ArzneimForsch DrugRes

Sonderdruck / Reprint

Arzneimittel-Forschung Drug Research

ECV · Editio Cantor Verlag · Aulendorf (Germany)
Arzneim.-Forsch./Drug Res. **48 (I),** 6, 701–706 (1998)

Effects of Branched-Chain Amino Acid-Enriched Nutrient Mixture on Natural Killer Cell Activity in Viral Cirrhosis

Kunio Takegoshi[a], Hiroshi Nanasawa[b], Hiroyuki Itoh[b], Toshihumi Yasuyama[c], Yasukazu Ohmoto[d], and Kota Sugiyama[e]

Takegoshi Internal Medicine Clinic[a], Department of Gastroenterology, Takaoka City Hospital[b], Department of Gastroenterology, Social Insurance Takaoka Hospital[c], Toyama, Cellular Technology Institute, Otsuka Pharmaceutical Co., Ltd.[d], and Microbiological Research Institute, Otsuka Pharmaceutical Co., Ltd.[e], Tokushima (Japan)

Effects of Branched-Chain Amino Acid-Enriched Nutrient Mixture on Natural Killer Cell Activity in Viral Cirrhosis

Kunio Takegoshi[a], Hiroshi Nanasawa[b], Hiroyuki Itoh[b], Toshihumi Yasuyama[c], Yasukazu Ohmoto[d], and Kota Sugiyama[e]

Takegoshi Internal Medicine Clinic[a], Department of Gastroenterology, Takaoka City Hospital[b], Department of Gastroenterology, Social Insurance Takaoka Hospital[c], Toyama, Cellular Technology Institute, Otsuka Pharmaceutical Co., Ltd.[d], and Microbiological Research Institute, Otsuka Pharmaceutical Co., Ltd.[e], Tokushima (Japan)

Summary

A controlled study was performed in 18 viral cirrhosis patients to evaluate whether immune function, as indicated by natural killer (NK) cell activity, was improved by a branched-chain amino acid-enriched nutrient mixture (nutrient-mixture), Aminoleban EN. Five patients received the nutrient-mixture (100 g/day) for 2 to 6 weeks preceded by control periods. Five additional patients received the nutrient-mixture for 2 to 4 weeks, and the remaining 8 patients did not receive the nutrient-mixture. NK cell activity, CD16, CD8, CD11b, and amino acids were assayed before and after the administration of the drug in the nutrient-mixture-supplemented group, and two times with 1 to 6 month intervals in the control group. In the nutrient-mixture-supplemented group ($n = 10$), increasing NK cell activity, expressed as the ratio of values of post-treatment to that of baseline (ratio > 1.25) was detected in 7 (70%) patients, whereas in the control group ($n = 13$), it was detected in only 1 (7.7 %) ($p < 0.01$). While in the affected group (NK cell activity ratio > 1.25, $n = 7$), all patients had compensated liver cirrhosis, in the unaffected group (NK cell activity ratio < 1.25, $n = 3$), 2 of 3 patients had decompensated liver cirrhosis ($p < 0.02$). Laboratory data, indicating severity of liver cirrhosis, such as total bilirubin and albumin, showed better values ($p < 0.01$, $p < 0.05$ respectively), and baseline NK cell activity was low (8.7 ± 7.2 % vs 33.3 ± 13.0 %, $p < 0.05$) in the affected group than unaffected group. NK cell subpopulations such as CD16 (%), CD11b (%) and one of the populations of T cell such as CD8 (%) showed no significant change throughout the study. As for amino acids analysis, Fischer's ratio was increased in the nutrient-mixture-supplemented group compared to the control group ($p < 0.05$), but none of the amino acids showed significant change. Thus the changes in NK cell activity were not explained by increase in NK cell subpopulations nor changes of amino acids. These results suggest that the branched-chain amino acid-enriched nutrient mixture increases NK cell activity moderately in patients who have compensated liver cirrhosis and shows lower values of baseline NK cell activity.

Zusammenfassung

Wirkungen einer mit verzweigtkettigen Aminosäuren angereicherten Nährmittelmischung auf die Zellaktivität natürlicher Killerzellen bei viraler Zirrhose

Bei 18 Patienten mit viraler Leberzirrhose wurde eine kontrollierte Studie durchgeführt, um zu untersuchen, ob die Immunfunktionen, wie durch die Aktivität der Killerzellen (natural killer cell = NK) angedeutet wird, durch die mit verzweigtkettigen Aminosäuren angereicherte Nährmittelmischung (Nährmittelmischung) Aminoleban EN verbessert wird. Fünf Patienten erhielten die Nährmittelmischung (100 g/Tag) nach jeweils entsprechenden Kontrollperioden (100 g/Tag) über einen Zeitraum von 2 bis 6 Wochen. Fünf weitere Patienten erhielten die Nährmittelmischung für 2 bis 4 Wochen, während die restlichen Patienten keine Nährmittelmischung erhielten. Die NK-Aktivität, CD16, CD8, CD11b sowie Aminosäuren wurden vor der Behandlung in der mit der Nährmittelmischung versorgten Gruppe und in der Kontroll-

gruppe zweimal in Intervallen von 1 bis 6 Monaten untersucht. In der mit der Nährmittelmischung versorgten Gruppe (n = 10) stieg die NK-Aktivität an, was sich in dem bei 7 Patienten (70 %) beobachteten erhöhten Verhältnis der posttherapeutischen Werte zu den Grundwerten (Verhältnis > 1,25) ausdrückte, während in der Kontrollgruppe (n = 13) ein solcher Anstieg nur bei einem Patienten (7,7 %) ($p < 0,01$) beobachtet wurde. Während bei allen Patienten in der Behandlungsgruppe (NK-Aktivität > 1,25, n = 7) eine Kompensation für die Leberzirrhose beobachtet wurde, fand sich in der Kontrollgruppe (NK Aktivität < 1,25, n = 3) bei 2 von 3 Patienten eine Dekompensation der Leberzirrhose ($p < 0,02$). In der Behandlungsgruppe fanden sich bessere Werte (jeweils $p < 0,01$, $p < 0,05$) für die die Schwere der Leberzirrhose reflektierenden Laborbefunde, wie zum Beispiel Gesamtbilirubin und Albumin-Konzentrationen, und eine geringere Grundaktivität der Killerzellen (8,7 % ± 7,2 % vs. 33,3 % ± 13,0 %) als in der Kontrollgruppe. Während der Untersuchung fanden sich jedoch keine signifikanten Unterschiede zwischen den Untergruppen der NK-Zellen, wie zum Beispiel CD16 (%), CD11b (%), und eine der T-Zellpopulationen, nämlich der CD8 (%). Die Analyse der Aminosäuren zeigte, daß das Fischersche Verhältnis in der mit der Nährmittelmischung versorgten Gruppe im Vergleich zur Kontrollgruppe ($p < 0,005$) erhöht war, während diese Veränderung allerdings für keine der Aminosäuren signifikant war. Daher können diese Veränderungen in der NK-Zellaktivität nicht durch die Untergruppen der NK-Zellen erklärt werden. Diese Ergebnisse deuten darauf hin, daß die mit verzweigtkettigen Aminosäuren angereicherte Nährmittelmischung bei Patienten mit kompensierter Leberzirrhose und verminderten Grundwerten für die Aktivität der NK-Zellen diese Aktivität in mäßigem Ausmaß steigerte.

Key words Aminoleban EN · Branched-chain amino acid · Liver cirrhosis · Natural killer cell · Nutrient mixture, branched-chain amino acid-enriched

Arzneim.-Forsch./Drug Res. **48 (I),** 701–706 (1998)

1. Introduction

An abnormal profile of plasma amino acids, low levels of branched-chain amino acids, high levels of aromatic amino acids, and high levels of tryptophan and methionine have been reported in advanced cirrhosis of the liver [1]. For cases of liver cirrhosis with chronic encephalopathy, branched-chain amino acids-enriched nutrient mixtures are specially prepared nutritional products that help to prevent hepatic encephalopathy and provide nutritional improvement with few adverse reactions [2, 3, 4].

In Japan, 76 % of the cases of hepatocellular carcinoma (HCC) are associated with cirrhosis [5], consequently, patients with viral cirrhosis are considered to be at high risk for HCC. It has yet to be proven whether the branched-chain amino acids-enriched nutrient mixture (nutrient-mixture), Aminoleban EN[1)], increases or decreases the incidence of HCC in liver cirrhosis. Because methionine, tyrosine, and phenylalanine concentrations are higher in HCC than they are in cirrhotic or normal livers [6], it seems reasonable to expect that a diet deficient in these amino acids may help to prevent HCC. The nutrient-mixture contains increased amounts of branched-chain amino acids, reduced amounts of aromatic amino acids, gelatin hydrolysate, casein, carbohydrate, lipid, glycyrrhizin, and other components. Although the inhibition of liver carcinogenesis by the nutrient-mixture has been observed in an animal experiment [7], there is little previous data on the use of branched-chain amino acids-enriched nutrient mixtures to prevent the emergence of HCC in cirrhosis patients.

[1)] Manufacturer: Otsuka Pharmaceutical Co. Ltd., Tokyo (Japan).

As the reduced immune function in liver cirrhosis is a serious risk factor for HCC emergence [8, 9], the normalization of immune function may be useful in preventing the emergence of HCC in viral cirrhosis. A controlled study was performed in viral cirrhosis to evaluate whether immune function, as indicated by NK cell activity, is improved by the nutrient-mixture. To the best of our knowledge, the immunomodulating effects of branched-chain amino acids-enriched nutrient mixtures have not been previously described in English language reports.

2. Materials and methods

2.1. Subjects

18 viral cirrhosis patients (7 males and 11 females, aged 49 to 79 yr, mean = 64.7 yr) and 5 healthy volunteers (3 males and 2 females, aged 24 to 38 yr, mean = 29.8 yr) were used as the subjects of this study. The diagnosis of liver cirrhosis was made by peritoneoscopy and needle biopsy or on the basis of clinical symptoms and signs accompanied by positive findings in imaging studies (CT, liver scintigraphy and sonography). Three patients were positive for hepatitis B surface antigen (HBsAg), and 15 patients were positive for hepatitis C virus-antibody (third generation) assayed by radioimmunoassay. None had a drinking history. 16 patients had compensated liver cirrhosis (Child A), and 2 patients had decompensated liver cirrhosis (Child C). On the basis of abdominal angiography or MRI, 4 patients had hepatocellular carcinoma (HCC). The size of the HCC in the four patients was 3.8 × 3.5 cm, 3.3 × 2.6 cm and 1.6 × 1.3 cm, 1.2 × 1.0 cm, respectively. Treatment of HCC was not conducted at the time of this study. None of the patients and healthy volunteers had a common cold or other infections at the time of this study. Informed consent was obtained from all the patients and the study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki.

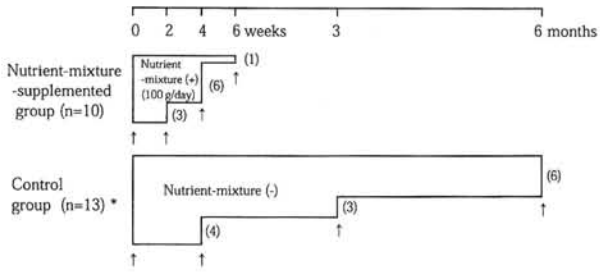

Fig. 1: Schedule of nutrient-mixture-supplemented diet in viral cirrhosis. (): Patient numbers. ↑: Immunological studies and amino acid analyses were performed. *: 5 patients out of 13 controls entered the nutrient-mixture-supplemented group after 3 to 6 month control periods.

2.2. Composition of the nutrient-mixture and schedule of study

The composition of Aminoleban EN, a soft-powder nutrient-mixture (Otsuka Pharmaceutical Co., Ltd., Tokyo), is shown in Table 1. In 100 g, the nutrient-mixture contains 13.0 g of free amino acids, 13.0 g of gelatin hydrolysate, 1.0 g of casein, 62.1 g of carbohydrate, 7.0 g of lipid, glycyrrhizin, and other components. The schedule of study is shown in Fig. 1. Five patients received the nutrient-mixture (100 g/day) for 2 to 6 weeks preceded by control periods. Five additional patients received the nutrient-mixture for 2 to 4 weeks, and the remaining 8 patients did not receive the nutrient-mixture. Diet was not restricted except for being supplemented with the nutrient-mixture.

NK cell activity, CD16, CD8, CD11b, and amino acids were assayed before and after the administration of the drug in the nutrient-mixture-supplemented group (n = 10), and two times with 1 to 6 month intervals in the control group (n = 13). Blood samples were taken early in the morning before breakfast. Clinical features and laboratory data of the nutrient-mixture-supplemented group and control group are shown in Table 2. Although cases of decompensated liver cirrhosis are not included in the control group, there are no significant differences between both groups.

To examine to what extent NK cell activity fluctuates day-to-day, the activity was assayed 5 times at one week intervals in 5 healthy volunteers. NK cell activity in each volunteer was 62.6 ± 5.6 %, 51.0 ± 12.1 %, 39.5 ± 2.1 %, 29.7 ± 7.0 %, 14.3 ± 3.3 % (mean value ± standard deviation), respectively, and the coefficient of variation ranged between 5.4 % and 24.3 %. Thus we regard values of NK cell activity, expressed as the ratio of values of post-treatment to that of baseline, over 1.25 as increasing, under 0.75 as decreasing, and between 0.75 and 1.25 as unchanged.

2.3. Preparation of peripheral lymphocytes

Peripheral blood mononuclear cells (PBMCs) were separated from heparinized venous blood by Ficoll-Paque (Pharmacia, Uppsala, Sweden) gradient centrifugation. After PBMCs were collected from the interface and washed 3 times in RPMI 1640 medium (GIBCO Laboratories, New York, USA), the concentration of PBMCs was adjusted to 1×10^6/ml with 10 % fetal calf serum (FCS) (GIBCO) in RPMI 1640.

2.4. Preparation of target cells

The K562 cell line, an erythroblastic cell line derived from human chronic myelogenous leukemia, was used as the target and the cells were cultured in RPMI 1640 with 10 % FCS. Before use, the cells were incubated with Na_2

Table 1: Nutrient and amino acid composition of the nutrient-mixture (/100 g).

Proteins (g)		Vitamins	
Amino acids	13,0	A	932 IU
Gelatin	13.0	B_1	0.199 mg
Casein	1.0	B_2	0.31 mg
Carbohydrates (g)		B_6	0.403 mg
Dextrin	62.1	B_{12}	1 µg
Lipids (g)		C	12.2 mg
Rice oil	7.0	D_2	93.2 IU
Amino acids (g)		E	16.9 mg
Valine	3.57	K_1	0.011 mg
Leucine	4.50	Pantothenic acid	2.18 mg
Isoleucine	4.08	Niacin	3.03 mg
Phenylalanine	0.33	Biotin	50 µg
Tyrosine	0.09	Folic acid	0.1 mg
Methionine	0.12	Choline	10.1 mg
Tryptophan	0.16	Electrolytes and others	
Arginine	1.76	Na	97.4 mg
Threonine	0.58	K	353,0 mg
Alanine	1.50	Ca	116,6 mg
Histidine	0.54	Mg	40.4 mg
Proline	1.96	Cl	437,9 mg
Serine	0.47	P	167,5 mg
Lysine	1.19	Fe	2.63 mg
Aspartic acid	0.89	Zn	1.71 mg
Glutamic acid	1.69	Cu	262 µg
Glycine	3.47	I	19.1 µg
Fischer's ratio	38.2	Mn	0.37 mg
		Glycyrrhizin	261 mg
		Energy	420 kcal

Table 2: Comparison of clinical features and laboratory data[a] between the nutrient-mixture-supplemented group (n = 10) and control group (n = 13).

	Nutrient-mixture-supplemented group (n = 10)	Control group (n = 10)
Age	67.6 ± 9.7	64.8 ± 7.7
Men/women	4/6	5/8
Type cirrhosis		
HCV positive	9 (90 %)	11 (84.6 %)
HBV positive	1 (10 %)	2 (15.4 %)
Childs' classification		
A	8 (80 %)	13 (100 %)
B	0	0
C	2 (20 %)	0
Hepatocellular carcinoma	3 (30 %)	2 (15.4 %)
Other medication (glycyrrhizin)	3 (30 %)	7 (53.8 %)
Total bilirubin (mg/dl)	1.62 ± 1.23	1.0 ± 0.57
Albumin (g/dl)	4.09 ± 0.45	4.16 ± 0.41
GOT (IZ/l)	77.8 ± 60.4	87.8 ± 77.3
GPT (IU/l)	64.3 ± 43.5	73.8 ± 67.6
CHo.E (ΔpH)	0.73 ± 0.19	0.6 ± 0.2
NH_3 (µg/dl)	71.2 ± 35.6	61.7 ± 28.2
RBC ($\times 10^4$/mm^3)	432.7 ± 57.8	414.3 ± 49.9
Platelet ($\times 10^4$/mm^3)	8.6 ± 4.1	9.6 ± 4.1
NK cell activity (%)	18.5 ± 15.8	33.8 ± 22.0
CD16 (%)	14.1 ± 9.4	15.9 ± 6.9
CD8 (%)	23.9 ± 7.8	25.8 ± 4.6
CD11b (%)	19.1 ± 10.5	13.0 ± 7.5
Fischer's ratio[b]	1.73 ± 0.65	1.86 ± 0.56
Valine (mmol/ml)	186.4 ± 35.1	191.7 ± 41.1
Methionine (mmol/ml)	46.9 ± 22.9	42.6 ± 12.7
Isoleucine (mmol/ml)	53.9 ± 10.4	53.7 ± 14.6
Leucine (mmol/ml)	100.9 ± 21.8	99.8 ± 24.9
Tyrosine (mmol/ml)	123.6 ± 38.6	117.4 ± 31.7
Phenylalanine (mmol/ml)	89.8 ± 29.5	76.8 ± 15.6
Arginine (mmol/ml)	92.6 ± 30.8	96.9 ± 26.3

[a] Data are shown as the mean ± standard deviation.
[b] Fischer's ratio: (valine + leucine + isoleucine)/phenylalanine + tyrosine) molar ratio.

$^{51}CrO_4$ (Amersham, Buckinghamshire, England; 100 μCi for 1×10^7 cells) in a water bath at 37 °C for 1 h. After being washed 3 times in RPMI 1640, the K562 cells were adjusted to 1×10^6/ml.

2.5. Assay of NK cell activity

The NK cell activity was measured by a 4-h chromium release assay. 200 μl of PBMCs and 10 μl of K562 cells were added to plastic microplates (Falcon, Oxnard, CA, USA) and then cultured in 5 % CO_2 at 37 °C for 4 h. The effector-target cell (E/T) ratio was 20 : 1. The maximal release (MR) was estimated by culturing the K562 cells in 2 % Triton X-100 (E. Merck, Darmstadt, Germany), and the spontaneous release (SR) was measured by culturing the K562 cells without PBMCs. All assays were performed in triplicate. After being incubated for 3.5 h, the microplates were centrifuged at 800 rpm for 5 min. 100 μl of supernatant was then removed and assayed using a gamma counter. The experimental release (ER) was calculated as the mean of the triplicate cultures. NK cell activity was expressed as ER-SR/MR-SR × 100 %.

2.6. Assay of subpopulations

The following monoclonal antibodies were used for assay of subpopulations: CD16, directed against the immunoglobulin Fc receptor, which is expressed on NK cells and neutrophils; CD8, directed against the MHC-class 1 receptor, which is expressed on cytotoxic T cells and NK cells; CD11b, directed against Mac-1, which is expressed on monocytes, neutrophils and NK cells. CD16, CD8 and CD11b were from Becton Dickinson (San Jose, Ca). Subpopulations of mononuclear cells after immunofluorescence staining were analyzed and separated with a FACS 440 (Becton Dickinson).

2.7. Assays of amino acids

Plasma amino acid concentrations were determined using a Hitachi amino acid analyzer (Type 835, Tokyo).

2.8. Statistical analysis

Statistical significance was determined by paired t-test.

3. Results

Changes of immune functions and amino acids analysis, expressed as the ratio of values of post-treatment to that of baseline, were compared between the nutrient-mixture-supplemented group and control group (Table 3). Increasing NK cell activity ratio (ratio > 1.25) was detected in more patients of the nutrient-mixture-supplemented group (7 of 10 patients, 70 %) than the control group (1 of 13 patients, 7.7 %) ($p < 0.01$). The nutrient-mixture-supplemented group can be divided in two, namely, those whose NK cell activity ratio was more than 1.25 (n = 7), the affected group, and those whose NK cell activity ratio was under 1.25 (n = 3), the unaffected group. Clinical features and laboratory data were compared between these two subgroups (Table 4). Although in the affected group, all 7 patients had compensated liver cirrhosis (Child A), 2 of 3 unaffected patients had decompensated liver cirrhosis (Child C) ($p < 0.02$).

Laboratory data, indicating severity of liver cirrhosis, such as total bilirubin and albumin, showed better values (1.0 ± 0.28 mg/dl vs 3.07 ± 1.44 mg/dl, $p < 0.01$, 4.29 ± 0.39 g/dl vs 3.63 ± 0.06 g/dl, $p < 0.05$, respectively), and baseline NK cell activity showed lower values (8.7 ± 7.2 % vs 33.3 ± 13.0 %, $p < 0.05$) in the affected group than in unaffected group. To ensure that the nutrient-mixture increases NK cell activity in liver cirrhosis when baseline NK cell activity is low, we compared increasing NK cell activity ratios between patients of the nutrient-mixture-supplemented group and those of the control group whose baseline NK cell activity was lower than 29 % (Table 5). Increasing NK cell activity was detected in more patients of the nutrient-mixture-supplemented group (6 of 7 patients, 85.7 %) than control group (1 of 6 patients, 16.7 %) ($p < 0.02$). There were no significant differences of clinical features and laboratory data between patients whose baseline NK cell activity was lower than 29 % and in those whose baseline NK cell activity was higher than 30 % in the present study (data not shown). These results suggest that the nutrient-mixture increases NK cell activity moderately in patients who have compensated liver cirrhosis and shows lower values of baseline NK cell activity.

To investigate the mechanism of the increase in NK cell activity induced by the administration of the nutrient mixture, the NK cell subpopulations, CD16 (%) and CD11b (%), and one of the popula-

Table 3: Effects of the nutrient-mixture on immune functions and amino acids in liver cirrhosis.

Changes of immune functions and amino acid analysis[a)]	Nutrient-mixture-supplemented group (n = 10)	Control group (n = 13)
NK cell activity ratio	3.44 ± 4.86	1.12 ± 0.58
1.25 <	7 (70 %)*	1 (7.7 %)*
0.75 ~ 1.25	2 (20 %)	11 (84.6 %)
0.75 >	1 (10 %)	1 (7.7 %)
CD16 ratio	2.83 ± 4.34	1.03 ± 0.5
1.25 <	6 (60 %)	3 (23.1 %)
0.75 ~ 1.25	2 (20 %)	8 (61.5 %)
0.75 >	2 (20 %)	2 (15.4 %)
CD8 ratio	1.06 ± 0.34	1.03 ± 0.5
1.25 <	2 (20 %)	1 (7.7 %)
0.75 ~ 1.25	8 (80 %)	11 (84.6 %)
0.75 >	0	1 (7.7 %)
CD11b ratio	1.04 ± 0.37	1.67 ± 1.12
1.25 <	4 (40 %)	6 (46.2 %)
0.75 ~ 1.25	4 (40 %)	5 (38.5 %)
0.75 >	2 (20 %)	2 (15.4 %)
Changes of Fischer's ratio	1.15 ± 0.14**	1.02 ± 0.11**
Valine ratio	1.17 ± 0.15	1.06 ± 0.15
Methionine ratio	1.06 ± 0.21	1.02 ± 0.26
Isoleucine ratio	1.11 ± 0.19	1.06 ± 0.16
Leucine ratio	1.03 ± 0.15	1.10 ± 0.21
Tyrosine ratio	1.00 ± 0.1	1.03 ± 0.15
Phenylalanine ratio	1.05 ± 0.1	1.02 ± 0.13
Arginine ratio	1.14 ± 0.25	1.05 ± 0.17

[a)] Expressed as ratio of values of post-treatment to that of baseline values. Values over 1.25 were regarded as increasing, under 0.75 as decreasing, and between 0.75 and 1.25 as unchanging. Values: mean ± standard deviation. * $p < 0.01$, ** $p < 0.05$. Increasing NK cell activity (ratio > 1.25) was detected in more patients of the nutrient-mixture-supplemented group (7 of 10 patients, 70 %) than control group (1 of 13 patients, 7.7 %) ($p < 0.01$). As for amino acids analysis, Fischer's ratio was increased in the nutrient-mixture-supplemented group compared to the control group ($p < 0.05$), but none of the amino acids showed significant change.

Table 4: Comparison of clinical features and laboratory data[a] between the affected group (n = 7) and unaffected group (n = 3).

	Affected group (n = 10)	Unaffected group (n = 10)
Age (years)	67.7± 8.5	67.3 ±14.6
Men/women	2/5	2/1
Type cirrhosis		
HCV positive	7 (100 %)	2 (66.7 %)
HBV positive	0	1 (33.3 %)
Childs' classification		
A	7 (100 %)[1]	1 (33.3 %)[1]
B	0	0
C	0	2 (66.7 %)
Hepatocellular carcinoma	1 (14.3 %)	2 (66.7 %)
Other medication (glycyrrhizin)	1 (14.3 %)	2 (66.7 %)
Total bilirubin (mg/dl)	1.0 ± 0.28[2]	3.07± 1.44[2]
Albumin (g/dl)	4.29± 0.39[3]	3.63± 0.06[3]
GOT (IU/l)	80.1 ±61.2	97.0 ±60.8
GPT (IU/l)	57.6 ±38.8	80.0 ±59.0
Cho.E (ΔpH)	0.78± 0.21	0.62± 0.06
NH_3 (μg/dl)	60.3 ±17.7	96.7 ±58.1
RBC ($\times 10^4/mm^3$)	436.6 ±64.2	423.7 ±50.2
Platelet ($\times 10^4/mm^3$)	10.2 ± 3.7	5.0 ± 2.8
NK cell activity (%)	12.0 ±11.0[3]	33.7 ±16.3[3]
CD16 ratio	1.58± 0.89	5.78± 7.99
1.25 <	4 (57.1 %)	2 (66.7 %)
0.75 ~ 1.25	1 (14.3 %)	1 (33.3 %)
0.75 >	2 (28.6 %)	0
CD 8 ratio	1.10± 0.28	0.97± 0.09
CD11b ratio	1.15± 0.36	0.81± 0.31
Fischer's ratio	1.99± 0.61	1.14± 0.19
Changes of Fischer's ratio	1.17± 0.16	1.10± 0.11
Tyrosine ratio	0.98± 0.1	1.03± 0.14
Phenylalanine ratio	1.1 ± 0.1	0.97± 0.07

[a] Data are shown as the mean ± standard deviation. [1] $p < 0.02$, [2] $p < 0.02$, [3] $p < 0.05$. Although in the affect group, all 7 patients had compensated liver cirrhosis (Child A), 2 of 3 unaffected patients had decompensated liver cirrhosis (Child C) ($p < 0.02$). Laboratory data, indicating severity of liver cirrhosis, such as total bilirubin and albumin, showed better values, and baseline NK cell activity was lower in the affected than unaffected group.

tions of T cells, CD8 (%), as well as amino acids were assayed in the nutrient-mixture-supplemented group and control group (Table 3). An increase in CD16 (ratio > 1.25) was detected in more patients of the nutrient-mixture-supplemented group than control group (60 % vs 23.1 %, $p < 0.1$), but the difference was not significant. CD8 and CD11b showed no significant changes in either group. As for amino acids analysis, changes of Fischer's ratio were more marked in the nutrient-mixture-supplemented group than control group (1.15 ± 0.14 vs 1.02 ± 0.11, $p < 0.05$), but the differences between the groups were not significant for any amino acids. Thus the changes in NK cell activity were not explained by the increase in NK cell subpopulations nor changes of amino acids.

Table 5: Effect of the nutrient-mixture on NK cell activity in liver cirrhosis whose baseline values of NK cell activity are lower than 29 % (n = 13).

Changes of NK cell activity[a]	Nutrient-mixture-supplemented group (n = 7)	Control group (n = 6)
NK cell activity ratio	4.41 ± 5.63	1.24 ± 0.21
1.25 <	6 (85.7 %)*	1 (16.7 %)*
0.75 ~ 1.25	0	4 (66.7 %)
0.65 >	1 (14.3 %)	1 (16.7 %)

[a] Expressed as ratio of values of post-treatment to that of baseline values. Values over 1.25 were regarded as increasing, under 0.75 as decreasing, and between 0.75 and 1.25 as unchanging. Values: mean ± standard deviation. * $p < 0.02$. Increasing NK cell activity was detected in more patients of the nutrient-mixture-supplemented group (n = 7) than control group (n = 6) ($p < 0.02$).

4. Discussions

The NK cell system is considered to have a role in the immunosurveillance network against carcinogenesis [10]. As NK cell activity is decreased in HCC compared with liver cirrhosis and normal controls [9, 11], increasing this activity may be of some benefit for patients who are suffering from HCC or who are at high risk of developing HCC. The results of the present study suggest that the nutrient-mixture increases NK cell activity in patients who have compensated liver cirrhosis and show relatively low values of baseline NK cell activity (Table 3, 4, 5). Norris et al. [12] reported that the consumption of a tyrosine- and phenylalanine-free diet supplemented with low-protein foods increased the NK cell activity in 6 of 9 healthy humans, although the mechanism of the increase is not known. This diet decreased fasting plasma tyrosine by up to 22 % without affecting plasma phenylalanine. They suspected that, in addition to the changes in amino acids, the changes in the intake of other nutrients (fat, copper, iron, vitamin E, and selenium) during the consumption of the formula diet could have contributed to the changes seen in immune function. As the nutrient-mixture contains increased amounts of branched-chain amino acids and reduced amounts of aromatic amino acids, changes of Fischer's ratio were more marked in the nutrient-mixture-supplemented group than control group (Table 3). However, in the present study, none of the amino acids showed significant changes after the administrations of nutrient-mixture. Reduced amounts of tyrosine and phenylalanine in the nutrient-mixture did not seem to play a role in increasing NK cell activity. Gelatin hydrolysate, which is one of the components of the nutrient-mixture and is made of collagens of bovine bone, seemed to play some role in increasing NK cell activity. It is reported that peptides derived from extracellular proteins, such as fibronectin, collagen and so on, increase both NK cell activity and NK cell subpopulations [13]. Although glycyrrhizin has been reported to have no effect on NK cell activity [14], it has also been reported to be an interferon-inducer [15] and immunopotentiator [16]. It is reported that glycyrrhizin increase NK cell activity in mice [17]. As the nutrient-mixture contains glycyrrhizin, 261 mg per 100 g, the role of glycyrrhizin in NK cell activity in the present study seemed important. Casein stimulates the phagocytic activity of macrophages and might be capable of playing a role in the proliferation and maturation of NK cells and T cells [18]. However

the nutrient-mixture contains a reduced amount of casein, 1.0 g per 100 g, thus the role of casein in NK cell activity in the present study seemed to be minimal. It has been reported that NK cell activity and lymphokine-activated killer activity were significantly higher when total parenteral nutrition contained half the fat of medium-chain triglycerides and half the amount of long-chain triglycerides in cancer patients [19]. As rice oil in the nutrient-mixture contains long-chain triglycerides only, the role of rice oil in NK cell activity in the present study seemed to be negligible. In the present study, although the reason why the nutrient-mixture has an increasing effect on NK cell activity in liver cirrhosis is unclear, gelatin hydrolysate, glycyrrhizin, casein, and other components of the nutrient-mixture which have not yet been reported to be immunopotentiators, could have contributed to the increase of NK cell activity.

As the results of our study suggested an increasing effect of the nutrient-mixture on NK cell activity in compensated liver cirrhosis but not in decompensated liver cirrhosis (Table 4), the immunomodulating effect of the nutrient-mixture in compensated liver cirrhosis and decompensated liver cirrhosis seemed to differ. It has been reported that, in in vitro experiments, the enhancement of NK cell activity by interferon decreased with the advance of liver diseases, and the reason given for this was the increased number of defective NK cells in advanced liver diseases [11]. It is supposed that the increasing effect of the nutrient-mixture on NK cell activity is not strong enough to increase NK cell activity in severely damaged liver cirrhosis.

As the results of our study also suggested an increasing effect of the nutrient-mixture on NK cell activity in patients with liver cirrhosis who show lower values of baseline NK cell activity (Table 4 and 5), the immunomodulating effect of the nutrient-mixture in those who show lower and higher values of baseline NK cell activity seemed to differ. It is reported that NK cell activity in liver cirrhosis decreases in relation to the severity of liver damage [11] and complication of hepatocellular carcinoma [9]. However, in the present study, there were no significant differences of clinical features and laboratory data between patients whose baseline NK cell activity was lower than 29 % and those whose activity was higher than 30 %. When the relative NK cell activity was measured at various effector-target cell (E:T) ratios, a plateau of maximum NK cell activity for each person was achieved at the higher E:T ratios [20]. This suggested an enhancement of NK cell activity by an immunopotentiator decrease at higher values of baseline NK cell activity. It is also reported that mild immunopotentiators, such as levamisole, improved the killing of lymphocytes from patients with hepatocellular carcinoma whose baseline NK cell activity was low, but did not produce a significant increase in normal NK cytotoxicity [21]. So it is supposed that the increasing effect of the nutrient-mixture is moderate.

Whatever the components of the nutrient-mixture responsible for increasing NK cell activity are, the changes in NK cell activity were not explained by increases in NK cell subpopulations but rather by the activation of each NK cell function. Analysis of the surface markers of NK cells, such as CD16 (%), CD11b (%) and one of the populations of T cell such as CD8 (%) showed no significant change throughout the study (Table 3). As for amino acids analysis, the value of Fischer's ratio was greater in the nutrient-mixture-supplemented group than the control group ($p < 0.05$), but there was no significant change for each amino acid.

In conclusion, our findings demonstrated for the first time the augmenting effect of the branched-chain amino acid-enriched nutrient mixture on NK cell activity in patients who have compensated liver cirrhosis and show lower values of baseline NK cell activity. Further studies are needed to determine whether the long-term administration of this nutrient mixture can also increase NK cell activity and be of benefit in decreasing the incidence of HCC in viral cirrhosis, and identify the exact components of the nutrient-mixture which increase NK cell activity and the mechanism behind this action.

5. References

[1] Fischer, J. E., Rosen, H. M., Ebeid, A. M., Surgery **80,** 77 (1976)

[2] Ichida, T., Shibasaki, K., Muto, Y., Nutrition **11,** 238 (1995)

[3] Okita, M., Watanabe, A., Nagashima, H., J. Nutr. Sci. Vitaminol. **31,** 291 (1985)

[4] Nompleggi, D. J., Bonkovsky, H. L., Hepatology **19,** 518 (1994)

[5] Liver cancer study group of Japan, Cancer **54,** 1747 (1984)

[6] Watanabe, A., Higashi, T., Sakata, T., Cancer **54,** 1875 (1984)

[7] Kurokawa, F., Yamashita, A., Okita, K., Jpn. J. Cancer Chemother. **19,** 612 (1992)

[8] Saibara, T., Maeda, T., Miyazaki, M., Hepatology **18,** 315 (1993)

[9] Chuang, W. L., Liu, H. W., Chang, W. Y., Cancer **65,** 926 (1990)

[10] Trinchieri, G., Adv. Immunol. **47,** 187 (1989)

[11] Sujino, H., Oshimi, K., Furukawa, R., J. Tokyo Wom. Med. Coll. **58,** 259 (1988)

[12] Norris, J. R., Meadows, G. G., Massey, L., Am. J. Clin. Nutr. **51,** 188 (1990)

[13] Lopez-Moratalla, N., Mar Calonge, M., Lopez-Zabalza, M., Biochim. Biophys. Acta **1265,** 181 (1995)

[14] Wada, T., Arima, T., Nagashima, H., Gastroenterol. Jpn. **22,** 312 (1987)

[15] Abe, N., Ebina, T., Ishida, N., Microbiol. Immunol. **26,** 535 (1982)

[16] Mori, K., Sakai, H., Suzuki, S., Tohoku J. Exp. Med. **158,** 25 (1989)

[17] Ito, K., Kumagai, K., Excepta Medica International Congress Series **641,** 460 (1984)

[18] Migliore-Samour, D., Jolles, P., Experientia **44,** 187 (1988)

[19] Sedman, P. C., Somer, S. S., Ramsden, C. W., Br. J. Surg. **78,** 1396 (1991)

[20] Pross, H. F., Banes, M. G., Rubin, P., J. Clin. Immunol. **1,** 51 (1981)

[21] Son, K., Kew, M., Rabson, A. R., Cancer **50,** 2820 (1982)

Correspondence: Kunio Takegoshi, M.D., Takegoshi Internal Medicine Clinic, 377-7 Nomura, Takaoka, Toyama 933-0014 (Japan)

Editors: Prof. Dr. Hans Georg Classen, Viktor Schramm. Editorial services: Waltraud Frey. Publisher: ECV · Editio Cantor Verlag für Medizin und Naturwissenschaften GmbH, P.O.B. 12 55, D-88322 Aulendorf (Germany), phone: (0 75 25) 9 40-0, fax: (0 75 25) 9 40-1 80; e-mail: redaktion@ecv.de; Internet: http://www.ecv.de. Printed by VEBU Druck GmbH, Am Reutele 18, D-88427 Bad Schussenried (Germany).

Delivery conditions: The journal is published monthly. Two volumes (I, II) per year. One volume includes 6 issues (supplementary issues free of charge). The journal is supplied by the publisher and by booksellers. Annual subscription rate (minimum 12 issues): 509.– DM plus postage; foreign annual subscription rate: 558.– DM plus postage. Sale per copy: 49.– DM plus postage. VAT always included. Free specimen copies are supplied by the publisher upon request.

Printed in Germany · ISSN 0004-4172