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• *CLINICAL RESEARCH* •

Improvement of regional cerebral blood flow after oral intake of branched-chain amino acids in patients with cirrhosis

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Abstract

AIM: To evaluate the effect of oral intake of branched-chain amino acids (BCAA) on brain perfusion in patients with liver cirrhosis.

METHODS: Single photon emission computed tomography scans were performed in 43 patients with cirrhosis and in 15 age-matched healthy subjects. Twenty-nine out of forty-three patients were randomly treated with either BCAA granules or placebo, and single photon emission computed tomography was performed before and after the treatment. We measured the regional cerebral blood flow values using a three-dimensional stereotaxic region of interest template.

RESULTS: Cirrhotic patients had regions of significant hypoperfusion in the bilateral central (right $P = 0.039$, $P<0.05$; left $P = 0.006$ $P<0.01$), parietal (right $P = 0.018$, $P<0.05$; left $P = 0.009$, $P<0.01$), angular (right $P = 0.039$, $P<0.05$; left $P = 0.008$, $P<0.01$), and left pericallosal segments ($P = 0.038$ P<0.05) as compared with healthy subjects. A significant increase in cerebral perfusion was observed 70 min after the oral intake of BCAA in the angular (right $P = 0.012$, $P<0.05$; left $P = 0.049$, $P<0.05$), temporal (right $P = 0.012$, $P<0.05$; left $P=0.038$, $P<0.05$), pericallosal segments (right $P = 0.025$, $P<0.05$; left $P = 0.049$, $P<0.05$) and left precentral ($P = 0.044$, $P<0.05$), parietal ($P = 0.040$, $P<0.05$) and thalamus ($P = 0.033$, $P<0.05$). No significant change in perfusion was observed in the placebo group.

CONCLUSION: Administration of BCAA rapidly improves cerebral perfusion.

Key words: Liver cirrhosis; Cerebral blood flow; Branched-chain amino acids

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INTRODUCTION

Beneficial effects of branched-chain amino acids (BCAA) supplementation on hepatic encephalopathy (HE) have been previously reported[1-3]. Recently, Marchestini *et al*[4] carried out a large multicenter, randomized controlled trial with BCAA-enriched dietary supplements in comparison with lactoalbumin or maltodextrin dietary supplementation. The results of this study showed reduced hospital admission rate in patients treated with BCAA compared with the control group[4]. Thus, BCAA therapy may be effective for the treatment of HE, which is a common cause of hospital admission.

Some cirrhotic patients with apparently normal mental status may have abnormalities in cognitive function when they are examined with sensitive and quantitative neuropsychological tests[5,6]. This group of patients is considered to have minimal HE[7]. This HE-associated cognitive impairment may be sometimes associated with a poor quality of life[8-10] and thus early diagnosis and treatment of this condition is important[8]. It has been reported that therapy with lactulose improves neuropsychological functions[11]. However, there are no data on whether BCAA supplementation ameliorates cerebral disturbance in cirrhotic patients.

Single photon emission computed tomography (SPECT) and positron emission tomography studies can demonstrate alterations in regional cerebral blood flow (CBF) and cerebral glucose metabolism in cirrhotic patients[12-19]. We previously reported that administration of solutions enriched with BCAA improves cerebral perfusion in patients with cirrhosis[20]. However, the BCAA-rich solutions used in this previous study in cirrhotic patients contained L-arginine, which is a precursor of nitric oxide, a potent vasodilator[21]. The dose of L-arginine used in previous study might have increased blood flow in the brain[21].

The main aim of the present study was to confirm regional differences in CBF in patients with liver cirrhosis and to evaluate whether alterations in CBF is reversed by the oral administration of BCAA. In this study, we used granules containing only BCAA and a three-dimensional stereotaxic region of interest template (SRT) for objective estimation of anatomically standardized CBF SPECT images[22,23].

MATERIALS AND METHODS

Subjects

Forty-three Japanese patients with liver cirrhosis (30 men and 13 women, mean age 63±8 years) were enrolled in this study. The diagnosis of cirrhosis was based on the results of liver function tests, ultrasonography, computed tomography imaging, laparoscopy and liver biopsy. The cause of liver cirrhosis was viral infection in the majority of patients (n = 37). No patient with alcoholic liver disease was included in the study. In six patients, the cause of liver cirrhosis was unclear. None of the patients had overt HE (grade I or more) at the time of the examination, and none of them exhibited neuropsychiatric signs or symptoms on standard bedside clinical assessment. Patients with focal brain lesions, severe brain atrophy, abnormalities on computed tomography or magnetic resonance images, or neurological or psychiatric disorders were excluded from the study. None of the patients were receiving psychoactive drugs. Twenty-nine out of forty-three patients were randomized into two groups: one group received BCAA granules orally (16 patients) and another group received placebo (13 patients). We have previously reported that the ratio of serum BCAA to tyrosine increases nearly twofold, 1 h after the administration of oral BCAA and that it decreases to basal values after 10 h[24]. The clinical and biochemical characteristics of the patients are summarized in Table 1.

Control SPECT images were obtained from 15 subjects (11 men and 4 women; mean age, 62±9 years) referred to our neurology department for minor subjective symptoms. These subjects were free of liver disease, neurological disorder or dementia and had normal brain magnetic resonance images. The control subjects were not taking any medication.

Informed consent was obtained from all subjects, and the study was performed in accordance with the Helsinki Declaration. The Ethics Committee of Mie University School of Medicine approved the protocol of this study.

Table 1 Patients' clinical characteristics

Age (yr)	63 ± 8	(48 - 74)
Sex ratio, M/F	30/13	
Etiology of cirrhosis, HBV/HCV/unknown	5/32/6	
Previous history of overt hepatic encephalopathy,		
None/Chronic	39/4	
Child-Pugh score	8.0 ± 2.2	(5 -13)
Laboratory examinations		
Platelet (10^4 μL)	7.5 ± 5.1	(8 - 31.4)
Albumin (g/dL)	2.9 ± 0.5	(1.9 - 3.9)
Total bilirubin (mg/dL)	2.0 ± 2.3	(0.5 - 13.8)
Cholinesterase (ΔpH)	0.36 ± 0.19	(0.11 - 0.97)
Plasma ammonia (μmol/L)	38 ± 23	(4 - 104)
Prothrombin time (%)	69.1 ± 12.8	(31.3 - 93.5)
BCAA to tyrosine ratio	3.3 ± 1.3	(1.32 - 6.95)
Neuropsychological test		
Trail making test (s)	54 ± 26	(28 - 160)
Digit symbol test (gross point)	35 ± 10	(12 - 54)

BCAA, branched-chain amino acids.

Scan acquisition and image processing

Each subject received 278 MBq of technetium-99 m L,L-ethyl cysteinate dimer (ECD) by intravenous injection in the morning after an overnight fast. Ten minutes after the injection of ECD, brain SPECT images were acquired using a three-head gammacamera system (GCA-9300A/DI, Toshiba, Tokyo, Japan) equipped with low-energy, high-resolution fanbeam collimators. The projection data were obtained using a matrix size of 128×128. SPECT images were reconstructed by filtered back-projection using a ramp filter follower by postprocessing with a Butterworth filter. Attenuation correction was performed using Chang's method[25]. The triple-energy window technique was employed for scatter correction. After baseline SPECT, the patients were orally treated with BCAA granules (8 g BCAA, 8 g protein, 32 kcal, Ajinomoto, Tokyo, Japan: L-isoleucine 1 904 mg, L-leucine 3 808 mg, L-valine 2 288 mg), or placebo (cornstarch, 8 g protein, 32 kcal). After 60 min, 278 MBq of ECD was intravenously administered, and 10 min later a second SPECT acquisition was performed. The methods are briefly summarized in Figure 1.

Image analysis

The spatial normalization was performed using linear and non-linear transformation and SPECT template in the statistical parametric mapping (SPM) 99 (Wellcome Department of Cognitive Neurology, London, UK)

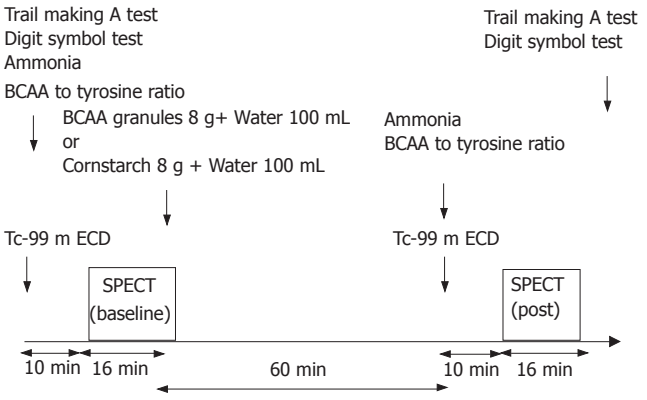

Figure 1 Study protocol. Baseline SPECT imaging was taken 10 min after the injection of 278 MBq ECD. After baseline SPECT, BCAA granules or placebo were orally administered. Sixty minutes later, another 278 MBq of technetium-99 m ECD was intravenously administered and a second SPECT acquisition was performed 10 min after the injection of ECD. Laboratory and neuropsychological tests were taken before and after the administration of BCAA or placebo. SPECT, single photon emission computed tomography; ECD, ethyl cysteinate dimer; BCAA, branched-chain amino acid

program. Smoothing was performed using 12 mm full width at half maximum Gaussian filter in SPM99. To obtain post-BCAA counts, baseline mean SPECT counts were subtracted from the second SPECT counts, multiplied by a correction factor, which is the coefficient of the decay of technetium-99 m between the baseline and post-treatment measurement. In each hemisphere, we estimated the regional CBF values of 270 constant regions of interest (ROI, three-dimensional SRT) grouped into 12 segments as follows: callosomarginal, 48 ROI; precentral, 45 ROI; central, 28 ROI; parietal, 14 ROI; angular, 8 ROI; temporal, 27 ROI; posterior cerebral, 33 ROI; pericallosal, 16 ROI; lenticular nucleus, 12 ROI; thalamus, 11 ROI; hippocampus, 17 ROI; and cerebellum, 11 ROI; and the segmental CBF was calculated as the area-weighed mean value for each of the 12 segments based on the regional CBF of each ROI[22,23].

Semiquantitative analysis was performed to obtain region-to-reference ratios for each segmental CBF value. The CBF value in cerebellum was selected as the reference region, because cerebellar abnormalities were not detected in SPECT images, computed tomography or magnetic resonance images.

Neuropsychological tests and laboratory examinations

The trail making A test (number connection test) and digit symbol test (revised Wechsler adult intelligence scale) were performed as neuropsychological tests. Laboratory examinations included plasma ammonia and BCAA to tyrosine ratio. These data were taken before and after the administration of BCAA or placebo (Figure 1).

Statistical analysis

Results were expressed as the mean±SD of the mean and range. The Mann–Whitney *U* test was used to evaluate the statistical difference in clinical or laboratory variables between BCAA and placebo groups and in CBF between patients and healthy subjects. The analysis of variance with Bonferroni's correction for multiple comparisons in the three groups was analyzed. The Wilcoxon was used to compare pre-, post-BCAA or -placebo values in the same group of patients. A $P<0.05$ was considered as statistical significance.

RESULTS

Baseline study

Cirrhotic patients ($n = 43$) had regions of significant hypoperfusion in the bilateral central (right, $P<0.05$; left, $P<0.01$), parietal (both, $P<0.05$), angular (right, $P<0.05$; left, $P<0.01$), and left pericallosal segments ($P<0.05$) as compared with healthy subjects ($n = 15$, Figure 2).

The influence of the clinical profile on regional CBF was also evaluated. There were no significant differences in cerebral perfusion between the mild (Child–Pugh A, $n = 13$), moderate/severe (Child–Pugh B+C, $n = 30$) liver dysfunction groups and healthy subjects (data not shown). In cirrhotic patients with hyperammonemia (i.e., more than 50 μm/L, $n = 15$), SRT showed regions of significant hypoperfusion in the right parietal ($P<0.05$) and left callosomarginal ($P<0.05$), central ($P<0.01$), angular ($P<0.01$) and pericallosal segments ($P<0.01$) as compared to healthy subjects (Figure 3A, right hemisphere is not shown). Likewise, in cirrhotic patients with severe decrease of serum BCAA to tyrosine ratio (i.e., <3, $n = 19$), SRT showed regions of significant hypoperfusion in the angular (right, $P<0.05$; left, $P<0.01$) and right parietal ($P<0.05$) and left callosomarginal ($P<0.01$), central ($P<001$) and pericallosal segments ($P<0.01$) as compared to healthy subjects (Figure 3B, right hemisphere is not shown).

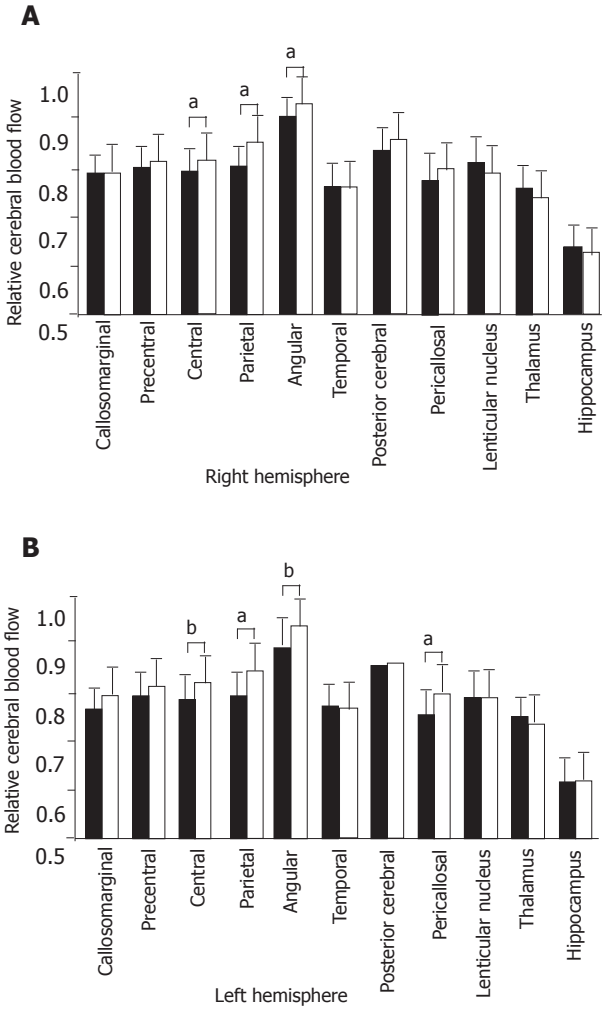

Figure 2 Relative CBF of different brain regions. Comparison of patients with liver cirrhosis (closed bars) and healthy subjects (open bars). Data were expressed as the mean±SD. [a]$P<0.05$, [b]$P<0.01$, [d]$P<0.001$. CBF, cerebral blood flow.

Abnormalities in neuropsychological tests [values more than two SD from the mean values for the age-matched healthy subjects at our hospital (i.e., more than 50 s on the trail making A test and less than 30 points on digit symbol test)] were considered to be indicative of minimal HE. Among cirrhotic patients, 10 showed abnormalities in both neurological tests and thus they were considered to have minimal HE. In patients with abnormalities in neurological tests ($n = 10$), SRT showed significant hypoperfusion in the left parietal ($P<0.01$), pericallosal ($P<0.01$), lenticular nucleus ($P<0.01$) and hippocampus ($P<0.05$) regions as compared to patients with grade-0 HE ($n = 14$, Figure 3C,

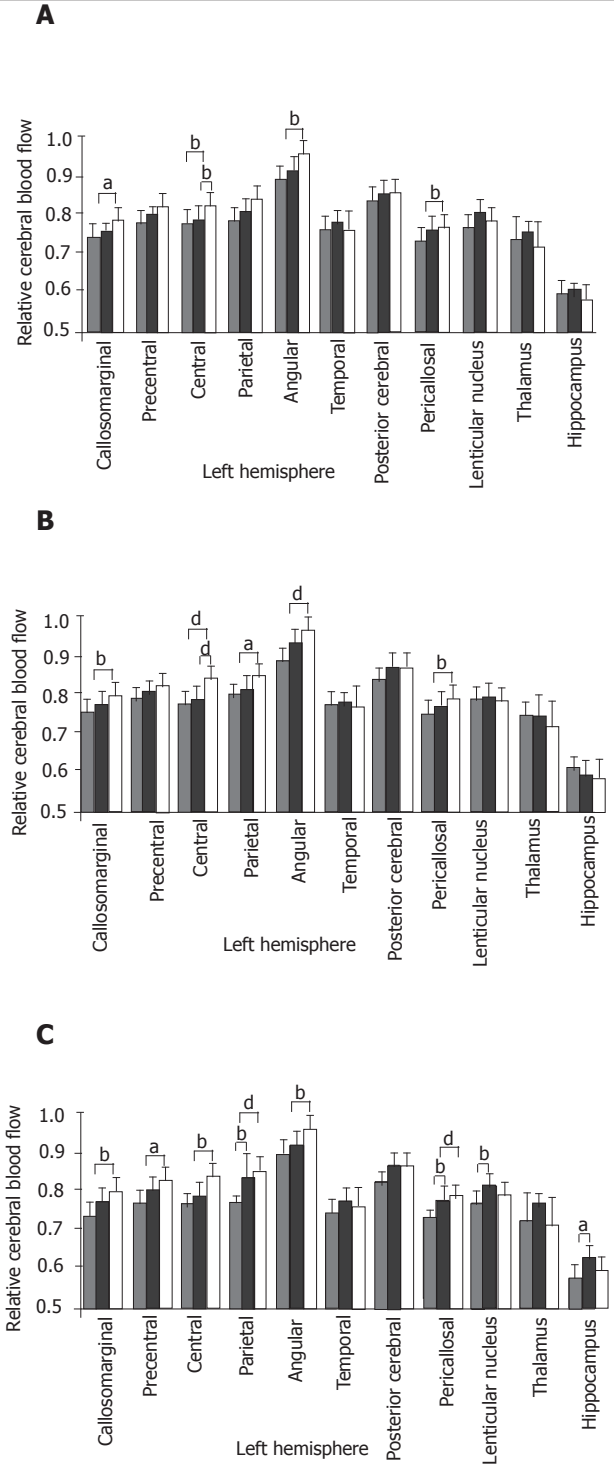

Figure 3 Relative CBF of different brain regions. **A:** Comparison of cirrhotic patients with hyperammonemia (gray bars), normal ammonemia (dashed bars) and healthy subjects (open bars); **B:** Comparison of cirrhotic patients with severe decrease in the levels of serum BCAA to tyrosine ratio (gray bars), with mild decrease in serum BCAA to tyrosine ratio (dashed bars) and healthy subjects (open bars); **C:** Comparison of patients with minimal encephalopathy (gray bars), with grade-0 encephalopathy (dashed bars) and healthy subjects (open bars).

right hemisphere is not shown).

Effect of BCAA

At entry, there was no difference in clinical or laboratory variables between BCAA (n = 16) and placebo (n = 13) groups (Table 2). A significant increase in cerebral perfusion was observed 70 min after oral intake of BCAA in the angular (both regions, P<0.05, Figure 4C), temporal (both regions, P<0.05, Figure 4D), pericallosal segments (both regions, P<0.05, Figure 4E) and left precentral (P<0.05, Figure 4A), parietal (P<0.05, Figure 4B) and thalamus (P<0.05, Figure 4F). In addition, after the administration of oral BCAA, the values of relative CBF improved in almost all segments, reaching values observed in healthy subjects. There were no significant differences in cerebral perfusion between cirrhotic patients after oral intake of BCAA and healthy subjects. No significant change in relative CBF values was observed in the placebo group (Figure 5). In the BCAA group, the serum BCAA to tyrosine ratio increased fourfold, 86 min after the administration of oral BCAA (P<0.01). No significant change in plasma ammonia levels or in neuropsychological tests was observed in BCAA and placebo groups (Table 3).

DISCUSSION

Functional imaging techniques such as CBF SPECT and positron emission tomography can demonstrate abnormalities in patients with cirrhosis[12-19]. In the present study, cirrhotic patients had regions of significant hypoperfusion in central, parietal, angular and pericallosal segments as compared to healthy subjects. These areas included parts of the frontal and parietal associated areas of the cortex and cingulum. Impaired flow and oxygen metabolism in the frontal, parietal and cingulate cortices in cirrhotic patients have also been reported[12-14,16-18]. Cognitive impairment, especially defect in attention is an important feature of HE[26,27]. The anterior cingulate gyri may provide an important connection between widely divergent aspects of attention and visual location[28]. The internal organization of the anterior cingulate gyri shows alternating bands of cells with close connections to the dorsolateral frontal cortex and the posterior parietal lobe[29]. The results of studies using N-13 ammonia positron emission tomography of cerebral ammonia metabolism in patients with cirrhosis and minimal encephalopathy coincide well with these regional differences[30]. This regional hypoperfusion may be the pathophysiological basis for the minimal cerebral dysfunction that is often detected by neuropsychological tests in patients with cirrhosis.

We found no significant relationship between cerebral perfusion and severity of liver disease, as assessed by the Child–Pugh scores. This observation may be due to the fact that non-hepatic factors, such as neurotoxins produced in the gut or toxic agents that cross the blood-brain barrier such as ammonia, contribute to impairment of cerebral function. Although no single metabolic derangement can account for the occurrence of HE, the plasma level of ammonia is believed to be an important causative factor of HE. Lockwood *et al*[16] reported regional metabolic abnormalities in cirrhotic patients with hyperammonemia. They also reported increased permeability of the blood-brain barrier to ammonia and suggested that ammonia might be responsible for cerebral dysfunction in HE[31]. In

Table 2 Clinical characteristics of BCAA and placebo groups

	BCAA group		Placebo group	
Age (yr)	64 ± 9	(50 - 72)	65 ± 8	(51 - 74)
Sex ratio, M/F	10/6		9/4	
Etiology of cirrhosis, HBV/HCV/unknown	1/11/4		0/12/1	
Previous history of overt hepatic encephalopathy, None/Chronic	14/2		12/1	
Child-Pugh score	8.1 ± 2.5	(5 - 13)	7.8 ± 2	(5 - 11)
Laboratory examinations				
Platelet (10^4 μL)	8.3 ± 6.8	(3.4 - 31.4)	7.6 ± 3.1	(2.8 – 12.3)
Albumin (g/dL)	2.8 ± 0.5	(2.1 - 3.9)	2.9 ± 0.4	(2.4 - 3.9)
Total bilirubin (mg/dL)	2.2 ± 3.2	(0.8 - 13.8)	1.9 ± 1.8	(0.5 – 6.9)
Cholinesterase (ΔpH)	0.32 ± 0.16	(0.12 - 0.64)	0.38 ± 0.12	(0.17 - 0.55)
Plasma ammonia (μmol/L)	35 ± 20	(9 - 79)	34 ± 18	(7 - 74)
Prothrombin time (%)	68.9 ± 14.4	(31.3 - 93.5)	71.1 ± 13.4	(51.8 – 89.2)
BCAA to tyrosine ratio	3.7 ± 1.2	(2.3 - 7)	3 ± 1.3	(1.3 – 5.6)
Neuropsychological test				
Trail making test (s)	49 ± 16	(34 - 77)	50 ± 16	(25 - 72)
Digit symbol test (gross point)	38 ± 10	(21 - 53)	37 ± 15	(19 - 63)

BCAA, branched-chain amino acids.

Table 3 Changes in laboratory and neuropsychological tests after BCAA and placebo administration

	BCAA group				Placebo group			
	Before		After		Before		After	
Laboratory variables								
Plasma ammonia (μM/L)	35 ± 20	(9 - 79)	31 ± 14	(8 - 54)	36 ± 20	(7 - 74)	37 ± 14	(17 - 58)
BCAA to tyrosine ratio	3.7 ± 1.2	(2.3 - 7)	16.7 ± 3.4[b]	(13.1 - 24.6)	3 ± 1.3	(1.3 - 5.6)	3.1 ± 1.3	(1.6 - 5.7)
Neuropsychological test								
Trail making test (s)	49 ± 16	(34 - 77)	47 ± 13	(30 - 72)	50 ± 16	(25 - 72)	51 ± 15	(29 - 75)
Digit symbol test (goss point)	38 ± 10	(21 - 53)	45 ± 14	(21 - 71)	37 ± 15	(19 - 63)	40 ± 14	(23 - 61)

[b]$P < 0.01$ BCAA, branched-chain amino acids

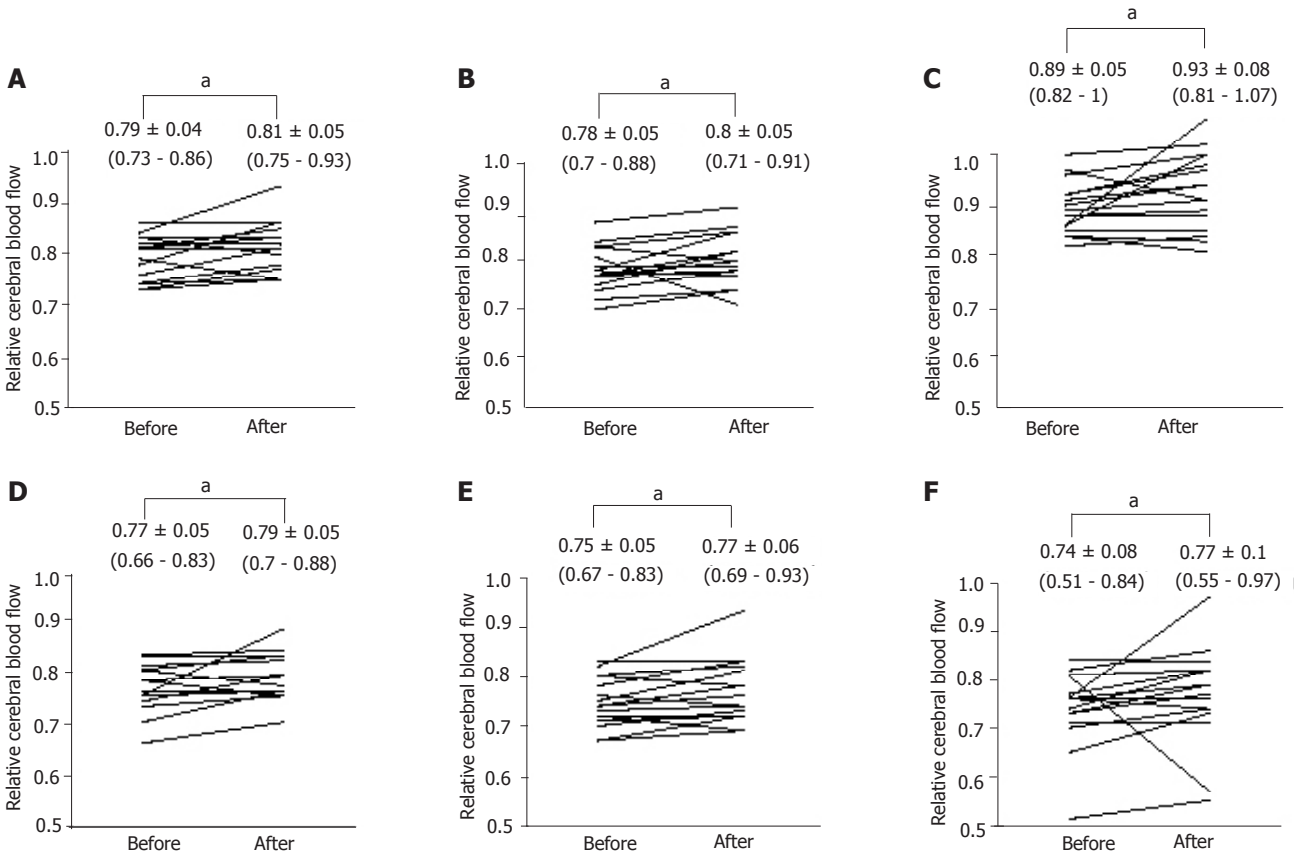

Figure 4 Changes in relative CBF of different brain regions. CBF was compared before and after BCAA. **A:** Left precentral; **B:** left parietal; **C:** left angular; **D:** left temporal; **E:** left pericallosal; **F:** left thalamus. Data were expressed as the mean±SD and range. [a]$P<0.05$V *vs* CBF, cerebral blood flow; BCAA, branched-chain amino acid.

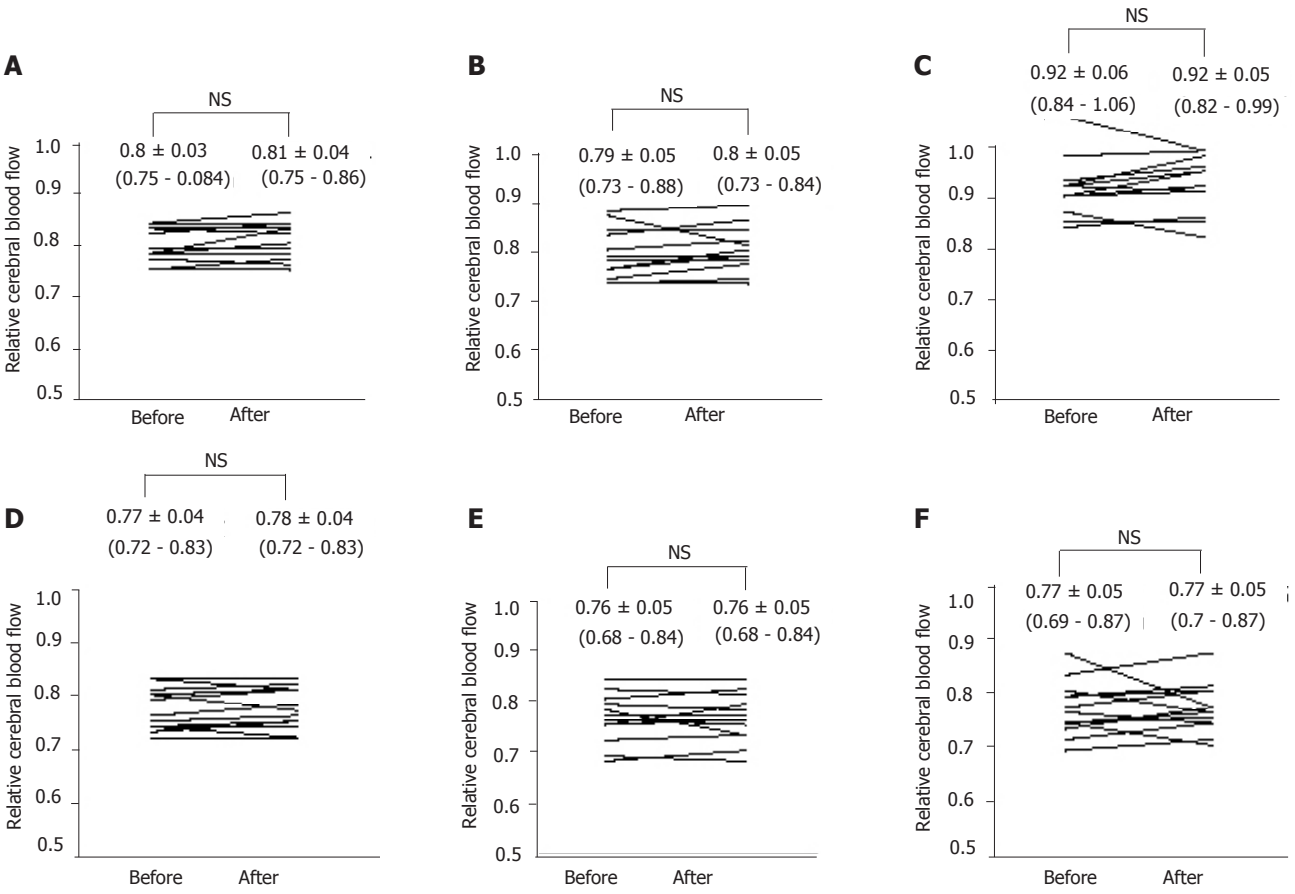

Figure 5 Changes in relative CBF of different brain regions. CBF was compared before and after placebo. **A:** Left precentral; **B:** left parietal; **C:** left angular; **D:** left temporal; **E:** left pericallosal; **F:** left thalamus. Data were expressed as the mean±SD and range. CBF, cerebral blood flow.

our patients with hyperammonemia, SRT analysis showed several segments, including the cingulum, with significant decreased perfusion as compared to healthy subjects. This finding supports the hypothesis of Lockwood *et al*. In addition, SPECT with SRT analysis showed a significant reduction in relative CBF values in cirrhotic patients with minimal HE as compared to patients with grade-0 HE and normal subjects. This result is also consistent with the observation of Lockwood *et al*[18]. Moreover, we found that the relative CBF values are significantly correlated with the baseline venous BCAA to tyrosine ratio. Relationship between CBF values and the serum BCAA to tyrosine ratio has not been previously assessed.

There are several studies in which stereotaxic ROI analysis was used[32,33], but in all of them the ROI values were transformed to fit the subjects' individual anatomical arrangements. Inter-individual anatomical variations may exist giving non-consistent relationship between ROI location and anatomy. In the present study, we used a fully automated regional CBF quantification software, SRT. This incorporates an anatomical standardization engine transplant into SPM99 and ROI for quantification on the Montreal Neurological Institute space of the magnetic resonance image anatomically standardized by SPM99. We believe that our results are valid due to the accuracy of the SRT analysis.

We took the cerebellum as reference for the semiquantitative analysis, because brain segments of cirrhotic patients exhibit variable perfusion values whose distribution may affect the validity of the uptake ratios. We previously reported that there is no alteration in cerebellar perfusion in cirrhotic patients as analyzed by SPM with ECD SPECT[13]. In addition, the absence of symptoms and the normal appearance of cerebellar perfusion in our patients led us to consider the cerebellum as the best reference to evaluate regional CBF. However, cerebellar hypermetabolism has been observed in cirrhotic patients with cerebellar degeneration[30], and thus it may be difficult to deal with methodological limitations in such region-to-cerebellar ratios as it was in this study. To evaluate the effect of BCAA, repeated SPECT studies are necessary. In the pre/post studies, the assumption is that the original distribution pattern is still the same during the second SPECT image. Moretti *et al*[3] reported that during the 50-120 min postinjection period, the regional structures are washing out at the same rate; however, there might be differences between cirrhotic and normal brain. This may be a potential pitfall in split-dose and sequential SPECT method with ECD.

The mechanism by which BCAA granules rapidly improve relative CBF in cirrhotic patients without overt HE is unknown. The rationale for BCAA therapy is based

on the results of studies showing that the use of solutions rich in BCAA reversed the abnormal blood levels of amino acids and led to mental recovery from acute HE in patients with cirrhosis[35,36]. Mental recovery is observed immediately after the treatment with BCAA solution. One of the proposed mechanisms of HE is the interference of cerebral metabolism by ammonia, including depletion of operational rates of tricarboxylic acid cycle by removing α-ketoglutarate for ammonia detoxification[37]. Decreased brain levels of glutamate have been reported in various models of HE. Glutamate is an integral component of malate-aspartate shuttle. It is therefore possible that reduced glutamate levels contribute to impaired cerebral energy metabolism in HE[38]. BCAA is known to cross rapidly the blood-brain barrier and to serve as an energy source in the brain[39,40]. It has been previously shown in animal models of chronic HE that decreased brain BCAA concentrations is normalized and that acceleration of ammonia metabolism occurs by stimulated glutamine synthesis, after intravenous infusion of BCAA[41]. Thus, it is possible that administration of BCAA blocks the vicious cycle of cerebral energy metabolism in HE by providing the amino group for glutamate synthesis from a-ketoglutarate in astrocytes.

In the present study, we found that cirrhotic patients increased thalamic perfusion after the administration of oral BCAA. According to current knowledge, information coming from the cortex passes through the striatopallidal system to the thalamus and then returns to the cortex. Alterations of neurotransmission within the pallidum and thalamus therefore may lead to impairment of cortical function, as in HE[42]. Catafau *et al*[12] reported that thalamic CBF increases in proportion to neuropsychological deficits as a compensatory effect. Supplementation with BCAA may be effective in the treatment of cirrhotic patients for improving thalamic CBF.

A number of studies have reported association between minimal HE and impairment of quality of life[5,6,8-10]. It has also been suggested that the ability to drive a car is impaired in patients with cirrhosis and minimal HE[43]. Therefore, it is important to initiate therapy to improve neuropsychological function. Protein restriction may not be beneficial for long-term therapy in patients with protein malnutrition. Oral BCAA supplementation may be one of the candidates for the initial treatment of minimal HE. In the present study, there was no significant change in the results of neuropsychological test after the administration of BCAA. Additional study is necessary to clarify the time course of regional CBF changes after oral administration of BCAA. Neuropsychological performance and cerebral perfusion after long-term oral administration of BCAA also needs further evaluation.

In conclusion, this study shows that patients with cirrhosis and no neurologic symptoms have widespread reduction in relative CBF and that this is restored after oral intake of BCAA. These findings suggest that oral supplementation with BCAA may be a therapeutic adjunct of conventional therapy for the treatment of cirrhotic patients for its beneficial action on regional CBF.

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