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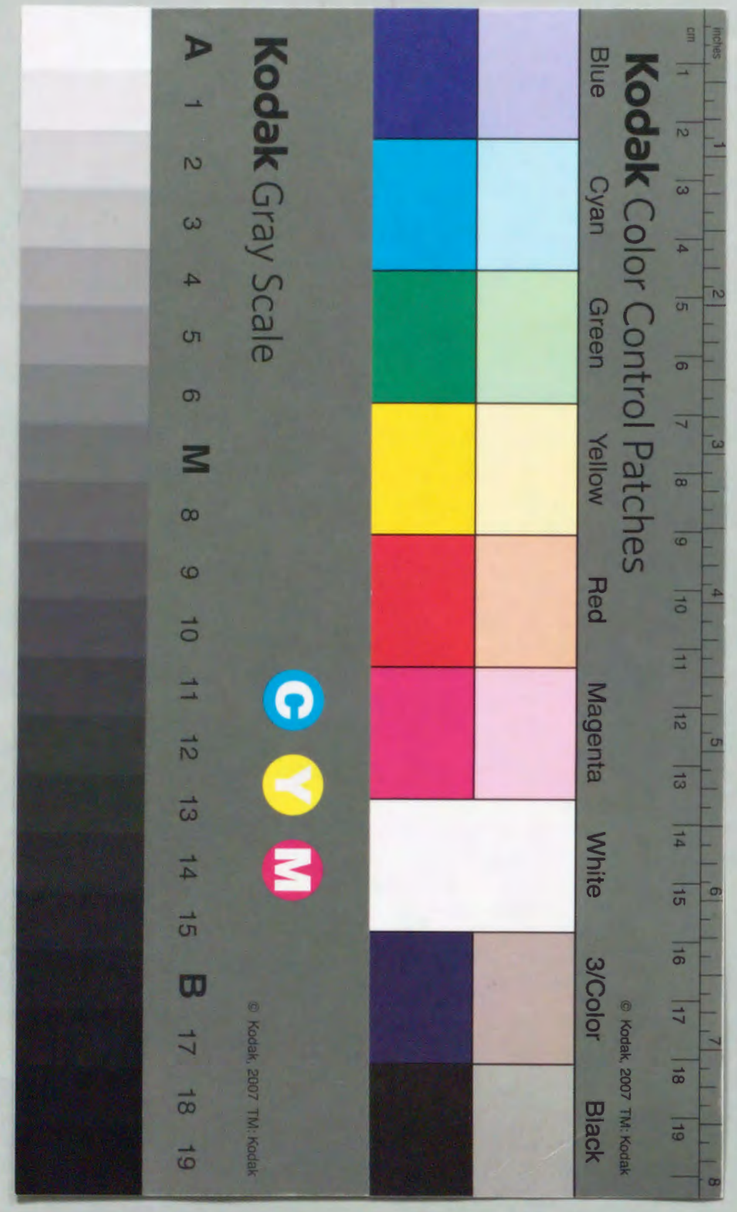

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MR imaging of hepatocellular carcinomas: effect of Cu and Fe contents on signal intensity

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Abstract

Background: To elucidate the metallic factors contributing to the signal intensities of hepatocellular carcinoma (HCC) on T1-weighted magnetic resonance (MR) images and to determine whether or not changes in signal intensity contribute to the diagnosis of histological grading of HCC.

Methods: In 35 patients immediately after surgery, the quantities of water, lipid, copper (Cu), iron (Fe), and manganese (Mn) were determined in HCCs and the surrounding hepatic parenchyma. The correlations among these findings, the histopathological findings, and the signal intensities of T1-weighted MR images were evaluated.

Results: Among the 35 HCCs, 12 (34%) were of high intensity, 14 (40%) were isointense, and 9 (26%) were of low intensity on T1-weighted images versus the surrounding hepatic parenchyma. The paramagnetic ions, which contributed to the signal intensity patterns, were assumed to be Cu in HCCs (30.5 ± 52.9 μg/g ww), and Fe in the livers (106.2 ± 86.8 μg/g ww) and HCCs (87.7 ± 49.1 μg/g ww). In 12 HCCs with high intensity, one was grade I, eight were grade II, and three were grade III according to Edmondson-Steiner's histopathological classification.

Conclusions: Signal intensity and signal intensity patterns alone cannot be signs of low-grade malignancy because of the Fe in livers and in HCCs.

Key words: Liver, neoplasms—Liver, metal—Magnetic resonance imaging—Hepatocellular carcinoma—Liver, signal intensity.

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Magnetic resonance (MR) imaging has facilitated the diagnosis of hepatocellular carcinoma (HCC). HCC displays a variety of signal intensities compared with those of the hepatic parenchyma on T1-weighted images. In 40% of HCCs, the signal intensities are grossly or partially higher than those of the surrounding hepatic parenchyma [1]. This is one of the characteristic features of HCCs, and the relatively high signal intensity of HCCs is reportedly a sign of a well-differentiated tumor, which is believed to be related to steatosis of cancer tissues [2–4]. However, it is not uncommon for HCCs of high intensity on T1-weighted images to show no signs of steatosis histopathologically, and high-intensity HCCs are not well differentiated [4]. Recently, the effect of combined metallic factors to the signal intensity has been suggested [5]. The purpose of the present study was to examine these metallic factors contributing to the T1 signal intensity of HCCs and to determine whether or not changes in signal intensity contribute to the diagnosis of histological grading of HCC.

Materials and Methods

Phantom Study

To clarify the correlation between the signal intensity on T1-weighted MR images and the metallic contents, phantom studies were performed.

Solutions of $CuSO_4$, $FeCl_3$, and $MnCl_2$ were used to set up phantoms to clarify whether or not the metal in the HCCs can contribute to the signal intensity on T1-weighted MR images. Each phantom consisted of 12–15 test tubes containing solutions of a metallic ion in different concentrations ranging from 1.0×10^{-5} to 0.1 mol/L dissolved in saline. T1-weighted images were obtained by using these phantoms under the same conditions and by using the same MR equipment with

body coil as that used in the studies of patients. The saturation plane utilized was coronal and no other softwares such as presaturation and respiratory compensation were used. The same kinds of metallic phantoms in different concentrations were scanned together. Saline without metallic ions was used as a control. The signal intensity of each phantom was determined as excess over that of the saline control. The differences among the phantoms and the saline control were used for the analyses.

Clinical Study

Thirty-five patients, 25 men and 10 women, whose mean age was 64 years (range 45–75 years old), with surgically proven HCCs were studied prospectively from July 1992 to October 1993. Hepatic lobectomy or segmentectomy was performed within 1 week after MR imaging. All tumors were histologically-proven HCCs. The tumors ranged from 1.0 to 14.0 cm and averaged 4.0 (±2.6) cm in greatest dimension. MR imaging was performed using a 1.5 T superconducting Signa MR system (GE, Milwaukee, WI) with body coil. Axial images were obtained with a 256 x 128 matrix and contiguous 10-mm-thick slices with a 2.5 mm intergap. Two spin-echo (SE) sequences (SE 600/20, four excitations and SE 2000/70, four excitations) were obtained. Only the T1-weighted images (SE 600/20) were used for analysis because all tumors were of high intensity on T2-weighted images (SE 2000/70). The saturation plane was axial, and respiratory compensation and presaturation were used.

Small portions of the HCCs and the surrounding hepatic parenchyma were obtained immediately after surgery to measure the content of water, lipid, and metals. To determine water content, the sample was dried at 80 °C for 24 h and weighed. The difference between the wet and dry weights was taken as the content (in %) of the sample. The lipid content was determined by the Soxhlet method. Lipid extracted by this method was weighed and expressed in milligrams per grams of tissue. To determine the contents of iron (Fe), copper (Cu), and manganese (Mn), the sample was placed in a Kjeldahl flask, to which 2 mol/L HNO_3, 70% $HClO_4$ and distilled water were added, and heated to evaporation. The metals were determined by atomic absorption and expressed in micrograms per gram of wet weight (μg/g ww). Immediately after surgery, the resected portion, segment, or lobe was cut horizontally in 10–12-mm-thick slices for comparison with the MR images. Specimens were obtained from the solid portion of the tumor, avoiding necrotic or hemorrhagic parts. Signal intensities of the HCCs and the surrounding hepatic parenchyma were measured from the same areas from which the specimens were obtained.

The results of MR imaging and the contents of metals in HCC were compared with the histopathological results according to Edmondson-Steiner's classification.

The Wilcoxon signed-rank test was used for statistical analysis of the contents in the HCCs and livers. For correlation of tumor sizes, Student's nonpaired t test was used.

Correlation of Clinical and Phantom Studies

The signal intensities and the metallic concentrations of the livers and the tumors were correlated with the results of the phantom studies. To determine whether the signal intensities of the livers and HCCs truly correlated with the metallic concentrations, the signal intensities of the clinical cases and the results of the phantom studies were compared. The differences [SI(in vivo)] between the signal intensities of the HCCs [SI(HCC)] and the livers [SI(liver)] were calculated as follows:

$$\mathrm{SI(in\ vivo)} = \mathrm{SI(HCC)} - \mathrm{SI(liver)} \quad (1)$$

The signal intensity (**SI**) was the function of relaxation time (**T1,2**), echo time (**TE**), repetition time (**TR**), proton density (ρ), and instrumental constant **K.**

$$\mathrm{SI} = \mathrm{K}\rho \cdot e^{-\mathrm{TE/T2}} \cdot (1 - e^{-\mathrm{TR/T1}}) \quad (2)$$

In the presence of two different metallic ions in the same solution, relaxation time of the metallic solution ($\mathbf{Ti_p}$, i = 1,2) is expected to be

$$(1/\mathrm{Ti_p}) = (1/\mathrm{Ti_o}) + \mathrm{Ri[C]} + \mathrm{Ri'[C']} \quad (3)$$

where $\mathbf{Ti_o}$ (i = 1,2) is the relaxation time of the control. **[C], [C']** are the concentrations of paramagnetic ions (mmol/L), and **Ri, Ri'** (i = 1,2) are relaxivity $(\mathrm{s \cdot mmol/L})^{-1}$ of each metallic ion. In the range where equation (3) holds, the signal intensity of the solution containing two kinds of paramagnetic ions in any composition can be calculated by using equations (2) and (3).

To analyze the in vivo signal intensities, matched calculations were made by using the metal concentrations found in HCC and liver as follows:

$$\mathrm{SI(phantom)} = \mathrm{SI(HCC - phantom)} - \mathrm{SI(liver - phantom)} \quad (4)$$

where SI(HCC - phantom) and SI(liver − phantom) are the signal intensities calculated by using equations (2) and (3) and the metal concentrations found in the corresponding tissues. The relaxation between SI(in vivo) in (1) and SI(phantom) in (4) was compared.

Results

Phantom Study

The signal intensity curves of three metals are shown in Fig. 1. The concentration of each paramagnetic ion showing the highest intensity was $3 \cdot 10^{-4}$ mol/L (Mn), $2 \cdot 10^{-3}$ mol/L (Fe), and $1 \cdot 10^{-2}$ mol/L (Cu). The concentration of each paramagnetic ion with the same intensity as that of the saline control was $1.6 \cdot 10^{-3}$ (Mn), $1.1 \cdot 10^{-3}$ (Fe), and more than $1 \cdot 10^{-1}$ mol/L (Cu).

Clinical Study

Among the 35 tumors, 12 (34%) were of higher and 9 (26%) were of lower intensity than the livers. Fourteen (40%) were isointense with liver. The differences in signal intensities between HCCs and livers [SI(HCC) − SI(liver)] were from 8 to 45 (average = 18) in high-intensity HCC, from 0 to −5 (average = −3) in isointensity HCC, and from −10 to −50 (average = −26) in low intensity HCC. The correlations between signal intensity and histological gradings are shown in Table 1.

The lipid contents of the livers and the HCCs ranged from 5 to 108 mg/g ww (53.0 ± 24.0) and from 14 to 107 mg/g ww (49.4 ± 29.6), respectively. Although fatty metamorphosis was microscopically observed in 11 HCCs, the distribution was minimal, and the extracted lipid content differences between the liver and HCC were less than 30 mg/g ww in all cases. Based on this, all 35 HCCs were used for the analysis of signal intensity. The water content of the livers and the HCCs

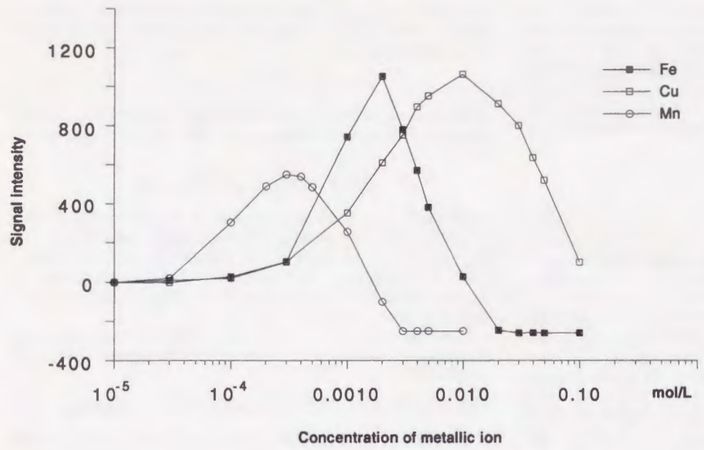

Fig. 1. Signal intensity curves of manganese (Mn), iron (Fe), and copper (Cu). The differences among the phantoms and the saline control are plotted.

Table 1. Relationship between histopathological grading and MR findings

Histopathological grading	No. (%) of tumors	MR findings High	Iso	Low
I	4 (11)	1	3	0
II	19 (54)	8	8	3
III	9 (26)	3	3	3
IV	3 (9)	0	1	2
Total (%)	35	12 (34)	15 (43)	8 (23)

ranged from 76.9 to 89.5% (83.9 ± 3.1) and from 76.0 to 90.7% (85.5 ± 3.4). No statistically significant difference was observed in the lipid and water contents between the livers and the HCCs.

Mn content of the livers ranged from 0.1 to 1.3 μg/g ww (0.6 ± 0.4) and from 0.1 to 1.2 μg/g ww (0.5 ± 0.3) in the HCCs. There was no statistically significant difference between the livers and the HCCs as to the content of Mn. The Mn contents of the livers and HCCs were too low to elevate the signal intensity.

Cu contents of the livers showed a minimal distribution ranging from 1.3 to 10.7 μg/g ww (4.1 ± 2.2), whereas HCCs exhibited wide variations ranging from 0.3 to 230.0 μg/g ww (30.5 ± 52.9). Statistically, the Cu contents of HCCs were greater than those of the liver ($p < 0.01$) (Fig. 2). Based on signal intensity curves, HCCs were categorized into three groups: one consisting of HCCs in which Cu exceeded 40 μg/g ww (n = 9); one consisting of HCCs in which Cu had accumulated to 10–39.9 μg/g ww (n = 8); and a third consisting of HCCs in which Cu had accumulated similarly to less than that in livers by less than 9.9 μg/g ww (n = 18).

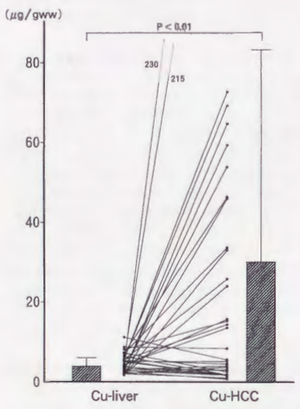

Fig. 2. Correlations of copper content in livers (Cu-liver) and hepatocellular carcinomas (Cu-HCC) (n = 35).

The noncancerous liver tissue had a low Cu content and did not differ significantly among cases. Correlations among Cu content, signal intensity, and histopathological grading are shown in Table 2. The nine HCCs with Cu contents greater than 40 μg/g ww were histological grades I (one) or II (eight) ($p < 0.05$). The HCCs with Cu content exceeding 40 μg/g ww (2.7 ± 1.3cm) were smaller than the HCCs with Cu content of less than 40 μg/g ww (4.5 ± 2.8cm) ($p < 0.01$).

Fe content in the livers and HCCs ranged from 2.0 to 366.0 μg/g ww (106.2 ± 86.8) and from 2.0 to 178.0 μg/g ww (87.7 ± 49.1), respectively. Fe content in the HCCs were statistically less than that in the liver ($p <$

Table 2. Relationship among Cu content, histopathological grading, and MR finding

Cu content (μg/g ww)	No. (%) of tumors	Histopathological grading I	II	III	IV	MR finding High	Iso	Low
<10	18 (51)	3	7	5	3	5	7	6
10 ≤ <40	8 (23)	0	4	4	0	5	2	1
≥40	9 (26)	1	8	0	0	2	5	2
Total (%)	35	4 (11)	19 (54)	9 (26)	3 (9)	12 (34)	14 (40)	9 (26)

* $p < 0.05$

0.0001) (Fig. 3). Based on signal intensity curves, HCCs were categorized into three groups; one consisted of HCCs in which Fe had accumulated to more than 30 μg/g ww (n = 24); one consisted of HCCs in which Fe had accumulated by 10 to 29.9 μg/g ww (n = 7); and the third consisted of HCCs in which Fe had similarly accumulated to less than that in livers by less than 9.9 μg/g ww (n = 4). Correlations among Fe contents, histopathological grading, and MR findings are shown in Table 3. No significant relation was seen in these correlations. No significant differences were observed between the sizes of the HCCs with more than 30 μg/g ww Fe content (3.8 ± 1.7 cm) and those with less than 30 μg/g ww Fe content (4.6 ± 3.2 cm).

Among 12 high-intensity HCCs, only two (17%) showed excessive Cu (more than 40 μg/g ww); the other 10 HCCs contained excessive Fe (more than 30 μg/g ww). Among nine low intensity HCCs, two (22%) contained excessive Cu and three (33%) contained excessive Fe. However, eight (89%) HCCs showed Fe content in the liver to be more than that in the HCCs.

Correlation of Clinical and Phantom Studies

From the results of the clinical study, the Mn content of the livers and the HCCs was less than 1.0 μg/g ww ($2 \cdot 10^{-5}$ mol/L). Although Mn is a paramagnetic ion contained in liver and HCCs, Mn was not expected to affect the signal intensity of livers and HCCs because of its low concentration.

The Cu concentrations in the liver were low and did not differ among cases; therefore, the paramagnetic ions that might contribute to the relative signal intensities were regarded to be Fe in the livers and Cu and Fe in the HCCs.

To clarify whether the signal intensities of the livers and the HCCs truly correlated with the Cu and Fe concentrations, signal intensities of the clinical cases and results of the phantom studies were compared.

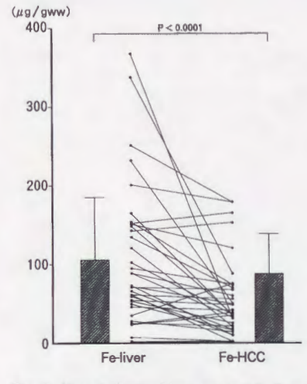

Fig. 3. Correlations of iron content in livers (Fe-liver) and hepatocellular carcinomas (Fe-HCC) (n = 35).

From the results of the phantom studies, the relaxivity of Fe and Cu and the constant were calculated as follows:

$$R1\,(Cu) = 0.45\,(s \cdot mmol/L)^{-1} \quad R2\,(Cu) = 0.71\,(s \cdot mmol/L)^{-1}$$

$$R1\,(Fe) = 2.7\,(s \cdot mmol/L)^{-1} \quad R2\,(Fe) = 8.0\,(s \cdot mmol/L)^{-1}$$

$$K\rho = 1819$$

The signal intensity curves calculated for Cu and Fe presented in the same solution are shown in Fig. 4.

The correlation between SI(in vivo) and SI(phantom) is shown in Fig. 5. SI(in vivo) and SI(phantom) showed a significant regression line: $y = 0.08x - 1.99$, $r^2 = 0.72$, and $p < 0.0001$.

Discussion

A Cu-containing enzyme is present in the liver as monoamino acid oxidase and superoxide dismutase and fulfills an important function as an essential metal. Cu is

Table 3. Relationship among Fe content, histopathological grading, and MR finding

Fe content (μg/g ww)	No. (%) of tumors	Histopathological grading				MR finding		
		I	II	III	IV	High	Iso	Low
<10	4 (11)	0	3	0	1	1	2	1
10 ≤ <30	7 (20)	0	4	2	1	1	1	5
≥30	24 (69)	4	12	7	1	10	11	3
Total (%)	35	4 (11)	19 (54)	9 (26)	3 (9)	12 (34)	14 (40)	9 (26)

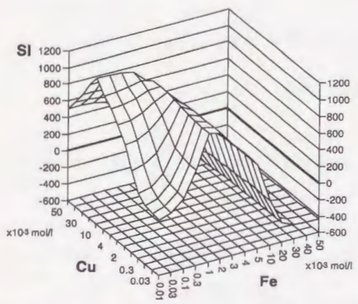

Fig. 4. Calculated three-dimensional signal intensity (SI) curves of mixed solutions of copper (Cu) and iron (Fe).

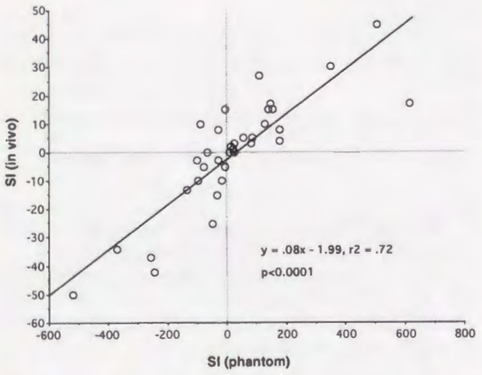

Fig. 5. Graphic demonstration of linear relationship of SI(phantom) and SI(in vivo): SI(phantom) SI(HCC - phantom) = SI(liver - phantom), SI(in vivo) SI(HCC) - SI(liver). SI(HCC - phantom): the signal intensities of Cu and Fe mixed solutions which were of the same concentrations as the hepatocellular carcinoma SI(liver - phantom): the signal intensities of Cu and Fe mixed solutions which had the same concentrations as the liver.

also present in the bile and becomes elevated in cases of biliary obstruction and liver cirrhosis [6]. Bile production is also known to be one of the cytological characteristics of well-differentiated (grade I or II) HCCs [7]. Cytoplasmic lipid storage and glycogen accumulation are frequently more prominent in small HCCs than in large ones [8]. These cytoplasmic characteristics, including the appearance of copper, are morphological expressions of altered cellular metabolism and are more conspicuous in the early than in the advanced stages of cancer. There is another explanation for the decrease of Cu in inverse proportion to the growth of HCC. Cu is excreted from normal liver cells mainly through the bile duct system [9]. This is absent in the nodules of HCCs. If HCC cells, similar to normal liver cells, retain the property of Cu uptake from serum during their early stages and lose it in proportion to the tumor growth, Cu may be accumulated more during early stages of HCCs [7].

Fe is known to be present in the liver, and the prevalence of HCCs is higher in cirrhotic livers with iron deposition than in livers without siderosis [10, 11]. Fe is an essential element for the growth of all cells including tumor cells. Human HCC cells develop faster in the presence of Fe supplements than in unsupplemented media [12]. These findings and other known associations of Fe overload with increased incidence of cancer indicate that iron supplementation in cancer might enhance tumor growth [13].

Based on pathological data, it had been assumed that neoplastic tissues, especially carcinomas, are unable to retain Fe [14], despite relatively high contents of ferritin [15]. However, recently, it was revealed that ferritin synthesis and secretion are stimulated by Fe and, as ferritin acts as an intracellular Fe store, cancer cells are able to take up Fe [16]. The Fe status of the HCC cells correlates with the intracellular levels of ferritin and may not relate to the histological grading of HCC. In the present study, the Fe contents of HCCs were statistically lower than those of the livers; however, no statistical correlation was observed between the Fe content of HCCs and histological grading. It is also known that Fe in the liver is Fe^{3+} rather than Fe^{2+} [17]. Fe^{3+} can be

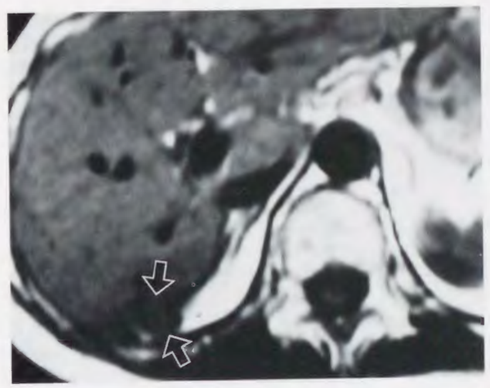

Fig. 6. T1-weighted MR image (600/20) shows the tumor to be of low intensity in the posterior-inferior segment of the liver. Although copper concentration in the hepatocellular carcinoma was relatively high (230 μg/g ww, $3.6 \cdot 10^{-3}$ mol/L), the signal intensity of the liver was more elevated by excessive iron in the liver (72 μg/g ww, $1.3 \cdot 10^{-3}$ mol/L). The signal intensities between the lesion and liver were -10 in SI(in vivo) and -18 in SI(phantom).

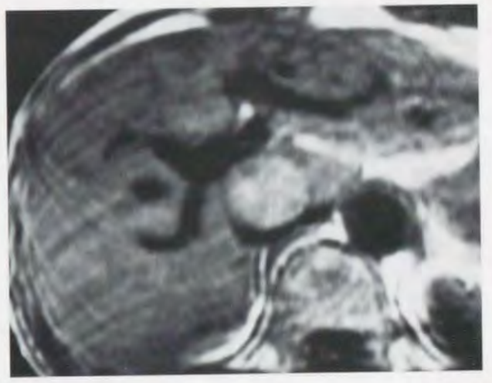

Fig. 7. Hepatocellular carcinoma with a high intensity at T1-weighted image (600/20). The copper concentration in the hepatocellular carcinoma was greater (72.0 μg/g ww, $1.0 \cdot 10^{-3}$ mol/L) than the iron concentration in the liver (7.3 μg/g ww, $1.3 \cdot 10^{-4}$ mol/L). Signal intensities between the lesion and liver were 27 in SI(in vivo) and 111 in SI(phantom).

paramagnetic and may play important roles in producing tissue hyperintensity due to T1 shortening.

In the present study, all nine HCCs with Cu contents of more than 40 μg/g ww were small and histologically low-grade HCCs (grade I or II). However, 22% (2/9) of them were demonstrated to be of low intensity. This is attributed to the high concentration of Fe in the liver and its increased signal intensity (Fig. 6).

Among the 12 high-intensity HCCs, only 17% (2/12) was supposed to have the signal intensity elevated by greater Cu content (more than 40 μg/g ww) in the HCC than that in the surrounding hepatic parenchyma (Fig. 7). The other 10 HCCs (83%) contained Fe of more than 30 μg/g ww. The signal intensity of these lesions were supposed to be related by Fe content in HCC (Fig. 8).

Two recent reports have described the relation between Cu and the MR findings. One reported a statistical significance between Cu content and the MR findings [1]; the other reported that there was no such statistical significance [18]. However, both reports disregarded the presence of Fe in HCCs and livers. Results of the present study indicate that Fe in HCCs and livers reflect as much as or more than Cu in HCCs for determining the signal intensity patterns of HCCs [5]. Even if a relatively large quantity of Cu is contained in an HCC, an elevated signal intensity of the liver caused by Fe displays the HCC as a low-intensity tumor. Even if a small amount of Cu is contained in an HCC, a large amount of Fe is displayed in the HCC as high intensity.

Inevitably the properties of a solution of a metallic ion will not behave identically with an intracellular protein-bound ion. The ratios between the protein-bound ion and the unbound ion in vivo are not known in the present cases. However, the signal intensities gained in vivo and in phantom studies demonstrated a significant regression line. Cu and Fe in vivo were suspected to influence signal intensities of HCCs and livers by ap-

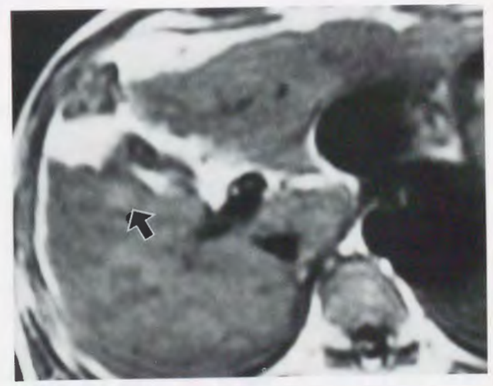

Fig. 8. T1-weighted MR image (600/20) shows a tumor of high intensity in the anterior segment of right lobe of the liver. Although the copper (25.2 μg/g ww, $0.4 \cdot 10^{-3}$ mol/L) concentration in the hepatocellular carcinoma was relatively low, excessive iron in the HCC (74.8 μg/g ww, $1.3 \cdot 10^{-3}$ mol/L) elevated the signal intensity to be greater than that in the liver (34.5 μg/g ww, $6.2 \cdot 10^{-4}$ mol/L). Signal intensities between the lesion and liver were 15 in SI(in vivo) and 140 in SI(phantom).

proximately one-twelfth of Cu and Fe in phantom solutions ($y = 0.08x - 1.99$).

Mn is known to be in pyruvate carboxylase, pyruvate kinase, and mitochondrial superoxide dismutase [19]. Although Mn is a paramagnetic ion and demonstrated elevations of signal intensity in concentrations lower than Fe and Cu, the Mn contents of livers and HCCs were too low to elevate the signal intensity [5].

In conclusion, the various signal intensities of HCCs in T1-weighted images are reflections of Cu in HCCs and Fe in livers and HCCs. Although Cu may elevate the signal intensity of HCCs and excessive Cu content is frequently observed in well-differentiated HCCs of grade I or II, the signal intensity patterns alone cannot be a sign of tumor with low-grade malignancy because Fe in the livers and HCCs also contributes to the T1 signal intensity.

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