4 It is recommended not to use ribavirin contraindicated in dialysis patients. (Evidence level: High, Recommendation level: Strong)
5 The therapeutic guidelines for patients with normal renal function mention the selection of drugs depending on the viral level and viral type and whether ribavirin should be used concomitantly. However, there is no recommendation for the selection of drugs according to the viral level or viral type for dialysis patients, in whom ribavirin administration is a contraindication.
6 In dialysis patients, treatment with pegylated interferon α monotherapy is more effective and less frequently causes adverse effects than treatment with standard interferon α monotherapy. (Evidence level: High, Recommendation level: Strong)
7 Interferon β can be used in dialysis patients at the standard dose
   - However, as its intravenous injection over a short period may cause adverse effects due to a rapid increase in its plasma concentration, it is recommended to administer it by intravenous drip infusion over 30–60 min for dialysis patients. (Evidence level: High, Recommendation level: Strong)
8 It is recommended that HCV-infected dialysis patients accepted for kidney transplantation be treated before transplantation. (Evidence level: High, Recommendation level: Strong)
9 It is recommended that treatment of HCV-infected kidney transplant recipients be considered only when the benefits of treatment clearly outweigh the risk of allograft rejection due to interferon therapy. (Evidence level: High, Recommendation level: Strong)

[Comments on treatments for HCV-infected dialysis patients]

1. Treatment with interferon (IFN) monotherapy

Monotherapy with standard interferon. Many of the studies of IFN therapy for dialysis patients have been case reports of a small number of patients, making its evaluation difficult. According to the reports of a relatively large number of patients published since 2000, the sustained virological response (SVR) rate varies widely from 19% to 62% (1–5).

The results of meta-analyses of treatments using IFNα monotherapy including these reports are reviewed. In the reports by Fabrizi et al., which covered 28 studies and 645 dialysis patients, the SVR rate by treatment using IFNα monotherapy was 39%, and the dropout rate from the treatment was 19% (6). According to the report by Gordon et al., which reviewed 20 studies and 459 dialysis patients, the SVR rate by IFNα monotherapy was 41%, and the dropout rate was 26% (7). Important factors that contributed to SVR were the administration of IFNα at 3 MU or above 3 or more times/week, a low HCV-RNA level, mild liver fibrosis, and a genotype other than genotype 1. In all meta-analyses, the effectiveness of IFN was similar or superior, but the dropout rate due to adverse effects was higher, in dialysis patients compared with patients with normal renal function. Since the treatment is discontinued more frequently due to cytopenia and psychiatric symptoms in dialysis patients than in patients with normal renal function, sufficient observation and management are needed.

Also, concerning the pharmacokinetics of IFNα2b, the AUC and Cmax are about two times higher, and the half-life is also prolonged in dialysis patients compared with patients with normal renal function. In dialysis patients, the dose must be reduced to a half of the usual dose for patients with normal renal function or below (8).

Monotherapy with IFNβ. As for studies on treatments using IFNβ monotherapy, there are data of 20 patients reported by Zeniya et al. in Japan. These patients, consisting of 60% genotype 1 (12/20) and 40% genotype 2 (8/20), in whom the HCV-RNA level was 15–150 KIU/mL, showed a high SVR rate of 90% (18/20) with no serious adverse effect such as depression during administration (9). There has been no other report of a large number of dialysis patients who underwent IFNβ therapy, and the SVR rate in dialysis patients is unclear. In Japan, however, IFNβ has been used widely in patients with normal renal function, and both its efficacy and safety are established.

Concerning the pharmacokinetics of IFNβ, the peak plasma concentration is higher in dialysis patients than in patients with normal renal function, but its half-life in dialysis patients does not differ markedly compared with that in patients with normal renal function, and there is no tendency for accumulation. Therefore, it is considered possible to administer IFNβ to dialysis patients at the same dose as in patients with normal renal function. Except, in dialysis patients, its intravenous injection over a short period has been reported to induce adverse effects such as headache, nausea, and a decrease in the blood pressure due to a rapid increase in its plasma concentration. Therefore, in dialysis patients, it is recommended to conduct IFNβ therapy by intravenous drip infusion over about 30–60 min (10–14).

2. Treatment with pegylated interferon (pegIFN) monotherapy

Effects of treatment with pegIFN monotherapy. There are 11 reports on treatment of dialysis patients using pegIFN monotherapy published by 2009, consisting of nine on pegIFN α-2a and 2 on pegIFN α-2b. The initial administration of pegIFN α-2a was made subcutaneously at 135–180 μg once a week, the SVR rate was 14–75%, and the dropout rate was 0–73% (15–25). Major adverse effects were fever, reduced appetite, malaise, cytopenia, and depression. The dropout rate was low in reports with a high SVR rate but high in those with a low SVR rate.

Comparison of effectiveness between standard IFNα monotherapy and pegIFNα monotherapy. A randomized controlled trial comparing standard IFNα monotherapy and pegIFNα monotherapy has been reported (25). Fifty hemodialysis patients were randomized to pegIFNα-2a and IFNα-2a therapies, the administration of pegIFNα-2a at 135 μg/week and IFNα-2a at 3 MU×3/week was continued for 24 weeks, and the results were evaluated by an intention-to-treat (ITT) analysis. In the pegIFNα-2a and IFNα-2a groups, the SVR rate was 48% and 20% ($P = 0.07$), fever was observed in 12% and 44% ($P = 0.03$), and dropout rate was 0% and 20% ($P = 0.04$), respectively, showing that pegIFN α-2a was more effective and less frequently caused adverse effects than the conventional preparation. Multivariate analysis indicated the use of a pegIFN α-2a preparation ($P = 0.02$) and an HCV-RNA level of less than 800 KIU/mL as factors contributing to SVR. Also, the SVR rate was 65% in the group that showed a rapid virological response (RVR) and 0% in the non-RVR group ($P < 0.001$). It was shown that SVR cannot be attained in patients in whom early negative conversion of HCV-RNA cannot be achieved either by pegIFN α-2a or IFN α-2a.

Pharmacokinetics. The pharmacokinetics after a single subcutaneous administration of pegIFN α-2a at 90 μg in patients with a creatinine clearance of 20 mL/min or above was the same as in healthy adults. However, when pegIFN α-2a was administered once subcutaneously at 45, 90, 135, and 180 μg, its plasma concentration increased in a dose-related manner, and the pharmacokinetics in dialysis patients after the administration at 135 μg was similar to that in healthy adults after the administration at 180 μg (26).

In a report about patients in Japan, Cmax and Tmax after a single administration of pegIFN α-2a at 90 μg were similar to those in healthy adults after the administration at 180 μg, but the disappearance of the drug from blood was delayed. The increase in the plasma concentration was insufficient by a single administration of pegIFN α-2a at 45 μg. Also, the pharmacokinetics on repeated administrations of pegIFN α-2a at 90 μg were similar to those in healthy adults at 180 μg (27). Therefore, the dose of pegIFN α-2a in dialysis patients must be reduced to 90–135 μg.

3. Treatment with combination of pegIFN and ribavirin

There were four reports on treatment of dialysis patients with a combination of pegIFN and ribavirin by 2009 (28–31). pegIFN α-2a was administered initially at 135 μg once a week, and pegIFN α-2b was administered at 50 μg once a week, by subcutaneous injection. The SVR rate was 29–97%, the dropout rate was 0–71%, and the treatment was often discontinued due to severe anemia requiring transfusion. However, in reports with a high SVR rate, the dropout rate was low, and modifications such as an increase in the dose of an erythropoiesis stimulating agent (ESA) and the administration of ribavirin every other day were made.

Also, there is a report that ribavirin is excreted through the kidneys, that its AUC increases three times or more in patients with a creatinine clearance of less than 30 mL/min compared with patients with normal renal function, and that it cannot be eliminated efficiently by hemodialysis (32), so its administration to dialysis patients is a contraindication.

4. Guidelines for IFN therapy in dialysis patients

(1) Drugs and administration methods
- Subcutaneous injection of pegIFN α-2a at 90–135 μg once a week over 24–48 weeks
- Subcutaneous or intramuscular injection of natural IFN α or recombinant IFN α-2b at 3–6 million units once a day, 3 times a week, over 24–48 weeks
- Intravenous drip infusion (30–60 min) of natural IFN β at 3–6 million unites once a day, 3 times a week, over 24–48 weeks

(2) Comments about the guidelines

In dialysis patients undergoing IFN therapy, the SVR rate is similar to, or higher than, in patients with normal renal function, but the dropout rate from the treatment is also high. Factors important for achieving SVR are a low viral level, a genotype other than genotype 1, use of pegIFN, rapid virological response, and no marked liver fibrosis.

While the SVR rate is high in patients in whom the treatment could be continued, the dropout rate is higher in dialysis patients than in patients with normal renal function because of cytopenia and psychiatric symptoms. For achieving SVR, it is important to complete the treatment by promptly using an ESA preparation at a high dose in patients showing anemia and by concomitantly using granulocyte colony stimulating factor (G-CSF) and reducing the dose of IFN in patients showing neutropenia.

There has also been a report that a low dropout rate and a high SVR rate were obtained in dialysis patients by ribavirin combination therapy with reduced dose and number of administrations. This approach is likely to be effective in patients treated again after no response to IFN monotherapy and genotype 1 patients showing a high viral level. However, as ribavirin accumulates and cannot be eliminated by hemodialysis, the drug is contraindicated for dialysis patients by its package insert, and we recommend not administering it to dialysis patients.

Therefore, we recommend IFNα or IFNβ monotherapy as an antiviral therapy for dialysis patients. Regarding the drug selection for antiviral therapy using IFNα alone, the results of an RCT that the SVR rate was high, that adverse effects were infrequent, and that dropout rate was low with a pegIFNα preparation have been reported. We recommend using pegIFNα for treating dialysis patients. Although there are pegIFNα-2a and pegIFNα-2b, treatment using pegIFNα-2a monotherapy is covered by medical insurance in Japan.

5. *Other treatments*

Drugs of suppressing inflammation in the liver. In patients with normal renal function, Stronger Neo-Minophagen C (SNMC) or ursodeoxycholic acid (UDCA, Urso) are administered as drugs of suppressing inflammation to those with liver dysfunction in whom IFN therapy cannot be performed or has been ineffective. RCTs and prospective studies in patients with normal renal function have provided little evidence of suppression of death and liver cirrhosis or liver cancer (33,34), and there is no evidence in dialysis patients. In addition, as no antiviral effect is observed in drugs of suppressing inflammation, they are administered with the objective of reducing ALT in patients with liver dysfunction.

Administration methods

1 Stronger Neo-Minophagen injection, intravenous injection at 40–100 mL per injection, at each dialysis
2 Urso (100 mg), 6–9 tablets/day, daily oral administration t.i.d.

Virus removal and eradication by DFPP (VRAD). VRAD is covered by insurance in patients receiving re-treatment with IFN, those with genotype 1B, and those with an HCV-RNA level of 100 KIU/mL or higher up to five times (there is no evidence regarding the amount of treated plasma or duration, interval, or number of VRAD).

A multi-facility collaborative prospective study in non-dialysis patients is in progress, and SVR is compared between groups undergoing PEG-IFN plus ribavirin (30 patients) and PEG-IFN plus ribavirin plus DFPP (74 patients) (35). In the patients in whom SVR could be evaluated, SVR was observed in 50.0% (29/58) in the PEG-IFN plus ribavirin group and 70.8% (17/24) in the PEG-IFN plus ribavirin plus DFPP group. While the SVR rate was higher in the group treated by combinations including DFPP, the increase was not significant. There is no report comparing SVR between IFN therapy and a combination therapy including DFPP in dialysis patients, and there is no evidence. However, ribavirin administration to dialysis patients is a contraindication, and as VRAD is expected to be effective as a concomitant treatment in re-treatment using IFN, evaluation by accumulation of clinical research is necessary for the future.

[Comments concerning HCV-infected recipients of kidney transplantation]

1. *HCV infection and kidney transplantation*

Fabrizi et al. performed meta-analysis of 10 clinical studies and 2502 kidney transplantation patients and reported the incidences of diabetes after kidney transplantation in HCV-antibody-positive and -negative patients (36). The incidence of diabetes in HCV-antibody-positive patients varied from 7.9–50.0% among reports but was significantly higher than in negative patients with an odds ratio of 3.97 (95% confidence interval = 1.83–8.61, P-value = 0.047). The authors suggested the possibility that this is related to the kidney graft survival rate in HCV-antibody-positive patients.

Mathurin et al. reported the survival rate and graft survival rate 10 years after kidney transplantation in 834 patients (128 were HBs-antigen-positive, 216 were HCV-antibody-positive) (37). The survival rate 10 years after kidney transplantation was 65 ± 5% in HCV-antibody-positive patients and 80 ± 3% in HCV-antibody-negative patients ($P < 0.001$), and the graft survival rate was 49 ± 5% and 63 ± 3%

($P < 0.0001$), respectively, both being lower in the HCV-antibody-positive patients.

2. IFN therapy before transplantation

Kamar et al. performed standard IFN therapy in five HCV-antibody-positive and HCV-RNA-positive hemodialysis patients (38). SVR was observed in 21 (38%), and 16 (76%) of them underwent kidney transplantation. All patients continued to be HCV-RNA-negative throughout an observation period of 22.5 months (2–88 months), with none having developed post-transplantation diabetes.

Cruzado et al. evaluated the occurrence of post-transplantation nephritis in 78 HCV-antibody-positive dialysis patients after kidney transplantation (IFN therapy was performed before transplantation in 15 and not in 63) (39). In those who underwent IFN therapy, 10/15 (67%) showed SVR, and only one patient (6.7%), who could not attain SVR, developed post-transplantation nephritis. In those who did not undergo IFN therapy, 12/63 (19%) developed post-transplantation nephritis. The frequency of post-transplantation nephritis was reduced by IFN therapy before transplantation.

Mahmoud et al. reported the effects of IFN therapy before transplantation on rejection and renal function after transplantation in 50 HCV-RNA-positive kidney transplantation patients (40). The patients consisted of 18 who underwent IFN therapy and 32 who did not, and the percentage of those who showed chronic rejection was significantly higher, and the renal function 5 years after transplantation was significantly lower, in the non-IFN therapy group.

Interferon therapy before transplantation is important to improve the kidney graft survival rate.

3. IFN therapy after transplantation

Fabrizi et al. carried out a meta-analysis concerning 12 studies (102 patients) in which standard IFN therapy and standard IFN plus ribavirin therapy were performed after kidney transplantation (41). The SVR rate was 18.0% (95% CI: 7.0–29.0%), and the dropout rate was 35.0% (95% CI: 20–50%). The most frequent adverse effect was kidney graft dysfunction. IFN therapy after transplantation was unsatisfactory in both efficacy and safety.

4. Guidelines for IFN therapy in kidney transplanted patients

In HCV-infected recipients of kidney transplantation, the post-transplantation incidence of diabetes is high, and the graft survival rate and survival rate are low. IFN therapy before transplantation reduces the incidences of post-transplantation diabetes, post-transplantation nephritis, and chronic rejection. However, IFN therapy after kidney transplantation is associated with a low SVR rate and a high dropout rate, and induces rejection of the kidney graft.

Therefore, in HCV-infected dialysis patients expecting kidney transplantation, IFN therapy should be performed before transplantation. Also, in HCV-infected recipients of kidney transplantation, IFN therapy is likely to induce rejection and should be performed only when the necessity surpasses the risk (fibrosing cholestatic hepatitis [FCH] etc.).

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PREVENTION OF HCV INFECTION AT HEMODIALYSIS FACILITIES

[Statements]

1 It is recommended to apply and implement a strict infection control procedure to prevent blood-borne infection of pathogens including HCV at hemodialysis facilities. (Evidence level: Very low, Recommendation level: Strong)
2 In addition to a strict infection control procedure, it is recommended to identify or isolate HCV-infected patients and to use special dialysis instruments (consoles) for them. (Evidence level: Very low, Recommendation level: Strong)
3 It is recommended that the infection control procedure includes hygienic cautions to effectively prevent direct transmission of pathogens between patients through blood or body fluid or via contaminated gloves, medical materials, or instruments. (Evidence level: Very low, Recommendation level: Strong)
4 In evaluating the results of HCV infection prevention measures at hemodialysis facilities, it is recommended to include observation of the state of

implementation of infection control measures, periodic surveillance of the state of infection, and review of infection control measures depending on the state of infection. (Evidence level: Very low, Recommendation level: Strong)

[Comments]

1. It is recommended to apply and implement a strict infection control procedure to prevent blood-borne infection of pathogens including HCV at hemodialysis facilities. (Evidence level: Very low, Recommendation level: Strong)

The occurrence of nosocomial infection of HCV in dialysis facilities has been documented by epidemiological and viral molecular biological researches (1,2). The most frequent patient-to-patient transmission of HCV is caused by contamination of the drugs administered and the surface of instruments and materials in the dialysis facility including gloves due to manipulations violating the infection control procedure (1,2). With the current equipment, transmission of infection in the dialysis instruments is unlikely (3). Other causes of nosocomial infection include direct contact between patients and medical actions outside the dialysis facility such as transfusion (4), but their frequency is considered to be low. Therefore, for the prevention of HCV infection, it is required to determine and observe effective infection control procedures and to periodically review them and make necessary modifications (5–8). In Japan, the Manual Regarding the Standard Dialysis Procedure and Prevention of Nosocomial Infections in Dialysis Medicine (7) prepared with a Grant-in-Aid for Health and Welfare Science by the Ministry of Health, Labour and Welfare is used widely as a manual of infection control procedures at dialysis facilities.

2. In addition to a strict infection control procedure, it is recommended to identify or isolate HCV-infected patients and to use special dialysis instruments (consoles) for them. (Evidence level: Very low, Recommendation level: Strong)

Since infection experiments cannot be performed due to ethical restrictions, we must depend primarily on the results of observational studies. In Japan, the prevalence of HCV infection is clearly higher than in Western countries (9). On the basis of the results of a multi-facility observational study (9) that the incidence of new HCV infection is high at facilities with a high prevalence of HCV infection and that it is lower at facilities with a larger number of stations for isolated dialysis and the results of an observational study (10) that infection is less frequent at facilities that isolate HCV-infected patients than at those that do not isolate them, we recommend isolation of HCV-infected patients or the use of dedicated HD machines. While this statement differs from the CDC guidelines of the United States (5), these are considered to be necessary infection control measures from the high prevalence of HCV infection in Japan, poorer prognosis of HCV-positive dialysis patients (11), and statement of the German clinical nephrology working group in 2006 (8).

3. It is recommended that the infection control procedure includes hygienic cautions to effectively prevent direct transmission of pathogens between patients through blood or body fluid or via contaminated gloves, medical materials, or instruments. (Evidence level: Very low, Recommendation level: Strong)

According to the Ministry of Health, Labour and Welfare, each hospital must have an "Infection Control Manual" independently prepared by the Infection Control Committee. However, it is difficult for a small facility to prepare a manual, survey the state of infection, and continue its modification. Therefore, the "Manual Regarding the Standard Dialysis Procedure and Prevention of Nosocomial Infections in Dialysis Medicine" (7) was prepared with a Grant-in-Aid for Health and Welfare Science by the Ministry of Health, Labour and Welfare and with the cooperation of the Japanese Association of Dialysis Physicians, Japanese Society for Dialysis Therapy, Japan Association for Clinical Engineering Technologists, and Japan Academy of Nephrology Nursing as a manual of infection control procedure at dialysis facilities (8) and is used as a model of individual hospital manuals (12). In addition, there has been a report of the observation that the incidence of new HCV infection was reduced by its implementation (13).

There are reports that the risk of infection does not increase by the reuse of the dialyzer if it is handled by a professional agent or dedicated machines are operated by strict observance of reliable infection control procedures. In Japan, however, there is no professional agent or dedicated machine, and dialyzers, the cost of which is covered by insurance, are not permitted to be reused. Since infection is expected to increase unless dialyzers are reused with sufficient caution under these conditions (10), it is recommended not to reuse them.

4. In evaluating the results of HCV infection prevention measures at hemodialysis facilities, it is recommended to include observation of the state of implementation of infection control measures, periodic surveillance of the state of infection, and review of infection control measures depending on the state of infection. (Evidence level: Very low, Recommendation level: Strong)

According to the results of inspection of the dialysis operation at nine dialysis facilities in Spain in November 2003, the staff of the dialysis facilities wore gloves in 93% of the manipulations requiring gloves, but the hands were washed 36% of the times after, and only 14% of the times before, contact with patients (14). On direct observation of how infection control manipulations were implemented after an outbreak (15), problems including (i) poor compliance with hand-washing, (ii) poor compliance with glove changes particularly in emergency hemostasis of arteriovenous fistula, (iii) carrying a channel contaminated with blood in the dialysis room without containing it in a bag, (iv) neglect of periodic decontamination of blood-contaminated dialysis system, and (v) neglect of replacement of a contaminated pressure transducer protector were revealed, but these problems are hardly detected by interviews with the staff (16).

In evaluating the results of HCV infection prevention measures at hemodialysis facilities, it is recommended to observe the state of implementation of infection control measures, periodically survey the state of infection, and review infection control measures depending on the state of infection.

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Branched-Chain Amino Acids as Pharmacological Nutrients in Chronic Liver Disease

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Branched-chain amino acids (BCAAs) are a group of essential amino acids comprising valine, leucine, and isoleucine. A low ratio of plasma BCAAs to aromatic amino acids is a physiological hallmark of liver cirrhosis, and BCAA supplementation was originally devised with the intention of normalizing amino acid profiles and nutritional status. However, recent studies on BCAAs have revealed that, in addition to their role as protein constituents, they may have a role as pharmacological nutrients for patients with chronic liver disease. Large-scale, multicenter, randomized, double-blinded, controlled trials on BCAA supplementation have been performed in Italy and Japan, and results demonstrate that BCAA supplementation improves not only nutritional status, but also prognosis and quality of life in patients with liver cirrhosis. Moreover, accumulating experimental evidence suggests that the favorable effects of BCAA supplementation on prognosis may be supported by unforeseen pharmacological actions of BCAAs. This review summarizes the possible effects of BCAAs on albumin synthesis and insulin resistance from clinical and basic viewpoints. We also review the newly discovered clinical impact of BCAAs on hepatocellular carcinoma and the prognosis and quality of life of patients with liver cirrhosis. (HEPATOLOGY 2011;54:1063-1070)

The liver is a central organ for regulating metabolism, and a variety of metabolic disorders are frequently seen in patients with chronic liver disease.[1,2] Decreased serum ratio of branched-chain amino acids (BCAAs) to aromatic amino acids (AAAs) is a hallmark of liver cirrhosis and is caused by several factors, including reduced nutritional intake, hypermetabolism, and ammonia detoxification in skeletal muscle.[3] Low serum BCAA/AAA ratio reduces biosynthesis and secretion of albumin in hepatocytes,[4] and is also associated with the prognosis of patients with chronic liver disease.[5]

BCAAs have aliphatic side chains with a branch point, and comprise valine (Val), leucine (Leu), and isoleucine (Ile) (Fig. 1). BCAAs are not only a constituent of protein, but also a source of glutamate, which detoxifies ammonia by glutamine synthesis in skeletal muscle.[3] Clinical studies have demonstrated that intravenous administration of BCAA improves hepatic encephalopathy with hyperammonemia.[6] Although dairy products and vegetables contain high BCAA content, increased consumption of these foods does not affect plasma BCAA levels in patients with cirrhosis.[7] The guidelines of the American Society for Parenteral and Enteral Nutrition and the European Societies for Clinical Nutrition and Metabolism currently recommend BCAA supplementation only for patients with cirrhosis with chronic hepatic encephalopathy unresponsive to pharmacotherapy.[8,9] A series of subsequent clinical trials and *in vitro* and *in vivo* studies suggest the possibility of more expansive utility of BCAA supplementation in liver disease.

Abbreviations: BCAA, branched-chain amino acid; BCATm, mitochondrial BCAA aminotransferase; DC, dendritic cell; GLUT, glucose transporter; IGF, insulin-like growth factor; IL, interleukin; Ile, isoleucine; Leu, leucine; MAPK, mitogen-activated protein kinase; mRNA, messenger RNA; MSUD, maple syrup urine disease; mTOR, mammalian target of rapamycin; NK, natural killer; PI3K, phosphatidylinositol 3-kinase; QOL, quality of life; Val, valine.

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triple therapy increases side-effects, especially severe anemia and skin rash.[7–11] Second-wave protease inhibitors, such as TMC435, which not only improve antiviral efficacy but also decrease side-effects, have been developed and are undergoing clinical trials.[12] Also, IFN-free regimens, such as protease inhibitor and polymerase inhibitor combination therapy, have been developed.[13,14] In Japan, HCV carriers are increasing in an aging population, and large numbers of patients are ineligible for triple therapy with telaprevir due to potential anemia. That is why re-treatment with PEG IFN plus ribavirin is a possible choice for patients who failed to achieve SVR to previous antiviral therapy or patients ineligible for triple therapy with telaprevir who must wait until next-generation antiviral therapies, such as triple therapy with second-wave protease inhibitors or IFN-free regimens, become commercially available.

As for re-treatment with PEG IFN plus ribavirin, some studies have been reported but the subjects and treatment protocols were varied.[15–20] According to past reports, the previous treatment response is associated with the efficacy of the re-treatment[17,20] and the SVR rates in re-treatment ranged 4–23%.[16–18] Recently, host factors, such as single nucleotide polymorphisms (SNP) located near the interleukin (IL)-28B gene, and virus factors, such as the amino acid substitutions in the HCV core region, were revealed to have a strong impact on SVR in PEG IFN plus ribavirin combination therapy for naïve CH-C patients.[21–26] Moreover, response-guided therapy which extends treatment duration until 72 weeks for patients with a slow virological response can raise the SVR rate for naïve CH-C patients.[27–29] However, the value of IL-28B SNP has been uncertain in re-treatment and the most appropriate treatment duration in re-treatment is still unclear. Although it remains obscure which factors are associated with SVR in re-treatment with standard PEG IFN plus ribavirin therapy as pointed out above, some patients do respond to re-treatment and it is very important to be able to identify them. Such findings will be valuable for optimizing the antiviral treatment for CH-C patients by making it possible to decide which patients should be considered for re-treatment with PEG IFN plus ribavirin therapy and which should wait for next-generation antiviral treatment.

In the present study, we tried to determine which patients could benefit from re-treatment and to identify the factors associated with SVR in re-treatment, including the host genome SNP and treatment duration.

METHODS

Patients

THIS RETROSPECTIVE, MULTICENTER study was conducted by the Study Group of Antiviral Therapy for Difficult-to-Treat Chronic Hepatitis C supported by the Ministry of Health, Labor and Welfare, Japan. This study was conducted with 143 CH-C patients, 113 patients (genotype 1, $n = 86$; genotype 2, $n = 27$) who had previously completed PEG IFN-α-2b plus ribavirin combination therapy but had failed to attain SVR, and 30 patients (genotype 1, $n = 22$; genotype 2, $n = 8$) who had previously discontinued this combination therapy due to adverse events.

Treatment

For the previous treatment, patients had been treated with PEG IFN-α-2b (PEGINTRON; MSD, Whitehouse Station, NJ, USA) plus ribavirin (REBETOL; MSD). For re-treatment with PEG IFN plus ribavirin, patients were treated PEG IFN-α-2a (PEGASYS; Roche, Basel, Switzerland) plus ribavirin (COPEGUS; Roche) or PEG IFN-α-2b plus ribavirin. In principle, as a starting dose, PEG IFN was given once weekly at a dose of 180 μg of PEG IFN-α-2a and 1.5 μg/kg of PEG IFN-α-2b and ribavirin was given at a total dose of 600–1000 mg/day based on bodyweight (bodyweight, ≤60 kg, 600 mg; 60–80 kg, 800 mg; ≥80 kg, 1000 mg), according to the standard treatment protocol for Japanese patients and the decision of the investigator at the participating clinical center. Dose modification followed, as a rule, the manufacturer's drug information on the intensity of the hematological adverse effects.

Laboratory tests and virological assessment

Examination of peripheral blood, transaminase and the serum HCV RNA level were tested at the start of treatment, weeks 4, 12 and 24, end of treatment (EOT), and 24 weeks after the treatment. Sequences of the IFN-sensitivity determining region (ISDR) and the core region of HCV were determined at start of the previous treatment, and the number of mutations in the ISDR, the amino acid substitutions at core 70 and 91, glutamine (Gln) or histidine (His) at core 70 and methionine (Met) at core 91, were analyzed. Genetic polymorphisms located near the IL-28B gene (rs8099917) and ITPA gene (rs1127354) were determined. As for the IL-28B gene, homozygosity for the major sequence (TT) was defined as having the IL-28B major allele, whereas homozygosity (GG) or heterozygosity (TG) of the minor sequence was defined as having

the IL-28B minor allele. As for the ITPA gene, homozygosity for the major sequence (CC) was defined as having the ITPA major allele, whereas homozygosity (AA) or heterozygosity (CA) of the minor sequence was defined as having the ITPA minor allele. The serum HCV RNA level was quantified using the COBAS AMPLICOR HCV MONITOR test ver. 2.0 (detection range, 6–5000 KIU/mL; Roche Diagnostics, Branchburg, NJ, USA) or COBAS TaqMan HCV test (detection range, 1.2–7.8 $\log_{10}$ IU/mL) and qualitatively analyzed using the COBAS AMPLICOR HCV test ver. 2.0 (lower limit of detection, 50 IU/mL). When the serum HCV RNA level quantified by the COBAS TaqMan HCV test was less than 1.7 $\log_{10}$ IU/mL, which was equivalent to 50 IU/mL of HCV RNA, that case was judged as HCV RNA negativiation against the lower limit of detection of the COBAS AMPLICOR HCV test.

Definition of virological response

A rapid virological response (RVR) was defined as undetectable serum HCV RNA level at week 4, partial early virological response (p-EVR) as a more than 2-log decrease in the HCV RNA level at week 12 compared with the baseline, complete EVR (c-EVR) as undetectable serum HCV RNA at week 12, late virological response (LVR) as detectable serum HCV RNA at week 12 and undetectable at week 24, and SVR as undetectable serum HCV RNA at 24 weeks after the treatment. Relapse was defined as undetectable serum HCV RNA at the EOT but a detectable amount after the treatment. Patients without p-EVR or without clearance of HCV RNA at week 24 were considered to be showing non-response (NR), and treatment was stopped in both the previous treatment and this re-treatment. A patient who attained HCV RNA negativiation during the re-treatment continued to be treated for 48 weeks or 72 weeks according to response-guided therapy or the decision of the investigator at the participating clinical center.

Statistical analysis

Baseline data of the patients are expressed as means ± standard deviation or median values. In order to analyze the difference between baseline data or the factors associated with SVR, univariate analysis using the Mann–Whitney *U*-test or χ^2-test and multivariate analysis using logistic regression analysis were performed. A two-tailed *P*-value of less than 0.05 was considered significant. The analysis was conducted with SPSS ver. 17.0J (IBM, Armonk, NY, USA).

RESULTS

THE PATIENT FLOW in this study is shown in Figure 1. Among the patients who had previously discontinued PEG IFN-α-2b plus ribavirin combination therapy, two patients underwent splenectomy to increase platelet count prior to re-treatment, 25 completed re-treatment of PEG IFN plus ribavirin combination therapy and 15 achieved SVR (genotype 1, $n = 11$; genotype 2, $n = 4$).

All of the patients who completed previous treatment also completed re-treatment and the baseline characteristics of those patients are shown in Table 1. Of the 86 genotype 1 patients, 54 were relapsers and 32 had shown NR to previous treatment. Of the 27 patients with genotype 2, 25 were relapsers and two had shown NR to previous treatment. Thirty-seven patients with genotype 1 and 14 patients with genotype 2 were assessed as IL-28B genotype, and 27 patients with genotype 1 and 10 patients with genotype 2 were assessed as ITPA genotype. There was no significant difference in the baseline characteristics between the previous treatment and the re-treatment with respect to peripheral blood cell counts, amino transaminase level and serum HCV RNA at the start of treatment (Table 1).

The baseline characteristics of patients with genotype 1 according to antiviral efficacy of the previous treatment are shown in Table 2. Among those with NR in the previous treatment, the rate of the minor allele of IL-28B was significantly higher than those with relapse in the previous treatment ($P < 0.01$). For genotype 1, the HCV RNA negative rate on re-treatment was 20% (17/86) at week 4, 61% (52/85) at week 12 and 76% (65/86) at week 24, and the SVR rate was 48% (41/86). The factors associated with SVR were assessed by univariate analysis and the factors of relapse after previous treatment and the serum HCV RNA level at the start of re-treatment were selected as being significant (Table 3). The SVR

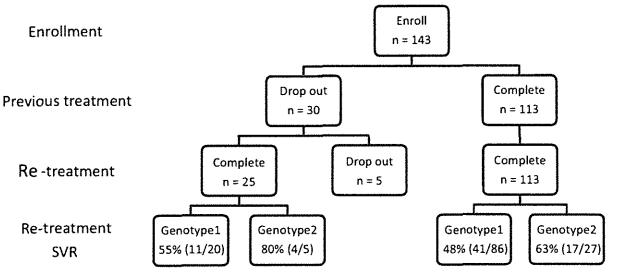

Figure 1 Patient flow for this study. SVR, sustained virological response.

Table 1 Baseline characteristics of patients and treatment factors in previous treatment and re-treatment

Factor	Genotype 1		Genotype 2	
No.	86		27	
Sex: male/female	46/40		15/12	
Effect of previous treatment: relapse/NR	54/32		25/2	
	Previous treatment	Re-treatment	Previous treatment	Re-treatment
PEG IFN type: α-2a/α-2b	0/86	41/45	0/27	6/21
Age (years)	58.1 ± 8.3	60.0 ± 8.5	58.9 ± 8.2	60.0 ± 8.1
White blood cells (/mm^3)	4779 ± 1383	4610 ± 1443	5195 ± 1473	4724 ± 1266
Neutrophils (/mm^3)	2478 ± 930	2355 ± 1071	2561 ± 827	2389 ± 941
Hemoglobin (g/dL)	13.7 ± 1.2	13.5 ± 1.7	14.4 ± 1.3	14.0 ± 1.2
Platelets (×10^4/mm^3)	16.0 ± 5.9	16.6 ± 6.2	18.0 ± 5.7	16.8 ± 5.2
ALT (IU/L)	75 ± 51	73 ± 72	57 ± 46	42 ± 32
Histology: activity, 0–1/2–3	29/29		11/7	
Fibrosis, 0–2/3–4	45/14		17/1	
Serum HCV RNA (KIU/mL)	1600	850	1500	700
IL-28B SNP: rs8099917; TT/TG	26/11		10/4	
ITPA SNP: rs1127354; CC/CA	20/7		9/1	
Core 70: wild/mutant	11/11			
Core 91: wild/mutant	15/7			
ISDR: 0–1/≥2	15/1			

ALT, alanine aminotransferase; HCV, hepatitis C virus; IFN, interferon; IL, interleukin; ISDR, IFN-sensitivity determining region; NR, non-response; PEG, pegylated; SNP, single nucleotide polymorphism.

rates of relapsers were significantly higher than those of patients with NR in the previous treatment (relapse, 67%, 36/54 vs NR, 16%, 5/32, $P < 0.0001$). As for the serum HCV RNA level at the start of re-treatment, although the SVR rate of those patients with 5 $\log_{10}$ IU/mL or more of HCV RNA was 38% (26/69), all patients with less than 5 $\log_{10}$ IU/mL of HCV RNA attained SVR (11/11) ($P = 0.0001$). As for the IL-28B genotype, among the patients with the major allele, the p-EVR rate was significantly higher and the EOT response rate showed marginal significance compared to that with the minor allele (p-EVR rate, 100%, 23/23 vs 30%, 3/10, $P < 0.0001$, EOT rate, 92%, 24/26 vs 64%, 7/11, $P = 0.05$). There was no significant difference of the SVR rate between major and minor alleles (major, 65%, 17/26 vs minor, 36%, 4/11, $P = 0.15$).

Figure 2(a) shows the result of stratified analysis according to the previous treatment response and HCV RNA at the start of re-treatment. The significant difference in SVR observed between high (≥5 $\log_{10}$ IU/mL) and low (<5 $\log_{10}$ IU/mL) baseline viral loads was still found in both previous relapsers ($P = 0.02$) and previous non-responders ($P = 0.02$). In patients with a high baseline viral load, previous relapsers achieved a higher SVR rate than previous non-responders ($P < 0.0001$). Next, the results of stratified analyses according to IL-28B genotype and previous treatment response or HCV RNA at the start of re-treatment showed no significant difference in SVR rates between the IL-28B genotype in patients with relapse after previous treatment ($P = 0.63$) (Fig. 2b). All patients with less than 5 $\log_{10}$ IU/mL of HCV RNA achieved SVR despite their IL-28B genotype and the SVR rates of patients with 5 $\log_{10}$ IU/mL or more of HCV RNA did not differ between IL-28B genotypes (Fig. 2c). Multivariate analysis among the factors of relapse to previous treatment response, HCV RNA at the start of re-treatment and IL-28B genotype showed that relapse after previous treatment response bore the most predictable relationship to SVR in re-treatment ($P = 0.074$).

As for the efficacy of re-treatment according to treatment duration among patients with HCV RNA negativity during re-treatment, the SVR rate of 72-week treatment was significantly higher than that of 48-week treatment (72 weeks, 73%, 29/40, vs 48 weeks, 52%, 12/25, $P < 0.05$). This significant difference was especially found in patients who attained c-EVR but not RVR on re-treatment (72 weeks, 73%, 16/22, vs 48 weeks,

Table 2 Baseline characteristics of patients and treatment factors according to the virological response in previous treatment among patients with genotype 1

Factor	Relapser in previous treatment		NR in previous treatment	
No.	54		32	
Sex: male/female	28/26		18/14	
	Previous treatment	Re-treatment	Previous treatment	Re-treatment
PEG IFN type: α-2a/α-2b	0/54	29/25	0/32	12/20
Age (years)	58.1 ± 8.1	60.3 ± 8.4	57.9 ± 8.9	59.6 ± 8.8
White blood cells (/mm^3)	4917 ± 1290	4692 ± 1035	4546 ± 1520	4462 ± 1993
Neutrophils (/mm^3)	2618 ± 846	2479 ± 805	2225 ± 1033	2105 ± 1454
Hemoglobin (g/dL)	13.9 ± 1.2	13.7 ± 1.6	13.5 ± 1.3	13.1 ± 1.9
Platelets (×10^4/mm^3)	17.1 ± 6.3	17.7 ± 6.1	14.1 ± 4.7	14.7 ± 6.2
ALT (IU/L)	75 ± 57	70 ± 76	75 ± 39	78 ± 64
Histology: activity, 0–1/2–3	20/18		9/11	
Fibrosis, 0–2/3–4	31/8		14/6	
Serum HCV RNA (KIU/mL)	1600	980	1550	800
IL-28B SNP: rs8099917; TT/TG	24/5		2/6	
ITPA SNP: rs1127354; CC/CA	15/6		5/1	
Core 70: wild/mutant	6/6		5/5	
Core 91: wild/mutant	9/3		6/4	
ISDR: 0–1/≥2	9/0		6/1	

ALT, alanine aminotransferase; HCV, hepatitis C virus; IFN, interferon; IL, interleukin; ISDR, IFN-sensitivity determining region; NR, non-response; PEG, pegylated; SNP, single nucleotide polymorphism.

38%, 5/13, $P < 0.05$) but not in patients who attained RVR or LVR (Fig. 3).

In genotype 2, the HCV RNA negative rate on re-treatment was 59% (16/27) at week 4, 85% (23/27) at week 12 and 93% (25/27) at week 24, and the SVR rate was 63% (17/27). The two patients with NR in previous treatment did not attain SVR with re-treatment. The factors associated with SVR were assessed by univariate analysis and only the factor of younger age at the start of re-treatment showed marginal significance ($P = 0.06$) (Table 4). Among the patients with RVR on re-treatment, the SVR rates were similar at 75% (6/8) to those with 24-week and 48-week treatment.

DISCUSSION

PAST STUDIES HAVE revealed that the factors of age, sex, progression of liver fibrosis, value of HCV RNA, number of mutations in the ISDR, amino acid substitutions in the core region, drug adherence and treatment duration show association with HCV eradication in PEG IFN plus ribavirin combination for naïve patients with CH-C.[3–5,25–33] Recently, the IL-28B genotype has been reported to be the most powerful factor associated with the antiviral effect of this combination therapy.[21–25] While the predictive factors for SVR in PEG IFN plus ribavirin combination therapy for naïve patients have been actively analyzed, those factors for patients who had already experienced this therapy are still unclear. Especially needing assessment is the correlation between IL-28B SNP or the previous treatment response and the antiviral effect in re-treatment. In this study, we tried to determine which factors could most effectively predict the antiviral effect in re-treatment.

In the present study, patients with relapse after the previous treatment and patients with a low serum HCV RNA level at the start of re-treatment showed significantly different results in this study of re-treatment of CH-C patients who had previously failed to attain SVR with PEG IFN plus ribavirin therapy. This result was similar to those of the EPIC[3] study on relapse and NR[17] and the SYREN trial of NR.[18] On the other hand, there was no significant difference between the influence of the IL-28B genotype and SVR. More specifically, if the previous treatment response was the same, there was no difference regardless of the IL-28B genotype. Considering this result, in re-treatment, the previous treatment response was a more effective predictive factor than IL-28B genotype. However, further investigation is needed to clarify the association between IL-28B

Table 3 Factors associated with a sustained virological response in re-treatment with PEG IFN plus ribavirin in patients with genotype 1

Factor		SVR	Non-SVR	*P*-value
No. of patients		41	45	
Age (years)		60.2 ± 7.1	59.9 ± 9.6	0.71
Sex: male/female		24/17	22/23	0.40
Serum HCV RNA (log IU/mL)		5.8 ± 1.4	6.4 ± 0.6	0.11
Serum HCV RNA: <5 log/≥5 log		11/28	0/43	<0.001
White blood cells (/mm^3)		4656 ± 1029	4566 ± 1763	0.42
Neutrophils (/mm^3)		2443 ± 804	2259 ± 1301	0.16
Hemoglobin (g/dL)		13.5 ± 1.6	13.4 ± 1.8	0.80
Platelets (×10^4/mm^3)		16.9 ± 5.7	16.3 ± 6.7	0.36
ALT (IU/L)		68 ± 69	78 ± 75	0.43
IL-28B SNP: TT/TG		17/4	9/7	0.15
ITPA SNP: CC/CA		13/3	7/4	0.39
Core 70: wild/mutant		5/4	6/7	1.00
Core 91: wild/mutant		7/3	8/5	1.00
ISDR: 0–1/≥2		9/0	6/1	0.44
PEG IFN: α-2a/α-2b		16/25	25/20	0.14
PEG IFN dose (μg/kg per week)	α-2a	2.91 ± 0.77	2.74 ± 0.69	0.61
	α-2b	1.25 ± 0.39	1.20 ± 0.32	0.59
Ribavirin dose (mg/kg per day)		9.34 ± 2.72	9.64 ± 3.20	0.51
1st treatment virological response	Relapse/NR	36/5	18/27	<0.001

ALT, alanine aminotransferase; HCV, hepatitis C virus; IFN, interferon; IL, interleukin; ISDR, IFN-sensitivity determining region; NR, non-response; PEG, pegylated; SNP, single nucleotide polymorphism; SVR, sustained virological response.

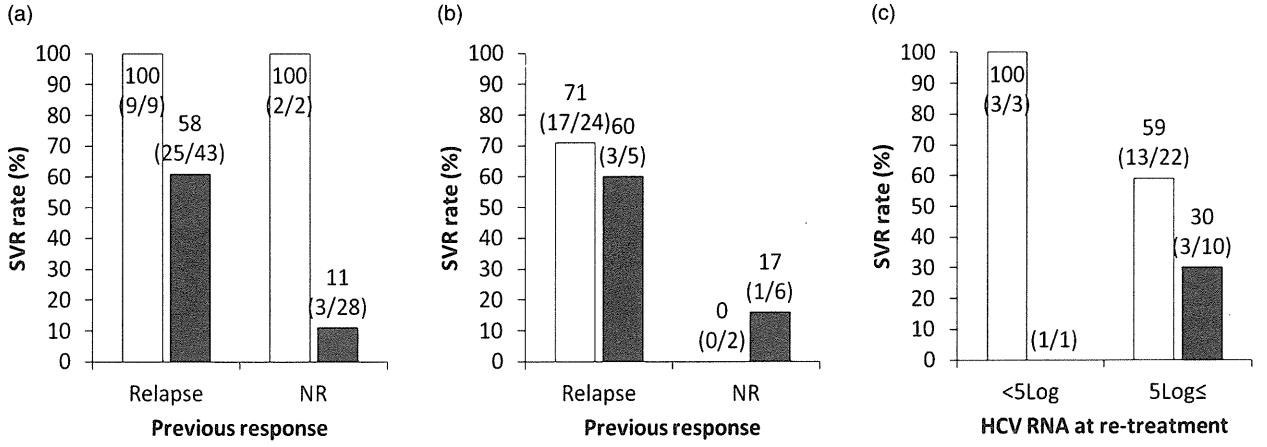

Figure 2 Sustained virological response (SVR) rates according to previous virological response, hepatitis C virus (HCV) RNA at start of re-treatment and genotype of interleukin (IL)-28B single nucleotide polymorphism (SNP) in patients with genotype 1. (a) Stratified analysis of previous virological response and HCV RNA at start of re-treatment. □, HCV RNA <5 log IU/mL at start of re-treatment; ■, HCV RNA ≥5 log IU/mL at start of re-treatment. (b) Stratified analysis of previous virological response and genotype of IL-28B SNP. □, Patients with major allele of IL-28B SNP; ■, patients with minor allele of IL-28B SNP. (c) Stratified analysis of HCV RNA at start of re-treatment and genotype of IL-28B SNP. □, Patients with major allele of IL28B SNP; ■, patients with minor allele of IL-28B SNP.

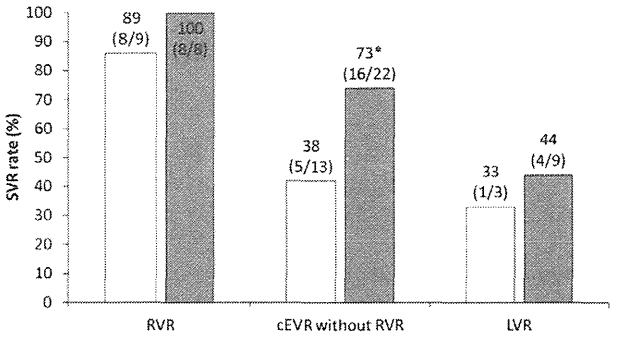

Figure 3 Sustained virological response (SVR) rates according to virological response in re-treatment and treatment duration in patients with genotype 1. □, Patients treated for 48 weeks; ▩, patients treated for 72 weeks. RVR, rapid virological response; cEVR, complete early virological response; LVR, late virological response. $^*P < 0.05$; compared to 48 weeks of treatment.

genotype and antiviral effect of re-treatment because of their small number in this study. In this study, only one patient with the minor allele of IL-28B and NR in previous treatment could start and continue with the increased dose of PEG IFN (from 1.37 μg/kg in the previous treatment to 1.79 μg/kg in re-treatment) and ribavirin (from 10.3 mg/kg per day in the previous treatment to 11.1 mg/kg per day in re-treatment) and attained SVR by extended treatment. If the drug adherence does not improve, patients with the minor allele of IL-28B who show NR in the previous treatment should be treated with new drugs.

The next question is how the patients should be re-treated in order to attain SVR on re-treatment. In this study, the patients with a low serum HCV RNA level (<5 $\log_{10}$ IU/mL) at the start of re-treatment showed a significant rate of cure on re-treatment, and this is almost the same result as that previously reported.[16,17] In this study, the two patients with NR in the previous treatment and with less than 5 $\log_{10}$ IU/mL of HCV RNA level (20 KIU/mL and 52 KIU/mL of HCV RNA) at the start of re-treatment attained SVR. On the other hand, even if the previous treatment response was a relapse, the SVR rates were 58% (25/43) among the patients with 5 $\log_{10}$ IU/mL or more of HCV RNA. Because the HCV RNA level changed after the antiviral treatment, it is important to not miss the timing of when the HCV RNA level is low.

With respect to treatment duration among patients with HCV RNA negativiation during re-treatment, 72 weeks of treatment significantly increased the SVR rate compared to 48 weeks. This result was almost the same as that of the REPEAT study.[16] In our present study, the SVR rate among the patients with c-EVR but not RVR in re-treatment was significantly high by 72 weeks of treatment. On the other hand, the SVR rates among the

Table 4 Factors associated with a sustained virological response in re-treatment with PEG IFN plus ribavirin in patients with genotype 2

Factor		SVR	Non-SVR	*P*-value
No. of patients		17	10	
Age (years)		57.7 ± 8.8	63.7 ± 5.1	0.06
Sex: male/female		7/10	8/2	0.11
Serum HCV RNA (log IU/mL)		5.4 ± 1.4	6.1 ± 0.8	0.15
Serum HCV RNA: <5 log/≥5 log		5/11	1/9	0.35
White blood cells (/mm^3)		5049 ± 1355	4171 ± 910	0.10
Neutrophils (/mm^3)		2556 ± 1064	1999 ± 404	0.24
Hemoglobin (g/dL)		14.1 ± 1.3	13.8 ± 1.6	0.51
Platelets (×10^4/mm^3)		17.9 ± 5.4	14.8 ± 4.3	0.17
ALT (IU/L)		38 ± 19	48 ± 47	0.71
IL-28B SNP: TT/TG		6/2	4/2	1.00
ITPA SNP: CC/CA		5/1	4/0	1.00
PEG IFN: α-2a/α-2b		4/13	2/8	1.00
PEG IFN dose (μg/kg per week)	α-2a	3.23 ± 0.34	2.24 ± 2.25	1.00
	α-2b	1.32 ± 0.28	1.18 ± 0.23	0.21
Ribavirin dose (mg/kg per day)		10.4 ± 2.21	10.1 ± 1.31	0.44
1st treatment virological response	RVR/non-RVR	4/13	3/7	1.00

ALT, alanine aminotransferase; HCV, hepatitis C virus; IFN, interferon; IL, interleukin; ISDR, IFN-sensitivity determining region; PEG, pegylated; RVR, rapid virological response; SNP, single nucleotide polymorphism; SVR, sustained virological response.

patients with RVR in re-treatment were similar between the patients with 48 weeks and 72 weeks of treatment. Thus, patients with c-EVR but not RVR in re-treatment should be re-treated for a longer period. In order to attain better SVR, extended treatment duration is generally recommended for patients with on-treatment LVR, whereas standard treatment duration is considered to be sufficient for patients with on-treatment c-EVR. However, the present study revealed that, even if patients achieved c-EVR on re-treatment, 72 weeks of treatment seems to be better than 48 weeks for treatment-experienced patients. The majority of naïve patients showing on-treatment c-EVR could eradicate HCV with 48 weeks of treatment while some could not. In a treatment-experienced setting, patients who are able to respond early but not eradicate HCV would be selected, and therefore extended treatment may be needed.

With genotype 2, the SVR rate was relatively high (63%). The patients who could not attain SVR in re-treatment (two patients) showed NR in the previous treatment. Thus, the patients with genotype 2 and showing NR in previous treatment seemed to be difficult to treat and could be treated with other drugs. Among the patients with RVR in re-treatment, the SVR rates were similar among those with RVR in re-treatment between 24 weeks and 48 weeks of treatment. The effectiveness of extended treatment for the patients with genotype 2 in re-treatment could not be demonstrated because of their small number in this study. Further investigation is needed to clarify this.

In conclusion, this study shows that the efficacy of re-treatment for genotype 1 patients who failed to show SVR to previous treatment with PEG IFN plus ribavirin could be predicted from the previous treatment response and a low HCV RNA level at the start of re-treatment. Re-treatment for 72 weeks led to clinical improvement for genotype 1 patients with c-EVR and without RVR on re-treatment.

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Branched-Chain Amino Acids as Pharmacological Nutrients in Chronic Liver Disease

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Branched-chain amino acids (BCAAs) are a group of essential amino acids comprising valine, leucine, and isoleucine. A low ratio of plasma BCAAs to aromatic amino acids is a physiological hallmark of liver cirrhosis, and BCAA supplementation was originally devised with the intention of normalizing amino acid profiles and nutritional status. However, recent studies on BCAAs have revealed that, in addition to their role as protein constituents, they may have a role as pharmacological nutrients for patients with chronic liver disease. Large-scale, multicenter, randomized, double-blinded, controlled trials on BCAA supplementation have been performed in Italy and Japan, and results demonstrate that BCAA supplementation improves not only nutritional status, but also prognosis and quality of life in patients with liver cirrhosis. Moreover, accumulating experimental evidence suggests that the favorable effects of BCAA supplementation on prognosis may be supported by unforeseen pharmacological actions of BCAAs. This review summarizes the possible effects of BCAAs on albumin synthesis and insulin resistance from clinical and basic viewpoints. We also review the newly discovered clinical impact of BCAAs on hepatocellular carcinoma and the prognosis and quality of life of patients with liver cirrhosis. (HEPATOLOGY 2011;54:1063-1070)

The liver is a central organ for regulating metabolism, and a variety of metabolic disorders are frequently seen in patients with chronic liver disease.[1,2] Decreased serum ratio of branched-chain amino acids (BCAAs) to aromatic amino acids (AAAs) is a hallmark of liver cirrhosis and is caused by several factors, including reduced nutritional intake, hypermetabolism, and ammonia detoxification in skeletal muscle.[3] Low serum BCAA/AAA ratio reduces biosynthesis and secretion of albumin in hepatocytes,[4] and is also associated with the prognosis of patients with chronic liver disease.[5]

BCAAs have aliphatic side chains with a branch point, and comprise valine (Val), leucine (Leu), and isoleucine (Ile) (Fig. 1). BCAAs are not only a constituent of protein, but also a source of glutamate, which detoxifies ammonia by glutamine synthesis in skeletal muscle.[3] Clinical studies have demonstrated that intravenous administration of BCAA improves hepatic encephalopathy with hyperammonemia.[6] Although dairy products and vegetables contain high BCAA content, increased consumption of these foods does not affect plasma BCAA levels in patients with cirrhosis.[7] The guidelines of the American Society for Parenteral and Enteral Nutrition and the European Societies for Clinical Nutrition and Metabolism currently recommend BCAA supplementation only for patients with cirrhosis with chronic hepatic encephalopathy unresponsive to pharmacotherapy.[8,9] A series of subsequent clinical trials and *in vitro* and *in vivo* studies suggest the possibility of more expansive utility of BCAA supplementation in liver disease.

Abbreviations: BCAA, branched-chain amino acid; BCATm, mitochondrial BCAA aminotransferase; DC, dendritic cell; GLUT, glucose transporter; IGF, insulin-like growth factor; IL, interleukin; Ile, isoleucine; Leu, leucine; MAPK, mitogen-activated protein kinase; mRNA, messenger RNA; MSUD, maple syrup urine disease; mTOR, mammalian target of rapamycin; NK, natural killer; PI3K, phosphatidylinositol 3-kinase; QOL, quality of life; Val, valine.

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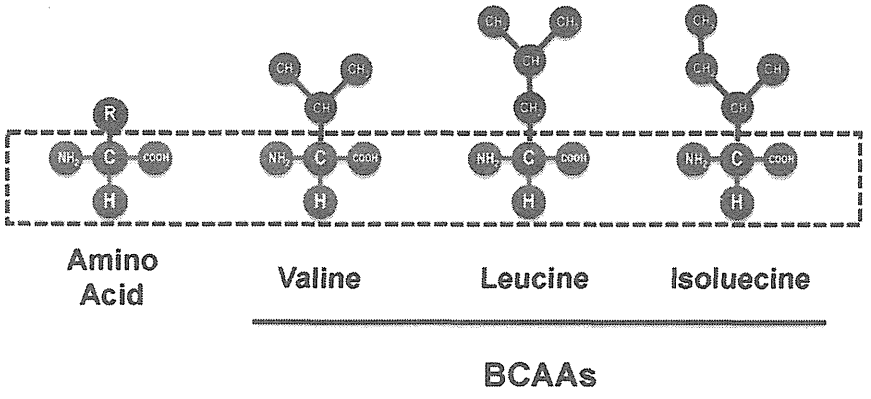

Fig. 1. Chemical structure of BCAAs. The dotted rectangle indicates the basic amino acid structure. The generic BCAA has an aliphatic side chain with a branch point. R, residue.

The liver carries out four main functions in protein metabolism: formation of plasma proteins, amino acid interconversion, deamination of amino acids, and urea synthesis (for ammonia excretion). Among the many other functions of the liver, it is responsible for the metabolism of hormones that have discordant effects on protein metabolism, including insulin, androgens, and glucagon. It is thus not surprising that cirrhosis is associated with altered circulating amino acid profiles, with decreased serum BCAA levels seen in patients even with compensated cirrhosis.[10] It is widely believed that the changes in amino acid metabolism not only occur as an epiphenomenon of liver disease but also play a role in the pathogenesis of many of the complications of cirrhosis, such as encephalopathy,[11] hypoalbuminemia with edema, and insulin resistance.[12-14] The potential of BCAA supplementation to alter the metabolic basis and frequency of complications of cirrhosis is suggested by studies indicating that BCAAs may inhibit hepatocarcinogenesis and improve immune function and oxidative stress *in vitro* and *in vivo*.[15-19] Clinical studies have further demonstrated that BCAA supplementation may improve the quality of life (QOL) and prognosis in patients with liver cirrhosis.[16,20,21]

Nutritional aspects of BCAAs on hepatic encephalopathy, liver regeneration, or hepatic cachexia have been well reviewed.[22,23] In this article, we review the recently identified pharmaceutical aspects of BCAAs on pathological conditions and complications associated with chronic liver disease from both the clinical and basic research viewpoints. We also summarize side effects of BCAA supplementation (Supporting Text).

Albumin Synthesis

BCAAs, particularly Leu, activate the mammalian target of rapamycin (mTOR) and subsequently up-regulates the downstream eukaryotic initiation factor 4E-binding protein-1 and 70-kDa ribosomal protein S6 kinase, which regulate messenger RNA (mRNA) translation and synthesis of albumin in cultured rat hepatocytes (Fig. 2).[4,12,24] Leu also stimulates the nuclear import of polypyrimidine-tract–binding protein, which binds to albumin mRNA and increases its translation in HepG2 cells (Fig. 2).[25] Consistent with these *in vitro* studies, BCAA supplementation has been found to activate the mTOR signaling cascade and increase albumin synthesis in animal models of cirrhosis.[26]

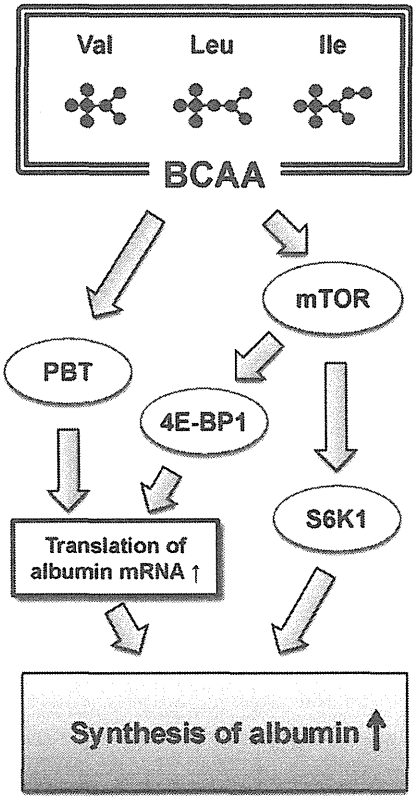

Fig. 2. Molecular mechanisms for BCAA-induced albumin synthesis. BCAA activates the mTOR and subsequently up-regulates the downstream molecules, eukaryotic initiation factor 4E-binding protein-1 (4E-BP1) and 70-kDa ribosomal protein S6 kinase (S6K1), which regulate mRNA translation and synthesis, respectively. BCAAs also stimulate the nuclear import of polypyrimidine-tract-binding protein (PBT), which binds with albumin mRNA and increases albumin translation.

Muto et al. conducted a multicenter, randomized, controlled trial in which 622 patients with cirrhosis were administered BCAAs at 12 g/day for 2 years. In that study, serum albumin levels in the BCAA group were significantly higher than in the nutrient intake-matched control group.[16] However, in another randomized, controlled study by Marchesini et al., BCAA treatment did not result in a significant increase in serum albumin levels.[15] Although the reason for this discrepancy remains unclear, a possible explanation is the difference in the BCAA/AAA ratio among the participants in the two studies. Approximately 45% of enrolled patients were Child-Pugh class A in the former study,[16] whereas all the patients were Child-Pugh class B or C in the latter study.[15] The BCAA/AAA ratio decreases along with progression of liver cirrhosis.[27] Because the BCAA/AAA ratio is positively correlated with the synthesis and secretion of albumin,[4] and the response to BCAA treatment,[27] a low BCAA/AAA ratio may be a reason for the discrepancy in results between the studies. In addition, the majority of other randomized, controlled trials have demonstrated that BCAA supplementation results in a significant increase in serum albumin levels in patients with cirrhosis (Supporting Table 1). The aggregate of the evidence suggests that BCAA administration may increase serum albumin levels in patients with liver cirrhosis.

Insulin Resistance

BCAAs are thought to affect glucose metabolism.[28] Recently, She et al. knocked out the gene of mitochondrial BCAA aminotransferase (BCATm), which catalyzes the first step of BCAA catabolism, leading to a significant elevation in the serum BCAA level. In $BCATm^{-/-}$ mice, fasting blood glucose and fasting serum insulin levels were decreased by 33% and 67%, respectively, and the Homeostasis Model Assessment for Insulin Resistance index was significantly lower than that of wild-type mice.[14] Similarly, treatment with Leu or Ile has been reported to improve insulin sensitivity in mice fed a high-fat diet.[29,30]

Supplementation with BCAAs enhances glucose metabolism in skeletal muscle, adipose tissue, and liver; however, the molecular mechanisms in each organ are different. In skeletal muscle, BCAAs promote glucose uptake through activation of phosphatidylinositol 3-kinase (PI3K) and protein kinase C and subsequent translocation of glucose transporter 1 (GLUT1) and GLUT4 to the plasma membrane (Fig. 3).[13,31] In adipose tissue, Leu enhances insulin-induced phosphorylation of Akt (protein kinase B) on Ser473 and Thr308 and mTOR on Ser2448, ultimately increasing glucose uptake (Fig. 3).[32] In the liver, BCAAs up-regulate the liver X receptor α (LXRα)/sterol regulatory element binding protein-1c (SREBP1c) pathway and subsequently activate liver-type glucokinase and GLUT2. In addition, BCAA suppresses hepatic expression of glucose-6-phosphatase, which catalyzes the final steps of gluconeogenesis (Fig. 3).[33] Recently, BCAA supplementation has been reported to improve insulin resistance by increasing oxidation of free fatty acids. BCAAs increase peroxisome proliferator-activated receptor α

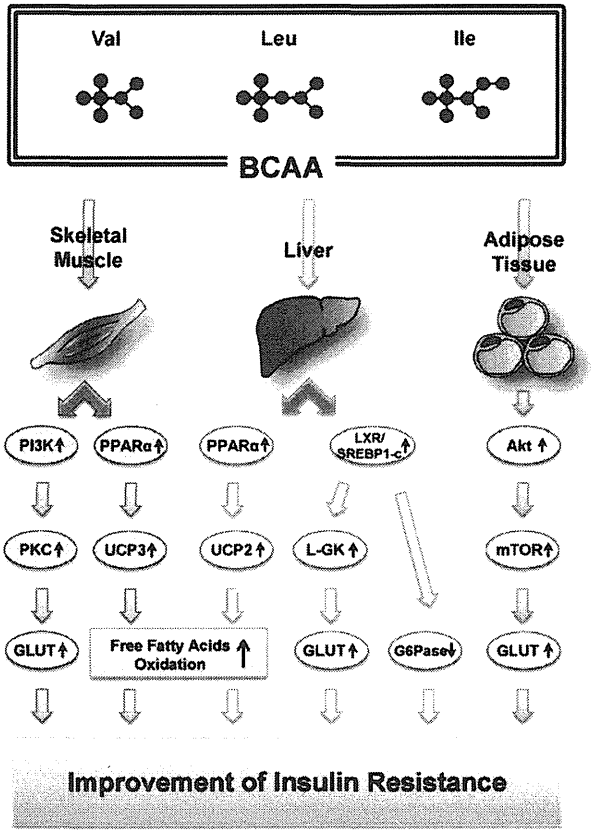

Fig. 3. Distinctive molecular pathway for BCAA-induced improvement of insulin resistance in insulin target organs. BCAAs improve glucose metabolism by acting on insulin target organs such as skeletal muscle, adipose tissue, and the liver. However, the molecular mechanisms in each organ differ. In the skeletal muscle, BCAAs promote glucose uptake through activation of PI3K and protein kinase C and subsequent translocation of GLUT1 and GLUT4 to the plasma membrane. In the adipose tissue, BCAAs, especially Leu, augment insulin-induced phosphorylation of Akt and mTOR, and consequently increase the glucose uptake. In the liver, BCAA activates the liver X receptor α (LXR)/sterol regulatory element binding protein-1c (SREBP1-c) pathway and subsequently up-regulates liver-type glucokinase (L-GK) and GLUT2. In addition, LXR/SREBP-1c activation suppresses hepatic expression of glucose-6-phosphatase (G6Pase), which catalyzes the final steps of gluconeogenesis. BCAAs also increase peroxisome proliferator-activated receptor (PPAR) α expression and subsequent uncoupling proteins 2 (UCP2) in liver and UCP3 in muscle. Up-regulation of UCP2 and UCP3 expression increases oxidation of free fatty acids and improves insulin resistance.

expression and subsequent expression of uncoupling proteins 2 in liver and uncoupling proteins 3 in muscle (Fig. 3).[34,35] These recent studies have revealed distinct cross-talk mechanisms between BCAAs and the insulin signaling cascade in insulin target organs.

Previous clinical studies have reported that BCAA infusion decreases plasma glucose levels in patients with advanced liver cirrhosis.[36] Furthermore, oral BCAA supplementation reduces both blood glucose[37,38] and insulin resistance in patients with chronic liver disease.[18,39] However, these studies had small sample sizes and/or were lacking in adequate controls. A randomized, controlled trial is required to definitively evaluate the effects of BCAA supplementation on insulin resistance in cirrhosis.

Hepatocellular Carcinoma

Clinical studies have reported that long-term oral supplementation with BCAAs is associated with decreased frequency of development of hepatocellular carcinoma (HCC) and HCC recurrence after treatment with radiofrequency ablation in patients with cirrhosis.[17,40] Recent animal studies have also suggested an antihepatocarcinogenic activity of BCAAs.[41,42] Animals used in these studies were, however, obese diabetic mice with insulin resistance.[41,42] Because insulin resistance is closely linked to hepatocarcinogenesis,[43] it is possible that BCAAs may inhibit hepatocarcinogenesis through amelioration of insulin resistance. Indeed, suppression of hepatocarcinogenesis is accompanied with significant reduction in insulin resistance in BCAA-treated animals.[41,42] A randomized, controlled trial demonstrated that BCAA supplementation reduces the frequency of development of HCC, but the effect was only evident in patients with cirrhosis who are obese and have hepatitis C virus infection (approximately 30% reduction in the development of HCC in 3 years).[17] Because patients who are obese and infected with hepatitis C virus frequently have insulin resistance,[44,45] these findings also support the hypothesis that BCAAs suppress hepatocarcinogenesis through amelioration of insulin resistance.

Insulin is a carcinogenic factor with mitogenic and cell proliferative effects through activation of mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase pathway.[46] Insulin also cross-reacts with insulin-like growth factor 1 (IGF-1) receptor and further activates the Raf/MAPK kinase/MAPK cascade.[47] Moreover, excess insulin binds to IGF-binding proteins, resulting in increased levels of free serum IGF-1 (Fig. 4).[48] Thus, insulin resistance/hyperinsulinemia enhances hepatocarcinogenesis through multiple pathways. Possible mechanisms for BCAA-induced inhibition of HCC development include: (1) BCAA activation of the insulin signaling cascade through up-regulation of PI3K[2,13,18] with reduction of serum insulin levels (Fig. 4) and (2) inhibition of the IGF/IGF-1R axis by suppressing the expressions of IGF-1, IGF-2, and IGF-1 receptor mRNA (Fig. 4).[41]

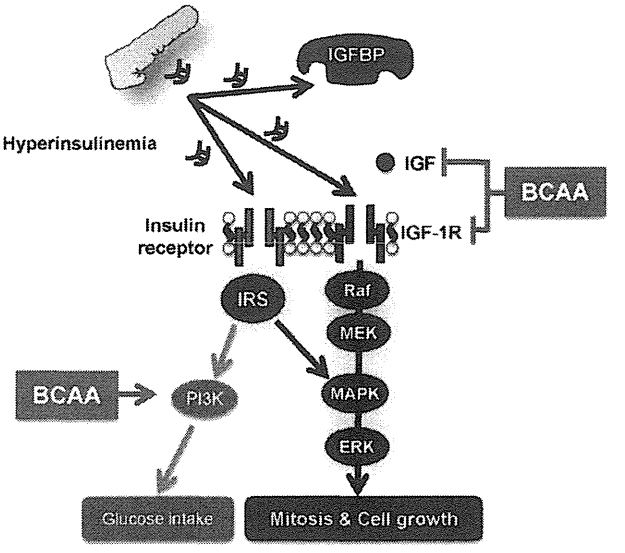

Fig. 4. Molecular mechanisms of the association between hyperinsulinemia and HCC and of BCAA-induced inhibition of hepatocarcinogenesis. As an adaptive response to insulin resistance, pancreatic beta cells secrete excess insulin. Insulin activates mitosis and cell growth through activation of the insulin receptor substrate (IRS)/mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) pathway. Insulin also cross-reacts with IGF-1 receptor (IGF-1R) and further activates the Raf/MAPK kinase (MEK)/MAPK cascade. Furthermore, excess insulin binds to IGF-binding proteins (IGFBP), resulting in increase in the level of free serum IGF-1. BCAA activates the insulin signaling cascade via up-regulation of PI3K and improves glucose uptake and reduces the serum insulin levels. BCAA also suppresses the IGF/IGF-1R axis through down-regulation of IGF-1, IGF-2, and IGF-1R mRNA expressions, leading to inhibition of mitosis and cell growth.

Besides activation of intracellular insulin and IGF-1 signaling cascade, insulin causes angiogenesis,[42] migration of HCC,[49] and epithelial mesenchymal transition of hepatocytes.[50] Because BCAAs reduce insulin resistance, BCAAs may suppress angiogenesis, migration, and epithelial mesenchymal transition of hepatocytes. BCAAs are also known to attenuate insulin resistance-induced expression of endothelial growth factor and eventually suppress hepatic neovascularization.[42] Thus, the diverse effects of BCAAs on insulin resistance may suppress hepatocarcinogenic activity.

In addition, BCAAs are reported to affect immune function *ex vivo* and *in vivo* studies (Supporting Table