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Branched-chain amino acid-enriched supplementation improves insulin resistance in patients with chronic liver disease

TAKUMI KAWAGUCHI[1,2], YUMIKO NAGAO[1], HISAKO MATSUOKA[1], TATSUYA IDE[1,2] and MICHIO SATA[1,2]

[1]Department of Digestive Disease Information and Research; [2]Division of Gastroenterology, Department of Medicine, Kurume University School of Medicine, Kurume 830-0011, Japan

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Abstract. Increased insulin resistance is a therapeutic target in patients with chronic liver disease. Branched-chain amino acids (BCAA) have been reported to improve insulin resistance in *in vivo* experiments. Thus, we investigated the effects of BCAA on insulin resistance in patients with chronic liver disease. Twelve patients with chronic liver disease were enrolled. Each patient was given one sachet of a BCAA-enriched supplement after breakfast and another at bedtime. The effects of the BCAA-enriched supplementation on insulin resistance were examined 30, 60 and 90 days after administration by the homeostasis model assessment method for insulin resistance (HOMA-IR) and for ß cell function (HOMA-%B). The HOMA-IR and HOMA-%B values were elevated at baseline, however, these parameters showed no significant changes after administration of the BCAA-enriched supplement in the overall patient population. By stratification via gender, patients in the male group showed a significantly greater elevation in the HOMA-IR value compared to the female patients at baseline. After the administration, the HOMA-IR and HOMA-%B values were significantly decreased only in the male group (9.4±4.8 vs. 2.4±0.7, 657±345 vs. 126±36, respectively; $P<0.05$). We found that there was a gender difference in chronic viral liver disease-related insulin resistance. Moreover, a BCAA-enriched supplement improved insulin resistance and ß cell function in male patients with chronic viral liver disease. Thus, a BCAA-enriched supplement may be a useful therapeutic agent for decreasing insulin resistance in male patients with chronic viral liver disease.

Correspondence to: Dr Michio Sata, Department of Medicine and Department of Digestive Disease Information and Research, Kurume University School of Medicine, 67 Asahi-machi, Kurume 830-0011, Japan
E-mail: takumi@med.kurume-u.ac.jp

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Introduction

The liver is one of the major target organs of insulin and chronic liver disease is associated with insulin resistance (1-3). Increased insulin resistance is related to the progression of hepatic fibrosis (4), development of hepatocellular carcinoma (HCC) (5,6) and reduction in long-term survival (6,7). Thus, the increase in insulin resistance is an important therapeutic target in patients at any stage of chronic liver disease.

Insulin resistance is treated by dietary modification, physical activity and/or drugs (8). However, a sufficient energy intake is required for patients with liver cirrhosis and dietary restrictions may lead to a decrease in liver function (9,10). Although physical activity is not restricted in compensated cirrhotic patients (11), such individuals often complain of fatigue, thus adequate exercise is not always possible. Biguanides and thiazolidinediones are insulin sensitizing agents and are currently utilized for the reduction of insulin resistance (12,13). However, it is not always possible to use these drugs in cirrhotic patients due to adverse effects, including lactic acidosis, fluid retention and severe hepatotoxicity (14,15).

Decreases in serum branched-chain amino acids (BCAA) levels are often seen in patients with chronic liver disease and lead to a decline of detoxified ammonia and albumin production. Therefore, BCAA are used for the treatment of hepatic encephalopathy and hypoalbuminemia (16,17). Previously, BCAA have been reported to modulate insulin signaling in an *in vivo* study. BCAA cause glucose up-take in the skeletal muscle, adipocytes and hepatocytes in rodents and in a rat model of liver cirrhosis (18-21). In addition, BCAA are known to up-regulate the mammalian target of rapamycin (mTOR), which cross-talks with intracellular insulin signaling (22,23). Taken together, these previous studies imply that BCAA improve glucose metabolism through the reduction of insulin resistance. In this study, we examined the effects of BCAA on insulin resistance in patients with chronic viral liver disease.

Materials and methods

A prospective, consecutive-patient entry study was conducted. Eligibility criteria were chronic viral liver disease with

Table I. Contents of one sachet of BCAA-enriched supplement (Aminofeel®).

Substance	Amount
BCAA	3200.0 mg
Valine	800.0 mg
Leucine	1600.0 mg
Isoleucine	800.0 mg
Calcium	22.1 mg
Magnesium	12.6 mg
Zinc	5.0 mg
Copper	0.2 mg
Selenium	49.6 μg
Chromium	14.4 μg
Pantothenic acid calcium	6.8 mg
Vitamin A	315.0 μg
Vitamin B1	2.4 mg
Vitamin B2	2.6 mg
Vitamin B6	2.4 mg
Vitamin B12	10.0 μg
Folic acid	0.2 mg
Vitamin C	40.0 mg
Vitamin D3	3.0 μg
Vitamin E	6.4 mg
Vitamin K2	29.6 μg
Niacin	12.0 mg

Table II. Characteristics of patients.

	Normal range	All patients
n		12
Age	n/a	64.3±2.4
BMI (kg/m²)	18.5-25.0	24.3±0.6
Fat%BW (%)	20-27	32.8±2.4
Muscle%BW (%)	40-50	36.0±1.6
Visceral fat area (cm²)	<100	118.2±14.9
Hemoglobin (g/dl)	14.0-18.0	13.6±0.4
White blood cell (μl)	4000-9000	4358±414
Platelet count (x10⁴/μl)	13-36	15.3±1.6
AST (U/l)	12-33	43.1±5.6
ALT (U/l)	8-42	39.8±7.3
LDH (U/l)	119-229	190.8±13.0
γ-GTP (U/l)	10-47	49.7±8.3
Total protein (g/dl)	6.7-8.3	7.7±0.2
Albumin (g/dl)	4.0-5.0	3.8±0.1
BTR	4.4-10.1	5.1±0.4
BCAAs (μmol/l)	344-713	435.6±27.5
Tyrosine (μmol/l)	51-98	90.1±7.9
Cholinesterase (U/l)	214-466	155.8±16.1
Total bilirubin (mg/dl)	0.3-1.5	0.9±0.1
Total cholesterol (mg/dl)	128.0-220.0	178.5±9.1
Fasting glucose (mg/dl)	80.0-109.0	104.5±6.4
HbA1c (%)	4.3-5.8	5.5±0.2
IRI (μU/ml)	5.0-20.0	22.8±9.7
HOMA-IR	<5.4	5.5±2.1
HOMA-%B	>156.5	326.4±159.1
Zinc (μg/dl)	80-130	82.9±6.1

The values are expressed as mean ± standard error. BMI, body mass index; BW, body weight; AST, aspartate aminotransferase; ALT, alanine aminotransferase; LDH, lactate dehydrogenase; γ-GTP, γ-glutamyl transpeptidase; BTR, BCAAs tyrosine ratio; BCAAs, branched-chain amino acids; HbA1c, hemoglobinA1c; IRI, immunoreactive insulin; HOMA-IR, homeostasis model assessment method for insulin resistance; HOMA-%B, homeostasis model assessment method for ß cell function; n/a, not applicable.

sufficient food intake and serum albumin concentration >3.5 g/dl and <4.0 g/dl. Patients with hepatic encephalopathy, ascites, HCC or renal failure were excluded. A total of 12 patients with HCV-related chronic liver disease (n=11), or HBV-related chronic liver disease (n=1) were enrolled in this study from August 2006 to June 2007 at Kurume University Hospital. The diagnosis of liver disease was based on clinical, serological, imaging and/or histological evidence. The patients were treated as outpatients and no therapeutic interventions such as changes in eating habits and physical activity were made in the patients' life-style after entering the study. Informed consent for participation in the study was obtained from each patient. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a prior approval by the Ethics Committee of the Kurume University School of Medicine. None of the subjects were institutionalized.

Study design. Each patient was given one sachet of a BCAA-enriched supplement (Aminofeel®, Seikatsu Bunkasya Co. Inc, Chiba, Japan) after breakfast and another at bedtime. The contents of this supplement are summarized in Table I. Using blood biochemical tests, effects on liver function including glucose metabolism, derived from the administration of the BCAA-enriched supplement were examined at 30, 60 and 90 days.

Measurements of body composition and visceral fat area. Body fat and skeletal muscle were evaluated by an eight-polar direct segmental multifrequency-bioelectrical impedance analyzer (DSM-BIA; InBody 3.2, Biospace, Tokyo, Japan) before and after 90 days of administration of the BCAA-enriched supplement and were expressed as fat%body weight (fat%BW) and muscle%body weight (muscle%BW), respectively. Visceral fat area was measured by a DSM-BIA

Table III. Effects of BCAA-enriched supplementation on body composition, protein, lipid and glucose metabolism.

	Before	Administration of BCAA-enriched supplement		
		30 days	60 days	90 days
BMI	24.3±0.6	n/a	n/a	24.2±0.8
Fat%BW	32.8±2.4	n/a	n/a	32.6±2.4
Muscle%BW	36.0±1.6	n/a	n/a	36.1±1.6
Visceral fat area	118.2±14.9	n/a	n/a	110.9±6.2
BCAAs (μmol/l)	435.6±27.5	512.5±45.3	506.6±24.3	527.3±47.1
Tyrosine (μmol/l)	90.1±7.9	84.2±7.0	77.0±7.0[a]	89.1±7.1
BTR	5.1±0.4	6.6±0.8[a]	7.2±0.9[b]	6.4±0.7
Zinc (μg/dl)	82.9±6.1	108.3±6.9[a]	106.8±5.6[a]	103.3±6.9[a]
ALT (U/l)	39.8±7.3	43.8±7.0	42.5±7.5	42.1±6.1
Total protein (g/dl)	7.8±0.2	7.7±0.2	7.6±0.1	7.6±0.2
Albumin (g/dl)	3.8±0.1	3.9±0.1	3.9±0.1[a]	3.9±0.1
Cholinesterase (U/l)	155.8±16.1	154.1±13.8	170.7±18.3	170.1±18.5
Fasting glucose (mg/dl)	104.5±6.4	103.9±6.1	101.8±5.3	102.8±5.4
HbA1c (%)	5.5±0.2	5.5±0.2	5.5±0.2	5.4±0.3
IRI (μU/ml)	22.8±9.7	12.7±1.4	10.8±1.5	13.3±1.9
HOMA-IR	5.5±2.1	3.3±0.4	2.8±0.5	3.5±0.6
HOMA-%B	326.4±6.4	127.5±17.6	112.0±16.8	140.8±28.8
Total cholesterol (mg/dl)	178.5±9.1	172.8±9.4	178.6±10.0	173.1±7.2
Total bilirubin (mg/dl)	0.9±0.1	1.0±0.1	0.9±0.1	0.9±0.1

The values are expressed as mean ± standard error. Statistical comparisons between before and after 30, 60 or 90 days of the administration were performed by Wilcoxon's test. [a]P<0.05 and [b]P<0.01. BMI, body mass index; BW, body weight; BCAAs, branched-chain amino acids; BTR, BCAAs tyrosine ratio; ALT, alanine aminotransferase; HbA1c, hemoglobinA1c; IRI, immunoreactive insulin; HOMA-IR, homeostasis model assessment method for insulin resistance; HOMA-%B, homeostasis model assessment method for ß cell function; n/a, not applicable.

before and after 90 days of administration of the BCAA-enriched supplement. The accuracy of the DSM-BIA analyzer has been reported (24).

Laboratory determinations. Venous blood samples were obtained in the morning after an overnight fast. Complete blood cell counts and levels of serum aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, γ-glutamyl transpeptidase, total protein, albumin, BCAA, tyrosine, cholinesterase, total bilirubin, total cholesterol, immunoreactive insulin (IRI), zinc, plasma glucose and HbA1c were measured by standard clinical methods (Department of Clinical Laboratory, Kurume University Hospital) as previously described (25,26). BCAA tyrosine ratio (BTR) was calculated as BCAA/tyrosine.

Evaluation for insulin resistance and β cell function. Insulin resistance and ß cell function were evaluated on the basis of fasting levels of plasma glucose and insulin, according to the homeostasis model assessment (HOMA) method (27). The formulas used for the HOMA model are as follows: Insulin resistance (HOMA-IR) = fasting glucose (mg/dl) x fasting insulin (μU/ml)/405; ß cell function (HOMA-%B) = fasting insulin (μU/ml) x 360/[fasting glucose (mg/dl) - 63].

Statistical analysis. All data are expressed as mean ± standard error. Differences between the two groups were analyzed using the Mann-Whitney U test. Statistical comparisons between before administration of the BCAA-enriched supplement and after 30, 60, or 90 days, were performed by Wilcoxon's test. P-values <0.05 were considered significant.

Results

Patient characteristics. Patient characteristics prior to BCAA-enriched supplementation administration are summarized in Table II. Serum aspartate aminotransferase and γ-glutamyl transpeptidase levels were elevated in comparison to normal limits. Serum albumin and cholinesterase levels were decreased. Although the BMI value and the levels of fasting plasma glucose and HbA1c were within normal limits, the values of fat%BW, visceral fat area, serum IRI, HOMA-IR, and HOMA-%B were elevated.

Effects of BCAA-enriched supplement on body composition, protein, lipid and glucose metabolism. The effects on body composition, protein, lipid and glucose metabolism as a result of administering the BCAA-enriched supplementation are summarized in Table III. There were no significant changes on body composition between before and after 90 days

Table IV. Characteristics of male and female groups.

	Normal range	Male group	Female group	P
n		5	7	
Age	n/a	61.2±3.4	66.4±3.3	0.29
BMI (kg/m^2)	18.5-25.0	24.8±1.1	23.9±0.8	0.29
Fat%BW (%)	20-27	23.2±3.0	35.5±1.6	0.002
Muscle%BW (%)	40-50	42.9±1.7	34.0±0.9	0.002
Visceral Fat Area (cm^2)	>100	111.0±12.9	120.3±18.4	0.66
Hemoglobin (g/dl)	14.0-18.0	14.4±0.7	13.0±0.5	0.17
White blood cell (/μl)	4000-9000	4040±738	4585±508	0.46
Platelet count (x10^4/μl)	13-36	11.8±1.5	17.9±2.0	0.06
AST (U/l)	12-33	43.6±6.5	42.7±8.8	0.46
ALT (U/l)	8-42	51.8±15.7	31.8±4.5	0.46
LDH (U/l)	119-229	177.0±27.4	205.2±10.8	0.56
γ-GTP (U/l)	10-47	50.6±8.1	49.0±13.5	0.68
Total protein (g/dl)	6.7-8.3	7.9±0.3	7.6±0.2	0.46
Albumin (g/dl)	4.0-5.0	3.8±0.1	3.8±0.1	0.57
BTR	4.4-10.1	4.6±0.6	5.5±0.5	0.29
BCAAs (μmol/l)	344-713	509.1±38.7	383.1±23.7	0.03
Tyrosine (μmol/l)	51-98	115.0±38.7	72.3±4.8	0.02
Cholinesterase (U/l)	214-466	171.6±36.4	144.6±11.5	0.80
Total bilirubin (mg/dl)	0.3-1.5	1.0±0.3	0.8±0.1	0.80
Total cholesterol (mg/dl)	128.0-220.0	160.2±9.9	191.5±12.0	0.10
Fasting glucose (mg/dl)	80.0-109.0	105.2±14.9	104.0±5.0	0.51
HbA1c (%)	4.3-5.8	6.0±0.4	5.2±0.1	0.17
IRI (μU/ml)	5.0-20.0	40.4±22.0	10.2±1.8	0.04
HOMA-IR	<5.4	9.4±4.8	2.7±0.6	0.03
HOMA-%B	>156.5	657.6±345.8	89.8±14.4	0.17
Zinc (μg/dl)	80-130	81.8±10.9	83.7±7.8	0.46

The values are expressed as mean ± standard error. Differences between the two groups were analyzed using the Mann-Whitney U test. BMI, body mass index; BW, body weight; AST, aspartate aminotransferase; ALT, alanine aminotransferase; LDH, lactate dehydrogenase; γ-GTP, γ-glutamyl transpeptidase; BTR, BCAAs tyrosine ratio; BCAAs, branched-chain amino acids; HbA1c, hemoglobinA1c; IRI, immunoreactive insulin; HOMA-IR, homeostasis model assessment method for insulin resistance; HOMA-%B, homeostasis model assessment method for ß cell function; n/a, not applicable.

of the administration of the BCAA-enriched supplement. Although serum BCAA levels were not significantly increased, the BTR value was significantly increased 30 and 60 days after administration of the BCAA-enriched supplement. The serum zinc level was significantly increased 30, 60 and 90 days after the administration and the serum albumin level was also significantly increased 60 days after the administration. There were no significant changes in fasting plasma glucose, HbA1c, IRI levels, HOMA-IR or HOMA-%B values after administration of the BCAA-enriched supplement.

Characteristics of the male and female groups. Characteristics of the male and female groups before administration of the BCAA-enriched supplement are summarized in Table IV. Fat%BW value was significantly higher in the female group than that in the male group. Muscle%BW value was significantly higher in the male group than that in the female group. Serum BCAAs and tyrosine levels were significantly higher in the male group than those in the female group. Although fasting plasma glucose and HbA1c levels were not significantly different between the two groups, serum IRI level and HOMA-IR values were significantly higher in the male group than those in the female group prior to supplement administration.

Effects of BCAA-enriched supplement on body composition, protein and lipid metabolism in the male and female groups. The effects of administering the BCAA-enriched supplement on body composition, protein and lipid metabolisms in the male and female groups are summarized in Tables V and VI, respectively. There were no significant changes on body composition between before and after 90 days of administration of the BCAA-enriched supplement.

Table V. Effects of BCAA-enriched supplementation on protein and lipid metabolism in the male group.

		Administration of BCAA-enriched supplement		
	Before	30 days	60 days	90 days
BMI	24.8±1.1	n/a	n/a	24.4±2.1
Fat%BW	23.2±3.0	n/a	n/a	23.1±3.1
Muscle%BW	42.9±1.7	n/a	n/a	42.8±1.9
Visceral fat area	111.0±12.9	n/a	n/a	109.1±14.1
BCAA (μmol/l)	509.1±38.7	569.4±98.0	488.9±35.4	437.4±44.0
Tyrosine (μmol/l)	115.0±10.0	103.7±6.8	94.2±11.5[a]	102.0±9.8
BTR	4.6±0.6	5.7±1.2	5.5±0.7[a]	4.4±0.5
Zinc (μg/dl)	81.8±10.9	116.0±15.0[a]	97.4±4.0	87.4±6.6
ALT (U/l)	51.8±15.7	55.6±14.6	54.0±16.7	53.2±13.2
Total protein (g/dl)	7.9±0.3	7.7±0.2	7.7±0.2	7.7±0.2
Albumin (g/dl)	3.8±0.1	3.9±0.1	3.9±0.1	4.0±0.1[a]
Cholinesterase (U/l)	171.6±36.4	166.0±31.3	201.8±38.8	203.2±39.0
Total cholesterol (mg/dl)	160.2±9.9	157.2±13.9	162.4±12.7	163.0±11.8
Total bilirubin (mg/dl)	1.0±0.3	1.2±0.3	1.0±0.3	1.1±0.3

The values are expressed as mean ± standard error. Statistical comparisons between before and after 30, 60 or 90 days of the administration were performed by Wilcoxon's test. [a]P<0.05. BMI, body mass index; BW, body weight; BCAAs, branched-chain amino acids; BTR, BCAAs tyrosine ratio; ALT, alanine aminotransferase; n/a, not applicable.

Table VI. Effects of BCAA-enriched supplement on protein and lipid metabolism in the female group.

		Administration of BCAA-enriched supplement		
	Before	30 days	60 days	90 days
BMI	23.9±0.8	n/a	n/a	24.3±1.8
Fat%BW	35.5±1.6	n/a	n/a	35.3±1.4
Muscle%BW	34.0±0.9	n/a	n/a	34.1±1.0
Visceral fat area	120.3±18.4	n/a	n/a	111.4±4.2
BCAAs (μmol/l)	383.1±23.7	471.9±34.8[a]	519.1±34.6[a]	591.6 ± 66.3[a]
Tyrosine (μmol/l)	72.3±4.8	70.3±7.3	66.2±6.0	79.9±8.9
BTR	5.5±5.0	7.3±1.0	8.5±1.3[a]	7.7±9.0
Zinc (μg/dl)	83.7±7.8	102.9±5.7	113.6±8.5	114.6±8.8[a]
ALT (U/l)	31.1±4.5	35.3±5.0	34.3±4.0	34.1±4.9
Total protein (g/dl)	7.6±0.2	7.6±0.2	7.7±0.2	7.7±0.2
Albumin (g/dl)	3.8±0.1	3.9±4.7	3.9±2.4	3.9±0.1
Cholinesterase (U/l)	144.6±11.5	145.6±10.1	148.4±11.5	146.4±11.3
Total cholesterol (mg/dl)	191.6±12.0	183.9±11.5	190.1±13.5	180.3±8.7
Total bilirubin (mg/dl)	0.8±0.1	0.8±0.1	0.8±4.2	0.7±0.1

The values are expressed as mean ± standard error. Statistical comparisons between before and after 30, 60 or 90 days of the administration were performed by Wilcoxon's test. [a]P<0.05. BMI, body mass index; BW, body weight; BCAAs, branched-chain amino acids; BTR, BCAAs tyrosine ratio; ALT, alanine aminotransferase; n/a, not applicable.

Significant increases in serum BTR value and zinc level were seen in the two groups after the supplement administration (Tables V and VI). In addition, the serum BCAA level was significantly increased in the female group (Table VI). The serum albumin level was also significantly increased, though only in the male group (Table V).

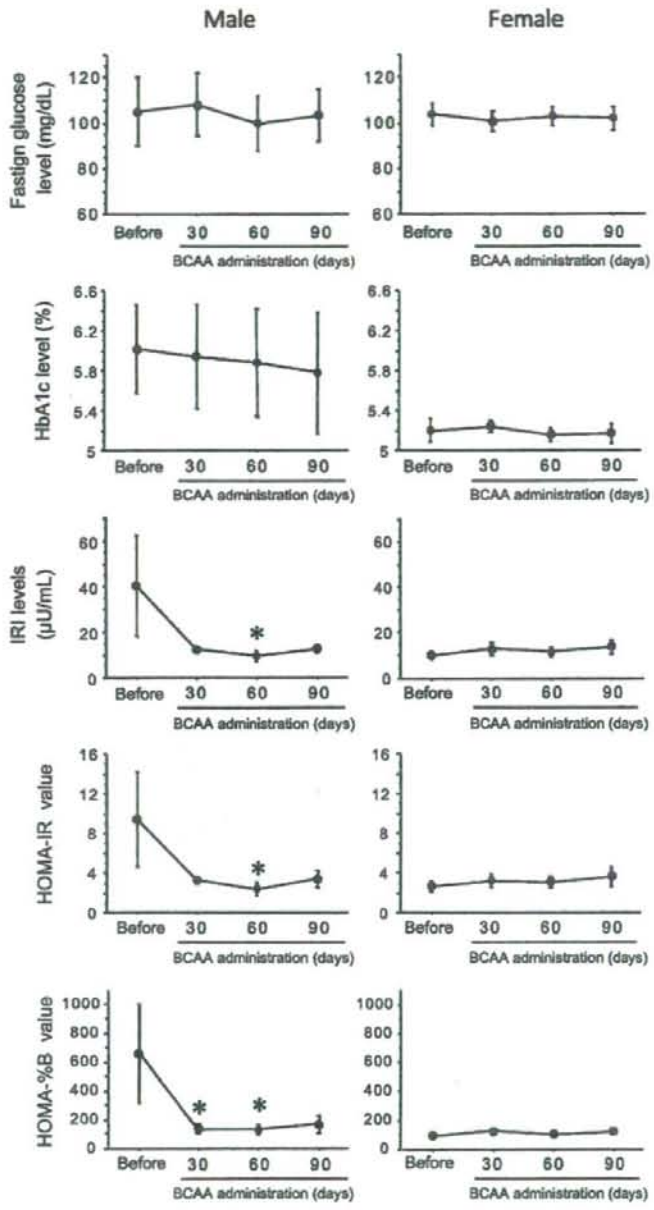

Figure 1. The effects of BCAA-enriched supplementation on glucose metabolism in the male and female groups. Glucose metabolism was evaluated by the fasting glucose level, HbA1c value, serum IRI level, HOMA-IR and HOMA-%B values. The values are expressed as mean ± standard error. Statistical comparisons between before and after 30, 60 or 90 days of the administration was performed by Wilcoxon's test. *P<0.05. HbA1c, hemoglobinA1c; IRI, immunoreactive insulin; HOMA-IR, homeostasis model assessment method for insulin resistance; HOMA-%B, homeostasis model assessment method for ß cell function.

Effects of BCAA-enriched supplementation on glucose metabolism in the male and female groups. Although there were no significant changes in the fasting plasma glucose and HbA1c levels during the administration of the BCAA-enriched supplement in the two groups, the serum IRI level was significantly decreased in the male group after the supplement administration (Fig. 1). Similarly, a significant decrease was seen in the HOMA-IR and HOMA-%B values in the male group, though not in the female group (Fig. 1).

Discussion

In this study, we demonstrated that there was a gender difference in chronic viral liver disease-related insulin resistance. Moreover, BCAA-enriched supplementation caused decreases in the serum IRI level, the HOMA-IR and HOMA-%B values without changes in body composition including visceral fat, suggesting the direct effects of the BCAA-enriched supplement on glucose metabolism among the male patients with chronic viral liver disease.

Increased insulin resistance is known to exist in pre-cirrhotic patients (2) and is a risk factor for the progression of hepatic fibrosis (4), development of HCC (5,6) and a reduction in long-term survival (6,7). Similar to previous reports (2,6,25,26,28), the enrolled patients in this study exhibited chronic hepatitis with increased insulin resistance. Since BCAA are known to modulate insulin signaling (18-21), we examined the effects of the BCAA-enriched supplementation on insulin resistance. In our study, the serum IRI level and the HOMA-IR and HOMA-%B values were all reduced after the supplement administration. However, the reduction in these parameters was not statistically significant. We previously reported on a gender difference in chronic viral liver disease-related insulin resistance (6). Therefore, we examined the effects of the BCAA-supplement on insulin resistance by stratification via gender.

Male cirrhotic patients show a significantly greater increase in insulin resistance compared to female cirrhotic patients (6). Similarly, increased IRI levels and HOMA-IR values were seen only in the male group in this study. Although the reason for the gender difference in insulin resistance is unclear, one possibility is that tyrosine is involved in the development of insulin resistance. The serum tyrosine level in our study of the male group was significantly increased compared to the female group. A synthetic enzyme of tyrosine, henylalanine hydroxylase, is known to be regulated by testosterone, a sex hormone (29). Tyrosine is the precursor of epinephrine, which causes peripheral and hepatic insulin resistance (30). The serum tyrosine level has a positive correlation with insulin resistance (31). Thus, the gender difference in the tyrosine production pathway and the metabolite of tyrosine may have been responsible for the increased insulin resistance in the male group among our patients.

BCAA-enriched supplementation decreased the serum IRI level and the HOMA-IR and HOMA-%B values without changes in body composition including visceral fat area. These data suggest that the BCAA-enriched supplementation improves insulin resistance and ß cell function. In this study, insulin resistance was significantly reduced 60 days after administration of the BCAA-enriched supplement. It remains unclear, however, how the supplement improved insulin resistance. Since the supplement used in this study contains trace elements such as zinc, chromium and selenium, which are known to decrease insulin sensitivity (32-34), it is reasonable to assume that these trace elements may contribute to improve insulin resistance. Alternatively, changes in the constitution of amino acids may be involved in the improvement in insulin resistance. Moreover, tyrosine is the precursor of epinephrine, which causes peripheral and hepatic insulin resistance (30). In this study, changes in serum tyrosine level and BTR reflected changes in insulin resistance. Insulin resistance was significantly reduced 60 days after the administration of the BCAA-enriched supplement. Likewise, the serum tyrosine level was significantly decreased and BTR was significantly increased 60 days after the administration of the BCAA-enriched supplement. Thus, a decrease in the serum tyrosine level and changes in the constitution of amino acids may contribute to a reduction in insulin resistance. In good agreement with our results, Vlasakova *et al* reported that BTR shows a negative correlation with insulin resistance (31). Although the reason for changes in the constitution of amino acids remains uncertain, a possibility is that BCAA activate mTOR, which in turn, promotes protein synthesis (35). Desai *et al* reported that BCAA cause the incorporation of tyrosine into protein as well as albumin renewal (36). In our study, a significant increase in serum albumin level was seen following the decrease in serum tyrosine level and an increase in BTR. Taken together, these findings suggest that the BCAA-enriched supplementation seems to lead to a dramatic reduction of insulin resistance through both changes in the constitution of amino acids and effects of trace elements.

A limitation of this study was the small sample size. However, leucine is reported to improve insulin resistance in *in vivo* experiments (37). In human subjects, two cases which showed BCAA improved insulin resistance were recently reported (38). These previous reports suggest the effects of the BCAA-enriched supplement on glucose metabolism. In order to confirm the significance of the BCAA-enriched supplement on insulin resistance in patients with LC, a large-scale multicenter clinical study is required.

This study revealed that the BCAA-enriched supplementation improved insulin resistance and ß cell function in male patients with chronic viral liver disease. Thus, the BCAA-enriched supplementation may be one of the useful therapeutic agents for insulin resistance in male patients with chronic viral liver disease.

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Graves' ophthalmopathy and tongue cancer complicated by peg-interferon α-2b and ribavirin therapy for chronic hepatitis C: A case report and review of the literature

YUMIKO NAGAO[1], YUJI HIROMATSU[2], TADASHI NAKASHIMA[3] and MICHIO SATA[1,4]

[1]Department of Digestive Disease Information and Research, [2]Division of Endocrinology and Metabolism, Department of Medicine, [3]Department of Otolaryngology, [4]Division of Gastroenterology, Department of Medicine, Kurume University School of Medicine, Asahi-machi, Kurume, Fukuoka 830-0011, Japan

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Abstract. Hepatitis C virus (HCV) infection induces not only chronic liver disease, but also extrahepatic manifestations such as thyroid disease and oral cancer. Thyroid dysfunction is also a complication known to be associated with interferon (IFN) therapy for HCV infection. We report on a 69-year-old Japanese man who developed Graves' ophthalmopathy and tongue cancer (malignant transformation of leukoplakia) while receiving peg-interferon (Peg-IFN) α-2b and ribavirin (RBV) treatment for chronic hepatitis C. This patient had no history of thyroid disease before the combination therapy, but did have bilateral leukoplakia of the tongue. The leukoplakia lesions did not change until 20 weeks after the start of the combination therapy, and ophthalmopathy was not diagnosed until 47 weeks later. As ophthalmopathy is considered to be a severe adverse event induced by Peg-IFN α-2b plus RBV, therapy was discontinued after 47 weeks. The patient received a partial glossectomy to remove the malignant neoplasm as well as extraocular muscle surgery for the ophthalmopathy, and was treated with an antithyroid agent and steroids. In conclusion, it is necessary to clinically examine organs other than the liver in patients with HCV infection.

Correspondence to: Dr Yumiko Nagao, Department of Digestive Disease Information and Research, Kurume University School of Medicine, 67 Asahi-machi, Kurume 830-0011, Japan
E-mail: nagao@med.kurume-u.ac.jp

Abbreviations: HCV, hepatitis C virus; IFN, interferon; Peg-IFN, peg-interferon; RBV, ribavirin; HCC, hepatocellular carcinoma; MRI, magnetic resonance imaging; HBsAg, hepatitis B surface antigen; TSH, thyroid stimulating hormone; FT_3, free tri-iodothyronine; FT_4, free thyroxine; TPOAb, thyroid peroxidase antibodies; TgAb, thyroglobulin antibodies; anti-HCV, HCV antibody; RBC, red blood cell; Hb, hemoglobin; WBC, white blood cell; AST, aspartate aminotransferase; ALT, alanine aminotransferase; γ-GTP, γ-glutamyl transpeptidase; LDH, lactate dehydrogenase; TRAb, TSH receptor antibody; TSAb, thyroid stimulating antibody; hTRAb, human TSH receptor antibody

Key words: hepatitis C virus, peg-interferon, ribavirin, Graves' ophthalmopathy, extrahepatic manifestation, oral squamous cell carcinoma

Introduction

Hepatitis C virus (HCV) frequently causes persistent infection, which leads to chronic liver disease and hepatocellular carcinoma (HCC). HCV-related HCC represents 80% of all HCC cases in Japan (1) and primary liver cancer, 95% of which is HCC-related, ranks third in men and fifth in women as the cause of death from malignant neoplasms. Interferon (IFN) α monotherapy for chronic hepatitis C infection leads to a sustained virological response in only 10-15% of HCV-infected patients (2,3). A substantial improvement in response of approximately 2-fold over IFN monotherapy was noted using the combination of IFN α plus ribavirin (RBV) (4,5). Recently, a combination treatment of peg-interferon (Peg-IFN) plus RBV has been adopted as standard care for patients with chronic hepatitis C, as it is associated with significant improvements in the rate of sustained virological response (50%) as compared to IFN α plus RBV or Peg-IFN α alone (6).

HCV infection has also been associated with extrahepatic manifestations and immune-mediated phenomena (7), including mixed cryoglobulinemia (8), thyroid disease (9), Sjögren's syndrome (10), porphyria cutanea tarda (11), lichen planus (12), oral cancer (13,14) and type 2 diabetes mellitus (15). The incidence of HCV infection in oral squamous cell carcinoma in Japanese patients has been reported as being 16.7-24.0% (13,14).

The side effects of IFN therapy for HCV have been well documented (16,17). Flu-like symptoms such as fever, chills, muscle ache, nausea, vomiting and fatigue are common side effects of treatment. Depression and related symptoms, such as anxiety, irritability, insomnia and mental confusion, are not rare and, in patients with a previous history, may be significant. Withdrawal rates in IFN-based combination studies due to side effects have ranged from 6 to 7% (5). Various side effects have been reported in patients treated with this cytokine, including the appearance or exacerbation

of underlying autoimmune diseases and the development of a variety of organ- and non-organ-specific autoantibodies (18). Auto-immune thyroid disease is a common side effect of IFN treatment of viral hepatitis C, affecting 2-19% of IFN-treated patients (19). We previously reported the case of a patient with chronic hepatitis C who developed worsening lichen planus lesions during treatment with IFN plus RBV (20), and the case of a patient who developed oral cancer after IFN therapy (21).

We now describe a patient with chronic hepatitis C infection who developed hyperthyroidism, Graves' ophthalmopathy and malignant transformation of tongue leukoplakia during combination therapy with Peg-IFN α-2b and RBV. This patient was treated successfully.

Case report

A 67-year-old Japanese man, diagnosed in 1998 with chronic hepatitis C, consulted the Digestive Disease Center of Kurume University on June 6, 2003 for treatment of his chronic liver disease. The patient had received a right upper lobectomy for lung tuberculosis at the age of 23 (in 1958), and had been administered blood transfusions of 600 ml during the procedure. Hypertension was noted at the age of 67, and antihypertensive treatment was started at 68. Hemangioma of the right middle finger was diagnosed at 69. For 20 years, he smoked 50 cigarettes a day, though he had not smoked for the last 30 years. His alcohol consumption was 500 ml of beer daily for 49 years. His family history was non-contributory.

Periodic blood tests and abdominal ultrasound exams were conducted by a hepatologist at Kurume University. As the patient's aminotransferase levels were in the normal range, he was monitored regularly for chronic hepatitis C. On July 30, 2004, at the age of 69, his aminotransferase levels were found to be elevated and a liver biopsy revealed chronic active hepatitis, diagnosed as F2A2 according to the new Inuyama classification (22). As of June 14, 2005, for a period of 1-3 months, the patient was treated by a family doctor with a combination of peg-IFN α-2b (Peg-Intron®; Schering-Plough, Kenilworth, NJ, USA) (40-100 μg/week) plus RBV (Rebetol®; Schering-Plough) (300-800 mg/day). During this time, he was examined by a hepatologist once.

At the start of Peg-IFN α-2b plus RBV therapy, laboratory data regarding hepatitis virus markers indicated that the patient was negative for hepatitis B surface antigen (HBsAg), but positive for HCV antibody (anti-HCV) and HCV RNA. Both free thyroxine (FT_4) and thyroid stimulating hormone (TSH) levels before Peg-IFN plus RBA therapy were within normal ranges (Table I).

In March 2006, while undergoing Peg-IFN plus RBV therapy, the patient experienced double vision. He did not consult a family doctor or a hepatologist, but was examined by an ophthalmologist, and then by a neurosurgeon who prescribed magnetic resonance imaging (MRI) followed by a neurological examination at Kurume University Hospital on May 9, 2006. Thyroid function tests on February 10, 2006 revealed suppressed TSH at 0.016 μIU/ml (normal value 0.21-3.85) and elevated free tri-iodothyronine (FT_3) at 4.1 pg/ml (normal value 1.9-3.5), but the hepatologist did not diagnose thyroid disease. Over the next 3 months, thyroid function tests revealed hyperthyroidism of autoimmune etiology, indicated by the following laboratory values from a test taken on May 16, 2006: TSH, 0.007 μIU/ml (normal value 0.21-3.85); FT_3, 4.6 pg/ml (normal value 1.9-3.5); FT_4, 1.58 ng dl (normal value 0.88-1.56); positive thyroid peroxidase antibodies (TPOAb), 92.2 IU/ml (normal value <5); thyroglobulin

Table I. Laboratory data of patient with hepatitis C virus infection at the time of admission for Peg-IFN and RBV therapy.

Laboratory assay	Value	Unit	Standard value
RBC	483	$x10^4/mm^3$	430-570
Hb	16.0	g/dl	14.0-18.0
Ht	46.9	%	40.0-52.0
WBC	63	$x10^4/mm^3$	40-90
Plt	17.4	$x10^4/mm^3$	13.0-36.0
AST	32	U/l	13-33
ALT	32	U/l	8-42
LDH	170	U/l	119-229
ALP	209	U/l	115-359
γ-GTP	90	U/l	10-47
TP	7.21	g/dl	6.70-8.30
Alb	4.11	g/dl	4.00-5.00
ChE	160	IU/l	107-233
TC	140	mg/dl	128-256
TB	1.14	mg/dl	0.00-1.50
DB	0.12	mg/dl	0.00-0.60
BUN	15.3	mg/dl	8.0-22.0
Crea	0.72	mg/dl	0.60-1.10
Na	139	mEq/l	138-146
K	4.0	mEq/l	3.6-4.9
Cl	104	mEq/l	99-109
Fe	190	μg/dl	80-170
UIBC	68	μg/dl	180-274
Ferritin	167.7	ng/ml	23.0-183.0
CRP	0.04	mg/dl	0.00-0.40
IgA	225	mg/dl	103-409
IgM	65	mg/dl	40-221
IgG	1856	mg/dl	918-1742
FT_4	1.24	ng/dl	0.88-1.56
TSH	2.970	μIU/ml	0.210-3.850
AFP (L3)	3.3	ng/dl	0.0-8.7
HbA1c	5.1	%	4.3-5.8
HBsAg	Negative		
Anti-HBc	Negative		
Anti-HCV	Positive		
HCV RNA level	2400	KIU/ml	
HCV genotype	1b		

June 14, 2005.

Table II. Laboratory data of patient with hepatitis C virus infection at time of admission for Graves' ophthalmopathy.

Laboratory assay	Value	Unit	Standard value
RBC	376	$x10^4/mm^3$	430-570
Hb	11.9	g/dl	14.0-18.0
Ht	35.8	%	40.0-52.0
WBC	43	$x10^4/mm^3$	40-90
Plt	18.3	$x10^4/mm^3$	13.0-36.0
AST	12	U/l	13-33
ALT	9	U/l	8-42
LDH	122	U/l	119-229
ALP	233	U/l	115-359
γ-GTP	17	U/l	10-47
TP	7.47	g/dl	6.70-8.30
Alb	3.94	g/dl	4.00-5.00
ChE	135	IU/l	107-233
TC	117	mg/dl	128-256
TB	0.43	mg/dl	0.00-1.50
DB	0.06	mg/dl	0.00-0.60
BUN	13.1	mg/dl	8.0-22.0
Crea	0.69	mg/dl	0.60-1.10
Na	141	mEq/l	138-146
K	4.1	mEq/l	3.6-4.9
Cl	104	mEq/l	99-109
CRP	0.88	mg/dl	0.00-0.40
Glucose	107	mg/dl	80-109
HbA1c	4.4	%	4.3-5.8
CEA	1.1	ng/dl	0.0-5.0
SCC	LT1.0	ng/dl	0.0-1.5
FT_3	4.6	mg/dl	1.9-3.5
FT_4	1.58	ng/dl	0.88-1.56
TSH	0.007	μIU/ml	0.210-3.850
TgAb	8.5	IU/ml	0.0-9.0
TPOAb	92.2	IU/ml	0.0-5.0
TRAb	19.7	%	<15
TSAb	139	%	<180
hTRAb	7.0	IU/l	<1.0
RA	<15	IU/ml	0-30
ANA	Negative		
Anti-SS-A	Negative		
Anti-SS-B	Negative		
HCV RNA	Negative		

May 16, 2006.

antibodies (TgAb), 8.5 IU/ml (normal value <9). Anti-TSH receptor antibodies [TSH receptor antibody (TRAb), 19.7% (normal value <15); thyroid stimulating antibody (TSAb), 139% (normal value <180); human TSH receptor antibody (hTRAb), 7.0 IU/l (normal value <1.0)] were positive. He had bilateral ocular disorders of supraduction and abduction, with bilateral conjunctival injection and periorbital edema. There was no tachycardia or exophthalmos (right, 12 mm; left, 12 mm). The size of the thyroid was normal according to ultrasonography. He was diagnosed with Graves' disease with ophthalmopathy by an endocrinologist. Table II shows laboratory data upon admission for Graves' ophthalmopathy, which was classified as IIa, IVc using the American Thyroid Association classification system for orbital changes in Graves' ophthalmopathy (23), with a clinical activity score of 3 (24). MRI of the orbits showed conspicuous enlargement of the bilateral inferior rectus muscles (Fig. 1). As these manifestations were regarded as a severe adverse event of Peg-IFN plus RBV therapy, the combined therapy was discontinued on May 2, 2006.

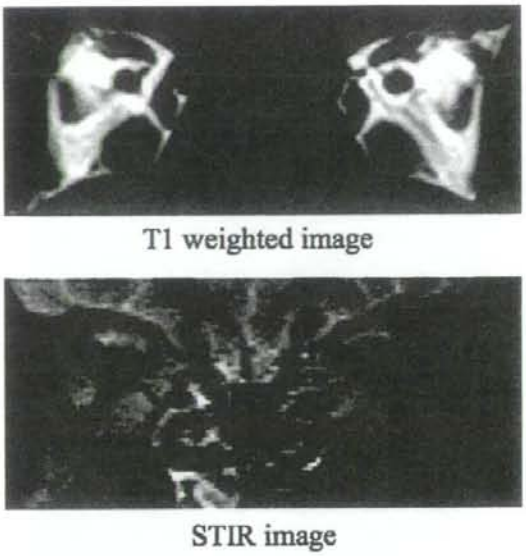

Figure 1. MRI of the orbits shows conspicuous enlargement of the bilateral inferior rectus muscles before steroid pulse therapy (coronal view).

Thiamazole (Mercazole®; Chugai Pharmaceutical Co., Ltd., Tokyo, Japan) (15 mg/day), an anti-thyroid drug, was administered as of May 19, 2006. After 4 weeks, the thyroid functions of the patient had normalized, but his ocular symptoms persisted. Consequently, methylprednisolone sodium succinate (Solu-Medrol®; Pfizer Inc., Tokyo, Japan) (1000 mg/day for 3 successive days, 3 courses) was started on July 11, 2006 as a steroid pulse therapy. Thiamazole dosage was reduced and terminated on August 12, 2006. The treatment was followed by oral prednisolone (Predonine®; Shionogi & Co., Ltd., Osaka, Japan) (20 mg daily) as of August 4, which was discontinued on October 15, 2006. Thyroid function improved and orbital edema and conjunctival injection were no longer apparent, but the double vision remained. The patient underwent extraocular muscle surgery on November 25, 2006. Fig. 2 illustrates the clinical course of the patient.

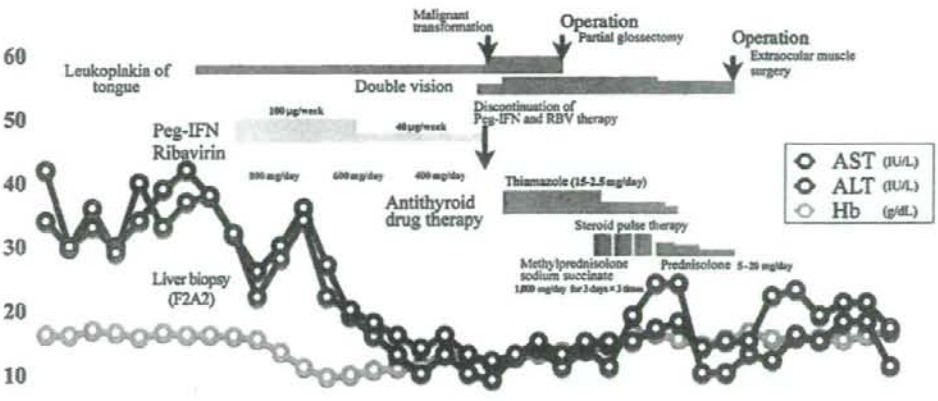

Figure 2. Clinical course of the patient.

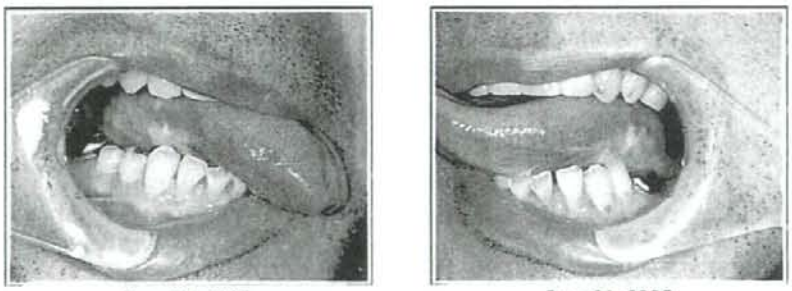

Figure 3. Bilateral oral leukoplakia of the tongue.

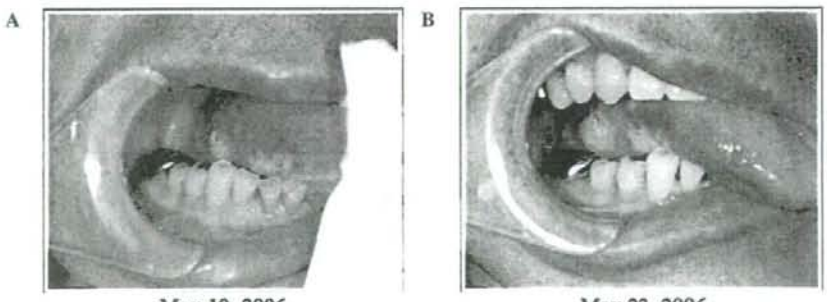

Figure 4. (A) Squamous cell carcinoma on the right lateral surface transformed from leukoplakia. (B) The mass exhibited a tendency for enhancement.

Table III. Cases of Graves' ophthalmopathy associated with IFN treatment for hepatitis C.

Year	Language	Refs.	Patient	Course
2000	French	33	62/man	Development of ophthalmopathy after IFN-α treatment
2002	English	34	47/man	Development of ophthalmopathy after treatment with a 6 month course of IFN-α and RBV
2002	French	35	49/woman	Development of ophthalmopathy after IFN-α treatment
2005	English	36	47/woman	Development of ophthalmopathy after IFN-α and RBV treatment
2007	English	37	50/woman	Exacerbation of ophthalmopathy during treatment with peg-IFN-α and RBV
2008	English	Our case	69/man	Development of ophthalmopathy during treatment with peg-IFN-α and RBV

The patient had symmetrically-located pre-cancerous leukoplakia on both lateral surfaces of the tongue before combination therapy with Peg-IFN α-2b plus RBV (Fig. 3). Cytodiagnosis of the tongue showed no evidence of malignancy, and the patient did not notice the lingual leukoplakia until they were discovered by us. The leukoplakia lesions remained unaltered during the combination therapy and for 20 weeks after it srarted. The patient did not have regular checkups after November 15, 2005 but, in April 2006, became aware of a mass at the right base of the tongue. Upon examination on May 9, 2006, the presence of a superficial mass on the right lateral surface of the tongue was confirmed. The mass measured 7 mm x 8 mm, had a granular surface and a hard-ened area, and was without pain (Fig. 4A). The Peg-IFN plus RBV therapy, which had been administered for 47 weeks, was stopped on May 2, 2006. The mass exhibited a tendency for enhancement (Fig. 4B), and there was no induration of the tumoral circumference and dysfunction. No cervical lymph node metastasis was detected. After a diagnosis of squamous cell carcinoma of the right tongue (T1N0M0, stage I), tumor resection of the tongue was performed at the Department of Otolaryngology of the Kurume University School of Medicine on June 6, 2006.

During Peg-IFN plus RBV therapy, the patient developed Graves' ophthalmopathy due to hyperthyroidism and tongue cancer resulting from oral leukoplakia. Serum HCV RNA was negative 6 months after the therapy ended, and the case was judged to be one of sustained virological response. Since that time, the patient has been monitored regularly by a hepatologist, an oral surgeon, an otolaryngologist, an endocrinologist and an ophthalmologist. To date, there has been no local recurrence of tongue cancer or late metastasis, and no double vision.

Discussion

IFN therapy for chronic HCV infection has been associated with thyroid dysfunction. The incidence of thyroid dysfunction ranges from 0.6 to 34.3% (25,26) with a mean of 6.6% (27), while in patients treated with IFN α and RBV combination therapy the incidence is higher (12.1%) (28). Recent research indicates that Peg-IFN in combination with RBV does not aggravate thyroid disease in the hepatitis C population (29).

Hypothyroidism is induced more frequently than hyperthyroidism during IFN therapy (3.8 vs. 2.8%), and females appear to be more susceptible to IFN-induced thyroid disorders than males (8.2 vs. 4.8%) (27). Factors predictive of dysthyroidism include female gender and the presence of thyroid autoantibodies before IFN treatment (27,30). TPOAb is considered to be more useful than TgAb in monitoring immunological response in patients treated with IFN (31). Koh *et al* reported that the risk of developing thyroid dysfunction in thyroid antibody-positive patients appears to be 46.1%, whereas only 5% of those who are thyroid antibody-negative at baseline develop thyroid dysfunction (27). They conclude that risk factors for developing thyroid dysfunction with IFN therapy are female gender, receipt of higher doses of IFN for longer durations, and the presence of thyroid autoantibodies prior to or during treatment. However, based on 138 eyes in 105 cases treated with eyelid surgery for Graves' ophthalmopathy, Inoue *et al* reports that the percentage of men with thyroid dysfunction increases as patients age (32).

As shown in Table III, few reported cases of Graves' ophthalmopathy have developed or been exacerbated following IFN treatment for hepatitis C (33-37). The mechanisms by which IFN induces thyroid autoimmunity remain unknown, but infectious agents have long been suspected to trigger thyroid autoimmunity, and HCV has shown the strongest association with autoimmune thyroid disease (38). HCV induces thyroid disease as an extrahepatic manifestation (9). Negative-strand HCV RNA has also been detected in the thyroid (39). IFN receptor activity results in the activation of the JAK-STAT pathway, leading to the activation of numerous IFN-stimulated genes. These effects can induce thyroid autoimmunity, and recent data have suggested that both the immune-mediated and direct thyroid-toxic effects of IFN play a role in its etiology (38). Our previous study found that the expression of thyrotropin receptor (TSH-R) mRNA in orbital fat tissue from patients with Graves' ophthalmopathy significantly correlated with orbital fat volume and the severity of ophthalmopathy (40). These results suggest that the expression of TSH-R in the orbit may play a role in the pathogenesis and clinical manifestations of ophthalmopathy.

Because the symptoms of hypothyroidism, such as fatigue, decreased appetite and depression, and the symptoms of hyperthyroidism, such as nervousness, irritability, fatigue and weight loss, can both be attributed to hepatitis C under IFN therapy, the diagnosis of thyroid disease in these patients may be delayed. This in turn may lead to the development of adverse effects induced by HCV therapy (38).

Our previous large-scale epidemiological survey showed that the incidence of oral pre-cancerous lesions and leukoplakia

was significantly higher in patients with HCV infection (41). Oral leukoplakia are well established as one of the best examples of pre-malignancy in humans. The rate of malignant transformation of these lesions is 3-20% (42). Furthermore, our study suggests the presence and elevation of HCV RNA in oral cancer and OLP tissues (43). Multi-center studies in Japan found that the presence of anti-HCV and HCV RNA was significantly higher in patients with squamous cell carcinoma of the head and neck than in control subjects (14). It has also been demonstrated that oral cancer patients often have carcinoma of the stomach (18%) and liver cancer (16%) as double cancers. Double-cancer patients have significantly higher HCV infection rates than controls (44). In the present case, the patient developed malignant transformation of leukoplakia after testing negative for HCV RNA during Peg-IFN plus RBV therapy. Whether the therapy was the trigger for malignant transformation is unknown.

In conclusion, our patient had Graves' ophthalmopathy, a rare side effect of IFN therapy for hepatitis C, and tongue cancer during Peg-IFN plus RBV therapy. To the best of our knowledge, this is the fifth case of ophthalmopathy newly-induced by IFN therapy (33-36). Thyroid function and pre-existing thyroid autoantibodies should be closely monitored for chronic hepatitis C with IFN therapy. In addition, when patients with HCV infection undergo follow-up, it is important to detect extrahepatic lesions early, refer the patient to specialists and start treatment earlier as well. Finally, we emphasize that medical professionals should perform regular follow-ups, including specialized clinical examinations, on patients with HCV infection.

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Analysis of factors interfering with the acceptance of interferon therapy by HCV-infected patients

Yumiko Nagao[1ABDEF], **Yutaka Kawakami**[2ABE], **Tamotsu Yoshiyama**[2B], **Michio Sata**[1AFG]

[1] Department of Digestive Disease Information & Research, Kurume University School of Medicine, Kurume, Fukuoka, Japan
[2] Pfizer Global R&D, Tokyo Laboratories, Pfizer Japan Inc., Shibuya-ku, Tokyo, Japan

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Authors' Contribution:
A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
G Funds Collection

PI

Summary

Background: Interferon (IFN) therapy, an antiviral agent, contributes to the prevention of occurrence of hepatocellular carcinoma (HCC) and to improvement in long-term prognosis. However, IFN therapy is not well-implemented in Japan. The present study was conducted to analyze factors preventing the implementation of IFN therapy.

Material/Methods: Questionnaires were sent to patients with hepatitis C virus (HCV)-related liver disease who were treated at 7 clinics (by non liver-specialists) and 1 hospital (by liver specialists) and by their attending physicians.

Results: Of 139 patients for whom attending physicians recommended IFN therapy, 92 (66.2%) agreed to receive the treatment. The proportions of patients who agreed to receive IFN therapy were 74 (86.0%) out of 86 hospital patients and 18 (34%) out of 53 clinic patients. In logistic regression analysis, the adjusted odds ratios on treatment facilities, sex and complications were 18.06, 3.65, and 3.63 respectively, indicating that there were significant differences. Female patients more than male patients declined IFN therapy because of worries over the adverse reactions of IFN therapy.

Conclusions: Multivariate analysis showed that factors contributing to the risk that a patient would not consent to receive IFN therapy included a) treatment facilities, b) sex, and c) the presence or absence of complications. It is also essential to devise measures to create cooperation between hospitals and clinics, and to improve communication between physicians and patients.

key words: **hepatitis C virus • interferon therapy • chronic hepatitis C • hepatocellular carcinoma • liver specialist • non liver-specialist**

Abbreviations: **anti-HCV** – anti-bodies to HCV; **HCC** – hepatocellular carcinoma; **HCV** – hepatitis C virus; **IFN** – Interferon

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Author's address: Yumiko Nagao, Department of Digestive Disease Information & Research, Kurume University School of Medicine, 67 Asahi-machi, Kurume 830-0011, Japan, e-mail: nagao@med.kurume-u.ac.jp

BACKGROUND

Hepatocellular carcinoma (HCC) is the third most common cause of death from cancer in men and the sixth most common cause in women [1]. An increase in the number of cases of HCC has occurred in the United States over the past two decades [2]. The age-specific incidence of this cancer has progressively shifted toward younger people. Similarly, the number of deaths in Japan from HCC keeps increasing. This trend is expected to continue until 2015 [3]. In Japan, ~80% of HCCs are caused by hepatitis C virus (HCV) and ~10% by hepatitis B virus (HBV). The increase in the number of HCC patients due to HCV contributes to the increase in the deaths in Japan from HCC.

It is presumed that between 1 and 2 million Japanese people are chronically infected with HCV [3]. Because many such people are unaware that they are infected, carriers may develop liver cirrhosis and HCC, and this poses a serious problem. In April 2002, the Ministry of Health, Labour and Welfare began targeting area residents for hepatitis virus screening as part of urgent comprehensive measures for identifying hepatitis C and other infections. Since 2002, antibodies to HCV (anti-HCV) and HBs antigens have been tested in Japanese individuals who receive a basic health check up. This is part of the Elderly Health Project whose goal is to re-test them every 5 years between ages 40 and 70.

The national compliance rate for this health check during 4 years from 2002 to 2005 was about 27% (~5.1 million people). The HCV infection rate at that time was 0.9% (~47,000 people). However, only 6,160 HCV carriers in fact received treatment at secondary medical facilities, while 16% (969/6,160) of carriers were treated with interferon (IFN) at secondary medical facilities during the 4 years. These statistics suggest that not many patients or residents are actually treated with IFN despite the fact that IFN can get rid of HCV [4]. Currently, creation of a network for post-health screening treatment has been in progress.

IFN therapy for chronic hepatitis C is the only treatment for completely eliminating HCV. In recent years, the standard therapy has been the combination of pegylated interferon (Peg-IFN) and ribavirin. Following 1-year administration of this combination, the treatment was found to be markedly effective in~50 to 60% of all HCV-infected patients, including those with conventionally intractable genotype 1b • high titer [5]. It has been demonstrated that IFN therapy contributes to the prevention of occurrence of HCC and to improvement in long-term prognosis [6–9].

Why is IFN therapy for HCV carriers in Japan not used more widely? Reasons remain unclear because no systematic investigation has been conducted.

In our previous study, we sent questionnaires to both 254 pairs of HCV carriers and their attending physicians in different areas in Japan in which we discussed the future state of medical care in which IFN therapy would be used more widely [10]. There was a great difference among types of medical facilities in the proportions of patients who opted to receive IFN therapy. Whereas 78.2% of patients of liver specialists agreed to IFN therapy, the proportion was only 15.7% for patients of non liver-specialists.

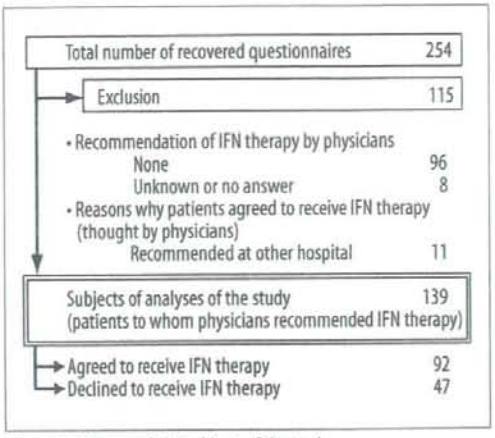

Figure 1. Diagram of 139 subjects of the study.

In the present study, patients who were recommended to receive IFN therapy were defined as "patients who ought to receive IFN therapy." Then, we looked for factors that caused patients who ought to receive IFN therapy to not receive it. That is, we looked for factors interfering with the introduction of IFN therapy. The geographical area where our investigation was conducted was one where we have been conducting successive epidemiological investigations on liver diseases and extrahepatic manifestations since 1990 [11–17].

MATERIAL AND METHODS

Subjects

Between October 1, 2005 and February 28, 2006, unregistered questionnaires were sent to HCV carriers who had been treated at a key hospital in A City, Fukuoka Prefecture and all clinics in H Town in A City and their attending physicians, and 254 pairs of answers were recovered. Subject medical organizations were 7 clinics without liver specialists and 1 hospital where many liver specialists authorized by the Japan Association for the Study of the Liver work full time. We mailed questionnaires directly to these 8 medical organizations. A database for the results of our investigation was compiled at the Office of Pharmaceutical Industry Research (OPIR)/Japan Pharmaceutical Manufacturers Association (JPMA).

The 254 patients were divided into groups depending on whether or not their physicians recommended any of the following IFN therapy: IFN monotherapy, Peg-IFNα-2a monotherapy, IFNα-2b plus ribavirin, and Peg-IFNα-2b plus ribavirin. As shown in Figure 1, 139 patients to whom physicians recommended IFN therapy were selected for the analysis of factors influencing the decision of patients whether or not to receive IFN therapy. Excluded from our analyses were 96 patients to whom physicians did not recommend IFN therapy, and 8 patients for whom it was unclear whether or not physicians recommended IFN therapy, or who did not respond to the questionnaire. Also excluded were 11 patients who received IFN therapy after recommendations from other hospitals. Of 139 patients analyzed, 92 consented to receive IFN therapy and 47 did not.

Table 1. Items of investigation by questionnaires sent to both physicians and patients.

1. Patients' background
(1) Patients' attributes (age, sex, joining the patient advocacy group for liver disease)
(2) Diagnosis of liver diseases and complications
(3) Nutritional instruction for liver diseases (received, not received)
(4) Health foods and folk medicines (taken, not taken)
(5) Treatment other than IFN therapy (treated, not treated)
2. IFN therapy
(1) Explanation of IFN therapy (given, not given). If yes, when
(2) Implementation of IFN therapy (received, not received)
(3) Frequency of IFN therapy (*)
(4) The nearest place where IFN therapy was given (*)
(5) Reasons why patients decided to receive IFN therapy (*)
(6) The latest therapeutic effects of IFN therapy
(7) Reasons why IFN therapy was discontinued (*)
3. Factors for which IFN therapy was not performed
(1) IFN therapy was recommended (yes, no)
(2) Reasons why IFN therapy was recommended Reasons why IFN therapy was not recommended (*)
(3) Did patients decline IFN therapy? (yes, no)
(4) Reasons why patients declined IFN therapy
4. Comments (write what you think about liver diseases)

(*) Questions asked to physicians only.

The investigation was conducted in accordance with the "ethical guidelines on epidemiological studies" by the Ministry of Education and Science and the Ministry of Health, Labour and Welfare and observed the spirit of the Helsinki Declaration. Physicians at study facilities explained to patients the content and significance of the study and obtained consent in accordance with rules at each facility.

Items of investigation

Unregistered questionnaires asked patients and their attending physicians to respond to the following items.

1) Patients' background, 2) IFN therapy, and 3) factors determining the decision to not implement IFN therapy.

Items of investigation are listed in Table 1.

Statistical analysis

Crude odds ratios and adjusted odds ratios were calculated for factors possibly related to consenting to IFN therapy. Adjusted odds ratios were calculated using logistic regression analysis. Candidate factors for logistic regression analysis were selected by using a strategy that was recommended by Hosmer, DW, et al. [18], and secondary interactions among the selected factors were also assessed. Selection of factors for the final model was performed in a stepwise method, and the significance level for entering or removing of factors into or from regression models were both 0.15. The fitting of models was assessed using the Hosmer-Lemeshow test.

We tabulated reasons why patients declined IFN therapy, and therapeutic effects in patients who received IFN therapy.

All statistical analyses were conducted using SAS for Windows Version 8.2 (SAS Institute, Cary, NC, USA). The level of statistical significance was defined as 0.05.

PI

RESULTS

Patients' background

Table 2 lists clinical information for patients who were recommended to receive IFN therapy. Physicians recommended IFN therapy to 139 patients; 53 at clinics (non liver-specialists) and 86 at a hospital (liver specialists). For patients older than 60, 36 were recommended at clinics (67.9%) and 55 at a hospital (64.0%). The number of patients who joined the patient advocacy group for liver disease was zero at clinics and 13 (15.1%) at a hospital. The number of patients who were female were 30 (56.6%) at clinics and 45 (52.3%) at a hospital. The number of patients with concomitant medical complications were 36 (67.9%) at clinics and 65 (75.6%) at a hospital. Patients in the two groups were well-matched for baseline characteristics.

Univariate analysis

Of 139 subjects of analyses to whom physicians recommended IFN therapy, 92 (66.2%) agreed to receive the therapy (Table 2). Whereas 74 of 86 hospital patients (86.0%) agreed to receive IFN therapy, only 18 of 53 clinic patients (34.0%) did so.

In univariate analyses (Table 3), the crude odds ratio of treatment facilities (clinic/hospital) was calculated as 11.99, demonstrating a significant difference in the proportion agreeing to receive IFN therapy between clinic patients and hospital patients. As for other factors, the crude odds ratio for sex (female/male) was 1.96 and that for joining the Liver Society (or not) was 0.14, suggesting that the associations between these factors and the decision to receive IFN therapy were not statistically significant.

Multivariate analysis

According to multivariate analysis, three factors, treatment facilities (clinic/hospital), sex (female/male) and complications (yes/no), were identified as factors that influenced patients' decisions to receive IFN therapy. The adjusted odds ratios for these 3 factors were 18.06, 3.65 and 3.63, respectively, and each was statistically significant. Among all of the selected factors, the adjusted odds ratios were increased over the crude odds ratios. Factors of sex and complications were not statistically significant in the crude odds ratios but significant following multivariate adjustment.

Table 2. Clinical information of 139 patients to whom IFN therapy was recommended.

		Total n=139 (%)		Clinic (Non liver-specialist) n=53 (%)		Hospital (Liver specialist) n=86 (%)	
IFN therapy	Accepted	92	(66.2)	18	(34.0)	74	(86.0)
	Not accepted	47	(33.8)	35	(66.0)	12	(14.0)
Treatment facilities	Hospital (liver-specialist)	86	(61.9)				
	Clinic (non liver-specialist)	53	(38.1)				
Age	20–29 years old	2	(1.4)	0	(0.0)	2	(2.3)
	30–39	3	(2.2)	0	(0.0)	3	(3.5)
	40–49	10	(7.2)	4	(7.5)	6	(7.0)
	50–59	33	(23.7)	13	(24.5)	20	(23.3)
	60–69	44	(31.7)	14	(26.4)	30	(34.9)
	70–79	45	(32.4)	22	(41.5)	23	(26.7)
	80 years or older	2	(1.4)	0	(0.0)	2	(2.3)
Sex	Male	63	(45.3)	22	(41.5)	41	(47.7)
	Female	75	(54.0)	30	(56.6)	45	(52.3)
	No answer	1	(0.7)	1	(1.9)	0	(0.0)
Diagnosis of liver diseases (choose one)	Chronic hepatitis C alone	103	(74.1)	34	(64.2)	69	(80.2)
	Other than chronic hepatitis C alone	36	(25.9)	19	(35.8)	17	(19.8)
	No answer	0	(0.0)	0	(0.0)	0	(0.0)
Diagnosis of liver diseases (choose all applicable)	Chronic hepatitis C	117	(84.2)	41	(77.4)	76	(88.4)
	HCV-related liver cirrhosis	22	(15.8)	10	(18.9)	12	(14.0)
	HCC type C	7	(5.0)	4	(7.5)	3	(3.5)
	Asymptomatic HCV carrier	1	(0.7)	1	(1.9)	0	(0.0)
	History of HCV infection	3	(2.2)	2	(3.8)	1	(1.2)
	Others	7	(5.0)	4	(7.5)	3	(3.5)
	Uncertain	0	(0.0)	0	(0.0)	0	(0.0)
	No answer	0	(0.0)	0	(0.0)	0	(0.0)
Concomitant medical complications	No	36	(25.9)	15	(28.3)	21	(24.4)
	Yes	101	(72.7)	36	(67.9)	65	(75.6)
	Hypertension	68	(48.9)	27	(50.9)	41	(47.7)
	Diabetes mellitus	28	(20.1)	11	(20.8)	17	(19.8)
	Heart diseases	10	(7.2)	3	(5.7)	7	(8.1)
	Cerebrovascular diseases	4	(2.9)	1	(1.9)	3	(3.5)
	Thyroid diseases	7	(5.0)	1	(1.9)	6	(7.0)
	Rheumatism	0	(0.0)	0	(0.0)	0	(0.0)
	Stomatitis	2	(1.4)	0	(0.0)	2	(2.3)
	Others	33	(23.7)	7	(13.2)	26	(30.2)
	No answer	2	(1.4)	2	(3.8)	0	(0.0)
Patient advocacy group for liver disease	Joined	13	(9.4)	0	(0.0)	13	(15.1)
	Not joined	126	(90.6)	53	(100.0)	73	(84.9)

HCC – Hepatocellular carcinoma.