Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochemical and Biophysical Research Communications 303 (2003) 59–64
BBRC
www.elsevier.com/locate/ybbrc

Branched-chain amino acids promote albumin synthesis in rat primary hepatocytes through the mTOR signal transduction system

Chiori Ijichi,* Tsuyoshi Matsumura, Takashi Tsuji, and Yuzuru Eto

Pharmaceutical Research Laboratories, Ajinomoto Co., Inc, 1-1, Suzuki-cho, Kawasaki-ku, Kawasaki 210-8681, Japan

Received 29 January 2003

Abstract

The administration of branched-chain amino acids (BCAAs) to cirrhosis patients increases serum albumin levels and improves the blood Fischer's ratio. Although it has been reported that albumin synthesis in rat primary hepatocytes is diminished under lower Fisher's ratio conditions compared to normal Fischer's ratio conditions, the mode of action at the molecular level for these effects is still uncertain. It has been reported recently that the triggering signal for protein synthesis is transmitted through mTOR (mammalian target of rapamycin). We have had an interest in the mTOR signal transduction system. In the present study, we analyzed the mode of action of BCAA-induced albumin synthesis using rat primary hepatocytes. The BCAA mixture dose-dependently promoted the production of albumin, with leucine being the major effector half of which was inhibited by the mTOR inhibitor rapamycin. We also showed that only leucine induces P70 S6 kinase activation and 4E-BP1 phosphorylation which are mTOR's downstream translational effectors. These activations were completely inhibited by rapamycin. Our results suggest that BCAAs, especially leucine, promote the production of albumin in rat primary hepatocytes through an mTOR signal transduction system.

Keywords: Amino acids; Leucine; Cirrhosis; BCAA; Fischer's ratio; mTOR

The administration of an oral BCAA (branched-chain amino acid) pharmaceutical preparation intended for decompensated cirrhotic patients improves hypoalbuminemia [1–3]. In chronic hepatic failure patients [2,4], the change in the plasma amino acid composition was shown especially, the decrease in the Fischer's ratio (Fischer's ratio; BCAA/AAA), caused by the increase in the concentration of aromatic amino acids (AAA), and the decrease in the concentration of BCAA [5]. A positive correlation between plasma BCAA concentration (or Fischer's ratio) and albumin concentration was observed in decompensated cirrhotic patients. Oral BCAA administration to cirrhotic patients and to carbon tetrachloride induced liver cirrhosis-model rats not only raised the serum albumin levels but also improved the prognosis [1,6].

Hormones and nutrients are known to have an influence on albumin synthesis or secretion, based on studies with primary hepatocytes and liver perfusion experiments [7,8]. Among hormones, insulin and dexamethasone have been reported to increase the production of albumin, and among nutrients, glucose and amino acids affect the production of albumin [7,9]. The synthesis and secretion of albumin increased when rat primary hepatocytes were cultured with an appropriate Fischer's ratio (3.0) [10]. The addition of amino acids similar to the plasma composition was found to promote albumin synthesis in primary hepatocytes and in liver perfusion experiments, and the removal of amino acids decreased albumin synthesis. It was shown by liver perfusion experiments that albumin mRNA was translated by membrane-bound polysomes in the presence of amino acids [11,12]. In contrast, mRNA is dissociated from the ribosome and was not translated in the absence of amino acids.

Recently, it has been clarified that certain kinds of amino acids can trigger protein synthesis in various cells through a key molecule known as mTOR (mammalian target of rapamycin) [13–17]. Activated mTOR phosphorylates p70 S6 kinase, followed by activated p70 S6 kinase that phosphorylates ribosomal S6 protein, and as a result, increases the protein synthesis complex. Acti-

* Corresponding author. Fax: +81-44-210-5873.
E-mail address: chiori_ijichi@ajinomoto.com (C. Ijichi).

0006-291X/03/$ - see front matter © 2003 Elsevier Science (USA). All rights reserved.
doi:10.1016/S0006-291X(03)00295-X

vated mTOR also phosphorylates eIF4E-BP1 and promotes the formation of the protein synthesis initiation complex. On the other hand, amino acids appear to be indispensable for insulin to promote protein synthesis through mTOR [15]. It has also been shown that leucine activates mTOR in the hepatic carcinoma cell lines FAO and H4IIE [13,16]. Oral administration of leucine to food-deprived animals leads to an increase in protein synthesis associated with effects on p70 S6 kinase and eIF4E-BP1 in skeletal muscle, adipose tissue, and liver [18,19]. However, no report on the correlation between mTOR activation with amino acids and albumin synthesis has been reported. Therefore, we used rat primary hepatocytes to analyze the albumin synthesis promotion activity of BCAA by examining the activation of mTOR signal transduction.

Materials and methods

Preparation and culture of rat primary hepatocytes. Hepatocytes were obtained from the livers of 6-week-old male Wistar rats (Charles River Japan, Kanagawa, Japan) by the collagenase perfusion method [20]. The liver tissue was digested and suspended in Hanks' balanced salt solution (Gibco-BRL, Life Technologies, Grand Island, NY), and filtered with a 100-μm pore size nylon mesh. The cells were suspended in 45% Percol–HBSS solution and centrifuged for 10 min and the pelleted cells were washed three times with 30 ml RPMI1640 (Gibco-BRL, Life Technologies, Rockville, MD) medium containing 1% fetal calf serum (JRH Biosciences, Lenexa, KS). The cells were cultured at 37 °C for 16 h in a collagen type I coated six-well plate (Iwaki Grass, Tokyo, Japan) at a density of 1×10^5cells/0.2 ml/cm^2 in the same medium, washed with amino acid-deficient RPMI1640 medium, and then incubated with amino acid-deficient RPMI1640 medium for 2 h. Then, cells were incubated with the media containing indicated BCAAs and rapamycin (Wako Pure Chemical Industries, Osaka, Japan). The amino acid-deficient medium was made with the RPMI Selection Amine Kit (Gibco-BRL, Life Technologies, Rockville, MD) with osmotic pressure adjusted between 300 and 310 mOsm. At the end of the culture period, two photographs were taken at random at 100× magnification and the number of cells in each area was counted. All procedures involving care and use of animals were approved by Institutional Animal Care and Use Committee of Pharmaceutical Research Laboratories of Ajinomoto prior to conduct.

Quantitative analysis of albumin. Albumin was quantified using europium (Eu)-labeling reagent (Amersham Pharmacia Biotech, Buckinghamshire, UK). Rat albumin (Sigma Chemical, St. Louis, MO) was dissolved in 50 mM $NaHCO_3$ (1 mg/ml) and mixed with Eu-labeling reagent for 12 h. The Eu-labeled albumin was collected by a Superdex Hi Load 16/60 column (Amersham Pharmacia Biotech, Buckinghamshire, UK). Anti-human albumin antibody solution (DAKO A/S, Glostrup; Denmark, 1 μg/ml in 10 mM carbonic acid buffer) was poured into a 96-well Maxisorp plate (Nalge Nunc International, Rochester, NY) and kept at 4 °C for 12 h. The plate was washed once with TBS (20 mM Tris, pH 7.4, 0.15 M NaCl). A fourfold dilution of Blockase (Snow Brand Milk Products, Japan) was added at 100 μl/well and kept at room temperature for 2 h. Twenty microliters of the sample (1/3-diluted with DELFIA assay buffer, Amersham Pharmacia Biotech) and 30 μl of the europium-labeled albumin (10,000 counts) were mixed in a well, kept at room temperature for 1 h, and then washed five times with TBS-Tween (20 mM Tris, pH 7.4, 0.15 M NaCl, and 0.05% Tween 20). After the addition of 100 μl/well of DELFIA enhancement buffer (Amersham Pharmacia Biotech), the time-resolved fluorescence was measured using a microplate reader ARVO (EG & GWallac Oy, Turku, Finland).

Immunoprecipitation and p70 S6 kinase assays. The p70 S6 activity was determined in the immunoprecipitates using ribosomal subunits as substrate. Hepatocytes were incubated with amino acid-deficient media supplemented with or without 2 mM each of BCAAs and 25 ng/ml rapamycin for 20 min after amino acid starvation. Then, cells were lysed in ice-cold buffer A (50 mM Tris–HCl at pH 8.0, 1% Nonidet P-40, 120 mM NaCl, 20 mM NaF, 1 mM EDTA, 6 mM EGTA, 20 mM β-glycerophosphate, 0.5 mM dithiothreitol, 50 μM *p*-amidinophenylmethylsulfonyl fluoride hydrochloride, 1 μg/ml aprotinin, and 1 μg/ml leupeptin) and the extracts were centrifuged at 10,000*g* for 30 min at 4 °C. Aliquots of the supernatants were subjected to immunoprecipitation with p70 S6 kinase (C-18) antibody (SC-230, Santa Cruz Biotechnology) for 2 h, which were absorbed to protein G–Sepharose. This was washed twice with buffer A containing 0.5 M NaCl and twice with wash buffer (20 mM MOPS at pH 7.2, 10 mM β-glycerophosphate). The 40S S6 protein kinase reaction was started by adding the reaction mixture (50 mM MOPS at pH 7.2, 12 mM MgCl2, 2 mM EGTA, 1 mM dithiothreitol, 60 μM ATP, 10 mM β-glycerophosphate, 0.5 μM protein kinase inhibitor, 0.5 A_{260} subunits of 40S ribosomal subunits, and 5 μCi of [γ-^{32}P]ATP). This was incubated for 20 min at 30 °C and the reaction was terminated by adding the SDS sample buffer. The samples were separated by SDS–PAGE on 12% acrylamide gel and radioactivity incorporated into 40S S6 protein of each sample was visualized by autoradiography.

Examination of 4E-BP1 phosphorylation state. Hepatocyte lysates were solubilized in SDS sample buffer, separated by SDS–PAGE on 12% acrylamide gel, and transferred to nitrocellulose membrane. Electrophoretic mobility shift assays using 4E-BP1 (C-19) antibody (sc-6024 Santa Cruz Biotechnology) were visualized by ECL.

Measurement of protein synthesis by L-[^{35}S]methionine. Hepatocytes were cultured for 6 h in the culture medium with or without 2 mM leucine and 25 ng/ml rapamycin, supplemented with 10 μCi/ml L-[^{35}S]methionine. The cell pellet or culture supernatant precipitated by 10% TCA was dissolved in a small volume of SDS–PAGE sample buffer and separated by 10% polyacrylamide gel SDS electrophoresis. The radioactivity of each protein band was measured with a BAS-2000 Bio Imaging Analyzer (Fuji Photo Film, Tokyo, Japan).

Detection of albumin mRNA. Total RNA was extracted from cells with a total RNA Isolation Kit (Invitrogen, Groningen, The Netherlands) and detected using Taqman EZ RT-PCR core reagents (Perkin–Elmer Biosystems, Turku, Finland). The PCR was carried out by a standard method. The primers and probes used in the experiments were as follows. Rat albumin: forward primer, 5′-T G T C C C C A A A G A G T T T A A A G C T G-3′; reverse primer, 5′-T C T T T A T C T G C T T C T C C T T G T C T G G-3′; probe, 5′-Fam-A C C T T C A C C T T C C A C T C T G A T A T C T G C A C A C T-Tamra-3′. Rat β-actin: forward primer, 5′-C G T G A A A A G A T G A C C C A G A T C A-3′; reverse primer, 5′-A C A C A G C C T G G A T G G C T A C G T A-3′; probe, 5′-Fam-T T T G A G A C C T T C A A C A C C C C A G C C A-Tamra-3′. The amount of albumin mRNA and β-actin mRNA was compared with that of a time-zero control.

Statistical analyses. The results are given by means ± SE. The judgment of significant difference (less than 5%) was based on Dunnett's *t* test multiple analysis or Tukey–Kramer analysis.

Results

Albumin secretion activity of BCAA

Many reports suggested that the amino acid composition in a culture medium was important to the albumin secretion/production from primary hepatocytes [7,21].

In the present study, we examined whether branched-chain amino acids (BCAAs) promote albumin secretion in primary hepatocytes. The mixture ratio of BCAA was adjusted to leucine:isoleucine:valine = 2.0:1.0:1.2 (molar ratio) according to the results of a study in which variable effects on viability were observed in response to the oral administration of BCAAs to cirrhosis-model rats treated with carbon tetrachloride [6].

It was confirmed that the albumin secretion rate increases linearly for the first 6 h (data not shown) and constantly remained independent of the amino acid levels in the medium.

Therefore, it was thought that the levels of albumin secretion correspond directly to albumin synthesis. The albumin synthesis rate was increased to 1.5 times in medium with an RPMI composition (400 ng/h/dish) compared to those in the absence of amino acids (260 ng/h/dish). At this time, a 2 mM BCAA mixture (400 ng/h/dish) showed promotion effects equal to those obtained with the RPMI composition. There was no change in the number of cells after 6 h in any of the experiments (data not shown).

Fig. 1 shows the amount of albumin in the 6-h culture supernatant. The BCAA mixture promoted albumin synthesis in a dose-dependent manner from 0.1 to 0.5 mM, with production reaching a plateau (3484 ± 246 ng/6 h/dish) at a BCAA concentration of 0.5 mM. The concentrations of amino acids in the media and the cells were measured. No great difference was seen in the amino acid concentrations in the medium before and after the culture. However, the amount of intracellular leucine in cells cultured with the 2 mM leucine medium was 10 times as high as that in cells of the amino acid-deficient medium.

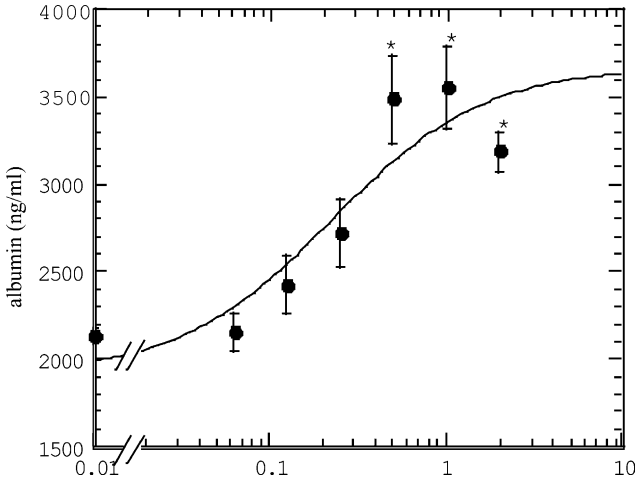

Fig. 1. Concentration-dependence of BCAA on albumin synthesis. After primary hepatocytes had been cultured for 6 h with an amino acid-deficient medium with the addition of a 0–2 mM BCAA mixture. The amount of albumin in the culture supernatant was measured in three independent experiments and the results are shown as means ± SEM. *$P < 0.05$ compared with the values without adding BCAAs.

Albumin-synthesis activity of leucine, isoleucine, and valine

The experiment shown in Fig. 2 was carried out to examine which amino acid has the most potent albumin-synthesis activity in a BCAA mixture. Among BCAAs, leucine appeared to be the most important amino acid, as 2 mM leucine showed activity equal to that of a 2 mM mixture of BCAAs. Isoleucine and valine showed slight promotion-inducing tendencies, although their effects were not significant.

The effects of other amino acids were examined. Our results (data not shown) showed no noticeable effect on albumin synthesis for histidine [21], proline [22], and alanine [23,24], all of which have been reported to have some positive effects on liver proliferation or liver injury.

Effect of the mTOR inhibitor rapamycin on the promotion of albumin synthesis by leucine

Experiments were carried out to clarify whether leucine promotes albumin synthesis through an mTOR signal transduction system. It is well known that rapamycin selectively binds to FKBP-12 and inhibits the activation of mTOR [13–16,25]. A decrease in p70 S6 kinase activity and the dephosphorylation of eIF-4EBP1 were observed when the mTOR activity was blocked [13–16]. As a result, the formation of the protein synthesis initiation complex was inhibited and the rate of

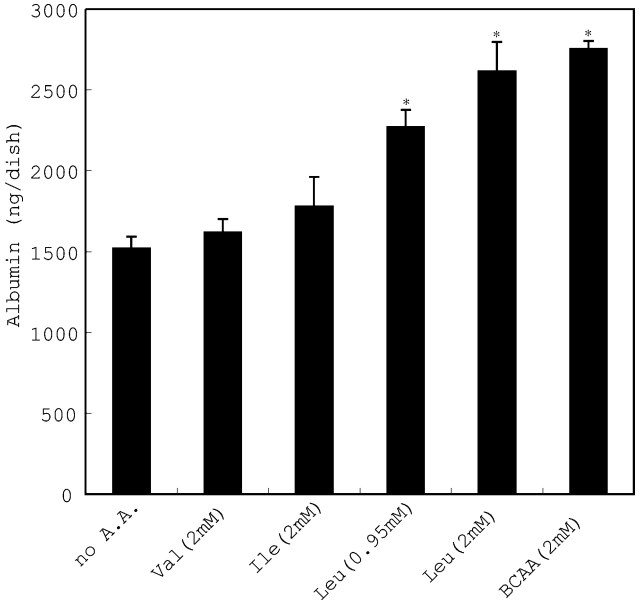

Fig. 2. Effect of each BCAA on albumin synthesis. The primary hepatocytes were cultured for 6 h with an amino acid-deficient medium with the addition of 2 mM leucine (also 0.95 mM), isoleucine, valine, or a 2 mM BCAA mixture, and the amount of albumin in the culture supernatant was measured. Three independent experiments were carried out. The results are shown as means ± SEM. *$P < 0.05$ compared with the values with no A.A.

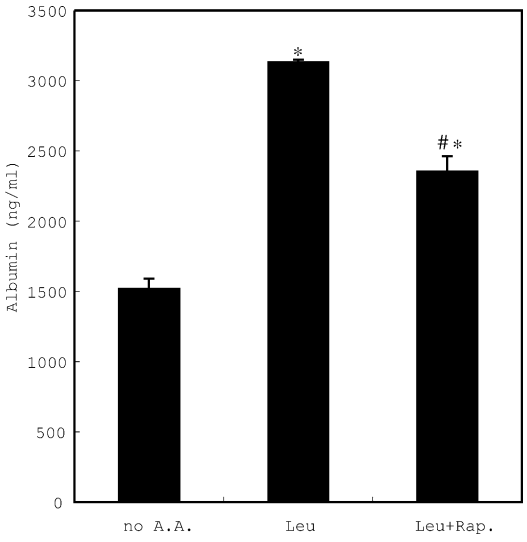

Fig. 3. Effect of the mTOR inhibitor rapamycin on albumin synthesis. The primary hepatocytes were cultured for 6 h in an amino acid-deficient medium with no additional amino acid, with an addition of 2 mM leucine, and 2 mM leucine with 25 ng/ml rapamycin, and the amount of albumin in the culture supernatant was measured. Three independent experiments were carried out and the results are shown as means ± SEM. $*P < 0.05$ compared with the values with no A.A. $\#P < 0.05$ compared with the values with Leu.

protein synthesis decreased. As shown in Fig. 3, 25 ng/ml rapamycin significantly inhibited the albumin-producing activity of leucine by 52%.

The newly synthesized proteins were measured to clarify whether leucine promotes only the synthesis of secreted proteins such as albumin or promotes all types of proteins. Newly synthesized proteins were measured using L-[^{35}S]methionine. The cell culture supernatant and cell extract were separated by SDS–PAGE and the radioactivity of each band was measured. The albumin in relation to total protein was 2.8% with no amino acids in the medium, 3.8% in 2 mM leucine, and 3.3% in 2 mM leucine + rapamycin. The rate of albumin synthesis rose with the amount of leucine by 1.4 times and rapamycin inhibited this activity by 58% (Table 1). It was also confirmed that only a small amount of albumin accumulated intracellularly, which suggests that the amount of albumin in the culture supernatant directly corresponded to the rate of albumin synthesis, which is similar to the results illustrated in Fig. 3. The synthesis of transferrin [26], another secreted protein, was also promoted by leucine and inhibited by rapamycin (0.48% in leucine, 0.41% in leucine with rapamycin, and 0.38% in medium with no amino acids). In contrast, no remarkable changes were observed in intracellular proteins.

Change in the amount of albumin mRNA

The amount of albumin mRNA and β-actin mRNA was quantified for primary hepatocytes cultured in the presence of leucine and rapamycin. Total mRNA was measured by quantitative RT-polymerase chain reaction and compared with the control sample prepared at the end of 2-h pre-culture in the absence of amino acids. There was no remarkable change in the amount of albumin mRNA after a 6-h culture with no amino acids in the medium, with 2 mM leucine, or with leucine and rapamycin. A similar result was obtained for β-actin mRNA with no amino acids in the medium, with 2 mM leucine, and with leucine and rapamycin.

Table 1
Ratio of newly synthesized albumin and transferrin

	No amino acid	Leu	Leu + rapamycin
Albumin	3.3	4.8	3.2
Transferrin	0.43	0.65	0.35
Total protein	100	100	100

Primary hepatocytes were cultured for 6 h in an amino acid-deficient medium with 10 μCi/ml L-[^{35}S]methionine containing no amino acid, 2 mM leucine, or 2 mM leucine with 25 ng/ml rapamycin. The TCA precipitate of the culture supernatant was separated by 10% polyacrylamide gel electrophoresis–SDS, and the radioactivity of the protein bands corresponding to albumin and transferrin was measured using BAS 2000 Bio Imaging Analyzer, and shown is the ratio to the radioactivity of total synthesized proteins.

Only leucine stimulates mTOR-signaling pathway

The ability of individual BCAA to stimulate p70 S6 kinase activity following amino acid withdrawal was examined using rat primary hepatocytes. As shown in Fig. 4A (upper panel), brief (2 h) withdrawal of amino acids from serum-deprived culture medium diminished the activity to basal level (lane 1) and the addition of leucine stimulated p70 S6 kinase activity (lane 2). However, the addition of isoleucine and valine did not stimulate p70 S6 kinase activity (lanes 3 and 4). Using the same lysates, we also monitored phosphorylation states of 4E-BP1 by the shift in the migration of 4E-BP1 polypeptides on SDS–PAGE. As with p70 S6 kinase, rat primary hepatocytes deprived of amino acids for 2 h exhibited a predominance of rapidly migrating bands (α and β bands), meaning lower phosphorylation state of 4E-BP1 (Fig. 4B, lane 1). Single addition of leucine diminished the proportion of more rapidly migrating bands (α and β bands) and increased that of the slower migrating band (γ band), meaning higher phosphorylation state of 4E-BP1 (Fig. 4B, lane 2). The readdition of isoleucine and valine did not change the phosphorylation states of 4E-BP1 compared to its culture after amino acid depletion (Fig. 4B, lanes 3 and 4). Rapamycin completely inhibited the activity of leucine to p70 S6 kinase and 4E-BP1 phosphorylation states (data not shown). We also examined the effect of other amino acids on p70 S6 kinase and 4E-BP1 phosphorylation states. No other amino acid exhibited the activity to these proteins except methionine, which stimulated these

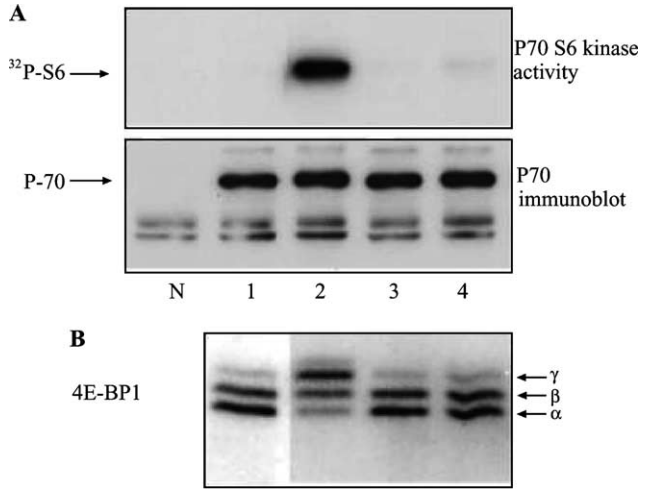

Fig. 4. Effect of each BCAA to stimulate p70 S6 kinase activity after amino acid withdrawal. After amino acid withdrawal for 2 h, rat primary hepatocytes were incubated for 20 min with no amino acids (lane 1), 2 mM leucine (lane 2), 2 mM valine (lane 3), and 2 mM isoleucine (lane 4). (A) P70 S6 kinase activity was determined as described under Materials and methods. Lane N means negative control and the lysate was immunoprecipitated using normal rabbit IgG. (B) The phosphorylation of 4E-BP1 was analyzed as described under Materials and methods.

effectors slightly (data not shown). Thus, in rat primary hepatocytes, only leucine among BCAAs stimulated the mTOR-signaling pathway. Consistent with our observations, the other reports have demonstrated that the readdition of leucine alone after amino acid depletion activates p70 S6 kinase activity in FAO hepatoma cells [13] and in H4IIE cells [16].

Discussion

Primary hepatocytes secrete albumin at a constant rate even when no amino acids are present and BCAA appears to promote the secretion in a dose-dependent manner from 0.1 to 0.5 mM. It is believed that the albumin synthesis is promoted at physiological concentrations because plasma BCAA concentrations of rats after 24 h fasting are approximately 0.4 mM [17]. These results suggest that BCAA administration to cirrhosis patients directly promotes liver albumin synthesis and increases plasma albumin levels.

In addition, the promotion of albumin synthesis was found to depend mainly on leucine in the BCAA mixture and only a slight promotion activity was observed with both isoleucine and valine. However, Anthony et al. [19] have reported that leucine administration for the food-deprived rats reduced circulating concentrations of isoleucine and valine compared with control rats. They discussed that this reduction of serum isoleucine and valine after leucine administration may reflect an increase in uptake of those amino acids to support rates of protein synthesis enhanced by leucine. For this, we are confident that oral administration of BCAA mixture, not only leucine, is of great benefit for cirrhosis patients who have hypoalbuminemia.

A specific mTOR inhibitor, rapamycin, inhibits the albumin synthesis promotion activity of leucine by half (Fig. 3) and completely inhibits the activation of P70 S6 kinase and the phosphorylation of 4E-BP1 in mTOR signaling pathway (Fig. 4). That is, leucine promotes protein synthesis in at least two signaling pathways, a rapamycin-sensitive pathway and an unknown rapamycin-insensitive pathway as shown in the skeletal muscle and adipose tissue [18,19,27]. In addition, based on the results of the protein-labeling experiments, a correlation between albumin synthesis and its accumulation in the culture supernatant was observed. These results suggested that leucine promotes the synthesis of secreted proteins such as albumin and transferrin, whereas it has no effect on the synthesis of intracellular proteins and the amount of albumin mRNA. Recently, Tomiya et al. [28] reported that leucine stimulates the secretion of hepatocyte growth factor (HGF) by hepatic stellate cells. It was indicated that leucine may stimulate protein production in hepatocyte indirectly in vivo through inducing factors such as HGF. The amounts of albumin mRNA showed no remarkable change in these experiments, therefore, it was deduced that the mTOR-dependent albumin-synthesis promotion activity of leucine is due to the stimulation of protein translation but not to the stimulation of mRNA transcription or stability.

BCAAs are essential in protein nutrition and are abundant in body proteins [29]. Among BCAAs, leucine was focused for its strong activity to stimulate protein synthesis through mTOR signaling pathway. Recently, it has been examined about the structural requirements for stimulation and inhibition of mTOR signaling pathway by leucine and its analogs [16,27]. The control mechanism with leucine upstream of the mTOR and a more detailed elucidation will be necessary to be clarified in the future.

Acknowledgments

We are grateful to Dr. Naohiro Terada, Dr. Kunio Matsumoto, Dr. Singo Niimi, and Dr. Masahiro Murata for their valuable advice and discussions. We also thank Ms. Taeko Inamura and Ms. Nao Mizuno for their technical assistance as well as Dr. Shoji Fujitani and Ms. Yuko Iida for their help in manuscript preparation.

References

[1] T. Yoshida, Y. Muto, H. Moriwaki, M. Yamato, Effect of long-term oral supplementation with branched-chain amino acid granules on the prognosis of liver cirrhosis, J. Gastroenterol. (Gastroenterol. Jpn.) 24 (1989) 692–698.

[2] Y. Muto, T. Yoshida, in: S. Osugi, A. Okada (Eds.), Effect of Oral Supplementation with Branched-chain Amino Acid Granules on Improvement of Protein Nutrition in Decompensated Liver Cirrhosis: A Cross-over Control Study in Parenteral and Enteral Hyperalimentation, Elsevier, Amsterdam, 1984, pp. 280–292.
[3] M. Kato, T. Yoshida, H. Moriwaki, Y. Muto, et al., Effect of branced-chain amino acid (BCAA) enriched-nutrient mixture on albumin metabolism in cirrhotic patients, Acta Hepatol. Jpn. 32 (1991) 692–699.
[4] Y. Muto, T. Yoshida, M. Yamato, Clinical assessment of nutritional status in patients with liver cirrhosis with special reference to plasma amino acid imbalance, J. Clin. Biochem. Nutr. 1 (1986) 89–95.
[5] J.E. Fisher, N. Yoshimura, A. Aguirre, J.H. James, M.G. Cummings, R.M. Abel, F. Deindoerfer, Plasma amino acids in patients with hepatic encephalopathy. Effect of amino acid infusion, Am. J. Surg. 127 (1974) 40–47.
[6] K. Kajiwara, M. Okuno, T. Kobayashi, N. Honma, T. Maki, M. Kato, H. Ohnishi, Y. Muto, H. Moriwaki, Oral supplementation with branched-chain amino acids improves survival rate of rats with carbon tetrachloride-induced liver cirrhosis, Digest. Dis. Sci. 43 (1998) 1572–1579.
[7] S.M. Hutson, C. Stinson-Fisher, R. Shiman, L.S. Jefferson, Regulation of albumin synthesis by hormones and amino acids in primary cultures of rat hepatocytes, Am. J. Physiol. 252 (1987) 291–298.
[8] L.J. Crane, D.L. Miller, Plasma protein induction by isolated hepatocytes, Mol. Cell. Biochem. 53/54 (1983) 89–109.
[9] K.E. Flaim, D.E. Peavy, W.E. Everson, L.S. Jefferson, The role of amino acids in the regulation of protein synthesis in perfused rat liver. II. Effect of amino acid deficiency on peptide chain initiation, polysomal aggregation, and distribution of albumin mRNA, J. Biol. Chem. 257 (1982) 2939–2946.
[10] M. Okuno, H. Moriwaki, M. Kato, Y. Muto, S. Kojima, Changes in the ratio of branched-chain to aromatic amino acids affect the secretion of albumin in cultured rat hepatocytes, Biochem. Bhiophys. Res. Commun. 214 (1995) 1045–1050.
[11] M. Kuwahata, T. Oka, K. Asagi, H. Kohri, A. Kato, Y. Natori, et al., Effect of branched-chain amino acids on albumin gene expression in the liver of galactosamine-treated rat, J. Nutr. Biochem. 9 (1998) 209–214.
[12] S.H. Yap, R.K. Strair, D.A. Shafritz, Identification of albumin mRNA in the cytosol of fasting rat liver and influence of tryptophan on a mixture of amino acids, Biochem. Biophys. Res. Commun. 83 (1978) 427–433.
[13] M.E. Patti, E. Brambilla, L. Luzi, E.J. Landaker, C.R. Kahn, Bidirectional modulation of insulin action by amino acids, J. Clin. Invest. 101 (1998) 1519–1529.
[14] Y. Iiboshi, P.J. Papst, H. Kawasome, H. Hosoi, R.T. Abraham, P.J. Houghton, N. Terada, Amino acid-dependent control of p70 s6k, J. Biol. Chem. 274 (1999) 1092–1099.
[15] K. Hara, K. Yonezawa, Q.P. Weng, M.T. Kozlowski, C. Belham, J. Avruch, Amino acid sufficiency and mTOR regulate p70 S6 kinase and eIF-4E BP1 through a common effector mechamism, J. Biol. Chem. 273 (1998) 14484–14494.
[16] K. Shigemitsu, Y. Tsujishita, H. Miyake, S. Hidayat, N. Tanaka, K. Hara, K. Yonezawa, Structural requirement of leucine for activation of p70 S6 kinase, FEBS Lett. 447 (1999) 303–306.
[17] E.F. Blommaart, J.J. Luiken, P.J. Blommaart, G.M. van Woerkom, A.J. Meijer, Phosphorylation of ribosomal protein S6 is inhibitory for autophagy in isolated hepatocytes, J. Biol. Chem. 270 (1995) 2320–2326.
[18] C.J. Lynch, B.J. Patson, J. Anthony, A. Vaval, L.S. Jefferson, T.C. Vary, Leucine is a direct-acting nutrient signal that regulates protein synthesis in adipose tissue, Am. J. Physiol. Endocrinol. Metab. 238 (2002) E503–E513.
[19] J.C. Anthony, F. Yoshizawa, T.G. Anthony, T.C. Vary, L.S. Jefferson, S.R. Kimball, Leucine stimulates translation initiation in skeletal muscle of postabsorptive rat via a rapamycin-sensitive pathway, J. Nutr. 130 (2000) 2413–2419.
[20] P.O. Seglen, Preparation of isolated rat liver cells, Methods Cell Biol. 13 (1976) 29–83.
[21] S.R. Kimball, M. Yancisin, R.L. Horetsky, L.S. Jefferson, Translational and pre translational regulation of protein synthesis by amino acid availability in primary cultures of rat hepatocytes, Int. J. Biochem. Cell Biol. 28 (1996) 285–294.
[22] T. Nakamura, H. Teramoto, A. Ichihara, L-Proline is an essential amino acid for hepatocyte growth in culture, Biochem. Biophys. Res. Commun. 122 (1984) 884–891.
[23] K. Maezono, K. Kajiwara, K. Mawatari, A. Shinkai, K. Torii, T. Maki, Alanine protects liver from injury caused by F-galactosamin and CCl4, Hepatology 24 (1996) 185–191.
[24] S. Ishizaki-Koizumi, I. Sonaka, S. Fujitani, S. Nishiguchi, Mechanisms of the protective effect of L-alanine to D-galactosamine-induced hepatocellular injury: comparative studies of L-alanine and pyruvate, Biochem. Biophys. Res. Commun. 291 (2002) 738–743.
[25] E.J. Brown, P.A. Beal, C.T. Keith, J. Chen, T.B. Shin, S.L. Schreiber, Control of p70 s6 kinase activity of FRAP in vivo, Nature 377 (1995) 411–446.
[26] T. Andus, V. Gross, T.A. Tran-Thi, G. Schreiber, M. Nagashima, P.C. Heinrich, The biosynthesis of acute-phase proteins in primary cultures of rat hepatocytes, Eur. J. Biochem. 133 (1983) 561–571.
[27] C.J. Lynch, Role of leucine in the regulation of mTOR by amino acids: revelations from structure–activity studies, J. Nutr. 131 (2001) 861S–865.
[28] T. Tomiya, Y. Inoue, M. Yanase, M. Arai, H. Ikeda, K. Tejima, K. Nagashima, T. Nishikawa, K. Fujiwara, Leucine stimulates the secretion of hepatic stellate cells, Biochem. Biophys. Res. Commun. 297 (2002) 1108–1111.
[29] A. Ichihara, BCA, HGF, and proteosomes, Biochem. Biophys. Res. Commun. 226 (1999) 647–651.