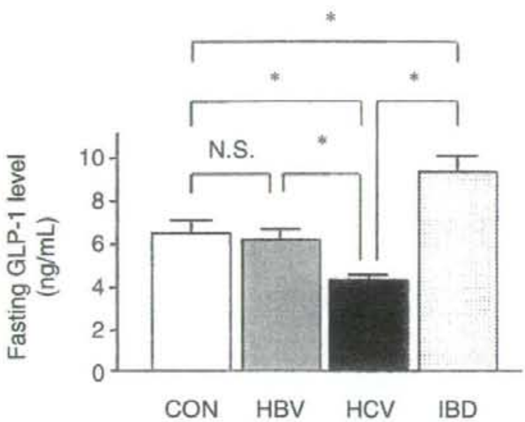

Figure 1 Fasting glucagon-like peptide-1 (GLP-1) levels in healthy controls (CON group, $n = 48$), patients with hepatitis B virus-related liver disease (HBV group, $n = 37$), patients with hepatitis C virus-related liver disease (HCV group, $n = 94$), and patients with inflammatory bowel disease (IBD; $n = 14$). Values are expressed as mean ± SEM. Comparisons between the groups were performed by ANOVA followed by Fisher's protected least significant difference. N.S., not significant. $*P < 0.01$.

Changes in fasting GLP-1 levels

The fasting serum GLP-1 levels were significantly decreased in the HCV group compared to those in the controls ($P < 0.01$), the HBV group ($P < 0.01$), or the IBD group, an intestinal disease control ($P < 0.01$) (Fig. 1).

GLP-1 content of the ileum in patients with HCV-related chronic liver disease

The GLP-1 content of the ileum was not significantly different between the control and the HCV groups (Fig. 2a). Immunostaining demonstrated that the GLP-1 intensity of L-cells was not significantly different between the controls and the HCV group (Fig. 2b).

Association between liver function and fasting GLP-1 levels in patients with HCV-related liver disease

In the HCV group, no significant difference was seen in the fasting GLP-1 levels among CH ($n = 9$), Child–Turcotte criterion A ($n = 54$), B ($n = 22$), and C ($n = 9$). No significant association was also seen between the fasting GLP-1 levels and albumin levels ($P = 0.08$, $r^2 = 0.001$), total bilirubin levels ($P = 0.07$, $r^2 = 0.04$), or platelet count in the HCV group ($P = 0.77$, $r^2 = 0.001$). Moreover, no significant correlations were found between the fasting GLP-1 levels and APRI, an indicator of liver fibrosis ($P = 0.82$, $r^2 = 0.001$) or serum IRI levels ($P = 0.09$, $r^2 = 0.003$).

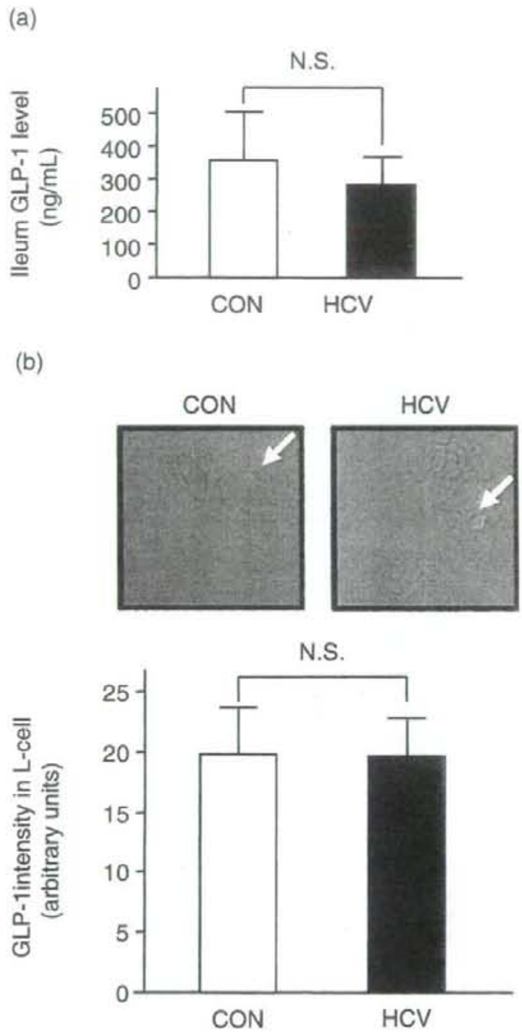

Figure 2 Ileum glucagon-like peptide-1 (GLP-1) content. Ileum GLP-1 content was evaluated by (a) enzyme immunoassay (control [CON] group, $n = 4$; HCV group, $n = 4$) and (b) immunostaining (CON group, $n = 4$; hepatitis C virus [HCV] group, $n = 4$). Representative photographs show GLP-1 expression of immunostaining in ileal tissue. Arrows show GLP-1 staining in endocrine L-cell of the ileum. Values are expressed as mean ± SEM. Comparisons between groups were made using the Mann–Whitney U-test. N.S., not significant. $*P < 0.05$.

DPPIV expression in the ileum, liver, and serum

In the ileum tissue, immunostaining showed that the DPPIV expression levels were increased in the HCV group compared to

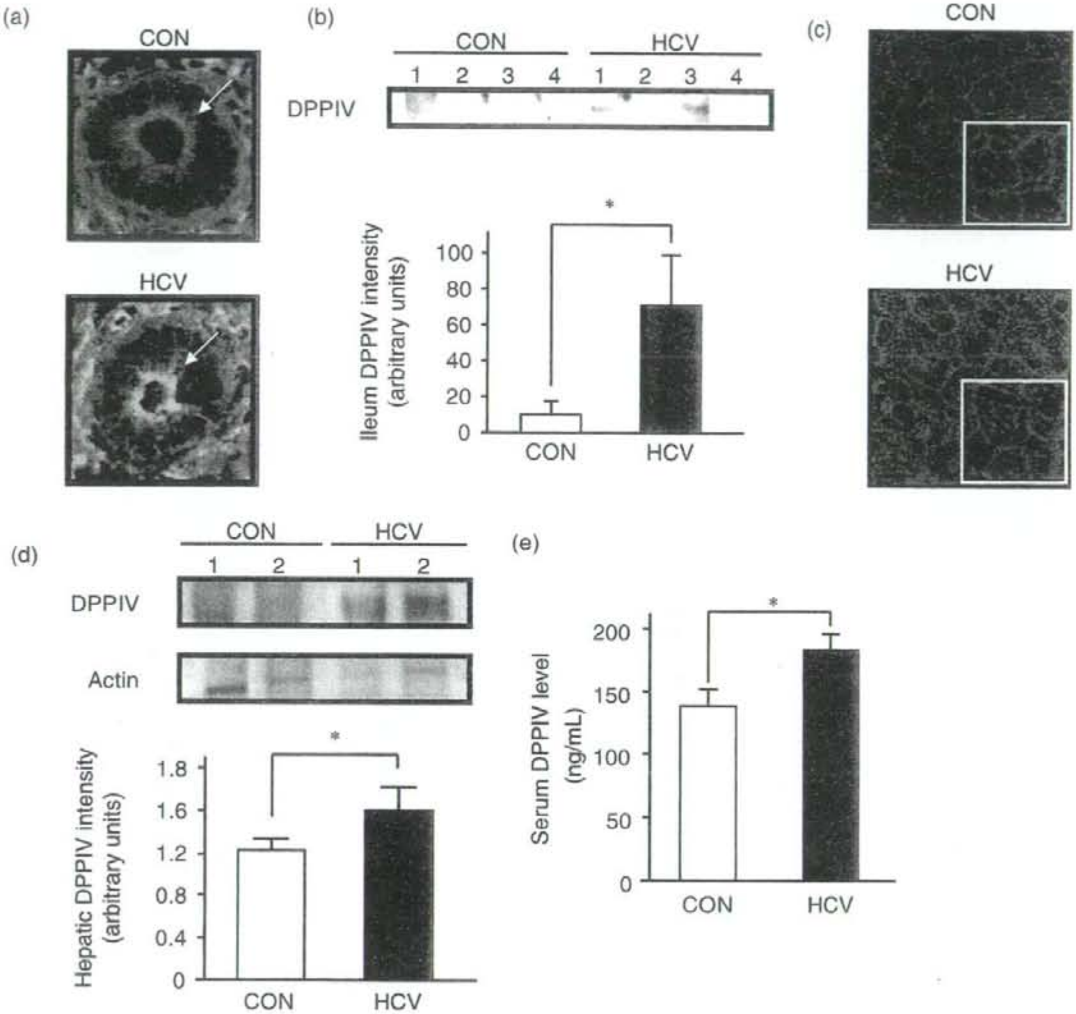

Figure 3 Dipeptidyl peptidase IV (DPPIV) expression in the ileum, hepatocytes, and serum. Ileum DPPIV expression was evaluated by (a) immunostaining and (b) immunoblotting (control [CON] group, $n = 4$; hepatitis C virus [HCV] group, $n = 4$). DPPIV expression in hepatocytes was evaluated by (c) immunostaining and (d) immunoblotting (CON group, $n = 5$; HCV group, $n = 4$). Representative photographs show the DPPIV expression of immunostaining and immunoblotting in the ileum or hepatocytes. (e) Serum DPPIV levels was measured by ELISA (CON group, $n = 10$; HCV group, $n = 19$). Values are expressed as mean ± SEM. Comparisons between groups were made using the Mann–Whitney *U*-test. $^*P < 0.05$.

that in the controls ($P < 0.05$)(Fig. 3a). Immunoblotting also showed a significant increase of DPPIV expression levels in the HCV group ($P < 0.05$) compared to that in the controls ($P < 0.05$) (Fig. 3b). In the hepatocytes, immunostaining showed that DPPIV expression levels were increased in the HCV group ($P < 0.05$) compared to the controls ($P < 0.05$) (Fig. 3c). Immunoblotting also revealed that the DPPIV expression levels were significantly increased in the HCV group than those in the controls ($P < 0.05$) (Fig. 3d). The serum DPPIV levels were significantly increased in the HCV group than that in the controls ($P < 0.05$) (Fig. 3e).

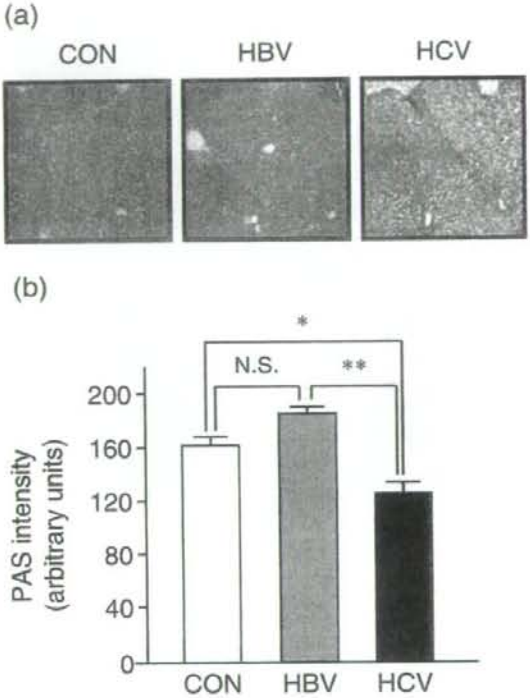

Figure 4 (a) Hepatic glycogen content was examined by periodic acid–Schiff (PAS) staining in healthy controls (CON; $n = 5$), patients with hepatitis B virus-related liver disease (HBV group, $n = 19$), and patients with hepatitis C virus-related liver disease (HCV group, $n = 20$). (b) PAS staining intensity was evaluated by measuring pixel intensities using NIH Image-J. Values are expressed as mean ± SEM. Comparisons between the groups were performed by ANOVA followed by Fisher's protected least significant difference. N.S., not significant. $*P < 0.05$; $**P < 0.01$.

Ability of glucose uptake and insulin secretion in patients with HCV-related chronic liver disease

The ability of glucose uptake and insulin secretion was examined by an OGTT. The HCV group ($n = 21$) showed persistent increases in glucose (208.5 ± 25.2 mg/dL) and insulin levels (63.2 ± 7.4 μU/dL) at 120 min, although Asian men with normal glucose tolerance showed decreases in glucose at 120 min.[26] The HOMA-β-values were within the normal range in the HCV group (97 ± 15.3; $n = 21$). Similarly, the insulinogenic index in the HCV group was within the normal range (1.0 ± 0.3; $n = 21$).

Glycogen accumulation levels in the liver

The glycogen accumulation levels of the liver specimens were significantly decreased in the HCV group than that in the controls ($P < 0.05$) or the HBV group ($P < 0.01$) (Fig. 4a,b).

Discussion

In this study, we demonstrated decreased serum GLP-1 levels and increased DPPIV expression in the liver, ileum, and serum in patients with HCV infection. We also showed impaired hepatic glycogen storage in patients with HCV infection.

Insulin resistance was significantly increased in the HCV group, which is in accordance with previous reports.[11,28] HCV genotype 3 is associated with insulin resistance.[1,2] Although we could not examined the HCV genotype for all of the enrolled patients, it was reported that the prevalence of HCV genotype 3 is only found in 1.3% of Japanese (5/379 patients).[4] In addition, there were no patients with HCV genotype 3 infection among the 158 patients with HCV infection in our previous investigation, which was conducted in an area similar to the one in the current study.[5] Thus, it seems that genotype is not a factor responsible for insulin resistance in this study. Insulin resistance can be also caused by the dysfunction of several organs. The gut is involved in the development of insulin resistance through the regulation of gut hormones, such as GLP-1.[13,14] In this study, we first demonstrated the decrease in the active form of serum GLP-1 levels in patients with HCV-associated insulin resistance. In contrast, increases in both insulin resistance and the active form of serum GLP-1 levels are seen in patients with type 2 diabetes mellitus and alcoholic liver disease.[29,30] This discrepancy suggests that HCV is involved in the downregulation of GLP-1.

GLP-1 mainly occurs in the terminal ileum.[13,14] In both immunostaining and ELISA, there was no significant difference in ileum GLP-1 expression levels between the controls and the HCV group. Thus, in patients with HCV infection, the downregulation of serum GLP-1 levels was not caused by a decrease in GLP-1 expression in the ileum. However, the liver is considered a major site for the regulation of serum GLP-1 levels and more than 40% of circulating GLP-1 is degraded in the liver.[18] Therefore, we investigated a relationship between serum GLP-1 levels and liver function. There was no correlation between the serum GLP-1 levels and the indicators of liver function. Thus, liver function also does not seem to be a responsible factor for the downregulation of serum GLP-1 in patients with HCV infection. It would be reasonable to assume that the downregulation of serum GLP-1 levels reflects negative feedback resulting from the increased insulin resistance in HCV-infected patients. However, there was no significant correlation between fasting serum GLP-1 levels and serum IRI levels. In addition, a previous study demonstrated that hyperinsulinemia does not inhibit GLP-1 secretion.[31] Thus, negative feedback does not seem to directly downregulate serum GLP-1 levels in HCV-infected patients.

DPPIV rapidly inactivates active GLP-1.[32] DPPIV localizes at intestinal brush-borders and membrane of hepatocytes.[33,34] In addition, the circulating soluble form of DPPIV exhibits enzymatic activity.[35] In this study, we found increased DPPIV expression of the ileum, liver, and serum in patients with HCV infection. Although we could not assess tissue DPPIV activity, DPPIV expression levels are positively correlated with its peptidase activity.[35] It seems therefore that the upregulation of DPPIV increases its peptidase activity, which subsequently causes a decrease in serum GLP-1 levels. The reason for the increased DPPIV expression in patients with HCV infection is unclear. However, DPPIV is also known as CD26, an immune-regulating molecule expressed on T cells.[36] A significant increase in DPPIV/CD26 expression is seen in a hepatoma cell line transfected with a HCV non-structural genome region.[37] HCV infects not only liver, but also extrahepatic tissues, including the intestine and lymphocytes.[38] Thus, it is possible that HCV directly upregulates DPPIV expression in the ileum, liver, and serum.

GLP-1 plays an important role in the early phase of insulin secretion.[39] Unexpectedly, in the HCV group, the HOMA-β-value, an indictor of basal insulin secretion, and the insulinogenic index, an indicator for early-phase insulin secretion, were equal to those of healthy Asian patients.[26] Although the serum GLP-1 levels were decreased, the downregulation of GLP-1 in patients with HCV infection did not seem to have a significant influence on the early phase of insulin secretion. Insulin secretion might be compensated by other stimuli of insulin secretion, including glucose-dependent insulinotropic polypeptide.

GLP-1 increases glycogen synthesis in hepatocytes by stimulating glycogen synthase α.[17,40] It is well known that hepatic glycogen storage is depleted in patients with cirrhosis. However, etiological differences in glycogen storage have never been investigated. Here, we demonstrated that glycogen content was significantly decreased in the HCV group than in the controls and the HBV group. The depletion of glycogen storage leads to an increase in free fatty acid (FFA) levels.[41] Serum FFA levels were increased in HCV-related chronic liver disease.[42] Increased FFA impairs intracellular insulin signaling, insulin receptor substrate-1 tyrosine phosphorylation, and results in increased insulin resistance.[41,43] These findings suggest that the downregulation of GLP-1 is involved in increased insulin resistance through the depletion of glycogen storage in hepatocytes.

The results of the ileum tissue experiments are limited, because the data were obtained from a small subgroup. Although all of the cases showed a similar trend, these patients may not be representative of the whole group. Furthermore, the significance of the DPPIV–GLP-1 regulatory pathways in HCV-related glucose intolerance is unclear. Thus, the comparison of the DPPIV–GLP-1 regulatory pathway with other factors linked to glucose intolerance, such as BMI, is required for further elucidation. Another limitation is patient selection. The majority of enrolled patients were in the early stage of liver cirrhosis (Child–Turcotte A). Moreover, patients who had been taking oral hypoglycemic agents or insulin injections were excluded from this study. Thus, we have to be cautious in the interpretation of data from a relatively selected population of HCV infection.

In conclusion, this study showed decreased serum GLP-1 levels and increased DPPIV levels in the ileum, liver, and serum in patients with HCV infection. Decreased GLP-1 levels may be due to an upregulation of DPPIV expression. A GLP-1 action, hepatic glycogen synthesis, was impaired in patients with HCV infection. Thus, the altered expression of GLP-1 and DPPIV may be responsible for HCV-associated glucose intolerance.

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Original Article

Suppressive effect of oral administration of branched-chain amino acid granules on oxidative stress and inflammation in HCV-positive patients with liver cirrhosis

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Aim: In chronic hepatitis C virus (HCV) infection, it is thought that both chronic persistent inflammation and oxidative stress contribute to the development of hepatocellular carcinoma (HCC), and it has been reported that long-term oral supplementation with branched-chain amino acid (BCAA) granules could inhibit liver carcinogenesis. However, the extent of the involvement of these factors remains obscure.

Methods: To clarify the involvement of inflammation and oxidative stress in the inhibition of liver carcinogenesis, we evaluated the effect of oral administration of BCAA granules on oxidative stress and inflammation in HCV-positive patients with liver cirrhosis.

Results: Twenty-seven patients were enrolled in the study: 18 of the patients were treated with BCAA granules (administered group) and nine were observed without BCAA granules (non-administered group). In the non-administered group, the production of oxidative stress, as indicated by urine 8-hydroxydeoxyguanosine (8-OHdG) and 15-F2t-Isoprostane (8-IsoPs), significantly increased with time, while in the administered group the levels of ferritin and 8-OHdG decreased significantly. Comparison of the two groups demonstrated that highly sensitive CRP, ferritin, 8-OHdG and 8-IsoPs were significantly reduced by taking BCAA granules. The time-course analysis showed that ferritin and highly sensitive CRP seemed to decrease first, followed by a decrease of 8-OHdG and 8-IsoPs.

Conclusion: These findings indicated that the administration of BCAA granules influenced microinflammation and the metabolism of iron in HCV-positive patients with liver cirrhosis, and subsequently seemed to reduce the production of oxidative stress, possibly leading to a decrease in the occurrence of HCC.

Key words: branched-chain amino acid, hepatitis C virus, liver cirrhosis, oxidative stress

INTRODUCTION

THERE ARE APPROXIMATELY 170 million people infected with hepatitis C virus (HCV) worldwide.[1] HCV is a major causative agent of liver disease, including chronic hepatitis, liver cirrhosis (LC) and hepatocellular carcinoma (HCC). It is estimated that 70–80% of HCV infections evolve into chronic infection. Once HCV develops into cirrhosis, HCC develops at an annual rate of 5–7%.[2] Nevertheless, the precise mechanism underlying HCV-associated HCC is not completely understood. HCV may contribute to the development of HCC by facilitating the accumulation of genetic damage as a result of continuous cell death followed by regeneration in the course of chronic hepatitis. If this is the case, HCV would only be associated indirectly with hepatocarcinogenesis. Another possibility is the direct involvement of HCV in hepatocarcinogenesis, whereby some products of the virus may be oncogenic and involved in cell transformation. Recently, the core protein of HCV has been shown to induce HCC in a transgenic mouse model and has been suggested to play a central role in the development of HCC in chronic

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hepatitis C.[3] However, it still remains unclear how the core protein influences the development of HCC. An additional possibility is the involvement of oxidative stress. Recently, numerous studies have shown that HCV infection itself causes oxidative stress to a greater degree than other inflammatory liver disease.[4,5]

Oxidative stress is defined as a disturbance of the normal balance between oxidants and antioxidants in the body. Under oxidative stress, reactive oxygen species (ROS) can modify macromolecules such as lipids, proteins and nucleic acids (DNA). If ROS-mediated DNA damage is not repaired, it leads to the production of genetic mutations as well as gross chromosomal alterations and contributes to cancer development in multistep carcinogenesis.[6]

Clinically, when long-term supplementation with branched-chain amino acids (BCAA) is given to patients with compensated or decompensated cirrhosis, their nutritional status, as indicated by hypoalbuminemia, ammonia metabolism and glucose metabolism, is improved.[7] Additionally, a recent study has shown that long-term oral supplementation with BCAA can inhibit liver carcinogenesis, especially in overweight or obese patients.[8]

Against this background, we hypothesized that when BCAA granules are administered orally to patients with LC, the improvement of hypoalbuminemia, glucose and ammonia metabolism, as well as the inhibitory effect on hepatocarcinogenesis, might be due to a reduction of oxidative stress by the BCAA supplementation. In the present study, we investigated the effects on oxidative stress of administering BCAA granules orally to patients with LC.

Table 1 Characteristics of HCV patients with LC

	P	C
Number of patients	18	9
Age	56–80	52–79
Median	70.2	66.8
Sex (male/female)	9/9	4/5
Routine parameters		
Uric acid (mg/dL)	5.44	5.8
NH3 (μg/dL)	64.44	68.56
T-protein (g/dL)	7.59	7.53
Alb (g/dL)	3.84	3.68
AST (IU/L)	68.39	67.44
ALT (IU/L)	49.94	46
LDH (IU/L)	228.29	230.44
ALP (IU/L)	338.12	325.67
T-chol (mg/dL)	153.94	168.56
FBS (mg/dL)	138.56	119.44
PT-INR	1.1	1.13
AFP (ng/mL)	33.39	16.06
Centrally measured parameters		
Ferritin (ng/mL)	65.93	45.23
Highly sensitive CRP (ng/mL)	1043.56	428.11
8-OHdG (ng/mL)	11.07	9.35
8-IsoPs (ng/mL)	373.96	338.57
Hepatocellular carcinoma	0	0

ALT, alanine transaminase; C, control patients; HCV, hepatitis C virus; LC, liver cirrhosis; P, patients who were orally administered branched-chain amino acid (BCAA) preparation thrice a day.

METHODS

Subjects, design and protocol

THE SUBJECTS INCLUDED in the present study were outpatients with compensated cirrhosis who had hypoalbuminemia despite adequate food intake.

A total of 27 subjects, 18 of whom were given a 4 g BCAA preparation (LIVACT Granules; Ajinomoto, Tokyo, Japan) administered orally three times daily after meals (administered group), and nine of whom were followed up without the BCAA granules as a control (non-administered group), were enrolled in the present study. All of subjects were confirmed to be HCV-positive and HBV-negative patients with liver cirrhosis and without other liver diseases, including HCC as shown by image techniques, and not to be have received any medication with BCAA granules, intravenous albumin administration, or enteral nutrition for hepatic insufficiency within 8 weeks before enrollment. Patients were followed up for at least 6 months and blood and urine samples were collected at treatment initiation (0 M) and at 1 month (1 M), 2 M, 3 M, 4 M and 6 M for as long as possible. Routine laboratory parameters as shown in Table 1, highly sensitive CRP, ferritin and oxidative stress markers (urine 8-hydroxydeoxyguanosine [8-OHdG] and urine 15-F2t-Isoprostane [8-IsoPs]) were evaluated.[9] Routine laboratory parameters were measured on every visit to hospital. Blood and urine samples were stored at −70°C until highly sensitive CRP, ferritin, urine 8-OHdG and urine 8-IsoPs were centrally measured at SRL MediSearch (Tokyo, Japan). The raw values (nanogram: ng/mL) of urine 8-OHdG and urine 8-IsoPs were conventionally corrected by urinary creatinine (mg/dL) to make it possible to compare them with each other.[9]

It was confirmed that the patients' medications had not been changed within 8 weeks before enrollment or during the administration of BCAA. The eligibility and exclusion criteria were made according to the manufacturer's instructions.

This study was a post-marketing clinical trial and was performed with the approval of the review board of Social Insurance Chukyo Hospital. Written informed consent to participate in this trial was obtained from all subjects.

Statistical analysis

The primary end point was the amount of change in oxidative stress markers. The *t* statistic was used to evaluate whether the study drug was useful for reducing oxidative stress. Data were further analyzed by the Mann–Whitney *U*-test. $P < 0.05$ was considered statistically significant.

RESULTS

THE CHARACTERISTICS OF the two groups of patients were comparable in terms of age, sex and underlying liver disease severity, as shown in Table 1.

To assess how the evaluated variables altered during the observation, the data from before (0 M) and at 6 months (6 M) were compared in each group (Table 2). The results showed that total cholesterol (T-chol) was significantly decreased and PT-INR was significantly prolonged, and also that urine 8-OHdG and 8-IsoPs were significantly increased at 6 M in the non-administered group. The decrease in T-chol and the prolongation of PT-INR might mean that the liver diseases were becoming more severe and that oxidative stress was increasing in the patients over time. In contrast, in administered group, urine 8-OHdG and serum ferritin at 6 M significantly decreased, suggesting that administering BCAA granules regularly might reduce oxidative stress. Considering that highly sensitive CRP was slightly, although not significantly, increased in the non-administered group and slightly decreased in the administered group during the observation period, it was considered necessary to compare the alterations between the non-administered group and the administered group. Therefore, the time-dependent alterations (Δp or $\Delta c = \text{value}_{6M} - \text{value}_{0M}$) of all parameters were compared between the two groups using the Mann–Whitney *U*-test. The results showed that ferritin, highly sensitive CRP, urine 8-OHdG and urine 8-IsoPs were significantly decreased by BCAA administration. Considering that ferritin reflects the body's iron storage[10] and that highly sensitive CRP can detect changes in microinflammation, our data suggested that taking

Table 2 Results of statistic analysis

	Uric acid	NH3	T-protein	Alb	AST	ALT	LDH	T-chol	FBS	PT-INR	AFP	Ferritin	H-CRP	8-OHdG	8-IsoPs
P (0 M)	5.44	64.44	7.59	3.84	68.39	49.94	228.29	154.94	138.56	1.10	33.29	65.03	1043.56	11.07	373.96
P (6 M)	5.70	75.22	7.63	3.87	63.44	48.56	213.92	160.33	122.89	1.11	34.88	53.15	351.28	0.03	337.89
P-value*	0.284	0.334	0.877	0.62	0.602	0.619	0.238	0.664	0.236	0.454	0.758	0.024	0.095	0.0003	0.518
C (0 M)	5.80	68.56	7.53	3.68	67.44	46.00	230.44	168.56	119.44	1.13	16.06	45.23	428.11	9.35	338.57
C (6 M)	5.61	80.89	7.29	3.59	59.33	41.11	211.89	158.00	120.56	1.16	26.21	44.87	591.44	10.64	512.41
P-value*	0.433	0.439	0.086	0.303	0.181	0.359	0.055	0.021	0.865	0.004	0.445	0.881	0.311	0.042	0.047
Mann–Whitney**	0.245	>0.10	0.291	0.194	0.857	>0.10	0.483	$P > 0.010$	0.799	$P > 0.05$	$P > 0.10$	0.037	0.041	0.00038	0.025

ALT, alanine transaminase; AST, aspartate aminotransferase; C, control patients; H-CRP, highly sensitive CRP; P, patients who were orally administered branched-chain amino acid (BCAA) preparation thrice a day.

*$P < 0.05$ versus data of 0 M for data of 6 M (T-test). **$P < 0.05$ versus data of ΔP ($P_{6M} - P_{0M}$) for ΔC ($C_{6M} - C_{0M}$) (Mann–Whitney *U*-test).

Data depict mean values.

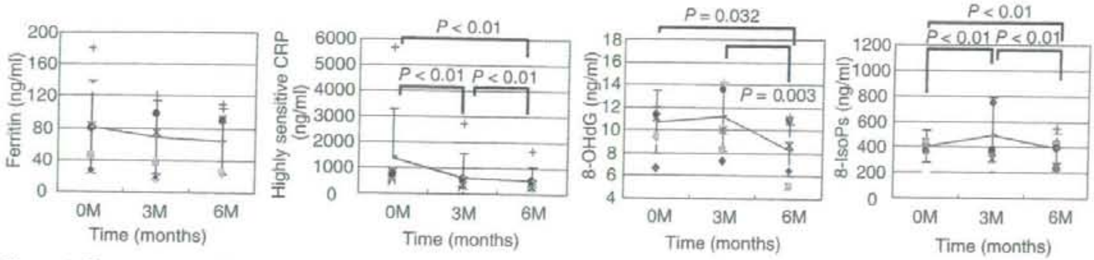

Figure 1 Time-course analysis of ferritin, highly sensitive CRP, 8-OHdG and 8-IsoPs. In seven patients of the administered group, markers of oxidative stress (8-OHdG and 8-IsoPs), ferritin and highly sensitive CRP were measured at 0 M, 3 M and 6 M. Each point represents the mean ± SD. Ferritin decreased slightly, although not significantly, and highly sensitive CRP significantly decreased over time. The values of 8-OHdG and 8-IsoPs did not decrease, but rather increased at 3 M and then significantly decreased at 6 M.

BCAA granules regularly reduced iron storage, microinflammation and oxidative stress.

Next, we evaluated how these parameters that were reduced by BCAA administration altered over time in seven patients of the administered group. As shown in Figure 1, ferritin decreased slightly although not significantly, while highly sensitive CRP significantly decreased with time. In contrast, urine 8-OHdG and 8-IsoPs were increased at 3 M and then significantly decreased at 6 M, compared to their levels before BCAA administration. Considering that it has been reported that hepatic iron accumulation was present in patients with chronic hepatitis C, the decrease of ferritin might indicate the improvement of not only hepatic iron accumulation, but also iron metabolism.[10] Therefore, these data may indicate that the reduction of oxidative stress was followed by an improvement of the iron metabolism and microinflammation.

DISCUSSION

THE AIM OF this study was to analyze the effects on oxidative stress of the oral administration of BCAA granules to HCV-positive patients with LC. In terms of the usefulness of BCAA granules, in the present study the level of albumin did not change significantly, which was probably due to the number of enrolled patients, although some papers have described BCAA granules as improving nutritional disorders associated with abnormal protein and amino acid metabolism in patients with LC.[4,11] Additionally, one *in vitro* study has shown that an increase in the molar ratio of BCAA to aromatic amino acids reduced the growth of HepG2 cells;[12] and recently Muto *et al.* reported that long-term supplementation with BCAA decreased the risk for HCC in patients with LC. These authors pointed out that hyperinsulinemia and peripheral insulin resistance are important mechanisms, although these mechanisms are not yet fully understood.[8] Under these circumstances, if BCAA granules did suppress the production of oxidative stress in HCV-positive patients with LC, it would be another potential mechanism by which the incidence of HCC in HCV–LC patients could be reduced. Therefore, in this study, which we believe is the first report on this topic, we focused on the effect of BCAA on oxidative stress. However, the present study was not a control study but a preliminary study; in the future a larger scale study will be required.

The risk of developing HCC is significantly increased in patients with chronic hepatitis. Hepatocarcinogenesis is considered a multi-step process, in a similar way to the carcinogenesis of other cancers, and is thought to involve at least two factors. One is HCV viral protein, especially the core protein that has been shown to alter the oxidant–antioxidant status of the liver,[4] and another is chronic inflammation. Regarding the former, several studies have investigated the possible role of oxidative injury in the pathogenesis of hepatitis C.[13,14] In particular, oxidative damage is evident in both HCV-infected patients and in HCV-transgenic mice in the absence of necroinflammatory changes or transaminoferase release.[4,15] Moreover, a direct relationship between HCV core protein and HCC has been reported recently.[3,16] Certainly, it is easily to imagine that when DNA damage caused by oxidative stress is not repaired, the accumulation of genetic damage leads to the development of hepatic neoplasm. It has actually been demonstrated that antioxidant treatment improved the liver injury of chronic hepatitis C patients in a prospective study.[17] Clinically, with oral administration of BCAA, we experienced not only that the nutritional status of such patients improved, but also that the incidence of HCC was reduced.[8] These findings prompted us to evaluate the effects of BCAA granules on the production of oxidative stress. The results, as expected, showed that urine 8-OHdG and 8-IsoPs were significantly reduced compared with those patients followed up without BCAA administration.

In addition to the substantial role of oxidative stress in HCV-associated hepatocarcinogenesis, chronic inflammation is also important. In asymptomatic patients who show normal alanine transaminase (ALT) levels despite having HCV-RNA, the possibility of progression of the disease to LC and HCC has been reported, but HCV-positive HCC is rarely observed in healthy carriers, and is mainly observed in patients with LC.[18] This means that chronic inflammation, creating a cycle of repeated hepatocyte destruction and regeneration, is very important to the development of HCC. On the other hand, chronic hepatitis in the absence of hepatitis virus infection, such as autoimmune hepatitis, carries a low risk for the development of HCC.[19] Based on these findings, it is thought that both HCV proteins and chronic inflammation contribute to the development of HCC additively or synergistically. In the present study, the level of ALT and aspartate aminotransferase (AST) was not significantly changed, but microinflammation, as indicated by highly sensitive CRP, not usual CRP, was found to be first reduced, followed by the reduction of oxidative stress by oral administration of BCAA granules.

However, oxidative stress is reported to be influenced by various things: alcohol consumption, supplementations such as vitamin A tablets, other inflammation and malignancy complications. In considering the mechanism by which the production of oxidative stress was reduced by the oral administration of BCAA granules, we experienced one interesting patient. This patient took iron supplementation for anemia from before entry into this study until 6 M after the enrollment. Despite administrating BCAA granules, the production of oxidative stress was not decreased in this patient, but rather gradually increased. When the patient stopped taking the iron supplements, the production of oxidative stress gradually decreased (data not shown). In the present study ferritin, which is considered to reflect the body's iron store, was shown to be significantly decreased, along with oxidative stress, in the administered group. There are some papers in the literature reporting that iron stimulates the production of ROS and that hepatic iron overload induces HCC.[20] These findings may suggest that BCAA is able to reduce the production of ROS by involving with the iron metabolism.

BCAA granules certainly seemed to reduce the production of oxidative stress and microinflammation, which could possibly lead to a decrease in the occurrence of HCC with long-term supplementation, but as the number of enrolled patients in this study was limited, in the future large-scale studies in which patients with LC, including patients without HCV infection, are administered BCAA granules, will be necessary.

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