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Oral administration of branched chain amino acids improves virus-induced glucose intolerance in mice

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Abstract

We investigated the therapeutic effect of branched chain amino acids (BCAA) on mice with glucose intolerance induced by encephalomyocarditis virus (EMCV). Male DBA/2 mice were divided into three groups: treated with BCAA, (such as valine, leucine, and isoleucine), untreated, and control. BCAA-treated and -untreated groups were inoculated intraperitoneally with the NDK25 variant of EMCV at 200 plaque-forming units per mouse. The BCAA-treated group was administered orally 0.9 g/kg/day of each BCAA from the day after viral inoculation. The control group neither received virus inoculation nor was treated with BCAA. One week after inoculation, oral glucose tolerance tests (OGTT) were performed. After the glucose loading at 1.5 g/kg of body weight, blood glucose levels in the untreated group were 92.0 ± 10.0 mg/dl at baseline, 224.6 ± 10.9 mg/dl at 30 min, and 169.4 ± 21.4 mg/dl at 60 min, which were significantly ($P < 0.05$) higher than those in the control group (62.7 ± 3.6 mg/dl, 167.2 ± 16.4, and 83.8 ± 6.0 mg/dl, respectively). Blood glucose levels in the BCAA-treated group were 54.5 ± 3.7 mg/dl at baseline, 145.2 ± 8.7 mg/dl at 30 min, and 128.7 ± 18.3 mg/dl at 60 min after the glucose loading, which were not significantly higher than those in the control group. Immunoreactive insulin levels at 30 min after the glucose loading were lower in the untreated group than in the control group at 1 week after virus inoculation. Histological investigations showed that the grade of insulitis in the pancreas of mice of the BCAA-treated group was lower than that of the mice of the untreated group. These results suggest that oral administration of BCAA is able to improve glucose intolerance induced by EMCV.

Keywords: Encephalomyocarditis virus; Glucose intolerance; Branched chain amino acid; Insulitis

1. Introduction

Type 1 diabetes mellitus results from the destruction of the pancreatic beta cells due to autoimmunity, genetic factors, and unknown environmental factors. Studies with animal models have implicated genetic factors, autoimmunity, and environmental factors, including viral infection, in the etiology of type 1 diabetes mellitus (Irvine, 1980; Yoon, 1988). Picorna viruses including coxsackie B4 virus are considered to be involved in the pathogenesis of type 1 diabetes mellitus in humans. Encephalomyocarditis virus (EMCV), a member of the picorna viridae, has been used extensively to study virus-induced diabetes in mice (Yoon et al., 1980). Insulin-dependent diabetes induced by the D variant of EMCV is due to the massive destruction of pancreatic β cells. In contrast, the NDK25 variants of EMCV cause glucose intolerance by causing the partial destruction of pancreatic beta cells in DBA/2 mice (Utsugi et al., 1992).

Siegel et al. (1980) reported that chronic administration of high-protein food improves glucose intolerance of streptozotocin-induced diabetic mice. The branched-chain amino

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acids, leucine, isoleucine, and valine, are indispensable amino acids of special interest. In a state of sepsis or bacterial infection, insulin resistance develops in skeletal muscle to spare glucose for use by other tissues (Black and Wilmore, 1983). Branched chain amino acids (BCAA) are utilized as an alternative energy source to synthesize glutamine and alanine. The decrease in BCAA levels is consistent with a decrease in plasma amino acid levels in a state of sepsis (Clones et al., 1980). BCAA administration has a therapeutic influence in patients with sepsis, whose amino acids are deranged (Freund et al., 1979).

Administration of BCAA is effective in rats with diabetes (Eizirik et al., 1987); however, the mechanism is still undetermined, and no one has reported the effect of BCAA on virus-induced diabetes. Thus, we administered BCAA to mice infected with EMCV to see whether BCAA have an inhibitory effect against virus-induced glucose intolerance.

2. Materials and methods

2.1. Animals and virus

Nine-week-old male DBA/2 mice (Charles River, Atsugi) were purchased and maintained on regular rat chow and tap water in a specific pathogen-free facility at the Gunma university, and cared for according to the guidelines of the Gunma University for Animal Care. The NDK25 variant of EMCV were obtained from Dr. Y. Seto, Keio University, Tokyo, Japan. Mice were inoculated intraperitoneally with 200 plaque-forming units of a NDK variant of EMC. Age- and sex-matched mice were inoculated intraperitoneally with the same amount of phosphate-buffered saline as control group.

2.2. Administration of BCAA

BCAA, including L-2-amino-3-methylbutanoic acid (valine, $C_5H_{11}NO_2$), L-2-amino-4-methylpentanoic acid (leucine, $C_6H_{13}NO_2$), and [2*S*,3*S*]-2-amino-3-methylpentanoic acid (isoleucine, $C_5H_{11}NO_2$), were kindly supplied by Ajinomoto (Yokohama, Japan). Before administration, 1 g of each BCAA was mixed and dissolved in 100 ml of distilled water. Mice were divided into three groups (6–8 mice in each group). For the BCAA-treated group, this BCAA cocktail was provided instead of drinking water, from the day after virus inoculation. The average daily doses of these amino acids were 0.9 g of each BCAA per 1 kg of body weight. Untreated and control mice were provided with distilled water.

2.3. Oral glucose tolerance tests

Glucose was orally administered at 1.5 g/kg body weight after an 8-h fast on 1, 2, 3 and 6 weeks after virus inoculation ($n = 6–8$ of each group). Before, 30 and 60 min after glucose loading, blood was collected from the tail vein or postorbital venous plexus to measure blood glucose, plasma insulin and amino acid concentrations. Blood glucose concentrations were determined by a glucose oxidase method, using a Fuji Dry Chem System (Medical System, Tokyo). The plasma levels of immunoreactive insulin were determined by radioimmunoassay using rat insulin as a standard. Plasma amino acid concentrations were determined by high-performance liquid chromatography (HPLC).

2.4. Insulin content of the pancreas

The pancreases were excised for insulin measurement and for histopathology at 1, 2 and 6 weeks after virus inoculation, and were extracted with acid ethanol as described (Utsugi et al., 1992). The insulin content of the pancreas was determined by radioimmunoassay as described above.

2.5. Histopathology

The pancreases were fixed in formaldehyde solution, embedded with paraffin, sectioned, and stained with hema-

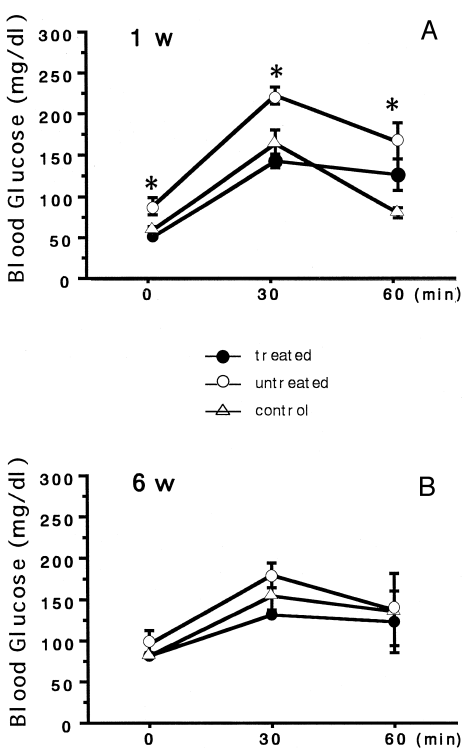

Fig. 1. Oral glucose tolerance tests in DBA/2 mice at 1 week (A) and 6 weeks (B) after virus inoculation. Mice infected with the NDK25 variants of EMCV showed glucose intolerance, and BCAA treatment improved blood glucose levels during OGTT. Results are shown as means ± S.E.M. for 6–8 animals, and were compared with ANOVA followed by Dunnett's test. * $P < 0.05$ vs. control. ●: BCAA-treated group, ○: untreated group, △: control.

toxylin-eosin. At least 5–10 islets per pancreas were assessed by two independent scorers unaware of the animal's prior treatment. The grade of insulitis was determined as follows: grade 0, intact islets; grade 1, less than 25%; grade 2, 25–50%; and grade 3, more than 50% of the islet area was infiltrated by mononuclear cells.

2.6. Statistical analysis

Data were analyzed by using the Stat view 4.5 software package for Macintosh computers (Abacus Concepts, Berkeley, CA), and presented as means ± S.E.M. For multiple comparison, the one-way analysis of variance (ANOVA) followed by Dunnett's test was used. Probability (P) value < 0.05 was considered significant.

3. Results

3.1. Oral glucose tolerance tests

One week after virus inoculation, blood glucose levels in untreated groups were 92.0 ± 10.0 mg/dl at baseline, 224.6 ± 10.9 mg/dl at 30 min, and 169.4 ± 21.4 mg/dl at 60 min after glucose loading, which were significantly ($P < 0.05$) higher than those in the control group (62.7 ± 3.6 mg/dl, 167.2 ± 16.4, and 83.8 ± 6.0 mg/dl, respectively) (Fig. 1A). The blood glucose levels in the BCAA-treated group were 54.5 ± 3.7 mg/dl at baseline, 145.2 ± 8.7 mg/dl at 30 min, and 128.7 ± 18.3 mg/dl at 60 min after glucose loading, which were not significantly higher than those in the control group. Blood glucose levels during oral glucose tolerance tests (OGTT) at 2 and 3

Table 1
Plasma amino acid concentrations from mice infected with the NDK25 variant of EMCV
Valine, leucine isoleucine, glycine, threonine, phenylalanine and tyrosine levels were significantly decreased in the untreated group compared with those in the control group. In the BCAA-treated group, the concentrations of valine, leucine, and isoleucine were similar to those of the control group. Results are shown as means ± S.E.M., and were compared with ANOVA followed by Dunnett's test.
Abbreviations: BCAA, branched chain amino acid.

Amino acids	BCAA-treated ($n = 6$)	Untreated ($n = 6$)	Control (nmol/ml) ($n = 4$)
Valine	220 ± 8	16 ± 16[a]	227 ± 14
Leucine	174 ± 4	113 ± 9[a]	151 ± 17
Isoleucine	114 ± 2	75 ± 6[a]	101 ± 10
Alanine	349 ± 10[b]	260 ± 16	200 ± 35
Glycine	232 ± 6	159 ± 8[a]	212 ± 8
Threonine	130 ± 6	102 ± 7	129 ± 9
Phenylalanine	85 ± 2	71 ± 6	73 ± 6
Tyrosine	87 ± 2	77 ± 6	70 ± 6
Arginine	125 ± 7[b]	99 ± 7[a]	58 ± 8

[a] $P < 0.05$, untreated group vs. control.
[b] $P < 0.05$, BCAA-treated group vs. control.

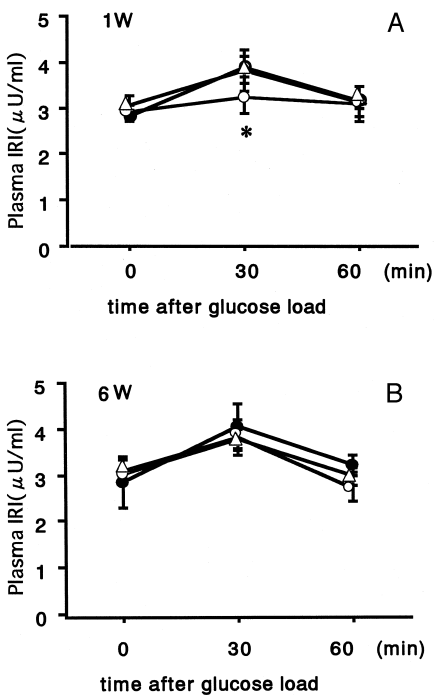

Fig. 2. Alternations of plasma immunoreactive insulin levels (IRI) during oral glucose tolerance tests at 1 week (A) and 6 weeks (B) after virus inoculation. The IRI levels at 30 min after the glucose loading in the untreated group was significantly ($^{*}P < 0.05$) lower than those in the control group at 1 week after virus inoculation. Results are shown as means ± S.E.M. for 6–8 animals, and were compared with ANOVA followed by Dunnett's test. $^{*}P < 0.05$ vs. control. ●: BCAA-treated group, ○: untreated group, △: control.

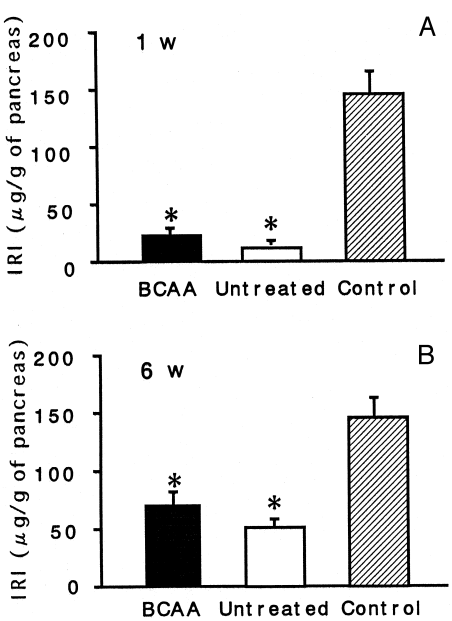

Fig. 3. Insulin content of the pancreas. The pancreases were excised and extracted with acid ethanol to measure insulin by radioimmunoassay at 1 week (A) and 6 weeks (B) after virus inoculation. The insulin content of the pancreas in the BCAA-treated group and the untreated group was significantly ($^{*}P < 0.05$) lower than that in the control group throughout the observation period. Results are shown as means ± S.E.M. for 6–8 animals, and were compared with ANOVA followed by Dunnett's test. $^{*}P < 0.05$ vs. control.

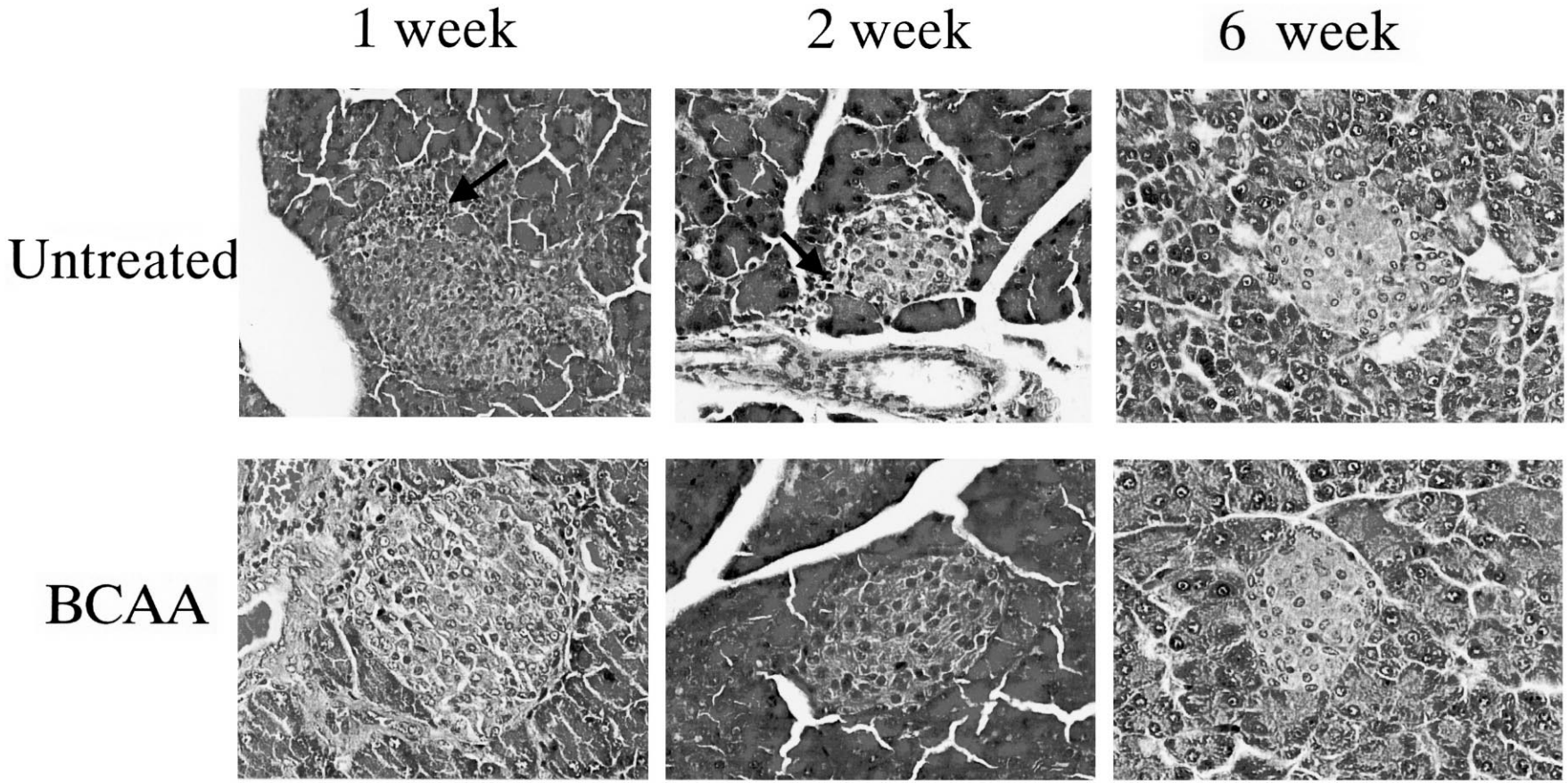

Fig. 4. Histological findings of the pancreas of mice infected with the NDK25 variant of EMCV. In BCAA-treated mice, mononuclear cell infiltration surrounding the islets was less than that seen in untreated mice. Arrows indicate mononuclear cells infiltrating into islets

weeks after virus inoculation demonstrated a pattern similar to that seen at 1 week after virus inoculation. At 6 weeks after inoculation, blood glucose levels during OGTT were not significantly different among the three groups (Fig. 1B). There were no significant differences in terms of body weight change between the BCAA-treated and -untreated groups.

IRI levels at 1 week after inoculation tended to be higher in the BCAA-treated group compared to the untreated group (Fig. 2A). The IRI levels at 30 min after glucose loading was significantly ($P < 0.05$) lower in the untreated group than in the control group. However, the IRI levels 6 weeks after inoculation showed no significant differences among the three groups (Fig. 2B).

3.2. Plasma amino acid concentrations

Amino acid concentrations in plasma were measured by HPLC at 1 week after virus inoculation. Levels of valine, leucine isoleucine, glycine, threonine, phenylalanine, and tyrosine were significantly ($P < 0.05$) decreased in the untreated group compared with the uninfected control group. In the BCAA-treated group, however, glycine, threonine and phenylalanine levels were slightly elevated compared with those of the untreated group and the concentrations of valine, leucine, isoleucine were similar to those of the control group (Table 1).

3.3. Insulin content of the pancreas

The insulin content of the pancreas was 15 ± 3 μg/g of pancreas in the untreated group and 23 ± 7 μg/g pancreas in the BCAA-treated group at 1 week after virus inoculation (Fig. 3A). These levels were significantly lower than that of the control group (145 ± 20 μg/g). After 6 weeks, the insulin content of the pancreas had recovered to 51 ± 7 μg/g in the untreated group and 61 ± 12 μg/g in the BCAA-treated group (Fig. 3B).

Table 2
Distribution of histopathological lesions in the pancreas of mice infected with the NDK25 variants of EMCV
The percentage of islets showing insulitis is expressed on a grade of 0–3. The grade of insulitis was determined as follows: grade 0, intact islets; grade 1, less than 25%; grade 2, 25–50%; and grade 3, more than 50% of the islet area infiltrated with mononuclear cells.
Abbreviations: BCAA, branched chain amino acid.

	Grade of insulitis (%)			
	0	1	2	3
BCAA-treated				
1W ($n = 8$)	55	36	9	0
6W ($n = 6$)	100	0	0	0
Untreated				
1W ($n = 8$)	56	31	8	5
6W ($n = 6$)	100	0	0	0
Control				
1W ($n = 8$)	100	0	0	0
6W ($n = 6$)	100	0	0	0

3.4. Histopathology

Histopathological investigations showed that mononuclear cells had infiltrated into the pancreatic islets in mice inoculated with virus. The grade of insulitis in the BCAA-treated group was significantly ($P < 0.05$) lower than that of the untreated group at 1 and 2 weeks after virus inoculation (Fig. 4, Table 2). There was no mononuclear infiltration of islets at 6 weeks after inoculation in any of the three groups (Fig. 4).

4. Discussion

This is the first study to demonstrate that oral administration of BCAA reduces the severity of insulitis in the pancreas and improves glucose intolerance in mice infected with EMCV.

The therapeutic effects of BCAA have been investigated in several different disorders. Intravenous BCAA treatment for liver cirrhosis is well known and established to improve Fisher's ratio and hepatic encephalopathy. Oral administration of BCAA is also effective for liver dysfunction (Yoshida et al., 1989). BCAA treatment also improves the glomerular filtration rate in patients with insulin-dependent diabetes mellitus (Rudberg et al., 1991). These effects are thought to be due to the anti-catabolic effects of BCAA (Maddrey, 1985) or to the improvement of protein malnutrition (Bianchi et al., 1992).

The EMC virus belongs to the picorna viridae as well as Coxsackie virus. Craighead and McLane (1968) first clarified that the M variant of EMC virus induces diabetes in mice. Yoon et al. (1980) found that the M variant included both a diabetogenic variant that absolutely causes diabetes, and a non-diabetogenic variant that never causes diabetes in mice. The difference between these two variants has been investigated by several researchers. The nucleotide sequence (Cohen et al., 1988), genomic differences (Bae et al., 1989), and the polyprotein sequence of these two variants (Bae et al., 1990) have been determined. Further, a single point mutation located on the major capsid protein VP1 is reported to be responsible for diabetogenicity (Jun et al., 1997), and EMCV-induced diabetes can be prevented by immunization with the recombinant viral outer coat protein 1 (VP1) (Jun et al., 1995). Subsequently, Fukuma et al. (1987) cloned a diabetogenic strain (termed DK27) that causes severe insulitis from the M variant of EMCV and a non-diabetic strain (termed NDK25) that causes glucose intolerance without a need for exogenous insulin for survival.

The NDK25 variant of EMCV induced glucose intolerance for 1–3 weeks after infection; however, 6 weeks after

infection, this effect tended to diminish (Utsugi et al., 1992).

Mononuclear cell infiltration of the pancreatic islets was observed at 1 week after virus inoculation. At 6 weeks after infection, however, the degree of insulitis and the insulin content of the pancreas tend to be restored. These results show that mice infected with the NDK25 strain could form an animal model for glucose intolerance in which exogenous insulin is not needed for survival. In this model, oral administration of BCAA improved glucose intolerance, and plasma IRI levels were maintained as the same levels as those before infection. Histopathologically, severe insulitis seemed to be prevented in the pancreas of the BCAA-treated group.

Eizirik et al. (1987) administered intraperitoneally a cocktail of BCAA to rats with diabetes induced by streptozotocin, and they observed that glucose intolerance was improved. They reported that administration of each individual amino acid had no therapeutic effect. Also in this report, they could not explain the therapeutic effect by insulin release stimulated by leucine (Lernmark, 1972) and/or isoleucine (Sener and Malaisse, 1981).

In conclusion, oral administration of a BCAA cocktail improves the glucose intolerance of mice injected with the NDK25 variant of EMCV and protects against the destruction of pancreatic islets.

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