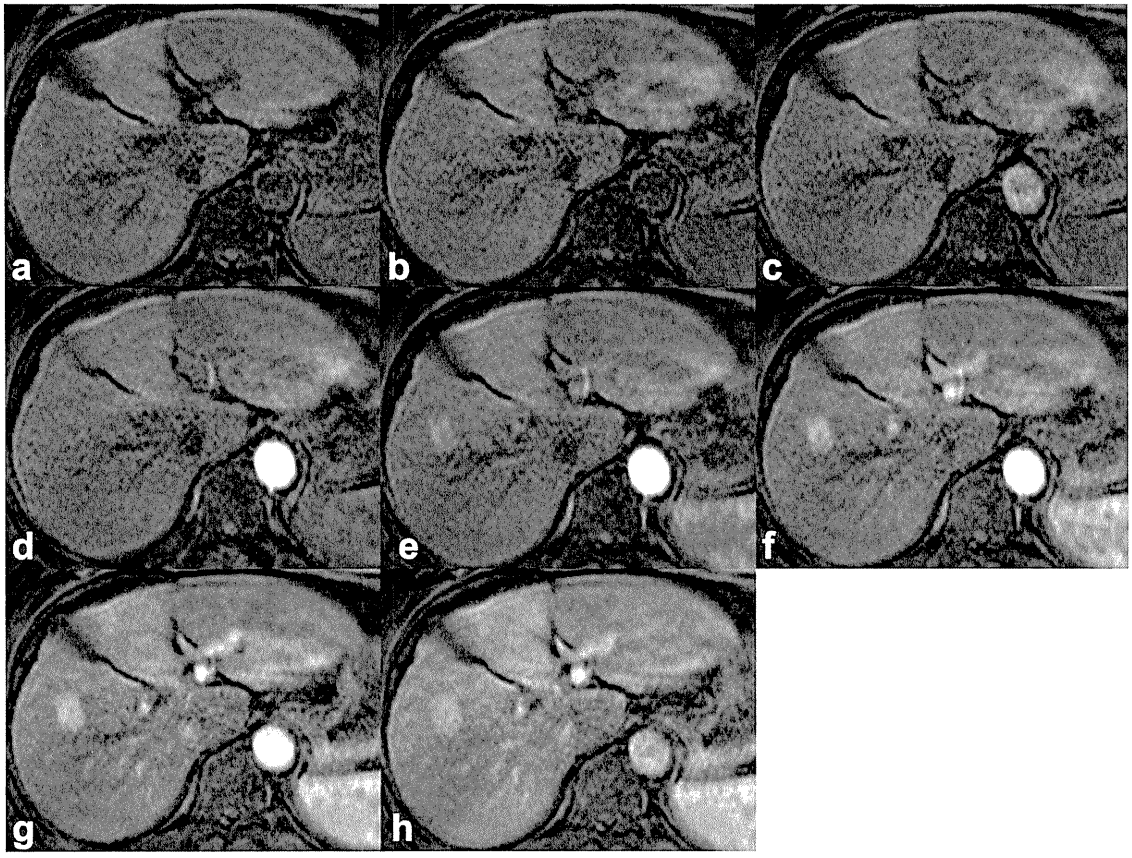

Figure 1. A 68-year-old man with a hypervascular HCC. First- to seventh-phase images obtained during dynamic MR imaging with Gadoxetate (gadolinium ethoxybenzyl diethylenetriaminepentaacetic acid; Gd-EOB-DTPA). Aortic enhancement is not seen in the first (**a**) or second (**b**) phases. Aortic enhancement is seen in the third phase (**c**). Aortic peak enhancement is seen in the fourth phase (**d**). HCC enhancement occurs in the fifth phase (**e**). Peak HCC enhancement appears in the sixth phase (**f**). HCC enhancement continues in the seventh phase (**g**), and liver parenchyma shows a gradual increase in enhancement; thus, this phase shows decreased liver-to-HCC contrast (**h**). In this patient, the duration of HCC enhancement is from the fifth to seventh phases. Aortic enhancement shows a rapid decrease, because of the small amount of contrast agent. Artifact caused by stomach motion is apparent in the parenchyma of the hepatic left lobe as a high-intensity area in the second (b) and third phases (c).

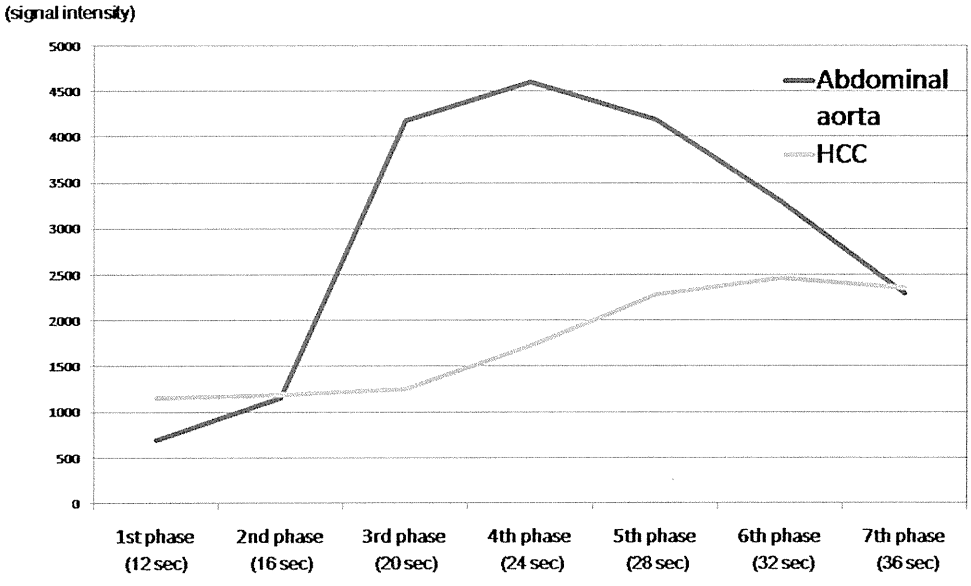

Figure 2. Time–intensity curve in the same patient as that shown in Figure 1. Aortic contrast arrival occurs at 16 s (second phase), while aortic peak enhancement occurs at 24 s (fourth phase). HCC enhancement occurs at 24 s, and peak HCC enhancement time is 32 s.

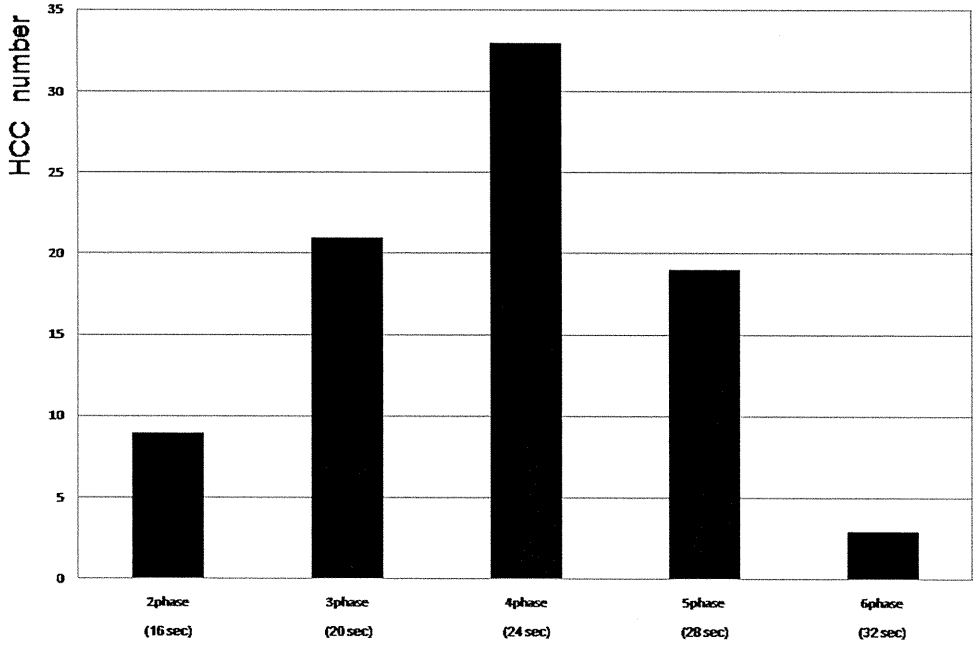

Figure 3. Beginning time of HCC enhancement in Gadoxetate-enhanced MRI. HCC enhancement began in the second phase in 9 nodules, third phase in 21 nodules, fourth phase in 33 nodules, fifth phase in 19 nodules, and sixth phase in 3 nodules.

the arterial phase, and time of peak HCC enhancement, were shown in Table 3. Only beginning time of HCC enhancement of patient with TACE (19.3 ± 5.4) was significantly earlier than that of patient without TACE (23.1 ± 6.5) ($P < 0.0071$).

DISCUSSION

Dynamic imaging such as CEUS, dynamic CT and MR imaging, are known as important modalities in the Association for the Study of Liver Diseases (AASLD) guideline (15). It is essential to detect hypervascularity on several imagings (tumor size of 1–2 cm; required 2 dynamic imaging modalities, whereas, tumor size greater than 2cm; required 1 dynamic imaging modality). In the present study, we investigated the optimal time delay after aortic contrast arrival for beginning HAP imaging, for the detection of HCC. Our most important finding was that the time delay from aortic contrast arrival of Gadoxetate to peak enhancement of HCC was 14.6 ± 4.6 s, as shown in Table 2. Therefore, to obtain optimal contrast between HCC and liver, the *k*-space center data sampling of MR imaging should be performed at approximately 14–15 s after aortic contrast arrival. Duration of HCC enhancement with Gadoxetate in the arterial phase was 17.4 ± 6.4 s. There was maximal variability of approximately 12–13 s (standard deviation × 2) in our patients. The mean duration (17.4 s) of HCC enhancement after Gadoxetate injection by visual analysis exceeded the standard deviation × 2. Thus, scanning time during the possible breathholding time should be optimized for the detection of arterial enhancement of HCCs.

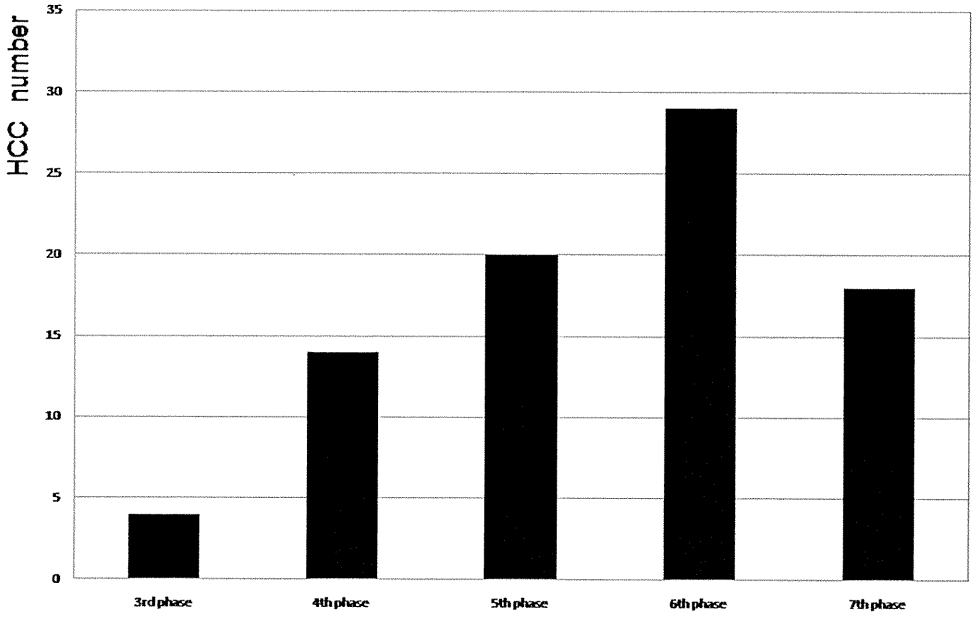

Figure 4. Time of peak HCC enhancement in Gadoxetate-enhanced MRI. Peak HCC enhancement is obtained between 20 and 36 s. Peak enhancement of 29 HCCs is obtained at 32 s.

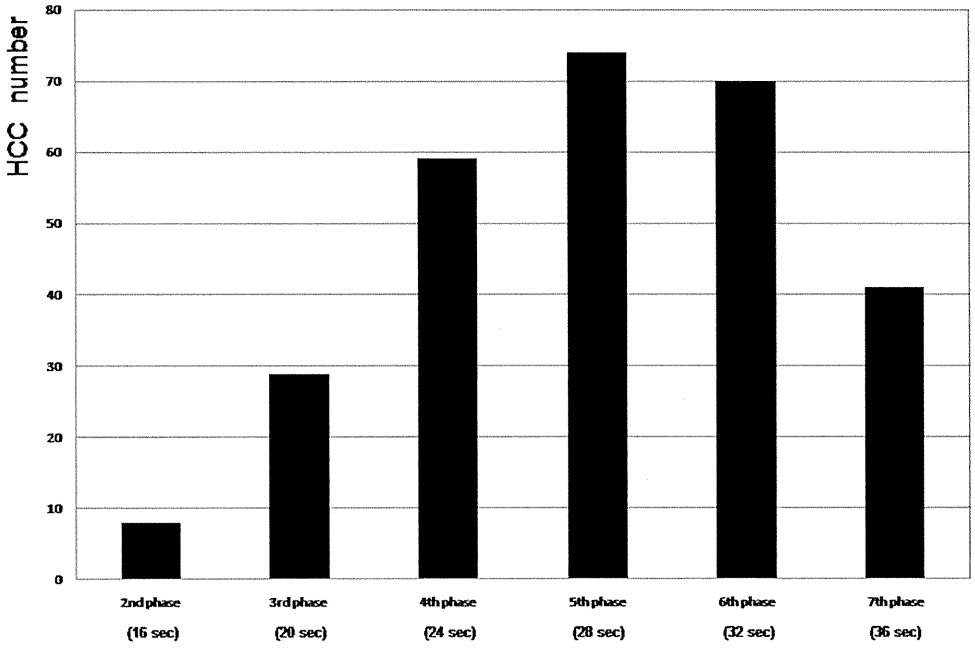

Figure 5. Number of enhancing HCCs in Gadoxetate-enhanced MRI. Enhancement is seen in most HCCs at 28 and 32 s. At 38 s (at the seventh phase), persistent enhancement remains in 41 HCCs.

We compared Gadoxetate-enhanced MR imaging with Gd-DTPA enhanced MR imaging with regard to the duration and peak intensity of aortic enhancement. The results suggest that aortic enhancement of Gd-DTPA is longer-lasting than that of Gadoxetate, and peak aortic enhancement of Gadoxetate was significantly lower than that of Gd-DTPA ($P < 0.000013$); this occurred because different injection volumes of contrast agent were used (0.2 mL/kg for Gd-DTPA versus 0.1 mL/kg for Gadoxetate), and the concentration of clinically used Gd in Gadoxetate (0.025 mmol/kg) is smaller than that in Gd-DTPA (0.1 mmol/kg). Therefore, optimal imaging timing of HAP is crucial. But, duration time of HCC enhancement of Gadoxetate was similar to that of Gd-DTPA.

Aortic contrast arrival time of Gadoxetate showed a wide range (15.1 ± 2.9 s). If the bolus tracking technique is used to indicate the starting point for HAP on dynamic MR imaging, it may be possible to overcome the problem of the varying time delays in aortic arrival, and thus improve the detection of hypervascular HCC. Aortic contrast arrival time was significantly different between Gadoxetate and Gd-DTPA ($P < 0.00013$). It is speculated that individual difference, such as cardiac function, may cause this difference.

Gadoxetate for liver MR imaging is provided commercially in 5- or 10-mL syringes. For example, a patient with body weight of 90 kg needs 9 mL of Gadoxetate, which leaves only 1 mL of contrast agent for the test bolus injection. In addition, contrast agent from the test bolus may affect liver intensity at image acquisition. Accordingly, a test bolus may be inappropriate for the detection of hypervascular HCC. Our study found that the time delay from aortic contrast

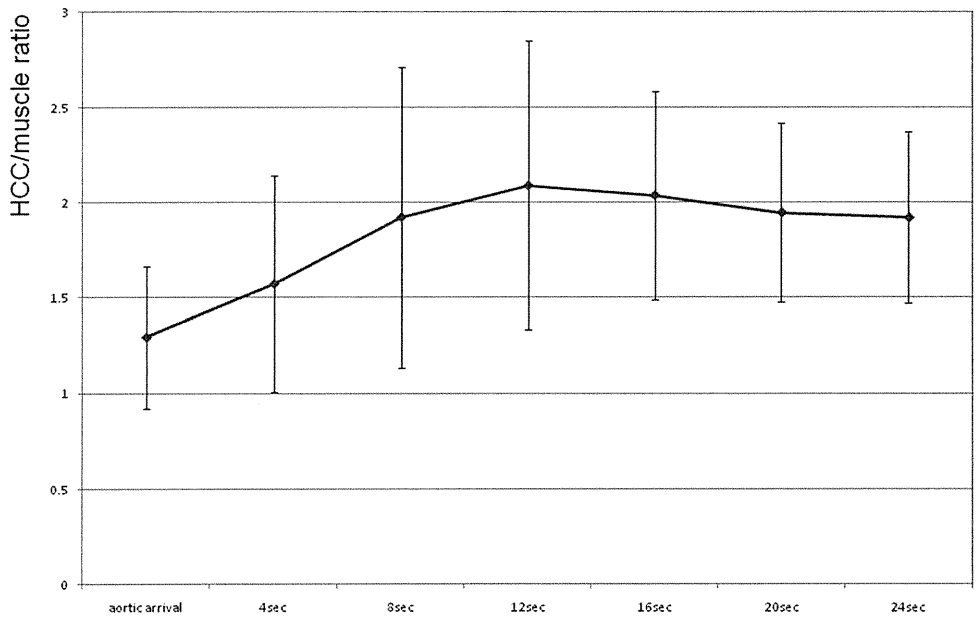

Figure 6. Time–intensity curve of HCC/muscle ratio from aortic contrast arrival in Gadoxetate-enhanced MRI. HCC shows higher and plateau enhancement at 12–28 s after aortic contrast arrival.

Table 3
Patients With or Without TACE Analyzed by Time–Intensity Curves After Gadoxetate Injection

Parameters	Patient with TACE	Patient without TACE	*P* value
Aortic contrast arrival time	14.8 ± 2.7 s	15.6 ± 3.2 s	NS
Time of peak aortic enhancement	20.5 ± 5.1 s	21.7 ± 4.7 s	NS
Beginning time of HCC enhancement	19.3 ± 5.4 s	23.1 ± 6.5 s	<0.0071
Duration time of HCC enhancement	18.4 ± 5.8 s	15.8 ± 7.2 s	NS
Time of peak HCC enhancement	29.1 ± 5.2 s	31.3 ± 3.1 s	NS
Time delay from aortic contrast arrival to peak enhancement of HCC	14.3 ± 4.9 s	15.0 ± 3.9 s	NS

*NS = not significant; TACE = transarterial chemo-embolization; HCC = hepatocellular carcinoma.

arrival to peak enhancement of HCC was 14.6 ± 4.6 s. Therefore, HCC can be imaged at peak enhancement by determining the aortic contrast arrival time using bolus tracking, confirming that the *k*-space center data sampling of MR imaging should be performed at peak enhancement time of HCC.

Sultana et al (16) stated that peak tumor–liver contrast during HAP occurred at 18 s after triggering (>100 HU), using 40 detector-row CT; this is longer than the time delay from aortic arrival of Gadoxetate found in the present study (14.6 ± 4.6 s). This discrepancy arose because a greater volume of contrast agent is used in CT than in MRI.

In CT scanning, the iodine dose is adjusted according to patient weight (17), and the patient is exposed to radiation. In contrast, MR imaging delivers no radiation exposure, and the image contrast is higher than that of CT; optimal imaging can be obtained when the time of *k*-space center filling corresponds to the time of peak tumor enhancement.

There are some limitations of the present study. First, although we evaluated only untreated HCCs, 30 of the 45 patients had a previous history of TACE, RFA, or TACE + RFA on Gadoxetate enhanced MRI. Untreated HCCs may have been affected by the previous embolization of the hepatic artery. From our result, the comparison between patients with TACE and those without TACE showed only beginning time of HCC enhancement significant difference ($P < 0.0071$), as shown in Table 3. But, both duration time of HCC enhancement and time of peak HCC enhancement between patients with TACE and without TACE did not show significant difference.

Second, the 4D THRIVE sequence with ultrafast acquisition ability has lower spatial resolution than conventional 3DFT-T1 weighted imaging such as THRIVE; however, the 4D image quality of THRIVE was sufficient to evaluate regional enhancement patterns.

Third, the scan time of each phase was 4 s; therefore, evaluation of HCC hemodynamics was not obtained in real time, as in angiography. In the present study, 4D THRIVE was used for both time–intensity analysis and actual diagnosis of the dynamic scans; therefore, we needed a balanced approach between time resolution and spatial resolution. Moreover, we think this sequence enables evaluation of regional enhancement patterns.

Fourth, in the seventh (last) phase at 38 s, TIC analysis revealed that persistent enhancement remained in 41 HCCs of Gadoxetate enhanced MRI. Thus, the scanning period for HAP used in the present study (seven phases) may be a little insufficient to obtain the endpoint of enhancement of HCCs, because of a limitation of breathhold keeping. In addition, initial aortic arrival may be insufficient at the first phase, because there were patients, who had very fast aortic contrast arrival. However, we could evaluate the usual arterial phase period by our protocol and believed to see the tendency of enhancement pattern.

In summary, bolus tracking system can help to obtain the peak enhancement of HCC on dynamic MR imaging with Gadoxetate. For optimal MR imaging of HAP, the *k*-space center data sampling should be performed at 14.6 ± 4.6 s after aortic contrast arrival.

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Original article

Recurrence-free survival more than 10 years after liver resection for hepatocellular carcinoma

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Background: High recurrence rates after liver resection with curative intent for hepatocellular carcinoma (HCC) remain a problem. The characterization of long-term survivors without recurrence after liver resection may help improve the therapeutic strategy for HCC.

Methods: A nationwide Japanese database was used to analyse 20 811 patients with HCC who underwent liver resection with curative intent.

Results: The 10-year recurrence-free survival rate after liver resection for HCC with curative intent was 22·4 per cent. Some 281 patients were recurrence-free after more than 10 years. The HCCs measured less than 5 cm in 83·2 per cent, a single lesion was present in 91·7 per cent, and a simple nodular macroscopic appearance was found in 73·3 per cent of these patients; histologically, most HCCs showed no vascular invasion or intrahepatic metastases. Multivariable analysis revealed tumour differentiation as the strongest predictor of death from recurrent HCC within 5 years.

Conclusion: Long-term recurrence-free survival is possible after liver resection for HCC, particularly in patients with a single lesion measuring less than 5 cm with a simple nodular appearance and low tumour marker levels.

Paper accepted 16 November 2010

Published online 25 January 2011 in Wiley Online Library (www.bjs.co.uk). **DOI:** 10.1002/bjs.7393

Introduction

Hepatocellular carcinoma (HCC) is a common malignancy in Japan, and often develops in virus-infected cirrhotic liver[1]. The high incidence of recurrence following treatment renders it difficult to cure this disease completely. On the other hand, long-term survival has been reported even beyond 10 years, with or without recurrence, after potentially curative liver resection[2–4]. However, there have been few reports regarding recurrence-free survival (RFS) for more than 10 years after liver resection with curative intent for HCC[5].

The Liver Cancer Study Group of Japan (LCSGJ) has conducted a nationwide survey of patients with primary liver carcinoma since 1969 to evaluate the clinicopathological characteristics and outcomes of these patients[6]. The large-scale registration system of the LCSGJ was used here to evaluate the characteristics of patients who survived without recurrence for at least 10 years after curative liver resection. These patients were compared with patients who died from recurrent HCC within 5 years in order to gain insight into the demography and biological behaviour of HCCs. In addition, such data might be important in determining follow-up strategies, and encouraging patients to undergo treatment, including surgical resection.

Methods

A nationwide follow-up survey of all patients with primary HCC was conducted by the LCSGJ. All patients with

primary malignant liver tumours diagnosed by imaging, preoperative clinical data, and/or histopathological studies at approximately 800 institutions in Japan were registered and followed prospectively every 2 years.

At the time of this analysis, the LCSGJ database contained 142 900 patients diagnosed with a liver tumour and 130 748 patients ultimately diagnosed with HCC. The present study enrolled 20 811 patients with HCC who had undergone liver resection with curative intent before 1993, and were registered in the JCSGJ database between 1988 and 2003 (from the 10th to the 17th surveillance). The indications for hepatic resection and operative procedures were based on both anatomical location of the tumour and liver function. Follow-up ended on 31 December 2003.

Patients who survived more than 10 years without recurrence of HCC and those who died from recurrent HCC within 5 years of liver resection were identified. Patients were further examined according to the degree of background liver damage, as advocated by the JCSGJ as an alternative to the Child–Pugh score (*Table 1*)[7]. The serological presence of hepatitis B antigen was considered evidence of hepatitis B infection, and that of hepatitis C antibody as an indicator of hepatitis C infection. Hepatic resections were classified according to the terminology of the Liver Cancer Study Group of Japan[7]. The macroscopic appearance of HCC was classified into six types: type 1 (simple nodular type), type 2 (simple nodular type with extranodular growth), type 3 (confluent multinodular type), type 4 (multinodular type), type 5 (others, including infiltrative, mass and diffuse types) and unknown[6,8]. Serum levels of α-fetoprotein (AFP) and des-γ-carboxyprothrombin (DCP) were measured as tumour markers. Microscopic portal vein invasion was defined as the presence of tumour emboli within the portal vein. Intrahepatic metastasis was classified into four groups: 0 (no intrahepatic metastasis), 1 (intrahepatic metastasis to the segment in which the main tumour is located), 2 (intrahepatic metastases to two segments), 3 (intrahepatic metastases of the three or four segments). Non-cancerous liver was classified microscopically as normal, or as having chronic hepatitis, fibrosis or cirrhosis.

Hepatic recurrence of HCC was diagnosed at each centre by ultrasonography and/or dynamic computed tomography. Distant metastases were diagnosed by computed tomography (lung) and scintigraphy (bone)[9].

Table 1 Degree of liver damage according to the Liver Cancer Study Group of Japan

	Degree of liver damage		
	A	B	C
Ascites	None	Controllable	Uncontrollable
Serum bilirubin (mg/dl)	> 2·0	2·0–3·0	< 3·0
Serum albumin (g/dl)	> 3·5	3·0–3·5	< 3·0
ICG-R15 (%)	< 15	15–40	> 40
Prothrombin activity (%)	> 80	50–80	< 50

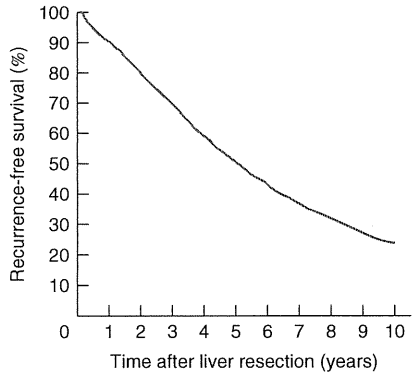
The degree of liver damage was classified as grades A, B and C based on the highest grade containing at least two of five items. Then, if two or more items scoring the same grade occur in the three grades, the higher grade is adopted as the degree of liver damage. ICG-R15, indocyanine green retention rate at 15 min.

Statistical analysis

Continuous data were expressed as mean(s.d.) and analysed by means of Student's t test. The χ^2 test was used to analyse the distribution of nominal variables, and the Wilcoxon rank sum test for analysis of ordered categorical variables. RFS curves were generated by the Kaplan–Meier method. A multivariable logistic regression model was used to investigate odds ratios. $P < 0{\cdot}050$ was considered statistically significant.

Results

Stratification according to the time of recurrence identified 281 patients who survived more than 10 years without recurrence of HCC (10-year RFS group), whereas 918 patients died from recurrent HCC within 5 years of liver resection. Median follow-up was 11·2 and 0·9 years respectively. The RFS rate at 10 years was 22·4 per cent after liver resection with curative intent (*Fig. 1*). Clinical



No. at risk	4977	3399	2253	1423	572	39
Cumulative recurrences	0	543	1047	1349	1533	1704
Cumulative deaths without recurrence	0	471	812	1110	1275	1339

Fig. 1 Recurrence-free survival after liver resection with curative intent for hepatocellular carcinoma

Table 2 Comparison of clinical data between recurrence-free survivors at 10 years and patients who died from recurrent hepatocellular carcinoma within 5 years

	10-year RFS ($n = 281$)	Died within 5 years ($n = 918$)	P§
Age (years)*	57·5(9·4)	60·8(8·5)†	< 0·001¶
Sex ratio (M : F)	219 : 62	755 : 162‡	0·115
Liver damage grade			< 0·001
A	212 (79·1)	553 (65·1)	
B	52 (19·4)	257 (30·3)	
C	4 (1·5)	39 (4·6)	
Unknown	13	69	
HBsAg-positive	82 of 255 (32·2)	179 of 812 (22·0)	< 0·001
HCV Ab-positive	103 of 198 (52·0)	356 of 474 (75·1)	< 0·001
AFP (ng/ml)			< 0·001#
< 20	140 (50·9)	272 (30·8)	
≥ 20 to < 400	73 (26·5)	345 (39·1)	
≥ 400 to < 1000	15 (5·5)	79 (9·0)	
≥ 1000	47 (17·1)	186 (21·1)	
Unknown	6	36	
DCP (mAU/ml)			< 0·001#
< 40	118 (69·4)	222 (50·5)	
≥ 40 to < 500	16 (9·4)	83 (18·9)	
≥ 500 to < 1000	36 (21·2)	135 (30·7)	
≥ 1000	0 (0)	0 (0)	
Unknown	111	478	
Operative method			0·270
> 1 segment	135 (48·2)	410 (44·9)	
Subsegment	71 (25·4)	216 (23·6)	
< 1 subsegment	74 (26·4)	288 (31·5)	
Unknown	1	4	

Values in parentheses are percentages unless indicated otherwise; *values are mean(s.d.). Data missing for †six and ‡one patients. RFS, recurrence-free survival; HBsAg, hepatitis B surface antigen; HCV Ab, hepatitis C antibody; AFP, α-fetoprotein; DCP, des-γ-carboxyprothrombin. §χ^2 test, except ¶Student's t test and #Wilcoxon rank sum test.

Table 3 Comparison of histopathological data between recurrence-free survivors at 10 years and patients who died from recurrent hepatocellular carcinoma within 5 years

	10 year RFS ($n = 281$)	Died within 5 years ($n = 918$)	P*
Maximum tumour size (cm)			0·009
< 2	91 (32·5)	198 (21·7)	
2–5	142 (50·7)	480 (52·6)	
> 5	47 (16·8)	234 (25·7)	
Unknown	1	6	
No. of tumours			< 0·001
1	253 (91·7)	675 (74·1)	
2	20 (7·2)	145 (15·9)	
≥ 3	3 (1·1)	91 (10·0)	
Unknown	5	7	
Macroscopic type			< 0·001
1	198 (73·3)	521 (60·2)	
2	32 (11·9)	174 (20·1)	
3	28 (10·4)	69 (8·0)	
4	6 (2·2)	66 (7·6)	
5	6 (2·2)	35 (4·0)	
Unknown	11	53	
Tumour differentiation			< 0·001
Well	52 (24·0)	95 (13·7)	
Moderate	133 (61·3)	427 (61·4)	
Poor	31 (14·3)	167 (24·0)	
Unclassified	1 (0·5)	6 (0·9)	
Unknown	64	223	
Vascular invasion			0·281
Yes	4 (1·4)	23 (2·6)	
No	272 (98·6)	875 (97·4)	
Unknown	5	20	
Intrahepatic metastases			< 0·001
0	258 (92·5)	673 (75·3)	
1	15 (5·4)	154 (17·2)	
2	6 (2·2)	62 (6·9)	
3	0 (0)	5 (0·6)	
Unknown	2	24	< 0·001
Non-cancerous liver			
Normal	35 (14·4)	50 (6·6)	
Chronic hepatitis/fibrosis	105 (43·2)	189 (25·1)	
Cirrhosis	103 (42·4)	514 (68·3)	
Unknown	38	165	

Values in parentheses are percentages. RFS, recurrence-free survival. *χ^2 test.

and histopathological characteristics of the two groups are compared in *Tables 2* and *3* respectively.

In the 10-year RFS group, at the time of liver resection the background liver damage was grade A in 79·1 per cent, grade B in 19·4 per cent and grade C in 1·5 per cent. Some 32·2 per cent of these patients were positive for hepatitis B virus antigens, whereas 52·0 per cent were positive for hepatitis C virus antibody. Serum levels of AFP and DCP were normal in 50·9 and 69·4 per cent of patients respectively. Surgical procedures comprised resection of less than a subsegment in 26·4 per cent, subsegmentectomy in 25·4 per cent and resection of more than one segment in 48·2 per cent of patients.

The maximum size of HCC at resection was less than 5 cm in 83·2 per cent of patients in the 10-year RFS group. Some 91·7 per cent of these patients had a single HCC at resection. HCCs in this group were of the single nodular type in 73·3 per cent, and 61·3 per cent were moderately differentiated; most showed no vascular invasion (98·6 per cent) or intrahepatic metastases (92·5 per cent). The non-cancerous tissue was cirrhotic in 46·5 per cent.

Comparison of the characteristics of patients who survived for at least 10 years without disease recurrence and those who died from recurrent HCC within 5 years revealed significant differences in age, degree of liver damage, positivity for hepatitis B antigen and hepatitis C antibody, serum levels of AFP and serum levels of DCP

(*Table 2*). Indeed, the 10-year survivors were younger, less frequently positive for hepatitis C and more frequently positive for hepatitis B. Levels of tumour markers (AFP, DCP) were lower in this group, whereas HCCs were smaller and fewer in number. There were also statistically significant differences in macroscopic appearance, tumour differentiation, intrahepatic metastasis and non-cancerous liver histology.

Table 4 Multivariable logistic regression analysis for death from recurrent hepatocellular carcinoma within 5 years

	Odds ratio	*P*
Age (years)		
≥ 60	1·00	
< 60	1·67 (1·06, 2·61)	0·026
Maximum tumour size (cm)		
< 2	1·00	
2–5	1·10 (0·63, 1·93)	0·728
> 5	2·56 (1·16, 5·65)	0·020
No. of tumours		
1	1·00	
≥ 2	1·99 (0·85, 4·62)	0·111
Macroscopic type		
1	1·00	
2	1·44 (0·75, 2·75)	0·270
3	0·76 (0·36, 1·62)	0·473
4	1·31 (0·36, 4·78)	0·687
5	1·68 (0·50, 5·67)	0·405
Tumour differentiation		
Well	1·00	
Moderate	1·59 (0·86, 2·92)	0·138
Poor	3·33 (1·46, 7·60)	0·004
Unclassified	1·01 (0·08, 12·67)	0·995
Vascular invasion		
No	1·00	
Yes	1·21 (0·25, 5·74)	0·813
Intrahepatic metastasis		
No	1·00	
Yes	2·34 (1·02, 5·37)	0·046
Non-cancerous liver		
Normal	1·00	
Chronic hepatitis/fibrosis	0·71 (0·30, 1·72)	0·450
Cirrhosis	2·25 (0·93, 5·40)	0·071
Liver damage grade		
A	1·00	
B or C	1·58 (0·96, 2·62)	0·075
AFP (units/l)		
< 20	1·00	
≥ 20 to < 400	1·96 (1·19, 3·25)	0·009
≥ 400 to < 1000	2·88 (1·19, 6·94)	0·019
≥ 1000	1·63 (0·86, 3·08)	0·134
DCP (units/l)		
< 40	1·00	
≥ 40 to < 500	2·73 (1·28, 5·41)	0·004
≥ 500 to < 1000	0·90 (0·39, 2·08)	0·804
≥ 1000	1·42 (0·76, 2·68)	0·273

Values in parentheses are 95 per cent confidence intervals. AFP, α-fetoprotein; DCP, des-γ-carboxyprothrombin.

Multivariable analysis revealed that tumour differentiation had the highest odds ratio related to death from recurrent HCC within 5 years, followed by raised levels of AFP and DCP (*Table 4*). When both the size and number of HCCs were categorized, the frequency of single HCC was significantly higher for any diameter of HCC in the 10-year RFS group than in patients who died from recurrent HCC within 5 years (data not shown).

Among patients whose levels of AFP (400–1000 units/l) and DCP (500–1000 units/l) were moderately raised, those with a single HCC had a lower risk of death from recurrent HCC than those with multiple tumours (data not shown). The number of HCCs yielded a higher odds ratio than the diameter of HCC in this specific group.

Discussion

The present study characterized tumour and patient factors among patients who survived without recurrence for 10 years after liver resection with curative intent for HCC. Although the characteristics of 10-year survivors after liver resection have already been investigated, there are few reports on 10-year RFS[2–5,10]. The present research was conducted as a nationwide large-scale comprehensive study of long-term recurrence-free survivors of HCC following liver resection in Japan.

In the present study, patients in the 10-year RFS group were younger with less background liver damage than patients who died from recurrent HCC within 5 years after liver resection. This was probably because there was less inflammatory change resulting from hepatitis C infection in the 10-year RFS group. The importance of underlying liver disease has been noted previously with regard to the degree of liver fibrosis and cirrhosis[10]. Underlying liver disease has more impact on patient survival than tumour factors[11]. Although two extreme HCC groups were compared in the present study (long-term RFS and short-term relapse), the present findings are of importance in determining possible factors associated with long-term RFS after curative liver resection.

Failure to detect latent intrahepatic HCC before surgery has no prognostic impact on the outcome or recurrence of HCC after liver transplantation[12,13]. The explanted diseased liver may show early HCCs that could not be detected before surgery, which can therefore appear as multicentric HCC on later examination. In the present study, patients in the 10-year RFS group had better liver function, despite a higher rate of positivity for hepatitis B surface antigen. Although the inflammatory activity in the resected liver was not investigated here, it was likely to have been lower in the remnant liver of the long-term survivors.

Tumour markers such as AFP or DCP have been reported to predict the early recurrence of HCC, even in the absence of microvascular invasion in the resected specimen[14,15]. The documentation of microvascular invasion depends on the slice width of the resected specimen and the number of slices investigated. Therefore, early recurrence can occur despite the absence of documented microvascular invasion. However, AFP or DCP levels are raised in nearly 60 per cent of patients with HCC, reflecting the biological behaviour of malignant tumours. The present data indicate that patients with no increase in AFP and DCP levels before surgery have a higher chance of survival without recurrence. In multivariable analysis, both tumour markers were independently associated with death due to recurrence after liver resection with curative intent. Furthermore, patients with a single HCC who had moderately raised AFP and DCP levels still had the prospect of surviving for longer after liver resection than those with high levels of tumour markers.

Considering the number and size of HCCs, a considerable percentage of patients in the 10-year RFS group had a single HCC (91·7 per cent) at the time of liver resection. Even with a raised AFP or DPC level, the risk of early death from recurrent HCC increased when there was more than one lesion. In other words, if a single HCC is found, a patient has an increased chance of surviving for longer after liver resection with curative intent.

Macroscopic HCC appearance was valuable for predicting 10-year RFS after curative liver resection, as shown previously[8]. HCCs of a contiguous multinodular type with clustering of small and contiguous nodules, and simple nodular types with extranodular growth carry a worse prognosis, most likely owing to microvascular invasion. In line with this, patients with these macroscopic types of HCC had a lower chance of long-term survival after liver resection in the present series.

The authors' group previously reported that anatomical resection has therapeutic value for treating patients with HCCs of 2–5 cm in diameter[16]. However, in the present study, this benefit of curative resection was not confirmed, even for HCCs with a diameter of 2–5 cm. This may have been because two extreme patient groups were compared. For example, even for HCC of 2–5 cm in size, the macroscopic appearance, vascular invasion, inflammatory status and fibrosis in the tumour-bearing liver may have been largely different between the two groups.

Acknowledgements

The authors acknowledge Professor T. Ichida, Department of Gastroenterology and Hepatology, Shizuoka Hospital, University of Juntendo, Shizuoka, Japan, and Professor O. Nakajima, Department of Pathology, Kurume University School of Medicine, Kurume, Japan, for helping to evaluate the study data. The authors declare no conflict of interest.

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Effect of Vitamin K2 on the Recurrence of Hepatocellular Carcinoma

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Hepatocellular carcinoma (HCC) is characterized by frequent recurrence, even after curative treatment. Vitamin K2, which has been reported to reduce HCC development, may be effective in preventing HCC recurrence. Patients who underwent curative ablation or resection of HCC were randomly assigned to receive placebo, 45 mg/day, or 90 mg/day vitamin K2 in double-blind fashion. HCC recurrence was surveyed every 12 weeks with dynamic computed tomography/magnetic resonance imaging, with HCC-specific tumor markers monitored every 4 weeks. The primary aim was to confirm the superiority of active drug to placebo concerning disease-free survival (DFS), and the secondary aim was to evaluate dose-response relationship. Disease occurrence and death from any cause were treated as events. Hazard ratios (HRs) for disease occurrence and death were calculated using a Cox proportional hazards model. Enrollment was commenced in March 2004. DFS was assessed in 548 patients, including 181 in the placebo group, 182 in the 45-mg/day group, and 185 in the 90-mg/day group. Disease occurrence or death was diagnosed in 58, 52, and 76 patients in the respective groups. The second interim analysis indicated that vitamin K2 did not prevent disease occurrence or death, with an HR of 1.150 (95% confidence interval: 0.843-1.570, one-sided; $P = 0.811$) between the placebo and combined active-drug groups, and the study was discontinued in March 2007. Conclusion: Efficacy of vitamin K2 in suppressing HCC recurrence was not confirmed in this double-blind, randomized, placebo-controlled study. (HEPATOLOGY 2011;54:532-540)

Hepatocellular carcinoma (HCC) is the third-leading cause of cancer death worldwide, claiming 600,000 victims each year. Because of advances in diagnostics and therapeutics, HCC can now be curatively treated, if detected at an early stage. Nevertheless, the long-term prognosis of HCC is not satisfactory, mainly because of its very frequent recurrence, which may occur after a long interval from initial "curative" treatment. Most cases of HCC develop in the liver with cirrhosis or advanced fibrosis.[1-4] Even if HCC nodules have been completely resected or ablated, the remaining liver retains the potential for *de novo* carcinogenesis.[5-7] In addition, precancerous lesions and microscopic metastasis may already exist in the remaining liver.

Adjuvant chemotherapy would be considered for other solid malignancies with high risk of recurrence. However, this is difficult in the case of HCC because few conventional chemotherapeutic agents are effective and hepatotoxicity can be of critical significance, as liver function is often already impaired. A randomized trial was performed with uracil-tegafur as postoperative

Abbreviations: AFP, alpha-fetoprotein; AFP-L3, alpha-fetoprotein lens culinaris agglutinin fraction-3; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BCLC, Barcelona Clinic Liver Cancer Staging System; CI, confidence interval; CT, computed tomography; DCP, des-gamma-prothrombin; DFS, disease-free survival; ECOG, Eastern Cooperative Oncology Group; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HRs, hazard ratios; IDMC, independent data monitoring committee; MRI, magnetic resonance imaging; RR, risk ratio.

adjuvant therapy, but did not improve recurrence-free survival, and overall survival appeared to be worsened.[8] Safety is clearly a prerequisite to the use of adjuvant therapy agents for HCC. Recently, a randomized trial with peretinoin, a retinoid, in patients with previously treated HCC was conducted. Although recurrence-free survival was higher with high-dose peretinoin than with placebo, there was no statistically significant difference in the predefined primary analysis.

In 2004, Habu et al.[9] reported that the incidence of development of HCC was reduced among cirrhotic women assigned to receive oral vitamin K2 (45 mg/day), originally for the prevention of osteoporosis, compared to controls (risk ratio [RR]: 0.13; 95% confidence interval [CI]: 0.02-0.99) with a limited number of subjects. Des-gamma-carboxy prothrombin (DCP), an abnormal prothrombin produced in vitamin K deficiency, is not only an HCC-specific tumor marker, but also a predictor of portal venous tumor invasion.[10] A number of findings *in vitro* have indicated that vitamin K may play a role in controlling cell growth, including inhibition of growth of HCC cells.[11-15] Vitamin K2 (menatetrenone) reportedly induced differentiation of human myeloid leukemia cells, as well as apoptosis in immature blast cells.[16-18] Vitamin K2 has been widely used for osteoporosis, and its long-term safety has been confirmed.[19-22] Thus, vitamin K2 would be an ideal adjuvant agent, if it could reduce HCC recurrence by preventing *de novo* carcinogenesis or suppressing tumor growth.

In fact, a few small-sized, controlled trials enrolling 45-61 patients have been performed to assess the effects of vitamin K2 on HCC recurrence. Mizuta et al.[23] reported that vitamin K2 reduced HCC recurrence with a multivariate-adjusted RR of 0.27 (95% CI: 0.12-0.60) and, possibly, improved survival. A preventive effect on HCC recurrence was also suggested by Kakizaki et al.,[24] who found an adjusted RR of 0.45 (95% CI: 0.10-2.05) for recurrence, although they failed to observe survival benefits. Another study failed to detect a reduction of HCC recurrence.[25] Although these previous results were inconsistent, considering the urgent need for prevention of HCC recurrence, we judged that the effect of vitamin K2 on HCC recurrence deserved evaluation in a larger scale, randomized, controlled trial. The present study was, therefore, performed as a multicenter, placebo-controlled, double-blind trial enrolling 548 patients at 31 study sites in Japan.

Patients and Methods

Patients. Candidate participants were those who had received curative treatment, in the form of local ablation or surgery, for primary HCC or first intrahepatic recurrence. Diagnosis of HCC was based on

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Received September 12, 2010; accepted May 3, 2011.

Financial support for this study was provided by Eisai Co., Ltd.

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View this article online at wileyonlinelibrary.com.

DOI 10.1002/hep.24430

Potential conflicts of interest: Kenichi Saito and Nozomu Koyanagi are employees of Eisai Co., Ltd. Drs. Shirator and Kudo are consultants for and received grants from Eisai. Drs. Shiina, Mizuta, Kojiro, Yamamoto, and Koike are consultants for Eisai. Dr. Koyanagi owns stock in Eisai. Dr. Omata is a consultant for, advises, and received grants from Eisai.

histopathologic examination or typical findings on dynamic computed tomography/magnetic resonance imaging (CT/MRI) (i.e., hyperattenuation in the arterial phase with washout in a later phase[26]). Inclusion criteria were the following: 20 years of age or older; performance status (Eastern Cooperative Oncology Group; ECOG) 0-2; and compensated liver function (albumin, ≥2.8 g/dL; total bilirubin, <2.0 mg/dL; prothrombin time activity, ≥40%). Exclusion criteria included the following: previous systemic or hepatic arterial chemotherapy; extrahepatic metastasis; portal vein invasion; interferon treatment within the previous 2 years or a sustained virologic response; uncontrollable encephalopathy, ascites, or plural effusion; a history of gastrectomy or extensive resection of the digestive tract; malabsorption of lipophilic agents, including a history of cholecystectomy; comorbidity with severe cardiovascular, hematological, or renal disease; a history of cancer other than HCC within 5 years; administration of warfarin; administration of vitamin K preparations within the previous 6 months; pregnant or breast-feeding women, or women with childbearing potential or intention; and ongoing participation in other clinical studies.

Assignment. The study was conducted as a multicenter, three-armed, randomized, placebo-controlled, double-blind, comparative, clinical study. Patients who met all criteria were enrolled and randomly assigned in double-blind fashion to receive 45 or 90 mg/day of oral vitamin K2 or a placebo with dynamic allocation, based on the modified minimization method by the registration center, which randomly allocated each patient a randomized study-drug number in the order of registration with a preset computer algorithm, adjusting for balance within each study site and across total registration, considering factors that may affect HCC recurrence (i.e., primary or recurrent HCC, medical ablation or surgical resection, hepatitis C virus (HCV)-related or -unrelated disease, and concomitant administration of glycyrrhizic acid).[27] The investigators, study sponsor, and patients remained blinded to the allocated drug during the study. The protocol was approved by the ethics committee of each participating institution. Patients were well informed of the details of the study and agreed to participate with written informed consent. This trial was conducted in conformity with CONSORT statements and in accord with the Declaration of Helsinki and good clinical practice and is registered as NCT00165633 at Clinicaltrial.gov.

Vitamin K2/Placebo Administration. Each patient took one of the identical capsules (Eisai Co., Ltd., Tokyo, Japan), containing 15 or 30 mg of menatetrenone, vitamin K2 with four isoprenoids, or a placebo, according to group assignment, three times a day after each meal. Medications for chronic hepatitis, such as glycyrrhizic acid and ursodeoxycholic acid, were continued but could not be newly commenced. Antiviral therapies (i.e., interferon, ribavirin, and nucleos(t)ide analogues, such as lamivudine) could not be administered during the study. Vitamin K2/placebo administration was discontinued when recurrent HCC was detected.

Sample Size. The sample size was determined based on previous reports on HCC recurrence among patients who received vitamin K2 and those who did not. Although a previous study reported an adjusted HR of 0.27 (95% CI: 0.12-0.60),[23] the study was conducted in a small number of subjects and the 95% CI ranged widely. We considered 30% risk reduction clinically significant, and the 30% risk reduction was conservatively adopted. Median disease-free survival (DFS) was considered to be 2 years in the placebo group, and the HR in the combined active drug groups was assumed to be 0.67-0.70. Assuming that DFS function followed an exponential distribution, a total of 240-360 events were required to detect the effect of vitamin K2 on DFS, with a one-sided significance level of 2.5%, power of 90%, and an allocation ratio of 1:2 (placebo group:combined active drug groups). To observe the number of events during the follow-up of 3-3.5 years, 180 patients were required in each group (540 in total), assuming loss of information in 5% patients.

DFS. The primary endpoint was DFS, defined as the interval between randomization and either diagnosis of HCC recurrence (i.e., intrahepatic lesions adjacent to or distant from previously treated nodules, and extrahepatic metastasis), cancer other than HCC, or death from any cause. Patients who survived without HCC recurrence or cancer other than HCC at the end of the study were censored on the day of last CT/MRI examination showing no recurrence.

Assessment of Recurrence. HCC recurrence was surveyed every 12 weeks with dynamic CT/MRI, together with ultrasonography. HCC-specific tumor markers, including alpha-fetoprotein (AFP), AFP lens culinaris agglutinin fraction-3 (AFP-L3), and DCP, were monitored every 4 weeks, and dynamic CT/MRI was additionally performed when recurrence was suspected by an increase in tumor marker levels. HCC recurrence was diagnosed by hyperattenuation in the arterial phase and hypoattenuation in the portal venous or equilibrium phase of dynamic CT/MRI. Tumor biopsy was performed when findings on CT/

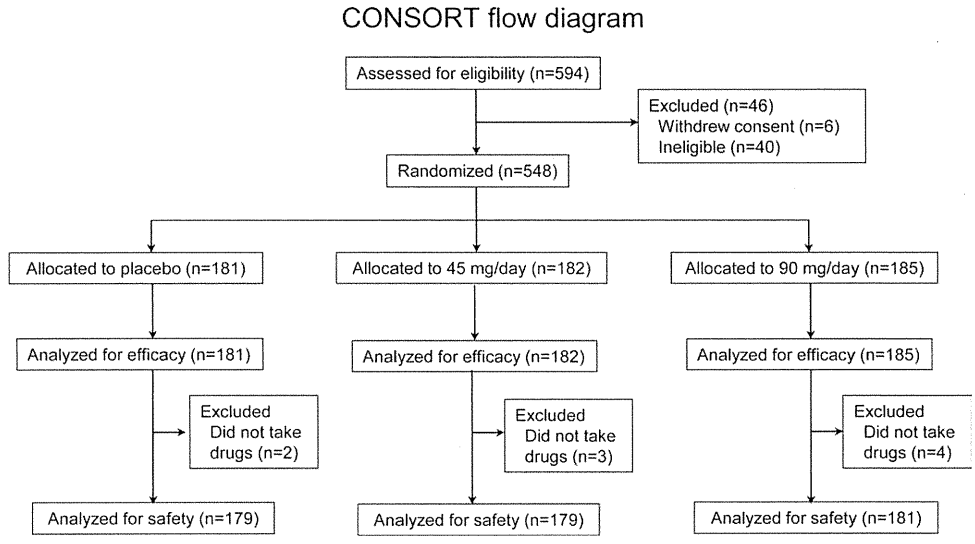

Fig. 1. CONSORT flow diagram.

MRI were equivocal. The presence of recurrence was finally judged by an independent review committee, which thoroughly reviewed the diagnostic imagings in blind fashion. The day of recurrence was defined at the time of first detection of recurrence.

Assessment of Safety. Safety was assessed at 4-week intervals by interview, physical examination, and laboratory tests. Adverse events were defined as any untoward or unintended events that occurred in a subject receiving a study drug. Serious adverse events were defined as those that resulted in death or required hospitalization. Adverse drug reactions were defined as adverse events possibly related to the study drug.

Statistical Analysis. The primary aim of this study was to confirm the superiority of active drug to placebo concerning DFS, and the secondary aim was to evaluate the dose-response relationship between the two active drug groups. DFS rate and median DFS were calculated using the Kaplan-Meier method. Superiority and dose-response relationship were evaluated by the log-rank test, using score statistics with contrast coefficients (−2, 1, and 1) and (0, −1, and 1), respectively, for placebo, 45-mg/day, and 90-mg/day groups. HRs were calculated using Cox's proportional hazards regression model. Adverse events and adverse drug reactions were tabulated based on groups and compared with placebo by Fisher's exact test.

Two interim analyses by the independent data monitoring committee (IDMC) were scheduled. The first was planned 1 year after the commencement of registration to assess safety. The second was planned when 160 events were recorded to assess significance of effect by the finding of $P < 0.005$ (one-sided) or futility. Alpha spending was, for this interim analysis, defined as 0.5% (one-sided), and the overall significance level of statistical tests for the primary aim was maintained at one-sided 2.5%, adjusted for multiplicity associated with interim analyses by the method of Lan and DeMets.[28] The rule for stopping for reasons of futility was defined as follows: The Bayesian predictive probability[29] of detecting a significant effect on observation of 360 events was less than 5%, or the number of events required to assure 50% conditional power exceeded 360. If the IDMC decided to continue the trial, the final required number of events (maximum, 360 events) was to be recalculated to assure 80% conditional power, with the overall significance level maintained for recalculation of the required number of events by Cui's method.[30]

Significance levels for homogeneity among the groups were two-sided 15%, and others were two-sided 5%.

Results

A total of 548 patients were enrolled at 31 study sites in Japan and randomly assigned between March 2004 and September 2005 (Fig. 1). Tumor biopsy was performed in 14 patients, whereas diagnosis was obtained radiologically in remaining patients.[26] Efficacy (i.e., DFS) was assessed among 548 patients (placebo group: 181; 45-mg/day group: 182; 90-mg/day group: 185). Safety was assessed among 539 patients, excluding nine patients who never took drugs. Two patients took drugs at a dose different from that allocated. They were included in the group of allocated dose in the efficacy analysis, but in the group of actually received dose in the safety analysis.