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Clearance of HCV Improves Insulin Resistance, Beta-Cell Function, and Hepatic Expression of Insulin Receptor Substrate 1 and 2

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OBJECTIVES: Hepatitis C virus (HCV) infection is linked to greater insulin resistance. Although HCV itself is a candidate for the development of insulin resistance, the effects of antiviral treatment on impaired glucose metabolism remain unclear. The aim of this study is to examine the effects of clearance of HCV on insulin resistance, beta-cell function, and hepatic expression of insulin receptor substrate (IRS)1/2, central molecules for insulin signaling.

METHODS: We analyzed 89 biopsy-proven patients with chronic HCV infection. Patients received interferon-α or interferon-α plus ribavirin for 6 months and were classified into three groups at 6 months after the conclusion of antiviral therapy according to their response to antiviral therapy: sustained responders (N = 29), relapsers (N = 12), and nonresponders (N = 48). Insulin resistance and beta-cell function were assessed by the homeostasis model assessment method (HOMA-IR and HOMA-%B, respectively). Hepatic expression of IRS1/2 was evaluated by immunoblotting and immunostaining in 14 sustained responders.

RESULTS: In nonresponders and relapsers, there were no significant changes in HOMA-IR and HOMA-%B values after antiviral therapy. On the other hand, in sustained responders, HOMA-IR values significantly decreased to 1.7 ± 0.8 from 3.1 ± 1.1 ($P < 0.05$) after antiviral therapy. Similarly, HOMA-%B values significantly decreased to 90.6 ± 10.0 from 113.7 ± 15.3 ($P < 0.05$). Immunoblotting showed a threefold increase in IRS1/2 expression after clearance of HCV. Immunostaining revealed that greater IRS1/2 expression was seen in hepatocytes.

CONCLUSIONS: We showed that clearance of HCV improves insulin resistance, beta-cell function, and hepatic IRS1/2 expression.

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INTRODUCTION

Chronic hepatitis C virus (HCV) infection is associated with a greater risk for the development of insulin resistance (1). Greater insulin resistance is more prevalent among patients with HCV infection compared with those with other liver diseases and with the general population (2). In patients with HCV infection, insulin resistance is involved in progression of hepatic fibrosis (3), the development of hepatocellular carcinoma (4, 5), extrahepatic manifestations (6), and prognosis (7). Thus, insulin resistance plays a crucial role in patients with HCV infection.

Insulin resistance can be caused by many factors. In general, obesity, inflammation, and various kinds of metabolic disorders are common factors for the development of insulin resistance. Similarly, body mass index (BMI), serum tumor necrosis factor-α (TNF-α) and hepatic iron concentrations, and hepatic steatosis are reported to be possible causative factors for the development of insulin resistance in patients with HCV infection (8–11). In addition to these factors, HCV itself is also known to have a variety of biological effects (12).

In HCV core transgenic mice, the development of insulin resistance is seen by 1 month of age, in the absence of either overt liver injury or excessive body weight gain (12, 13). Furthermore, even if liver function is restored by transplantation, postliver transplantation diabetes mellitus occurs more frequently among patients who undergo transplantation for HCV than for other conditions (14). Although precise mechanisms for HCV-associated insulin resistance have not been fully elucidated, we recently demonstrated the involvement of insulin receptor substrate 1 and 2 (IRS1/2), central molecules in insulin signaling. Downregulation of IRS1/2 is seen in

livers from HCV core transgenic mice as well as in patients with HCV infection (15). Thus, HCV itself is a candidate risk factor for the development of insulin resistance.

If HCV is a causal factor, then clearance of HCV might decrease insulin resistance just as histologic improvement of fibrosis and reduction in the risk of hepatocellular carcinoma are seen in patients with hepatitis C who have sustained response to interferon therapy (16, 17). The ability of antiviral therapy to improve glucose metabolism would support the notion that HCV causes insulin resistance in patients with HCV infection. Accordingly, we studied the effects of HCV clearance on insulin resistance, beta-cell function, and hepatic expression of IRS1/2.

MATERIALS AND METHODS

Materials

All reagents were purchased from Wako Pure Chemical Industries (Osaka, Japan) unless otherwise indicated.

Patients

We analyzed 89 patients with HCV infection. The diagnosis was based on elevated serum aminotransferase level, histological examination, consistent detection of anti-HCV, and HCV-RNA. Patients who coincided with other causes of liver disease such as chronic hepatitis B, autoimmune hepatitis, or alcoholic liver disease (greater than 80 g alcohol per day for at least 1-month duration prior to the onset of illness) were excluded, as were those who had been taking corticosteroids or those with a history of, or evidence of, pancreatitis or a pancreatic tumor. Clinical data collected before antiviral therapy included age, sex, and alcohol use. BMI was calculated as body weight in kilograms divided by the square of height in meters (kg/m^2). Informed consent for participation in the study was obtained from each subject. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in prior approval by the Ethics Committee of the Kurume University School of Medicine. None of the subjects was institutionalized.

Laboratory Determinations

Venous blood samples were taken in the morning after a 12-h overnight fast. Plasma glucose, serum aspartate aminotransferase, alanine aminotransferase, albumin, total bilirubin, and immunoreactive insulin (IRI) levels were measured by using standard clinical methods (Department of Clinical Laboratory, Kurume University Hospital). Beta-cell function and insulin resistance were calculated on the basis of fasting levels of plasma glucose and IRI, according to the homeostasis model assessment (HOMA) method (18). The formulas for the HOMA model are as follows: beta-cell function (HOMA-%B) = fasting IRI (μU/mL) × 360/(fasting glucose (mg/dL) − 63); insulin resistance (HOMA-IR) = fasting glucose (mg/dL) × fasting IRI (μU/mL)/405. HCV genotyping was performed according to Okamoto's method (19) and genotypes were classified according to Simmonds's classification system (20). An Amplicor-HCV-Monitor 1.0 (Roche Diagnostics K.K., Tokyo, Japan) was used to quantify HCV-RNA levels.

Liver Biopsy

Liver tissue was obtained by percutaneous ultrasound image-guided liver biopsy. The biopsies were performed by two staff gastroenterologists using a Pro-Mag™ Biopsy Needle (Medical Device Technologies Inc., Gainesville, FL), which has a biopsy specimen notch of 20.00 mm in width and 2.05 mm in diameter. More than 95% (vol/vol) of liver tissue was used for histological and immunostaining analyses. Less than 5% (vol/vol) of liver tissue was homogenized and 80 μg of protein was used for immunoblotting analysis.

Histological Data

For each patient, a liver biopsy specimen was fixed in 10% formalin buffer and stained with hematoxylin-eosin. Liver biopsy specimens were evaluated by a single, experienced pathologist who was unaware of the patients' clinical and laboratory data. The specimens were scored according to the METAVIR scoring system (21), which is suited for evaluation of chronic hepatitis C.

Treatment Outcome

All patients were treated with 3 to 10 million U of interferon-α (interferon-α2a, Nippon Roche K.K., Tokyo, Japan; interferon-α2b, Schering-Plough K.K., Osaka, Japan; or natural interferon-α, Dainippon Sumitomo Pharma Co., Osaka, Japan) by subcutaneous injection three times per week, or 6 to 10 million U of interferon-α2b plus ribavirin (600 to 1,000 mg daily, Schering-Plough Co) for 6 months. Patients were followed up until 6 months after the conclusion of antiviral therapy and classified into three groups: sustained responders (N = 29), who had undetectable serum HCV-RNA; relapsers (N = 12), who had undetectable HCV-RNA at the end of antiviral therapy but HCV-RNA relapse during follow-up; and nonresponders (N = 48), who had detectable HCV-RNA during and after treatment.

Immunoblotting

Liver tissue was homogenized on ice in 1 mmol/L $NaHCO_3$ containing protease inhibitors, stored at −80°C as previously described (22, 23). Equal amounts of protein (40 μg) from liver homogenates were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis on a 7.5% acrylamide gel. The resolved proteins were transferred electrophoretically onto polyvinylidene difluoride membranes (Amersham International, Buckinghamshire, UK). The membranes were incubated with an antihuman IRS1 polyclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA) or an antihuman IRS2 polyclonal antibody (Santa Cruz Biotechnology), and were subsequently incubated with an HRP-conjugated goat antirabbit IgG (Amersham International). The membranes were then incubated with chemiluminescence reagents (ECL kit, Amersham International) and immediately exposed on

radiograph film. Immunoblotting intensities were determined using NIH-Image J (developed at the National Institutes of Health and available from the Internet by an anonymous FTP from http://rsb.info.nih.gov/ij/download.html) as previously described (22, 23).

Immunohistochemistry

In 14 sustained responders, liver biopsy was performed before and after conclusion of antiviral therapy. Paraffin-embedded liver sections from patients with HCV infection were deparaffinized and subjected to immunohistochemical staining using a Vectastain ABC kit (Vector Laboratories, Burlingame, CA) with an antihuman IRS1 polyclonal antibody (Santa Cruz Biotechnology) or an antihuman IRS2 polyclonal antibody (Santa Cruz Biotechnology), and developed with 3,3′-diaminobenzidine (DAB). The primary antibodies for IRS1/2 were used at a 1:100 dilution. The specificity of IRS1/2 staining was confirmed by immunization using an excess amount of the N-terminal peptide of IRS1/2.

Statistical Analysis

All data are expressed as mean ± SD. The Wilcoxon's single-rank test was employed for analysis of paired samples. Statistical comparisons among multiple groups were performed by analysis of variance followed by Scheffe's *post hoc* test. P values < 0.05 were considered significant.

RESULTS

Characteristics of the Patients

Characteristics of the patients before antiviral therapy are summarized in Table 1. There was no significant difference in age or sex distribution among the groups. In sustained responders, higher infection rates of genotype 2 (62.0%) were seen compared with nonresponders (12.5%) or relapsers (16.7%). Although HCV viral load, hepatic fibrosis, and HOMA-IR were lower in sustained responders, BMI and hepatic necroinflammatory activity were not significantly different among the groups.

Changes in BMI, Insulin Resistance, and Beta-Cell Function After Antiviral Therapy

Changes in BMI, insulin resistance, and beta-cell function after antiviral therapy are summarized in Figure 1. In nonresponders (N = 48), BMI significantly decreased to 21.7 ± 1.6 kg/m^2 from 22.7 ± 2.3 kg/m^2 ($P < 0.01$) at the end of follow-up. However, there were no significant changes in HOMA-IR and HOMA-%B values at the end of follow-up compared with those before antiviral therapy (HOMA-IR 4.0 ± 1.7 *vs* 3.6 ± 1.2, $P = 0.11$, HOMA-%B 120.0 ± 26.1 *vs* 112.4 ± 24.1, $P = 0.09$) (Fig. 1A). In relapsers (N = 12) no significant differences were seen in BMI (21.8 ± 1.7 kg/m^2 *vs* 22.1 ± 1.6 kg/m^2, $P = 0.70$), HOMA-IR values (3.7 ± 1.2 *vs* 3.6 ± 1.2, $P = 0.69$), and HOMA-%B values (121.5 ± 13.3 *vs* 117.4 ± 17.4, $P = 0.24$) at the end of follow-up compared with those before antiviral therapy (Fig. 1B). In sustained responders (N = 29), there was no significant difference in BMI at the end of follow-up (22.6 ± 1.6 kg/m^2 *vs* 21.9 ± 1.9 kg/m^2, $P = 0.07$). On the other hand, HOMA-IR values significantly decreased to 2.2 ± 0.7 from 3.1 ± 1.0 ($P < 0.01$) by the end of follow-up. Similarly, HOMA-%B values significantly decreased to 92.6 ± 14.0 from 113.7 ± 21.3 ($P < 0.01$) (Fig. 1C).

Changes in Hepatic Expression of IRS1/2 in Sustained Responders

Immunoblotting demonstrated a significant increase in expression of IRS1/2 after antiviral therapy in livers from sustained responders (Fig. 2A). After antiviral therapy, mean IRS1 and IRS2 intensities showed a two- and threefold increase, respectively, compared with intensities before antiviral therapy (Table 2). In immunostaining, IRS1 occurred mainly in lymphocytes (Fig. 2B, left upper panel) before antiviral therapy, but occurred in hepatocytes after antiviral therapy (Fig. 2B, right upper panel). On the other hand, IRS2

Table 1. Characteristics of the Patients

	Nonresponders	Relapsers	Sustained Responders	*P* Value
N	48	12	29	
Age (yr)	61.7 ± 7.7	63.2 ± 6.1	58.5 ± 8.6	N.S.
Male/female	27/21	8/4	19/10	N.S.
BMI	22.7 ± 2.3	21.9 ± 1.7	22.6 ± 1.6	N.S.
Aspartate aminotransferase (U/L)	68.1 ± 36.3	75.7 ± 24.4	64.2 ± 30.4	N.S.
Alanine aminotransferase (U/L)	92.5 ± 35.1	86.3 ± 24.8	88.7 ± 30.4	N.S.
γ-glutamyltranspeptidase (U/L)	97.9 ± 44.6	88.0 ± 37.1	94.0 ± 34.9	N.S.
Total bilirubin (mg/dL)	0.84 ± 0.11	0.81 ± 0.20	0.85 ± 0.15	N.S.
Albumin (g/dL)	3.79 ± 0.26	3.71 ± 0.29	3.87 ± 0.28	N.S.
Genotype 1/2	42/6	10/2	11/18	0.008
Viral load (×10^3 copies)	485 ± 299	534 ± 254	309 ± 212	0.024
Necroinflammatory activity	2.04 ± 0.71	2.00 ± 0.74	1.90 ± 0.78	N.S.
Fibrosis	2.29 ± 0.74	2.25 ± 0.87	1.82 ± 0.81	0.046
HOMA-IR	3.95 ± 1.69	3.73 ± 1.21	3.07 ± 0.95	0.01

Data are expressed as mean ± SD or as number of patients.
BMI = body mass index; HOMA-IR = homeostasis model assessment for insulin resistance.

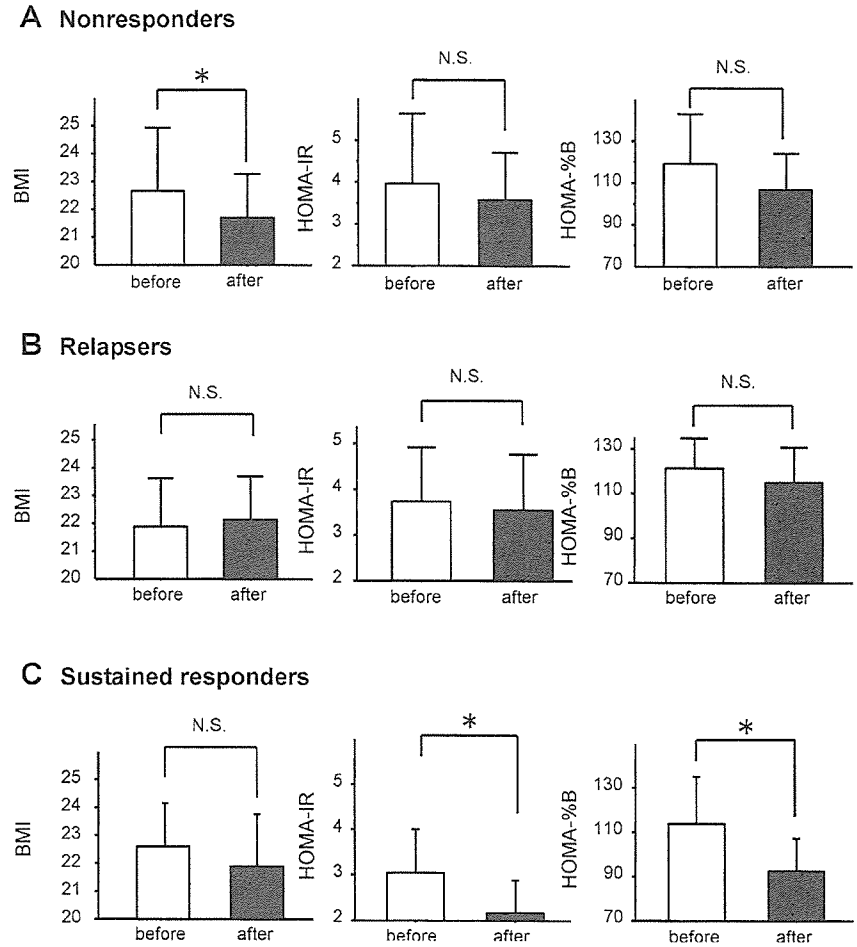

Figure 1. BMI, HOMA-IR, and HOMA-%B before and after antiviral therapy in nonresponders (N = 48; *A*), relapsers (N = 12; *B*), and sustained responders (N = 29; *C*). Data were obtained before antiviral therapy and 6 months after its conclusion. Data are expressed as mean ± SD. $^{*}P < 0.01$. N.S., not significant.

occurred in hepatocytes both before and after antiviral therapy (Fig. 2B, lower panels). After antiviral therapy, expression of IRS2 was upregulated mainly in periportal hepatocytes (Fig. 2B, right lower panel).

DISCUSSION

The present study demonstrates that clearance of HCV improves HOMA-IR, HOMA-%B, and hepatic expression of IRS1/2. These findings indicate that HCV itself is involved in the development of insulin resistance.

Insulin resistance can be caused by many factors. Obesity is a common factor for the development of insulin resistance (24). Although greater insulin resistance was seen in patients with chronic hepatitis C, BMI values were within normal limits in this study. Improved HOMA-IR was only seen in sustained responders and HOMA-IR remained unchanged in nonresponders, despite a decrease in BMI after antiviral therapy. In an epidemiologic study, Bahtiyar *et al.* reported that obesity is not associated with the development of insulin resistance in patients with HCV infection (25). In addition, the development of insulin resistance is seen by 1 month of age, in the absence of either overt liver injury or excessive body weight gain in HCV core transgenic mice (13) and serum HCV core protein levels are associated with HOMA-IR values in patients with chronic hepatitis C (15). Moreover, a significant increase in the incidence of diabetes was seen in subjects with high titers of HCV core compared with subjects with low titers of HCV core or anti-HCV-negative subjects at the population level during 7 yr of follow-up (26). Taken together, these findings suggest that HCV itself causes insulin resistance.

Interferon is known to induce insulin resistance. However, our results showed that interferon leads to a reduction in insulin resistance in sustained responders. Even in nonresponders or relapsers, interferon did not worsen insulin resistance. Interferon-induced insulin resistance is observed only in the early phase of treatment (27). Indeed, after 3 months of treatment, interferon-induced insulin resistance disappears (28).

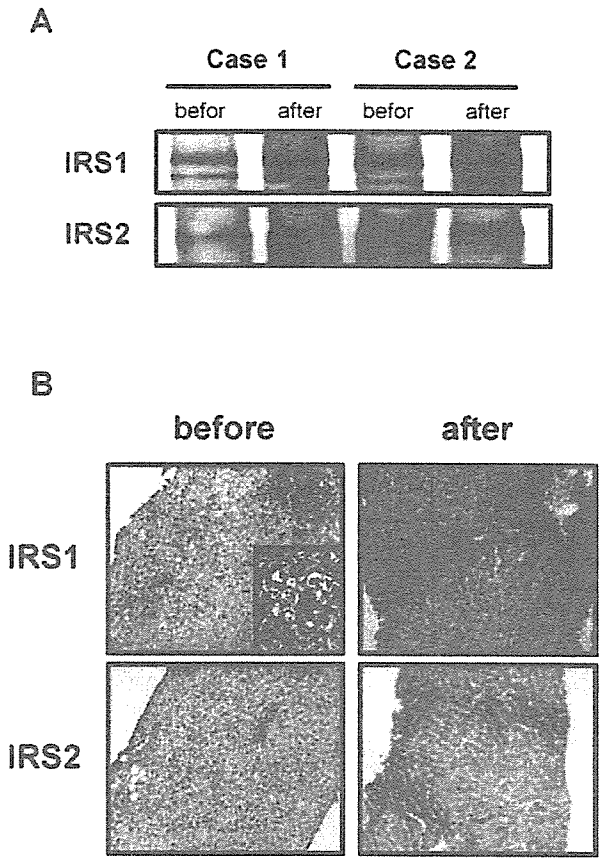

Figure 2. Protein expression levels of IRS1/2 before and after antiviral therapy in sustained responders. Immunoblotting for IRS1/2 (*A*). Proteins in liver extracts before and after antiviral therapy were immunoblotted with anti-IRS1 antibodies (upper panel) or anti-IRS2 antibodies (lower panel). Immunostaining for IRS1/2 (*B*). Liver sections before and after antiviral therapy were immunostained with anti-IRS1 antibodies (upper panels) or anti-IRS2 antibodies (lower panels). Expression of IRS1 and IRS2 were visualized by 3,3′-diaminobenzidine (brown). Expression of IRS1 in lymphocytes was shown. Original magnification ×400. Protein expression levels of IRS1/2 were examined in 14 sustained responders and representative immunoblotting and immunostaining images are shown.

Romero-Gomez *et al.* reported that improved insulin resistance during and after interferon therapy is correlated with a positive response to antiviral therapy (29), which is in good agreement with our findings. These finding also suggest the involvement of HCV in the development of insulin resistance.

Pancreatic beta-cells play a crucial role in maintaining glucose homeostasis. Although HCV infects not only liver but also pancreas (30), our results demonstrated that beta-cell function, especially the ability to secrete insulin, was preserved in patients with chronic hepatitis C. Because HOMA-%B was significantly decreased after antiviral therapy in sustained responders, increase in HOMA-%B seems to be an adaptation against greater insulin resistance. On the other hand, Narita *et al.* reported that beta-cell function is significantly decreased in patients with HCV infection (31). Although the reasons for this discrepancy are not clear, it could be explained by following reasons: First, BMI in the previous study is higher than that in our study. Second, patients who consumed alcohol were enrolled in the previous study, while we excluded the patients who had >80 g/day of alcohol. Obesity and alcohol consumption lead to a decrease in early-phase insulin secretion (32, 33). In addition, HCV core-transgenic mice exhibited a significant increase in early-phase insulin secretion compared with control mice (13). Thus, dysfunction of beta-cells does not seem to be responsible for HCV-associated insulin resistance.

Table 2. Hepatic Expression Levels of IRS1/2 Before and After Antiviral Therapy in Sustained Responders

	N	Before (Arbitrary Units)	After (Arbitrary Units)	*P*
IRS1	14	83.3 ± 47.9	156.8 ± 47.5	0.002
IRS2	14	31.7 ± 16.4	88.0 ± 33.8	0.001

Data are expressed as mean ± SD.

TNF-α is a causative factor for greater insulin resistance. However, there was no significant difference in serum TNF-α level between HCV patients with insulin resistance and without insulin resistance (34). Impairment of insulin receptor can cause insulin resistance. However, there was no significant change in hepatic insulin receptor between controls and HCV core-transgenic mice (15). Recently, we identified a molecular mechanism for HCV-associated insulin resistance. HCV core downregulates hepatic expression of IRS1/2 (15). Because IRS1 and IRS2 are central molecules in intracellular insulin signaling, downregulation of these molecules should decrease downstream insulin effects such as glucose uptake, thereby contributing to insulin resistance. In this study, we first demonstrated increases in hepatic expression of IRS1/2 after antiviral therapy in sustained responders. These findings support our proposed molecular mechanism that HCV directly downregulates hepatic expression of IRS1/2.

In conclusion, we showed that clearance of HCV improves HOMA-IR, HOMA-%B, and hepatic expression of IRS1/2. These findings indicate that HCV itself is involved in the development of insulin resistance in patients with HCV infection.

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STUDY HIGHLIGHTS

What Is Current Knowledge

- Greater insulin resistance and hyperinsulinemia are seen in patients with hepatitis C virus (HCV) infection.
- Insulin receptor substrate 1 and 2 (IRS1/2), central molecules in insulin signaling, are downregulated in livers from patients with HCV infection.

What Is New Here

- Clearance of HCV reduced insulin resistance and improved hyperinsulinemia.
- Hepatic expression of IRS1/2 was increased by clearance of HCV.

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CONFLICT OF INTEREST

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Potential competing interests: None

Case Report

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Simultaneous Hepatic Relapse of Non-Hodgkin's Lymphoma and Hepatocellular Carcinoma in a Patient with Hepatitis C Virus-Related Cirrhosis

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Key Words

Hepatitis C virus · Hepatocellular carcinoma · Non-Hodgkin's lymphoma · Radiofrequency ablation · Rituximab · THP-COP

Abstract

We report a 66-year-old man with hepatitis C virus (HCV)-related cirrhosis and simultaneous hepatic relapse of non-Hodgkin's lymphoma (NHL) and of hepatocellular carcinoma (HCC). Although the liver is frequently involved by NHL, hepatic colocalization of NHL and HCC is rarely detected by imaging techniques. HCV has been suggested to be lymphotrophic as well as hepatotrophic, and therefore has attracted speculation about a causative role in some cases of lymphoma. The patient had a past history of cutaneous diffuse large B cell lymphoma (DLBCL) in concurrence with HCC 32 months previously. Complete remission (CR) had been maintained for both diseases until February 2004, when ultrasonography and computed tomography (CT) showed multiple liver tumors. Two of these, appearing hyperattenuating in the arterial phase of contrast-enhanced CT, were diagnosed histopathologically as HCC, and treated with radiofrequency ablation. The other tumors, hypoattenuating in the portal phase CT, were diagnosed histopathologically as DLBCL, and treated with cyclophosphamide, tetrahydropyranyl-Adriamycin, vincristine and prednisolone (THP-COP) in combination with rituximab. CR was achieved for both DLBCL and HCC. Given the previously demonstrated immune system tropism and perturbation by HCV, the virus might have contributed to the occurrence of the NHL as well as the HCC.

Introduction

An Italian study reported B cell malignant diseases as the most frequent neoplasms associated with hepatocellular carcinoma (HCC) [1], but few such cases have involved colocalization of both within the liver. According to the report on the focal liver lesions detected by imaging techniques in 414 patients with non-Hodgkin's lymphoma (NHL) [2], only 1 case presented with simultaneous coexistence with NHL and HCC. We know of only four previously reported similar cases [3–6], all associated with hepatitis B virus (HBV) infection. How HBV

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and development of lymphoma are related is not known, although some reports have suggested a possible pathogenetic role of HBV in the development of hematologic malignant diseases [7, 8].

Hepatitis C virus (HCV) is well known as a causative agent of chronic hepatitis, which often progresses to liver cirrhosis and HCC in the chronically infected patients. On the other hand, HCV has been associated with various extrahepatic autoimmune diseases [9]. HCV, being lymphotrophic as well as hepatotrophic [10], has attracted speculation about a causative role in some cases of lymphoma [11, 12]. In particular, several investigators have reported an association between HCV and B cell NHL [13–16]. Chronic antigenic stimulation by HCV has been suspected to be related to the development of clonal B cell expansion [12, 17], although the mechanism is not clear. We present a patient with HCV-related cirrhosis who showed simultaneous intrahepatic relapses of HCC and of B cell lymphoma without extrahepatic involvement.

Case Report

A 66-year-old man with HCV-related cirrhosis was referred to our hospital in March 2004 for treatment of newly detected liver tumors. The patient had a 16-year history of HCV-related chronic hepatitis and also a history of treatment of cutaneous diffuse large B cell lymphoma (DLBCL) in concurrence with HCC diagnosed in May 2001. At that time the DLBCL additionally involved the bone marrow, and was assigned to stage IV according to the Ann Arbor staging system. Subsequently complete response (CR) had been maintained after chemotherapy. Further, radiofrequency ablation (RFA) of the HCC located in the left lobe of the liver had been performed successfully; no other lesion had been detected until shortly before the present admission.

In February 2004, ultrasonography and computed tomography (CT) showed multiple tumors in the right lobe of the liver. All tumors except two were hypoattenuating in the portal phase of contrast-enhanced CT (fig. 1a). The other two tumors located in segment 8, were hyperattenuating in the arterial phase (fig. 1b). Histologic examination of a percutaneous needle biopsy specimen obtained from a hypoattenuating tumor showed infiltration by abnormal lymphoid cells with large and sometimes irregularly shaped nuclei (fig. 2a). Immunohistochemical staining indicated that the lymphoid cells were positive for CD20 (fig. 2b), and the tumor was diagnosed as DLBCL.

In March 2004 the patient was admitted to our hospital for treatment. Physical examination on admission disclosed pallor, spider angiomata, and ascites. Laboratory data obtained on admission showed decreases of choline esterase (40 IU/l; normal range 107–233), albumin (3.0 g/dl; normal range 4.0–5.0), and total cholesterol (125 mg/dl; normal range 128–256) as well as an increase of total bilirubin (2.46 mg/dl; normal range 0.0–1.5). Serum concentrations of aspartate aminotransferase, alanine aminotransferase, and lactate dehydrogenase were normal, as was prothrombin time. Soluble interleukin 2 receptor was increased in serum (1,313 U/ml; normal range 220–530). The serum concentration of α-fetoprotein was normal (7.3 ng/ml; normal range 0–8.7), but PIVKA-II (protein induced by vitamin K absence or antagonist II) was increased (254 mAU/ml; normal range 0–40). No extrahepatic involvement by DLBCL was detected in CT, ^{67}Ga scintigraphy, or bone marrow examination.

Following the diagnosis of DLBCL, two courses of cyclophosphamide, tetrahydropyranyl-Adriamycin, vincristine and prednisolone (THP-COP) were given combined with a course of rituximab. As a result, all tumors except two disappeared or decreased greatly in size according to CT (fig. 1c). The two nonresponding tumors were those that were shown as hyperattenuating lesions in the arterial phase of contrast CT (fig. 1d). An abdominal angiogram demonstrated that these two tumors were hypervascular (fig. 3). Histologic examination of a percutaneous needle biopsy specimen obtained from one of these two tumors showed moderately differentiated HCC with a trabecular and pseudoglandular growth pattern (fig. 4). Chemotherapy for DLBCL was suspended, as it had compromised the patient's liver function and exacerbated ascites (fig. 1c). After improvement of liver function, RFA of the two HCC was performed successfully. A CR was attained for both DLBCL and HCC.

Discussion

In a Japanese study concerning extrahepatic primary cancers in 384 patients with HCC, no B lymphocyte-derived neoplasms were detected [18]. On the other hand, an Italian study of 317 patients with HCC found B cell-derived neoplasms to represent the most frequent cancers associated with HCC, accounting for 10 of 35 extrahepatic primary neoplasms, or 28.6% [1]. Disagreement between these two reports concerning the frequency of B cell neoplasms in patients with HCC is likely to involve the difference in ethnicity between study subjects. In Italy, B cell NHL is reported to show a frequent association with HCV. Accordingly, patients with HCV-related HCC are likely to be at increased risk for B cell-derived neoplasms.

As mentioned, the Italian study included 10 patients with B lymphocyte-derived neoplasms associated with HCC. These were varied: 7 cases of NHL, 2 cases of multiple myeloma, and 1 chronic lymphocytic leukemia [1]. These cases also showed a relatively nonspecific distribution pattern, that of double cancers with the B cell neoplasms involving essentially any part of the body. In our patient, DLBCL coexisted with HCC within the liver, with no extrahepatic involvement. According to the report on the focal liver lesions detected by imaging techniques in 414 patients with NHL [2], hepatic lymphomatous involvement was observed in 69 cases, and HCC in

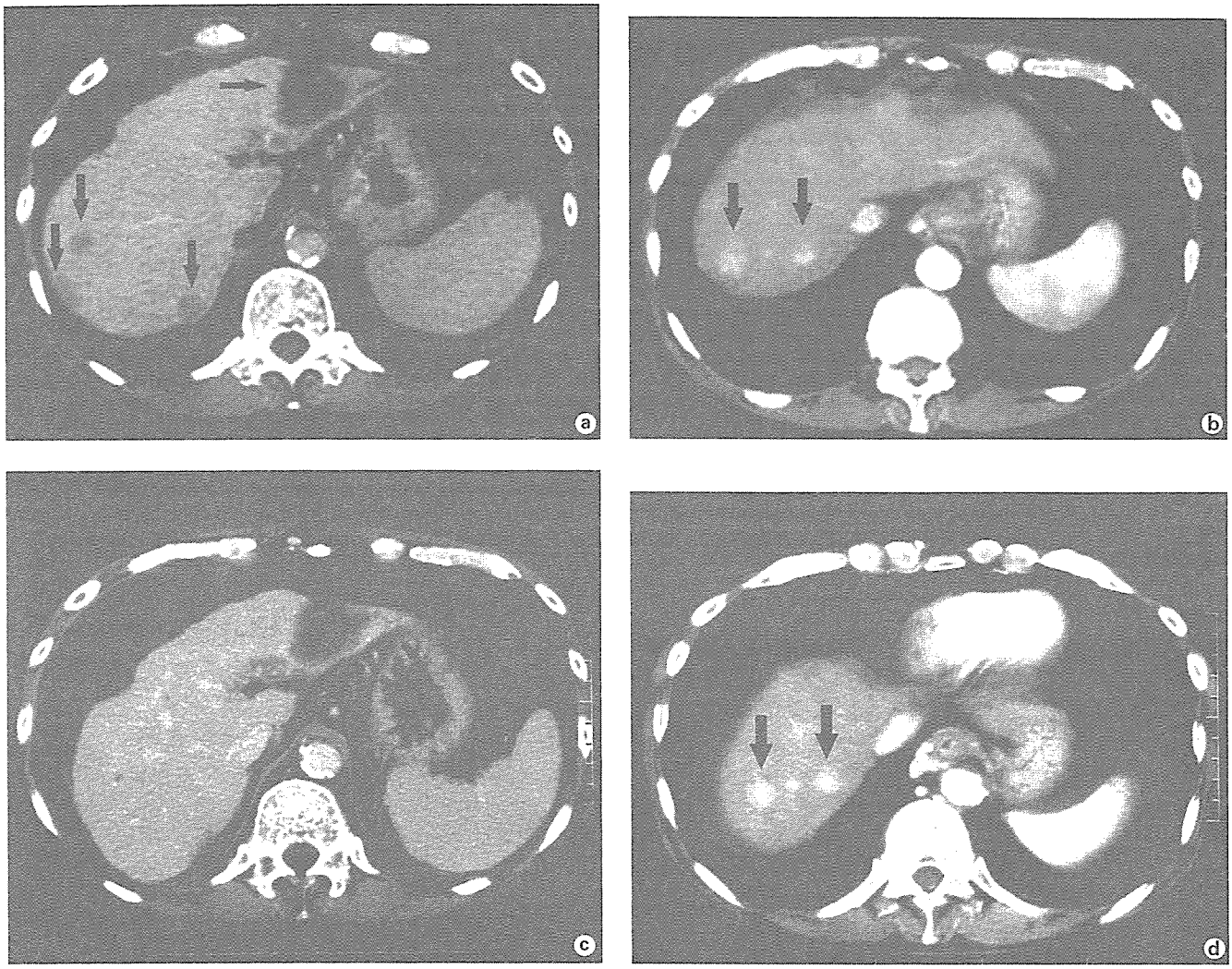

Fig. 1. CT findings during the patient's recent course. **a** Contrast-enhanced CT in the portal phase showed multiple hypoattenuating tumors in the liver (vertical arrows). The horizontal arrow in the left lobe indicates an area of necrosis where RFA had previously been performed. **b** CT in the arterial phase showed two hyperattenuating tumors (arrows) in hepatic segment 8. **c** CT in the portal phase after THP-COP with rituximab showed disappearance or shrinkage of the hypoattenuating tumors shown in **a**. Marked ascites can also be seen. **d** CT in the arterial phase after THP-COP with rituximab showed no shrinkage of the two hyperattenuating tumors shown in **b**.

7 cases, yet only 1 case presented with a simultaneous coexistence of NHL and HCC. That case was described by Cavanna et al. [6]. Although liver is the common site for lymphomatous involvement and occurrence of HCC, we rarely see such a case where the two tumors were simultaneously detected by imaging techniques as distinct hepatic mass lesions without extrahepatic involvement. We know of only 4 previously reported similar cases. Talamo et al. [3] were the first to report a case of simultaneous occurrence of primary hepatic lymphoma and HCC. Takeshima et al. [4] reported a patient with hepatic occurrence of mucosa-associated lymphoid tissue lymphoma together with HCC. These 2 cases showed no evidence of extrahepatic involvement by lymphoma, and they are considered to represent primary hepatic lymphoma, defined as confined to the liver with no evidence of lymphomatous involvement in the spleen, bone marrow, or other lymphoid structures. Shikuwa et al. [5] reported a case

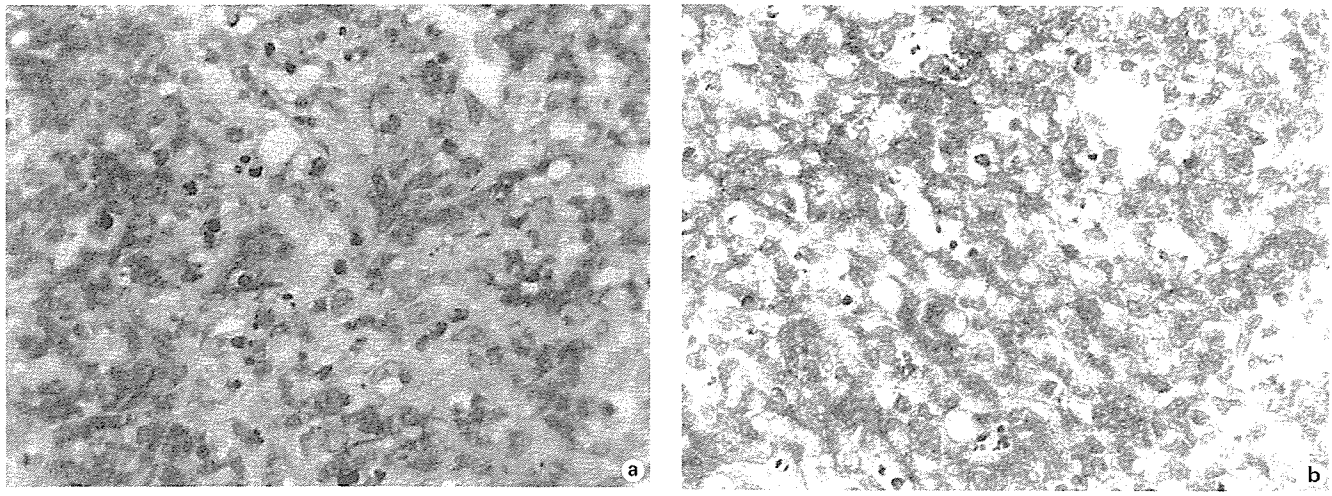

Fig. 2. Microscopic findings in a percutaneous needle biopsy specimen from a hypoattenuating lesion. **a** The liver showed infiltration by abnormal lymphoid cells with large, sometimes irregularly shaped nuclei. Hematoxylin and eosin. ×400. **b** The abnormal lymphoid cells were positive for CD20. Immunohistochemical staining. ×400.

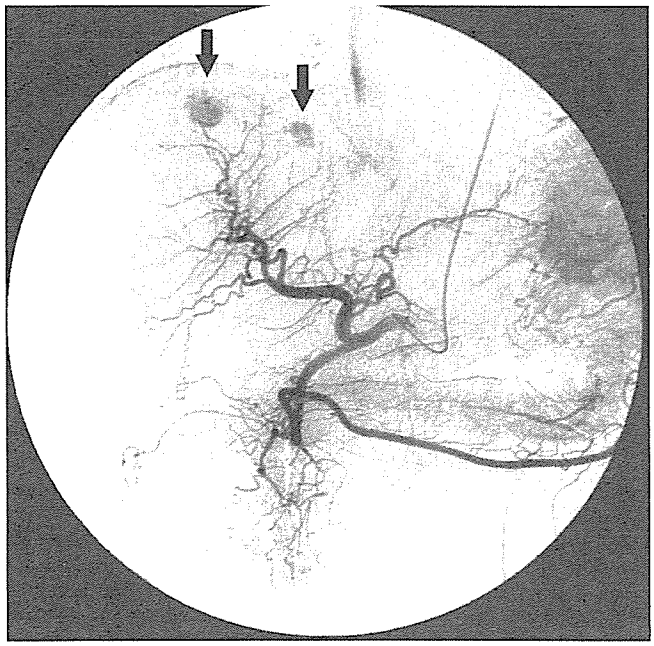

Fig. 3. An abdominal angiogram demonstrated two hypervascular tumors (arrows) in hepatic segment 8.

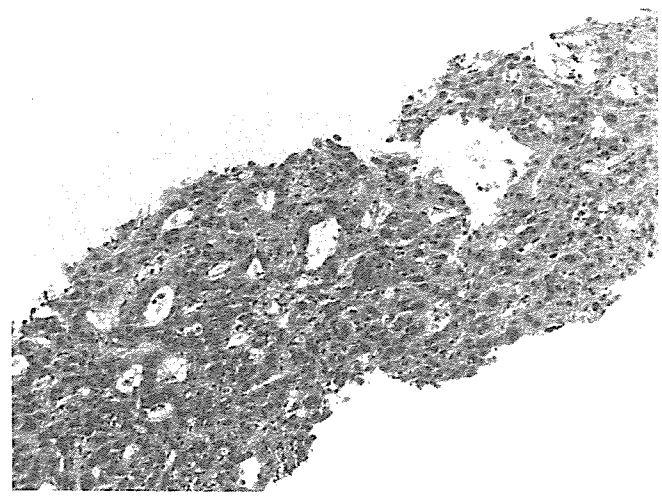

Fig. 4. Histologic findings in a percutaneous needle biopsy specimen from a hyperattenuating, hypervascular lesion showed moderately differentiated HCC with a trabecular and pseudoglandular growth pattern. Hematoxylin and eosin. ×100.

of colocalized HCC and malignant lymphoma, but autopsy demonstrated that the lymphoma involved the bone marrow in addition to the liver. Cavanna et al. [6] reported a case where NHL relapsed in liver without extrahepatic involvement in a patient with HCC. Our patient also presented with relapse, specifically simultaneous relapse of HCC and DLBCL with no extrahepatic involvement. It has been shown that metastatic cell sub-

populations can outgrow their nonmetastatic counterparts within the primary tumor and it was suggested that the metastatic potential of a primary tumor may increase during the course of its growth [19]. Rowbotham et al. [20] referred to the apparent predilection of tumors to invade the liver in patients with acute liver failure secondary to hepatic infiltration and thus suggested the presence of a phenotypic lymphomatous subtype with selective organ invasion, and additionally reported that over one quarter of patients with lymphoma had a history of previous treatment for the same disease. This fact suggests that chemotherapy might change the behavior of the tumors and enhance the properties which preferentially target and invade the liver. The appearance of such chemotherapy-induced lymphoma cell subpopulations as selectively invading the liver and recurrence of HCC may have resulted in the simultaneous colocalization of the two distinct tumors. Our case report demonstrates that it is important to pay attention to patients with HCV or with a previous history of malignant lymphoma at the diagnosis of hepatic mass lesions.

Importantly, all 4 cases reported prior to ours were associated with HBV infection. Although some reports have suggested a possible pathogenetic role of HBV in the development of hematologic malignant diseases [7, 8], a basis for a relationship between HBV and lymphoma occurrence is not clear. In distinction to the other cases, ours is associated with HCV, not HBV, infection. HCV is a well-known cause of chronic hepatitis, which in these chronically infected patients often progresses to cirrhosis and eventually HCC. On the other hand, HCV has also shown reported associations with various extrahepatic autoimmune diseases, such as mixed cryoglobulinemia, Sjögren's syndrome, renal disease, and neuropathy [9]. As a lymphotropic virus [10], HCV is suspected to contribute to the etiology of B cell NHL [11, 12]. A relationship between HCV and NHL has been demonstrated by many investigators in Italy [13, 14], the United States [15], and Japan [16]. Especially in Italy, a high proportion of HCV positivity has been reported among patients with NHL. Ascoli et al. [21] reported HCV-related extranodal B cell lymphomas of various types. The apparent relationship was supported by a report demonstrating regression in splenic lymphoma with villous lymphocytes in patients with HCV after treatment of the virus with interferon α [22]. In patients with type II mixed cryoglobulinemia, the most common immune disorder related to chronic HCV infection, the paraprotein is a monoclonal IgM rheumatoid factor indicative of clonal B cell proliferation [23]. Thus, chronic B cell stimulation by HCV-related antigens has been proposed as a causative factor in neoplastic transformation [12, 17], although details of the underlying mechanism remain unclear. Our patient had been infected by HCV for over 16 years; indeed, HCV might have caused his malignant lymphoma as well as HCC to result in a unique HCV-related simultaneous hepatic colocalization of HCC and NHL.

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Case Report

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Appearance-specific Satiety Increases Appetite and Quality of Life in Patients with Metastatic Liver Tumor: A Case Report

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Summary: Loss of appetite is frequently seen and is a main factor affecting quality of life (QOL) in patients with advanced cancer. The etiology for loss of appetite in patients with cancer is multifactorial. The sensory properties of food are factors regulating appetite. Changes in taste, smell and texture of foods influence food intake. The appearance of the food is also a notable factor in sensory-specific satiety. We described a 46-year-old Japanese woman with multiple metastatic liver tumors. Although there was no obvious factor for loss of appetite, she suffered from a loss of appetite and subsequent malnutrition. In order to improve the appearance of food, we reduced the diet to 1000 kcal/day from 1500 kcal/day. On the new diet, the patient's appetite significantly increased and patient's nutritional status was improved. Eating whole diet was an important achievement and increased in mental aspects of QOL. Arrangement for the appearance of food may be a simple and nontoxic therapeutic strategy for patients with cancer suffering a loss of appetite.

Key words advanced cancer, malnutrition, sensory-specific satiety, anorexia, nutritional status, SF-36

INTRODUCTION

Patients with advanced cancer often suffer from a loss of appetite, leading to malnutrition [1]. As many as 20% of patients with cancer die of the effects of malnutrition rather than of the malignancy [2]. Loss of appetite is also a main factor affecting quality of life (QOL). Thus, maintaining appetite is a valuable part of managing patients with advanced cancer.

The etiology for loss of appetite in patients with cancer is multifactorial [3]. Loss of appetite can result from systemic effects of cachexia and altered metabolism by tumor. Local effects of tumor, such as obstruction and pain, can also interfere with dietary intake. Furthermore, treatment for cancer may cause fatigue, taste changes, diarrhea, and nausea, which lead to a subsequent loss of appetite [4,5].

Currently prescribed appetite stimulatory drugs are corticosteroids, megestrol acetate, and metoclopramide. Although corticosteroids may increase appetite, their metabolic, infectious, and psychiatric side effects usually limit their use to the short term. Megestrol acetate has been found to improve appetite [6], but is associated with water retention and an increase in the risk of venous thromboembolism. Metoclopramide has been shown to improve chronic nausea [7], but it has no proven effect on appetite. Clearly, many patients are not helped by or cannot tolerate currently available treatments to stimulate appetite. A simple and nontoxic therapeutic strategy

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Abbreviations: MH, mental health; QOL, quality of life; RE, role-emotional; SCC, squamous cell carcinoma; SF, social functioning; SF-36v2, medical outcomes study 36-item short-form health survey version 2; VT, vitality.

for increasing appetite is needed.

The sensory properties of food regulate appetite. Several studies have been carried out to investigate the impact of food varying in sensory properties. Changes in taste, smell, and texture of foods can influence food intake [8,9]. An increase in the amount of pasta offered affects its' appearance, resulting in a decrease in the pleasure of eating [8]. The appearance of the food seems to be a notable factor in sensory-specific satiety. In other words, the appearance of food may influence appetite through visual information.

Patients with cancer are usually prescribed a high-calorie diet because cancer increases energy expenditure. High-calorie diet is accompanied by heavy appearance, which can provide a visual stress leading to a decrease in appetite. It is possible that the appearance of the food in a high-calorie diet spoils appetite in patients with advanced cancer.

In this report, we describe a case in which the appearance-specific satiety of food increased appetite and improved nutritional status and QOL as well. Arrangement for appearance of food may be a simple and nontoxic therapeutic strategy for patients with cancer suffering a loss of appetite.

CASE REPORT

A 46-year-old Japanese woman was referred to Kurume University Hospital for treatment of multiple metastatic liver tumors and obstructive jaundice. The patient was diagnosed at age 44 with cervical cancer and was treated with radiation and chemotherapy following a radical hysterectomy. Two years later, computed tomography revealed multiple hepatic metastases due to cervical cancer and a rapid growth of metastatic liver tumors. These tumors were treated with a continuous intrahepatic arterial injection of 5-fluorouracil (375 mg/day) and intermittent cisplatin (30 mg/day) via a reservoir system for 5 days [10]. With 2 periods of this regimen, the serum squamous cell carcinoma (SCC) antigen level (normal range $<$ 1.5 ng/ml) decreased from 119.4 ng/ml to 30.1 ng/ml. Metastatic liver tumors were responsive to the chemotherapy.

The patient did not show any troublesome adverse effects of the anticancer drugs administered, such as vomiting and the patient's liver function was preserved, however, she suffered from loss of appetite 2 weeks before admission, which resulted in 4 kg loss of body weight. The patient's body mass index, arm muscle circumference, and triceps skin-fold thickness decreased to 14.6 kg/m^2, 16.3 cm, and 8 mm, respectively. The percentage of the age-adjusted standard value for Japanese women was 65.3%, 80.5%, and 47%, respectively. Laboratory data showed decrease in total lymphocyte count and levels of hemoglobin and albumin (Table 1). Thus, physical examination and laboratory data indicated a state of severe malnutrition.

TABLE 1.
Characteristics of the patient on admission

	Value	% of standard value
Physiological measurements		
Height	158.5 cm	101.9%
Weight	36.5 kg	67.9%
Body mass index	14.5	65.3%
Arm muscle circumference	16.3 cm	80.5%
Triceps skin-fold thickness	8 mm	47.0%
Loss of body weight in 2 weeks	4 kg	
Laboratory data		(Normal range)
Hemoglobin	7.0 g/100ml	(11-15)
Total lymphocyte count	435/μl	(1200-3870)
Albumin	3.2 g/dl	(4.0-5.0)
Total cholesterol	246 mg/dl	(128-256)
Glucose	86 mg/dl	(80-112)
Aspartate aminotransferase (U/l)	22 U/l	(13-33)
Alanine aminotransaminase (U/l)	34 U/l	(6-27)
Total bilirubin (mg/dl)	2.2 mg/dl	(0.3-1.5)
Prothrombin time	96%	(60-130)

Note. Standard values of physiological measurements were referred to Japanese anthropometric reference data

Acute bowel toxicity is one of the common complications of chemotherapy that leads to malnutrition. Oral administration of glutamine reduces this anticancer drug-induced bowel toxicity. The patient was treated with glutamine via oral administration, however, her appetite did not increase. Granisetron is effective against nausea induced by anticancer drugs. Nevertheless, loss of appetite persisted after the patient received an infusion of 3 mg of granisetron. Corticosteroids and megestrol acetate are also known to increase appetite in patients with advanced cancer. On the other hand, the long term use of these agents is associated with decreased efficacy and increasingly unacceptable adverse effects. Therefore, corticosteroids and megestrol acetate were not used in our case.

The patient's energy requirement was estimated to be 1400 to 1600 kcal/day, based on the Harris-Benedict equation [11]. Accordingly, the patient was prescribed a diet of 2000 or 1500 kcal/day. She understood the importance of nutritional therapy, however, her actual intake was only about 600 kcal/day. The sensory properties of the food play important roles in controlling the patient's food intake. Appearance is one of the important sensory properties and heavy appearance of food spoils appetite. In order to improve the appearance of food, we reduced the diet to 1000 kcal/day (Fig. 1). On the new diet, the patient's appetite significantly increased and she ate not only the prescribed diet but also snack. She maintained an intake of more than 1000 kcal/day (Figs 2A and B). Then, we followed her nutritional status until Day 76. Although total lymphocyte count showed no change on the new diet, there was a significant increase in serum albumin level (Figs 3A and B).

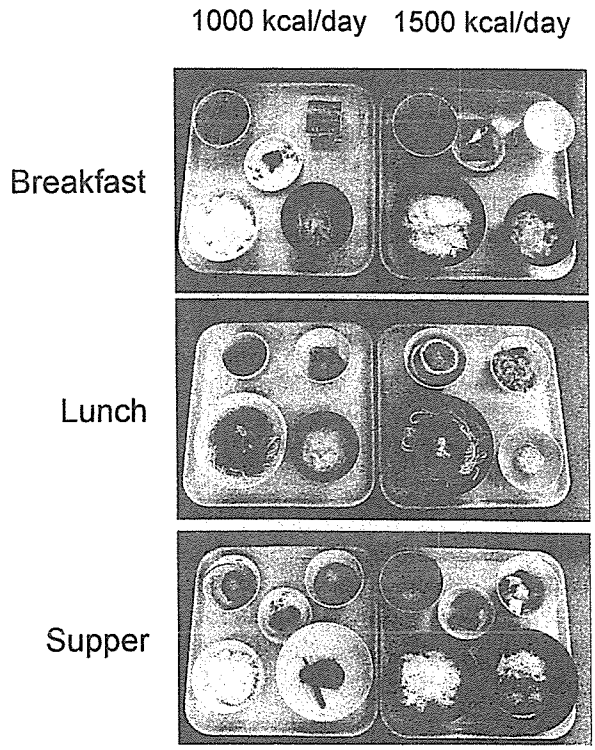


Fig. 1. Appearance of food in the 1000 kcal diet (left) and the 1500 kcal diet (right). A nutrient balance of 1000 kcal diet is similar to that of 1500 kcal diet.

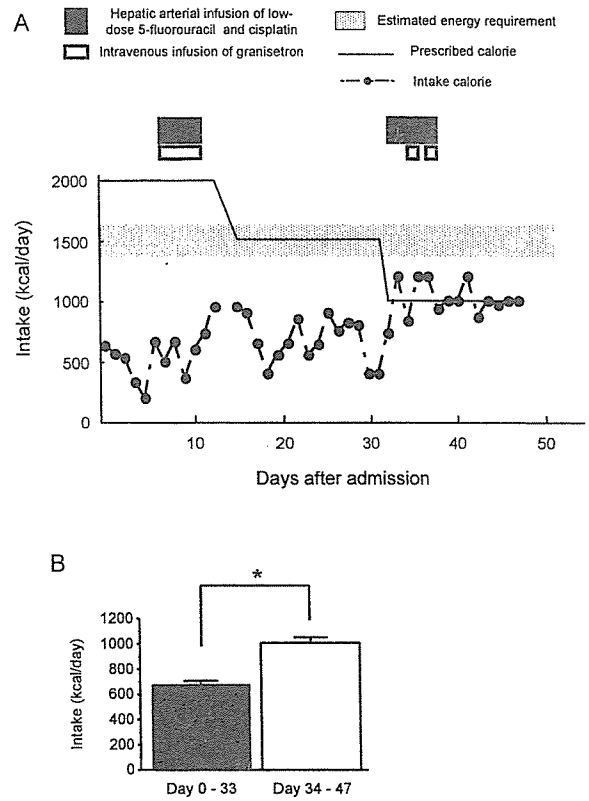
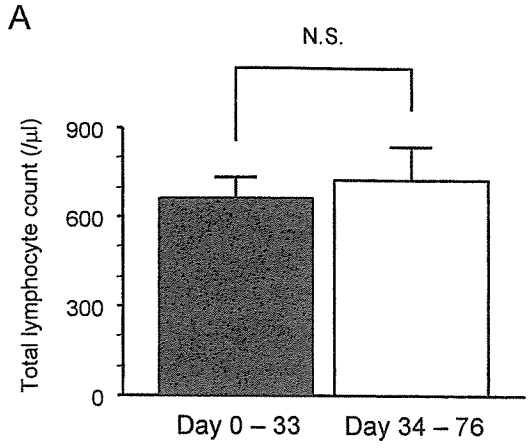


Fig. 2. (A) Time course of daily caloric intake. Metastatic liver tumors were treated with a continuous intrahepatic arterial injection of 5-fluorouracil (375 mg/day) and intermittent cisplatin (30 mg/day) via a reservoir system (■). Granisetron (3 mg) was administrated by intravenous infusion at 30 min before chemotherapy (□). The patient's energy requirement was estimated to be 1400 to 1600 kcal/day, based on the Harris-Benedict equation. (B) Caloric intake after improvement of the appearance of food in the prescribed diet. 2000 or 1500 kcal/day diet was prescribed during Day 0 to 33 (n=34; black bar). 1000 kcal/day diet was prescribed during Day 34 to 47 (n=14; white bar). Caloric intake was evaluated everyday and changes in caloric intake were statistically analyzed by the Mann-Whitney *U* test. *$P<0.01$.



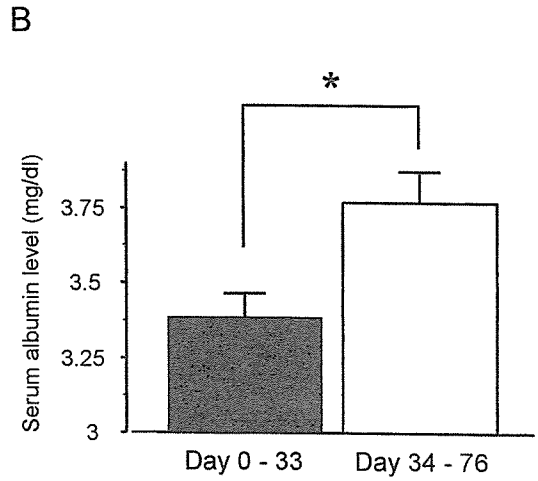
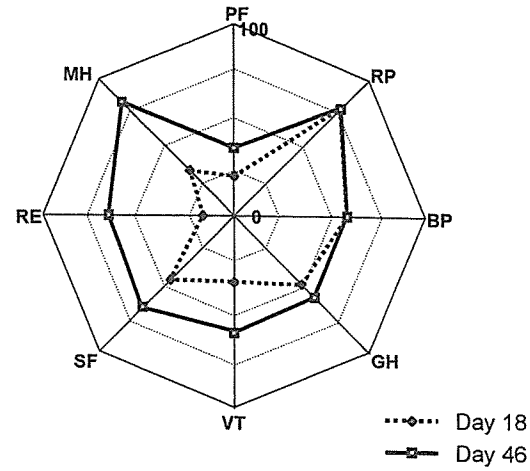


Fig. 3. Long-term effects on (A) total lymphocyte count, and (B) serum albumin levels after improvement of appearance of the food in the prescribed diet. 2000 or 1500 kcal/day diet was prescribed during Day 0 to 33 (black bar). 1000 kcal/day diet was prescribed during Day 34 to 76 (white bar). During Day 0 to 33, total lymphocyte count (n=5) and serum albumin levels (n=5) were measured. During Day 34 to 76, total lymphocyte count (n=4) and serum albumin levels (n=5) were measured. Changes in total lymphocyte count and serum albumin were statistically analyzed by the Mann-Whitney *U* test. N.S.; not significant. *P<0.05.



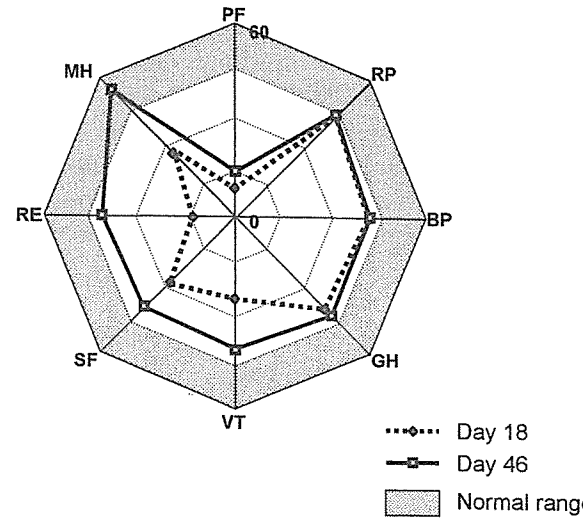


Fig. 4. Changes in SF-36v2 scores after improvement of appearance of the food in the prescribed diet. (A) actual score on a scale of 0 to 100. (B) Norm-based score. PF: physical functioning; RP: role-physical; BP: bodily pain; GH: general health; VT: vitality; SF: social functioning; RE: role-emotional; MH: mental health.

We also evaluated the effects of appearance-specific satiety on the patient's QOL by using the Medical Outcomes Study 36-Item Short-Form Health Survey version 2 (SF-36v2) on Day 18 and 46. By improvement in appearance of food, her scores for vitality (VT), mental health (MH), role-emotional (RE), and social functioning (SF) were markedly increased on a scale of 0 to 100 (Fig. 4A). In particular, the MH score reached to normal range (Fig. 4B). Appearance-specific satiety stimulated appetite and resulted in the improvement of nutritional status as well as in the QOL in this patient with metastatic liver tumors.

DISCUSSION

In this study, we describe a case in which the appearance-specific satiety of food increased appetite