SHORT-TERM EFFECTS OF BRANCHED-CHAIN AMINO ACIDS ON LIVER FUNCTION TESTS IN CIRRHOTIC PATIENTS

Pisit Tangkijvanich[1], Varocha Mahachai[2], Supeecha Wittayalertpanya[3], Vilailak Ariyawongsopon[2] and Sachapan Isarasena[2]

[1]Departmemt of Biochemistry, [2]Gastroenterology Unit, Department of Internal Medicine, [3]Department of Pharmacology, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand

Abstract. A randomized study was conducted in 29 ambulatory cirrhotic patients to determine the short-term effects of branched-chain amino acids (BCAA) on nutritional status, biochemical liver function tests and caffeine clearance. Each patient received a 4-week period of isonitrogenous and isocaloric regimens, either a standardized diet contained 40 g protein with supplementation of BCAA 150 g daily (group I) or only a standardized diet contained 80 g protein daily (group II). At the end of treatment, only group I showed significant improvements in transaminase levels as well as the caffeine clearance test compared with those of the pre-treatment levels. Nonetheless, significant improvements in nutritional parameters and additional liver function tests were not yet detected. We conclude that the short-term nutritional supplementation of BCAA is well tolerated and leads to improvement in hepatic metabolic capacity assessed by the caffeine clearance test.

INTRODUCTION

Malnutrition, particularly protein deprivation, is commonly found in patients with liver cirrhosis (Muller, 1995). In general, the prevalence and severity of malnutrition, whether assessed clinically or anthropometrically, are positively correlated with clinical stage of underlying liver disease. Furthermore, malnutrition has also been recognized as an independent prognostic factor for determining the clinical outcome in term of survival and complications (McCullough and Tavill, 1991). Cirrhotic patients who are severely malnourished tend to have uncontrolled ascites, to develop recurrent infections and to have higher mortality rates (Lautz *et al*, 1992). Treatment of malnutrition, therefore, not only improves the nutritional state and liver function, but may also improve the prognosis of the patients (Plauth *et al*, 1997).

Multiple factors are synergistically contributed to the development of malnutrition in cirrhotic patients. The main causes are an increased energy expenditure from hypercatabolic state, a reduced nutrient intake from nausea and anorexia or gastrointestinal maldigestion and malabsorption (Munoz, 1991). Consequently, this derangement leads to muscle wasting, clotting factor deficiency, reduced serum carrier protein levels and reduced branched-chain amino acids (leucine, valine, and isoleucine) (Fan, 1997).

Regarding protein supplement in cirrhotic patients, branched-chain amino acids (BCAA) have been extensively studied as treatment for hepatic encephalopathy and as nutritional therapy. Some, but not all studies, have suggested that oral or intravenous administration of BCAA-enriched solutions are more beneficial than standard amino acid formula in the treatment of hepatic encephalopathy (Wahren *et al*, 1983; Horst *et al*, 1984; Egberts *et al*, 1985; O'Keefe *et al*, 1987; Weber *et al*, 1990; Fischer *et al*, 1990). Similarly, its benefits as nutritional therapy in chronic liver disease have not been shown conclusively better than conventional formulations in achieving nitrogen balance (Rocchi *et al*, 1985; Okuno *et al*, 1985; Kanematsu *et al*, 1988; McCullough *et al*, 1989).

Our study was designed as a randomized trial to determine whether administration of supplemental BCAA to conventional diet for one month improved nutritional status and biochemical liver functions, as well as functional metabolic capacity assessed by caffeine clearance, in stable cirrhotic patients.

PATIENTS AND METHODS

Population study

Thirty ambulatory patients with documented liver cirrhosis verified by biopsy specimen and/or

Correspondence: Assoc Prof Supeecha Wittayalertpanya, Department of Pharmacology, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand. Tel: (662) 256 4481; Fax: (662) 256 4481; E-mail: wsupeech@pioneer.chula.ac.th

clear-cut clinical evidence were studied. All patients were in stable clinical conditions at the time of the study and were classified according to Child-Pugh classification system into Child A, B and C. None had clinical signs of hepatic encephalopathy, recent acute gastrointestinal bleeding, uncontrolled ascites or spontaneous bacterial peritonitis as well as hepatocellular carcinoma. In addition, they had no clinical evidence of severe, uncontrolled other medical illnesses, such as diabetes, renal failure, severe cardiopulmonary diseases and had no contraindication to use caffeine. All patients were informed as to the objective of the study and subsequently provided their consent.

Study protocol

The patients were randomized into two groups: group I received a standardized basal diet which contained 40 g protein and branched-chain amino acids 150 g (Aminolaban-EN, containing 40 g protein) daily for 4 weeks. Group II received only a standardized basal diet contained 80 gm protein daily for 4 weeks. The total caloric intake in each group was equally approximately 2,000 Kcal per day.

Before the onset and at the end of treatment, the body mass index (BMI) of each patient was measured and recorded in order to assess the overall nutritional status. Anthropometric measurements were performed using the mid-arm muscle circumference (MMC) to estimate the muscle mass. Blood was obtained during examinations to determine biochemical liver function tests, as well as markers of protein nutritional status such as blood urea nitrogen (BUN), albumin and transferrin. In addition, the caffeine clearance test was performed in order to assess the hepatic metabolic capacity before and after the treatment.

Caffeine clearance test

We assessed the caffeine clearance test in each patient's serum samples using a simplified method by two-point analysis as mentioned elsewhere (Wittayalertpanya *et al*, 1996). Briefly, each patient took a 3.5 mg/kg single dose of caffeine (anhydrous, BP grade, batch no. 71015, 0.35% aqueous solution) orally after an overnight fast. Blood samples were subsequently collected at 12 and 18 hours following caffeine administration. The sera were separated and stored at -20ºC until assayed. The patients were asked to abstain from smoking, caffeine-containing beverages, foods and medication that could inhibit caffeine metabolism, for example cimetidine, oral contraceptive and norfloxacin (Benowitz, 1990) at least 5 days before and during the test.

Five hundred microliters of each serum sample was deproteinized using 100 μl of zinc sulfate solution (10% W/V), mixed 10 seconds and followed by 750 μl of absolute methanol. Each sample was vortex-mixed for 30 seconds and then centrifuged for 5 minutes at 4,000 rpm. The supernatant was filtered and then 50 μl of this filtrate solution was injected into the HPLC system.

Caffeine clearance (Cl) was calculated by two point analysis using the equation CI=Kel x Vd. Kel was determined from the slope of two points and Vd was obtained from the mean value in each patient group classified by the previous report (Wittayalertpanya *et al*, 1996).

Statistical analysis

Comparisons between-group differences were made by the χ^2 or Fisher's exact test for categorical variables and by the Mann-Whitney test or unpaired Student's *t*-test when appropriate for quantitative variables. Paired *t*-test was used to compare the variables within-group differences. A p values less than 0.05 was considered significant.

RESULTS

During the follow-up period, one patient in group I was excluded from the study due to voluntary withdrawal. Among the remaining who completed the protocol, there were almost similar in clinical and laboratory parameters between groups as shown in Table 1. For example, no difference was observed in term of the patientsí mean age, sex, underlying etiologies and the severity of liver disease, graded as compensated cases (Child-Pugh score 5-7) and decompensated cases (Child-Pugh score 8-15) as well as pre-treatment caffeine clearance. In addition, the patientsí nutritional status before the treatment, assessed by the body mass index (BMI), the mid-arm muscle circumference (MMC) and the blood tests, were also comparable.

After the four-week treatment, in group I, significant improvements in the transaminase levels (SGOT, SGPT) as well as the caffeine clearance test were demonstrated as shown in Table 2. Serum albumin and transferrin, indicating nutritional status, also increased in this group but were not reached the significance. On the other hand, in

Table 1
Clinical characteristics of the patients in this study.

Characteristics	Group 1 (n=14)	Group 2 (n=15)	p-value
Sex (male : female)	10:5	12:3	NS
Age (years)	53.07 ± 10.58	53.20 ± 12.74	NS
Etiology of cirrhosis			NS
Alcoholic	6	7	
Post viral hepatitis	6	6	
Alcoholic and post viral hepatitis	0	1	
Cryptogenic	2	1	
Severity of cirrhosis (Child-Pugh score)			NS
Compensated (score 5-7)	9	9	
Decompensated (score 8-15)	5	6	
BMI (Wt/Ht^2)	23.69 ± 3.40	25.79 ± 4.14	NS
MMC (cm)	14.35 ± 3.87	15.05 ± 3.97	NS
Serum albumin (g/dl)	3.81 ± 0.86	3.66 ± 0.75	NS
Serum transferrin (mg/dl)	245.78 ± 70.18	253.04 ± 85.11	NS
BUN (mg/dl)	12.8 ± 3.55	13.13 ± 4.06	NS
Caffeine clearance (ml/min.kg)	0.27 ± 0.38	0.27 ± 0.36	NS

NS= not significant; BMI= body mass index; MMC= mid-arm muscle circumference

Table 2
Comparison of parameters between pre and post-treatment (group I).

	Pre-treatment	Post-treatment	p-value
BMI (Wt/Ht^2)	23.69 ± 3.40	23.67 ± 3.45	NS
MMC (cm)	14.35 ± 3.87	14.14 ± 3.91	NS
SGOT (U/l)	68.33 ± 3.87	45.23 ± 25.41	<0.05
SGPT (U/l)	57.53 ± 34.45	36.20 ± 23.87	<0.05
Alkaline phosphatase (U/l)	229.0 ± 78.07	232.0 ± 93.52	NS
Total bilirubin (mg/dl)	2.19 ± 1.86	1.99 ± 1.44	NS
PT (second prolonged)	2.27 ± 2.33	2.10 ± 1.67	NS
Total protein (g/dl)	7.27 ± 0.58	7.59 ± 0.66	NS
Serum albumin (g/dl)	3.81 ± 0.86	3.91 ± 0.82	NS
Serum transferin (mg/dl)	245.74 ± 70.18	256.61 ± 67.19	NS
Caffeine clearance (ml/min.kg)	0.27 ± 0.38	0.34 ± 0.40	<0.05

NS = not significant; BMI= body mass index; MMC= mid-arm muscle circumference

group II, the significant improvements were not found when compared between pre- and post- treatment (Table 3).

Table 4 showed the pre- and post-treatment caffeine clearance test in each group, classified according to Child-Pugh score. In group I, among compensated cirrhosis (Child-Pugh score 5-7), the mean value of the post-treatment the caffeine clearance was significantly higher than that of the pre-treatment. In contrast, among the compensated cases in group II and the decompensated cases (Child-Pugh score 8-15) in both groups, the caffeine clearance were not significantly different.

No patients in this study developed hepatic encephalopathy or other serious complications such as gastrointestinal bleeding, spontaneous bacterial peritonitis and hepatocellular carcinoma during and at the end of treatment. Likewise, treatment with

Table 3
Comparison of parameters between pre- and post-treatment (group II).

	Pre-treatment	Post-treatment	p-value
BMI (Wt/Ht^2)	25.79 ± 4.14	25.73 ± 4.0	NS
MMC (cm)	15.05 ± 3.97	14.94 ± 3.69	NS
SGOT (U/l)	75.20 ± 46.26	71.20 ± 41.61	NS
SGPT (U/l)	50.0 ± 35.79	47.60 ± 31.43	NS
Alkaline phosphatase (U/l)	253.0 ± 87.55	258.0 ± 108.48	NS
Total bilirubin (mg/dl)	2.75 ± 2.19	2.82 ± 2.81	NS
PT (second prolonged)	3.89 ± 3.48	3.06 ± 2.61	NS
Total protein (g/dl)	7.52 ± 0.76	7.59 ± 0.66	NS
Serum albumin (g/dl)	3.66 ± 0.71	3.68 ± 0.83	NS
Serum transferin (mg/dl)	253.04 ± 85.11	252.90 ± 68.97	NS
Caffeine clearance (ml/min.kg)	0.27 ± 0.35	0.29 ± 0.42	NS

NS = not significant; BMI= body mass index; MMC= mid-arm muscle circumference

Table 4
Pre and post-treatment caffeine clearance*.

	Pre-treatment	Post-treatment	p-value
Group I			
Compensated cirrhosis	0.43 ± 0.42	0.54 ± 0.39	<0.05
Decompensated cirrhosis	0.04 ± 0.09	0.03 ± 0.10	NS
Group II			
Compensated cirrhosis	0.34 ± 0.44	0.40 ± 0.32	NS
Decompensated cirrhosis	0.16 ± 0.17	0.10 ± 0.25	NS

*mg/min.kg; NS = not significant

BCAA was not accompanied by any adverse reaction, except for mild symptoms of the gastrointestinal system such as abdominal distension, diarrhea and loss of appetite in some patients.

DISCUSSION

Decreased branched-chain amino acid (BCAA, leucine, valine, and isoleucine) and increased aromatic amino acid (AAA: phenylalanine, methionine, tyrosine) levels in patients with chronic liver disease regardless of hepatic encephalopathy have been well documented by previous studies (Morgan *et al*, 1982; Munoz and Maddrey, 1986). These changes have been attributed to increased release of AAA from the necrosing liver, together with impaired AAA uptake and metabolized by the diseased liver (James *et al*, 1979). In contrast, BCAA undergoes predominantly peripheral metabolism in the muscles, which is enhanced in cirrhosis, resulting in a decrease in the concentration of BCAA (Fischer *et al*, 1975). Therefore, the administration of enteral BCAA to normalize the amino acid imbalance and improve the nutritional status seems to be beneficial for cirrhotic patients.

In our study, although the nutritional and biochemical parameters except for the transaminase (SGOT/SGPT) were not significantly changed after short-term BCAA treatment, the caffeine clearance was significantly improved in those treated with BCAA, particularly in compensated cases. The caffeine clearance test is a measure of intrinsic metabolic function that is not dependent upon hepatic blood flow (Wahllander *et al*, 1985; Lewis *et al*, 1992). In addition, the hepatic elimination of caffeine depends highly on CYPIA2 isoenzyme-mediated *N*-demethylation, which leads to a variety

of urinary methyxanthine metabolites (Shyu *et al*, 1996). Therefore, its clearance by the liver can be used as a sensitive and specific global indicator of hepatic functional impairment, as well as provide useful information regarding prognosis and effects of therapy. To that end, our results could be implied the potential short-term benefit of BCAA as nutritional supplementation to standard therapy in stable cirrhotic patients.

As mentioned above, it remains unclear whether administration of BCAA improves the hepatic function in cirrhotic patients with compare to conventional protein diet. Nonetheless, BCAA-enriched solution can promote hepatic protein synthesis and accelerate liver regeneration in animal models (Rigotti *et al*, 1986; Miwa *et al*, 1995). Furthermore, some clinical studies have shown that BCAA affects favorably the impaired protein nutritional state in cirrhosis by increasing the protein synthesis rate (O'Keefe *et al*, 1987; Kato *et al*, 1991). In a recent randomized study, some cirrhotic patients who had received BCAA for at least 6 months improved their cumulative survival rate (Yoshida *et al*, 1989). Moreover, according to Ichida *et al* (1995), long-term administration of enteral BCAA may lead to nutritional improvement with little adverse reactions as well as the improvement of the quality of life and performance status in some patients with decompensated cirrhosis.

Recently, the European Society for Parenteral and Enteral Nutrition (ESPEN) Consensus Group provided clear nutritional guidelines for the management of patients with chronic liver disease (Plauth *et al*, 1997). In patients with clinically stable cirrhosis an intake of 1.3 times the resting energy expenditure or 25 to 30 kcal/kg of non-protein energy plus 1.0 to 1.2 g/kg of protein daily is recommended. In protein-intolerant patients, dietary protein may need to be reduced to an intake of 0.5 g/kg/day. In this situation, positive nitrogen balance and improvement in nitrogen intake can be achieved by oral supplementation of BCAA at 0.25 g/kg/day without undue risk of encephalopathy (Marchesini *et al*, 1990).

In conclusion, although the administration of BCAA-enriched enteral nutrient for one month in patients with stable cirrhosis did not improve in nutritional parameters, it resulted in greater improvement in functional hepatic capacity, assessed by the caffeine clearance test, particularly in compensated cases. This improvement in the quantitative liver function test indicates that such treatment could be beneficial for patients with stable cirrhosis. However, additional studies are still required to assess short-term and long-term effects of BCAA on liver function, nutritional state and the prognosis of the patients.

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