weeks after starting sorafenib administration in the PD group revealed that the median value after 4 weeks was significantly higher than that after 2 weeks. Even in the PR + SD group, the median value after 4 weeks was higher than that after 2 weeks. There were no significant differences between AFP levels after 2 and 4 weeks; thus, one of the reasons for this phenomenon was unevenness of AFP levels owing to the small sample size in this study.

With regard to DCP, there have been numerous reports that the time course change in DCP following treatment for HCC reflects therapeutic efficacy [17–19]. However, in the present study, we found that both the actual and relative levels of DCP were elevated in >90% of the patients, not only in the PD group but also in the PR + SD group, both 2 and 4 weeks after starting sorafenib therapy. To our knowledge, there have been no comprehensive clinical reports regarding the time course changes in DCP following sorafenib treatment. In a case report by Nakazawa et al. [27], DCP levels were markedly increased following treatment, even in patients who achieved a complete response on the basis of image analysis. From basic research, Murata et al. [28] reported that culturing a liver cancer cell line (HepG2) under hypoxic conditions resulted in increased DCP production by the cells. One possible mechanism for the increased DCP levels following sorafenib administration is that sorafenib-mediated inhibition of angiogenesis places tumor cells under hypoxic conditions, subsequently leading to increased DCP production. Thus, the increase in DCP levels following sorafenib administration may reflect HCC cell ischemia. Based on our results, increases in DCP soon after the start of sorafenib administration, regardless of antitumor effect, are not useful for assessing the antitumor responses, as DCP may increase in response to the ischemia caused by sorafenib.

Assessment by image analysis is the gold standard for evaluating antitumor responses of anticancer drugs [4, 22, 23]. However, such image analysis can be difficult in patients with multiple HCC lesions, vascular invasion, extrahepatic metastases, or ischemic tumors. In particular, patients in whom therapy using sorafenib is indicated are often in advanced stages of disease. There are limitations in using only radiological criteria to evaluate sorafenib treatment.

Our results suggest that the determination of early changes in AFP is useful for evaluating both antitumor response and prognostic efficacy of sorafenib, as assessed by TTP and OS, in patients with advanced HCC. In patients with advanced HCC treated with sorafenib, it is important to evaluate therapeutic efficacy as early as possible, as appropriate and early evaluation of sorafenib therapy can avoid unnecessary adverse events and allow second-line therapy when sorafenib therapy is not effective. In addition, determination of early changes in AFP is useful for evaluating the efficacy of new molecularly targeted agents currently under development. At present, there is no effective second-line treatment and we could not confirm whether continuing sorafenib administration would prolong the survival of patients with elevated AFP. Therefore, we cannot conclude that sorafenib therapy should be stopped in the case of elevated AFP ratio after 2 or 4 weeks of treatment. However, when an effective second-line treatment becomes available, an elevated AFP ratio may be a good indicator for switching to second-line therapy.

On the other hand, with regard to early changes in DCP, caution is required when assessing the antitumor response of sorafenib, as DCP elevation can occur irrespective of therapeutic effects.

In conclusion, our results suggest that early evaluation of AFP after starting sorafenib therapy is useful for predicting antitumor response. In contrast, early elevation of DCP does not necessarily suggest treatment failure of sorafenib. Appropriate and early evaluation of efficacy of sorafenib by AFP determination can provide valuable information that may influence subsequent decisions regarding patient management, thus avoiding unnecessary adverse events and allowing the opportunity for second-line therapy.

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Disclosure Statement

The authors declare that they have no financial conflicts of interest.

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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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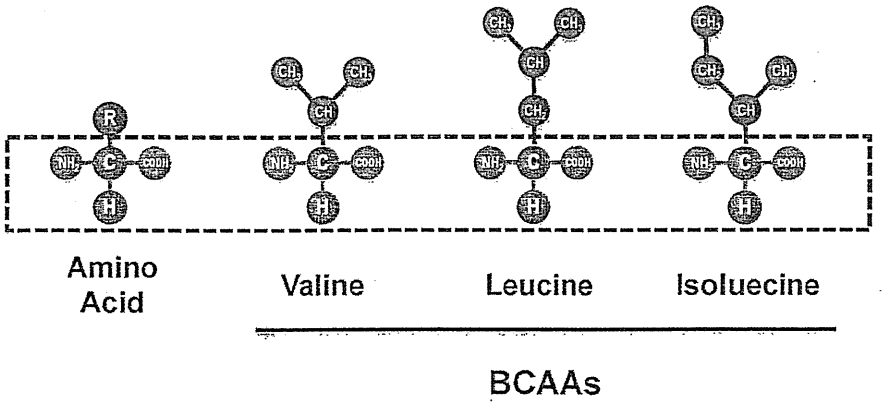

Fig. 1. Chemical structure of BCAAs. The dotted rectangle indicates the basic amino acid structure. The generic BCAA has an aliphatic side chain with a branch point. R, residue.

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]

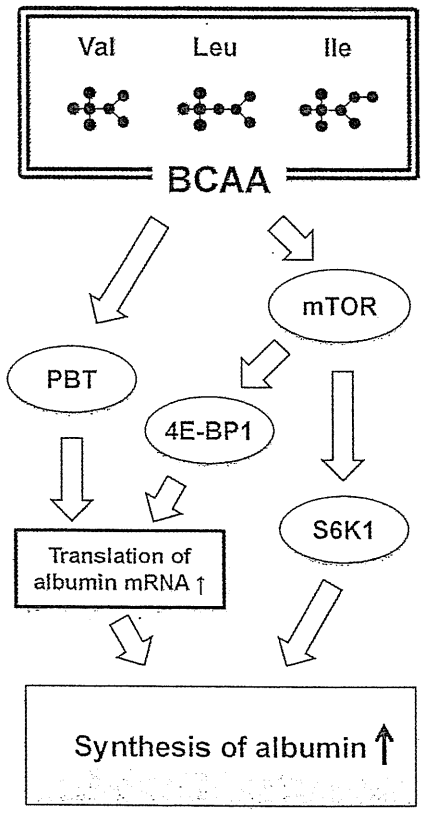

Fig. 2. Molecular mechanisms for BCAA-induced albumin synthesis. BCAA activates the mTOR and subsequently up-regulates the downstream molecules, eukaryotic initiation factor 4E-binding protein-1 (4E-BP1) and 70-kDa ribosomal protein S6 kinase (S6K1), which regulate mRNA translation and synthesis, respectively. 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

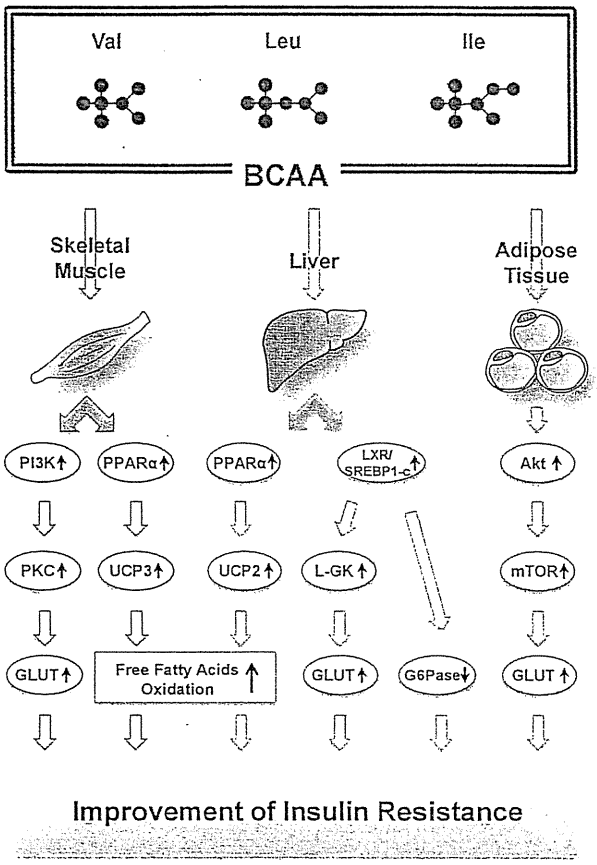

Fig. 3. Distinctive molecular pathway for BCAA-induced improvement of insulin resistance in insulin target organs. BCAAs improve glucose metabolism by acting on insulin target organs such as skeletal muscle, adipose tissue, and the liver. However, the molecular mechanisms in each organ differ. In the 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.

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

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]

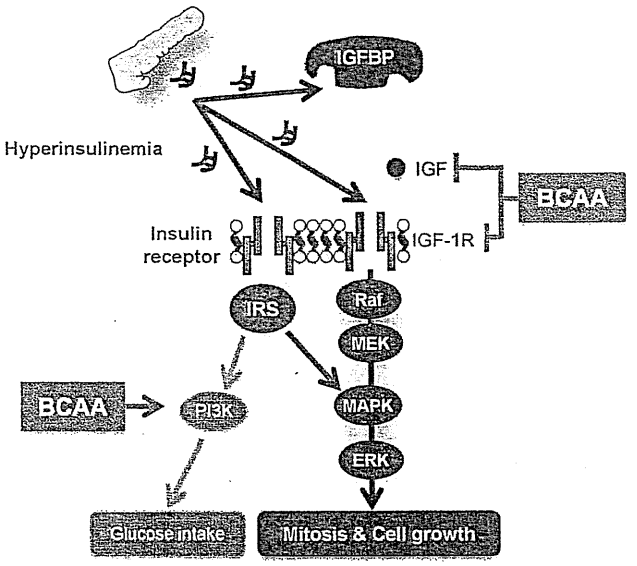

Fig. 4. Molecular mechanisms of the association between hyperinsulinemia and HCC and of BCAA-induced inhibition of hepatocarcinogenesis. As an adaptive response to insulin resistance, pancreatic beta cells secrete excess insulin. Insulin activates mitosis and cell growth through activation of the insulin 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

2). In patients with cirrhosis, BCAAs increase liver-associated lymphocyte counts and restore phagocytic function of neutrophils and natural killer activity of lymphocytes.[51] Moreover, BCAA treatment may suppress hepatic oxidative stress by modulating the redox state of albumin.[52,53] Serum albumin is divided into two forms, reduced and oxidized albumin, depending on the redox state at Cys34,[54,55] and the oxidized/reduced albumin ratio increases in patients with cirrhosis.[56,57] BCAA supplementation increases ratio of reduced albumin[52] and decreases iron-related oxidative stress in patients with cirrhosis,[53] suggesting that BCAAs may reduce the iron-induced oxidative stress through a qualitative alteration of serum albumin. Thus, BCAAs may suppress hepatocarcinogenesis partly by improvement of immune function and reduction of oxidative stress.

Mortality and Clinical Decompensation

Some reports suggest that oral BCAA supplementation improves survival in a rat model of cirrhosis and in decompensated patients with cirrhosis.[58-60] Marchesini et al. first performed a randomized, controlled trial exploring the usefulness of BCAAs in patients with cirrhosis.[15] One year of BCAA treatment significantly reduced the occurrence of the primary outcome (a composite of death, number of hospital admissions, and duration of hospital stay) compared to that in the lactalbumin-treated group.[15] Although this study shows the effectiveness of BCAA supplementation, the complications that contributed to the reduction of outcome incidence was not identified because of a small number of enrolled patients (n = 59 in BCAA group) and high dropout rate (15% in the BCAA group) due to poor compliance with the BCAA supplement.

Since 1996, a BCAA supplement formulation (L-Val:L-Leu:L-Ile = 1.2:2:1; Ajinomoto Pharmaceuticals, Tokyo, Japan) has been approved for use in cirrhosis in Japan. The supplement is in the form of small uniform granules, which reduces BCAA-induced stimulation of taste buds and contributes to improved compliance. Using these BCAA granules, Muto et al. performed a large (n = 314 in the BCAA group) randomized, controlled trial.[16] None of the patients discontinued the study because of poor compliance. A preplanned safety analysis revealed that BCAA granules significantly reduced the occurrence of the overall primary outcome (hepatic failure, variceal bleeding, development of liver cancer, and death from any cause) compared to that in the control diet group. Among individual events of primary outcome, the occurrence of hepatic failure was significantly less in the BCAA group compared to the control diet group (hazard ratio 0.45; 95% confidence interval 0.23-0.88; $P = 0.016$). On the basis of the results, the Data and Safety Monitoring Board concluded that the harm associated with the increased occurrence of primary outcome in the control diet group outweigh any potential benefits and the study was discontinued 10 months early due to safety concerns. Beneficial effects of BCAAs on clinical decompensation, including development of hepatic failure, are also reported in patients with cirrhosis accompanied with HCC.[61-63] Thus, the treatment with BCAA supplementation is now recommended in the guidelines for the treatment of liver cirrhosis by the Study Group for the Standardization of Treatment of Viral Hepatitis Including Cirrhosis from the Ministry of Health, Labour and Welfare of Japan.[64]

Quality of Life

Generally, the overall health status and QOL of patients with liver cirrhosis is poor.[65,66] Patients with cirrhosis frequently complain of fatigue and sleep disturbances. There is, however, no standard approach to the management of these symptoms in the absence of overt hepatic encephalopathy.[67] In a randomized study, BCAA-enriched supplements have been reported to improve weakness and easy fatigability compared to ordinary food.[20] BCAA-enriched supplementation has also been reported to improve the Epworth Sleepiness Scale score.[21] In large-scale randomized controlled trials, BCAA supplementation was found to significantly improve the Short Form-36 scores of general health perception compared to control groups.[15,16]

Although it is still unclear how BCAA supplementation provides relief from fatigue and sleep disturbances in patients with cirrhosis, there are at least three possible mechanisms. First, fatigue and sleep disturbances could be caused by minimal hepatic encephalopathy, and BCAA may ameliorate these symptoms by improving this condition.[68] Second, increased serum tryptophan levels are known to impair the QOL in various conditions involving malnourishment, including liver cirrhosis.[69] Tryptophan is a precursor for the neurotransmitter 5-hydroxytryptamine, which is associated with fatigue and sleep disturbances.[70] Because BCAAs compete with tryptophan for transport into the brain, these symptoms may be alleviated by supplementation with BCAAs.[71] Third, impaired cerebral blood flow is associated with fatigue and sleep disturbance[72] and is decreased in patients with liver cirrhosis.[73,74] BCAA supplementation is known to improve cerebral blood flow, possibly resulting in lessened fatigue and sleep disturbances.[75,76]

Muscle cramps are also associated with poor QOL in patients with liver cirrhosis,[77] and the frequency of muscle cramps has been reported to be dramatically reduced by BCAA supplementation over a period of 3 months (7.4 ± 2.0 versus 0.3 ± 0.5 times/week).[78] Muscle cramps are caused by a variety of factors, including diuretic treatment, reduction of circulating volume, and deficiency of vitamin E and taurine.[79] Amino acid imbalance decreases taurine production, and therefore, BCAA may inhibit muscle cramps, possibly through improvement of the imbalance and consequent restoration of taurine production.[78,79]

Conclusion

In this article, we have reviewed evidence for potential pharmaceutical properties of BCAAs on various physiological and clinical events associated with chronic liver disease. Evidence for beneficial effects of BCAA supplementation has yet to be fully validated, and improvement for low compliance of BCAA supplementation is still required. However, there is substantial evidence that depletion of serum BCAA levels is involved in the progression of liver disease and the development of clinically important sequelae. Pharmacological supplementation with BCAAs may be a promising therapeutic strategy for patients with liver cirrhosis.

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Recent advances of radiofrequency ablation for early hepatocellular carcinoma

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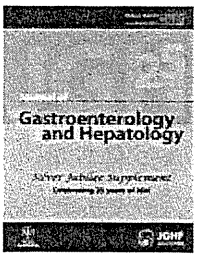

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Abstract

Hepatocellular carcinoma (HCC) is the third leading cause of death in the malignant neoplastic diseases in the world. Surgical operation is sometimes not indicated because of complicated liver cirrhosis and extrahepatic disorders. Radiofrequency ablation has been developed as a less invasive treatment for HCC since 1999, and long-term outcome has been shown. There are several complications which should be paid attention, and to improve the prognosis, combination treatment with transarterial chemoembolization should be discussed. Overall survival after between RFA and surgical resection should be compared prospectively. Establishment of staging system for treatment allocation of HCC and prevention of HCC recurrence is important issue to be examined.

Introduction

Radiofrequency ablation (RFA) has been utilized as a less invasive and curative treatment for the treatment of hepatocellular carcinoma (HCC), and the methods and procedure have been developed. In some countries, it has been chosen as first line treatment for early stage HCC. Long-term prognosis has been reported and the associated factors for the prognosis after RFA have been shown. Several complications were reported after RFA. The prognosis was compared between patients who were treated by between surgical resection and those treated by RFA. The recent developments and future perspective of RFA is discussed in this review.

Radiofrequency ablation method

Of all therapeutic apparatus compared and evaluated up to now, the RF 3000 generator system (Boston Scientific, Boston, USA) had the most positive therapeutic effects.[1] However, in many articles, an internally cooled single electrode was used.[2] When there was a risk of RFA incurred by

the hepatocellular carcinoma (HCC) location, the therapeutic effects were reduced, in particular the complete response rate was low in the vicinity of the gall bladder and the stomach and intestine, as well as the diaphragm, and in the vicinity of large blood vessels.[3] However, it has been reported that, although the therapeutic effects are not reduced when tumors exist in the vicinity of large blood vessels or adjacent to the extrahepatic organs, attention should be paid to the prevention and control of complications.[4] RFA with the use of artificial ascites for HCC adjacent to the diaphragm and to the stomach and intestine produced sufficient therapeutic effects, thereby improving the sonic window.[5]

When performing RFA, the use of a guiding needle with an external insulated sheath was useful because it allowed for precise tumor targeting.[6] The use of laparoscopic RFA has allowed a sufficient therapeutic effectiveness to achieve complete tumor ablation in all cases when the HCC nodule is located with bulging or at subcapsular area, as well as an adequate safety margin, compared to percutaneous RFA.[7] As shown in Fig. 1, extra-hepatic protruding HCC nodule is the most appropriate indication for laparoscopic RFA, and complete necrosis could be achieved after one treatment session under laparoscopic ultrasound guiding. When RFA was performed under laparoscopy, complete necrosis is usually observed.[8]

Figure 1. Hepatocellular carcinoma (HCC) nodule protruding from the liver surface is treated by laparoscopic radiofrequency ablation (RFA) under ultrasound guiding. (a) HCC nodule is directly observed under laparoscopy. (b) Under laparoscopic ultrasound guiding, RFA electrode is introduced to accurate position of the nodule, avoiding damage to diaphragm and intrahepatic vessels. (c) The entire HCC nodule was completely ablated by RFA. (d) Computed tomography (CT) scan before the treatment revealed hypervascular nodule with 2.6 cm in diameter at the surface of the liver. (e) After laparoscopic RFA, complete necrosis was confirmed by CT scan.

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Assessment of the therapeutic effect of RFA

Although the effect of RFA is, in general, evaluated by dynamic computed tomography (CT) scans taken 1 to 7 days after the procedure, it was possible to assess the therapeutic effect by multidetector row helical CT (MD-CT) immediately after RFA, thereby achieving shorter hospital stays.[9] As well, one report indicates that it was possible to assess the therapeutic effect by contrast-enhanced ultrasonography immediately after RFA.[10] Contrast-enhanced sonography with abdominal virtual

sonography was useful in monitoring the therapeutic effect and reducing the CT scan frequency.[11]

Prognosis after radiofrequency ablation

According to a report from a single institution in France, RFA was performed in 235 cases, with up to three HCC ≤ 5 cm in diameter, and achieved complete ablation in 222 cases. 67 cases were judged potentially resectable according to Barcelona Clinical Liver Cancer (BCLC) criteria; in these patients, RFA treatment produced 76% survival at 5 years. The factors contributing to survival were the prothrombin time and serum alpha-feto protein (AFP) levels. Conversely, the factors related to recurrence were multinodular tumors as well as the AFP level. In this report, RFA could be used as an effective first-line treatment in patients with a single nodule of 5 cm or less, a low serum AFP level, and well preserved liver function.[12]

According to a report from Italy, RFA was performed in 218 cases of single nodule HCC, measuring 2 cm or less in diameter, followed by an analysis of the prognosis. The 5-year survival rate was 68.5%, with a low 1.8% incidence of complications. Compared with resection, it was less invasive and could be conducted at a lower cost. It could therefore be considered the treatment of choice for resectable single HCC ≤ 2.0 cm.[13] In Japan, the prognosis of 1000 patients who had undergone RFA was analyzed; the 1, 3, and 5-year survival rates were 94.7%, 77.7% and 54.3%, respectively.[2] According to a report from China, the factors related to the prognosis after RFA were the tumor diameter, the number of tumors, the use of combination therapy with ethanol injection, the margin, and the Child-Pugh score.[14] According to the outcome of RFA treatment for a large single-institution series in Korea, the method had a local recurrence rate of 8.1% at 1 year and 11.8% at 3 years, and patient survival rates were 95.2% at 1 year, 69.5% at 3 years, and 58.0% at 5 years.[15] The five year survival after RFA was similar between Western and Eastern countries (Table 1).

Table 1. 5-year overall survival after r with operable HCC nodule

Overall survival	
Investigator	**Diameter of the nodule**
N'Kontchou G [16]	≤ 5 cm
Livraghi T [17]	≤ 2 cm
Tateichi R [18]	≤ 3 cm
Peng ZW [19]	≤ 5 cm
	≤ 3 cm
Choi D [20]	

Prognosis after RFA and the staging system

The Cancer of the Liver Italian Program (CLIP) score and BCLC scoring system more accurately predicted the prognosis than the Okuda score in patients with early-intermediate HCC, undergoing non-surgical therapy, such as RFA.[21] The results of an analysis in Japan demonstrated that, regardless of the CLIP score, the combination of transarterial chemoembolization (TACE)—RFA had the highest 5-year survival.[22] In Japan, where early-stage HCC is prevalent, the majority of cases are classified into CLIP stage 1 of CLIP scores and, as such, the Japanese integrated staging (JIS) score was proposed as a new early HCC staging system.[23] The results of the validation done in many cases demonstrated that the JIS score yielded a better prediction of the prognosis than the CLIP score.[24] It has also been reported from in Korea that the JIS score is the most appropriate score for predicting the prognosis.[25]

Tumor markers

The tumor marker relevant to the prognosis after RFA is des-gamma-carboxy prothrombin time (DCP) levels; wherein, high DCP levels predicted a poor prognosis after RFA.[16,17] However, the same institution also reported that a comparison of AFP, DCP and AFP-leptin 3 (AFP-L3) demonstrated that AFP-L3 was the most useful indicator of the overall survival and disease-free survival.[18] It was pointed out that the AFP mRNA levels in the blood after RFA are also an objective index of recurrence.[19] On the other hand, blood vascular endothelial growth factor (VEGF) levels

have also been reported to be related to the prognosis.[20]

Recurrence

Local tumor recurrence after RFA is 9.0% at 1 year and 17.7% at 3 years; therefore, local recurrence is relevant to the prognosis for survival.[26] Evaluation of the therapeutic effects of RFA by contrast enhanced CT scans or by enhanced magnetic resonance imaging (MRI) here demonstrated that the procedure provides good local control and the recurrence rate is low in cases in which the post-ablation margin was 0.4 cm or more and the tumor size was smaller than 2.5 cm.[27] The overall local recurrence rate after RFA was 12.8% and the tumor diameter of >2.5 cm was a significant independent factor.[28] However, another report indicates that even when local recurrence occurred, it did not adversely affect the survival prognosis.[29] Utilizing the RF 3000 generator system has been reported more positive effects than cool-tip electrode.[30]

On the other hand, the cumulative rate of intrahepatic distant recurrence was reported as 10.4% and 77.0% at 1 and 5 years, respectively. In a multivariate analysis, AFP and DCP values, as well as the safety margin, were significant independent factors.[31] The intra-hepatic distant recurrence was associated with multi-nodular lesions and hepatitis C virus (HCV), even after curative ablation was achieved.[32] Recurrence at a distant site is an important, poor prognostic factor.[33] Although it is possible to ensure long-term survival by carrying out repeat RFA after recurrence,[34] the more frequently recurrences occur, the higher the risk for subsequent recurrence becomes.[35] Histological grade is relevant to the therapeutic efficiency of RFA and also plays a part in determining survival.[36]

Prognosis and possible measures to improve survival after RFA

Long-term interferon maintenance therapy improved the survival in patients with HCV related HCC after RFA.[37] On the other hand, the administration of lamivudine after RFA for hepatitis B virus (HBV)-related HCC maintained the liver function and was also safe.[38,39] The administration of vitamin K for HCV-related HCC did not produce a chmenopreventative effect.[40] The oral administration of a branched-chain amino acid after RFA made it possible to maintain the serum albumin levels and it was also useful for improving the liver function.[41]

Resection *versus* RFA

With regard to the question of whether surgical resection or RFA is superior, two randomized comparisons have been reported—all from China. In these reports, the life prognoses of single HCCs of 2 cm or less diameter were randomly compared between RFA and resection. It was reported that

there would be no difference between the two, or that, for single HCC of 5 cm or less, there was no difference in terms of both disease-free survival and overall survival.[42,43] In Italy, a group of 109 patients who underwent RFA and a group of 91 patients who underwent resection were compared retrospectively; there was no difference in terms of the overall survival and disease-free survival, for HCC of 3 cm or less.[44] Likewise, a retrospective analysis conducted in Korea, compared a group of 55 patients who underwent RFA treatment for single HCC 4 cm or less and well-preserved liver function with a group of 93 patients who underwent resection; the authors concluded that there was no difference in terms of overall survival and recurrence-free survival at 1 year and 3 years after RFA.[45] When laparoscopic RFA was performed on patients with single HCC nodule with Child-Pugh A liver function, RFA and resection had similar survival rates.[46,47]

However, a case control study of resection versus RFA showed that recurrence, tumor diameter, and whether resection or RFA were performed, all affected overall survival. The authors concluded that a resection provided some advantages.[48] Furthermore, with regard to cases of HCC which are not suitable candidates for liver transplantation, a Markov model was used to compare the life-adjusted survival between resection and RFA. The survival rate in the resection group was 5.33 years, while in the RFA group it was 3.91 years. It was concluded therefore, that patients treated by a resection would have a better survival rates.[49] In another study, 79 cases of resection and 79 cases of RFA treated at two different institutions were compared. The result showed that resection would be better than RFA for tumors of 3 cm and larger in diameter with Child A score, but that the overall survival would be the same for surgery and RFA in the case of Child B score.[50]

Comparison between RFA and other ablations

A comparison between microwave coagulation and RFA for HCC, 2 cm or less in tumor diameter demonstrated that RFA was superior because it created a larger necrotic area, resulting in a lower local recurrence rate; this conferred better cumulative survival, while bile duct injury and pleural effusion occurred less frequently.[51]

Another study compared percutaneous ethanol injection (PEI) and RFA. This randomized controlled trial (RCT) conducted in Taiwan demonstrated that RFA required fewer treatment sessions to achieve complete tumor necrosis, and provided better overall survival.[52] Another RCT between PEI and RFA was conducted in Japan. The 4-year survival rates were 74% for RFA versus 57% for PEI, resulting in RFA treatment being associated with a lower risk of death and recurrence. There was no difference in frequency of adverse events.[53] Although it was not RCT, another study compared PEI and RFA and found that local recurrence rates after RFA were lower.[54] An RCT conducted in Italy compared RFA with PEI and found that complete response of RFA after one year was associated with a better outcome, though no survival advantage was observed.[55]

There have been three meta-analyses, based on RCT comparing the effects and complications

between RFA and PEI. Each found that RFA had better overall survival, while PEI had a higher local recurrence rate; thus RFA was superior in cancer-free survival rates.[56–58] No difference was observed in the complications between the two.

RCT was conducted to identify whether a combination of RFA and PEI would produce a better outcome than RFA alone. For tumors measuring between 3.1 cm and 5 cm in size, RFA + PEI improved patient survival, and overall recurrence was lower with combination treatment.[59]

Combined TACE and RFA treatment

The combination of transarterial embolization (TAE) and RFA or PEI was compared with TACE alone, and it was found that TACE + RFA had a better prognosis.[60] The results of a case-control comparison between RFA combined with TACE and RFA alone demonstrated that there was no difference in cases of single HCC ≤ 5 cm, but that the TACE + RFA combined treatment had a higher survival rate in cases of single HCC > 5 cm or multiple tumors.[61] The combination of TACE and RFA was technically successful in 88% of cases; such patients, complete the therapy after a single treatment session.[62] In addition, the combination of TACE and RFA produced high local control rates.[63] TACE and RFA has been performed for HCC immediately below the diaphragm, and found to be effective.[64] The combination of bland arterial embolization with RFA and a resection has also been compared; the overall survival was found to be similar in patients with single HCC measuring up to 7 cm in diameter.[65]

The extent of necrosis resulting from RFA increases when combined with hepatic arterial balloon occlusion.[66] Furthermore, combined treatment with balloon occlusion after transcatheter arterial infusion chemotherapy (TAI) is effective in expanding the necrotic area.[67] However, some researchers argue that this combination is not necessary because the effects of the combined therapy involving TACE and RFA, and that of RFA alone, for small HCC ≤ 3 cm, are the same.[68]

Complications

Data from 3891 cases were collected in a joint study conducted in Osaka, Japan. Complications were observed in 207 cases (7.9%), with 9 patients dying within 3 months. The causes of death in these cases were: liver failure in 3 cases, rapid progression in 3 cases, biliary injury in 1 case, gastrointestinal bleeding in 1 case, and myocardial infarction in 1 case.[69] Data for 255 cases in China have also been reported, with major complications observed in 31 cases (10%) as follows: 13 cases of liver failure, 10 cases of hydrothorax, 2 cases of tumor seeding, 1 case of upper gastrointestinal bleeding, and one each of intrahepatic abscess, bile duct injury, and cardiac arrest, 5 cases of hyperglycemia, and 11 cases of death due to liver failure.[70] A report from the United States noted that complications had been observed in 7 out of 91 cases as follows: 2 cases of hepatic abscess and one each of skin burn, hemorrhage, myocardial infarction, and liver failure.[71] According to the

results of a multicenter survey conducted in Korea, liver abscess (0.66%), peritoneal hemorrhage (0.46%), biloma (0.20%), ground pad burn (0.20%) and pneumothorax (0.20%) were reported as complications.[72]

Liver abscess and bile duct injury

Liver abscess is the most common complication—de Raere *et al.* observed 7 cases out of 350 sessions and a high risk of this complication among patients with a revious bilioenteroc anasomosis.[73] Likewise, Choi *et al.*[74] and Elias *et al.*[75] also reported that liver abscess was seen more often in cases of biliary abnormality, as well as after TACE treatment. In one report, cholangitis and liver abscess occurred simultaneously.[76] Attention should therefore be paid to the fact that the risk for liver abscess complication is high in cases of complicated anastomosis of the bile duct to the intestinal tract.

Biliary stricture was observed in 7 cases after the RFA procedure, with liver abscess as a complication in 3 cases.[77] It was reported that intraductal chilled saline perfusion by endoscope had been effective in preventing bile duct injury.[78]

Bleeding

A total of 4133 RFA treatments were performed in 2154 cases, with hemorrhagic complications occurring in 63 treatments (1.5%) as follows: hemoperitoneum (0.7%), hemothorax (0.3%) and hemobilia (0.5%). In addition, there were two deaths due to hemoperitoneum.[79] Poggi *et al.* reported only one case of bleeding that required surgery.[80] Attention has also been focused on bleeding which occurred in one case of subcapsular liver tumor, but there were no complications such as seeding.[81,82]

Intestinal injury

Two cases were reported in which colonic perforation occurred as a complication on the 8 th day after RFA. Attention should be paid to the fact that intestinal injury was indolently present.[83,84] Another report indicated the occurrence of duodenopleural fistula formation as a complication.[85]

Hepatic infarction

Hepatic infarction has been observed after RFA; the frequency is 1.8%.[86] The use of internally cooled electrodes is a risk. In addition, portal thrombosis has also been reported to occur.[73,87]

Seeding

A report given by Llovet *et al.* in 2001 on the high rate of seeding after RFA has received much attention. The risk factors included: subcapsular tumor localization, a high degree of poor differentiation, and a high baseline AFP.[88] Since then the risk of seeding after RFA, has been attiributed to subcapsular location, poorly differentiated tumors and high AFP levels.[89,90] However, a discrepancy exists between institutions, with some arguing that, in reality, seeding is exceptionally rare.[91,92] In order to prevent seeding, tract ablation should therefore be properly performed.

Other complications

In another report, pneumothorax occurred after RFA, and so careful attention is required for tumors adjacent to the diaphragm.[93] It has been reported that myoglobinuria occurs as a complication after RFA and that the serum creatinin level rises, making it necessary for attention to be paid thereto.[94] Another case was reported in which hemolysis occurred, thus inducing hemogeobinuria as a complication.[95] There have also been reports that rapid tumor progression occurred after RFA;[96,97] however, the actual frequency was low and it is therefore necessary to investigate whether or not it was indeed a complication associated with RFA.

We have done 1440 sessions of RFA to patients with early stage HCC from July, 1999 to December 2009. The complications have been analyzed as shown in Table 2. The complication rates were 1.8% and 1.9% when the patients were treated by laparoscopic or percutaneous RFA, respectively.