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Original Research Article

Effects of parenteral nutrition of ω-3 polyunsaturated fatty acid, arginine and glutamine on cellular immune status of patients following liver cancer surgery

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Abstract

Purpose: *To study the effects of parenteral nutrition (TPN), ω-3 polyunsaturated fatty acid (PUFA), L-arginine (Arg), and glutamine on cellular immunity of patients who have done the liver cancer (LC) surgery.*
Methods: *Seventy-five (75) LC patients were randomly divided into 5 groups (A - E; 15 cases each), group A, B, C, D and E, in which patients were treated with TPN, TPN + fish oil, TPN + Arg, TPN + glutamine, and TPN + ω-3 PUFA + Arg + glutamine, respectively. Before and after surgery, CD3 +, CD4 + and CD8 + were measured by antibody-sensitized erythrocyte rosette test, and IL-6, IL-10 and TNF-a were assayed with double-antibody sandwich enzyme-linked immunoassay (DAS-ELISA). IgA and IgM were measured nephelometrically.*
Results: *The levels of CD3 +, CD4 + and CD8 + in group A showed no obvious change after surgery ($p > 0.05$). However, CD3 + and CD4 + increased in groups B, C and D, while CD8 + decreased in group E ($p < 0.05$). IL-6 in group E was lower than that in any of the other four groups ($p < 0.05$). IL-10 in group A was lower than that in groups B, C and D, but lower than in group E ($p < 0.05$). The levels of TNF-a in groups B and C were lower than those in group A, but higher than that in group E ($p < 0.05$) but lower than in group D. IgA in group E was higher than in the other groups ($p < 0.05$), while IgM level in group E was lower than in groups A, B and C ($p < 0.05$).*
Conclusion: *Immunosuppressive status and cellular immunity of patients after liver cancer surgery may be improved by a combination therapy of TPN, ω-3 PUFAs, Arg and glutamine.*

Keywords: *Polyunsaturated fatty acid, Arginine, Glutamine, Parenteral nutrition, Hepatoma, Cellular immunity*

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INTRODUCTION

Patients with malignant tumors often suffer from malnutrition and immune disfunction. Trauma and post-operative stress response further lower immunity, which often result in poor outcomes and failure of operation [1]. Parenteral nutrition (TPN) can improve the nutritional status of

postoperative patients, but it cannot restore immunity. However, immune nutrition (the addition of immune nutrient preparations to TPN) can provide postoperative patients with adequate nutrition and inflammation elimination while boosting their immune levels [2]. ω-3 fatty acids and glutamine are common immune nutrients.

In this study, ω-3 unsaturated fatty acids, arginine and glutamine were selected as immune preparations, and the influences of which on cellular immune status of patients after liver cancer surgery were also investigated.

EXPERIMENTAL

General information on subjects

In this study, 75 liver cancer patients hospitalized from 2014 to 2016, were selected as study subjects, who were diagnosed with liver cancer through CT, serum AFP examination, and liver biopsy, and who were made up of 45 males and 30 females, aged 40 - 68 years (mean age, 45.8 ± 7.2 years). There were 35, 30 and 10 cases of tumors in the left liver, right liver, and the middle lobe of the liver respectively. Inclusion criteria were: absence of metabolic, infectious and inflammatory bowel diseases; normal liver and kidney functions; absence of other malignant tumors; non-usage of steroids and immunosuppressive agents within 1 month prior to the operation, and non-administration of chemotherapy. Additionally, each patient signed a written informed consent to the participation in the study [3]. Patients who did not meet these inclusion criteria were excluded from the study.

Study methods

The patients in this study were randomly divided into five groups (A – E), with 15 patients in each one. Patients in group A were treated with TPN, while those in group B, C, D and E received TPN + fish oil, TPN + Arg, TPN + glutamine and TPN + ω-3 PUFA + Arg + glutamine respectively. Twenty (20) patients undergoing open cholecystectomy were selected as control group (with normal immune levels). No significant difference was found in age, sex, number of T-cell sub-types, cellular inflammatory factors and immunoglobulin among the 5 experimental groups.

Composition and dosage of TPN

TPN was administered to patients after surgery through a central venous catheter placed across the subclavian vein during the operation. The total input procedure was 6 days. In accordance with the nutrition calculation rule, the daily supplemental energy was 30 kcal/kg and the nitrogen source intake was 0.20 g/kg/day, which could guarantee patients' the daily normal nutritional requirement. Compounded by a nutritionist, TPN contained 10 % Compound Amino Acid Injection (Wuxi Huarui Company), 20 % Lipofundin (fat emulsion; Braun, Germany) and intravenous glucose (50, 10 and 5 %); in addition to daily intake of adequate vitamins and trace elements. The immune nutrition preparations consisted of ω-3 fish oil, Fat Emulsion Injection (Austria Fresenius Kabi Austria GmbH), 25 g L-Arg/day (Shanghai Biochemical Pharmaceutical Factory), and 0.4 g glutamine/kg/day (Jiangsu Shenhua Pharmaceutical Co, Ltd), all of which were mixed prior to packaging and presentation.

Evaluation of biochemical indices

Following manufacturer's instructions, T-cell sub-types CD3 +, CD4 +, CD8 + were assayed with antibody sensitized erythrocyte rosette kits; cellular inflammatory factors IL-6 and IL-10 were determined by applying double antibody sandwich (DAS)-ELISA (Shenzhen Jingmei Company). TNF-a was assayed with TNF-a kit (Shanghai Xinyu Company), while IgA and IgM levels were determined by nephelometric assay by using kits (American Dade Behring Company).

Ethical approval for the human studies was given by Ethical Committee of Baoding Second Hospital (approval no. 20170101 and the experiments followed international guidelines for human studies [4].

Statistical analysis

The data are expressed in percentage, and differences were analyzed by t-test. Numerical data are presented as mean ± standard deviation (SD), and were analyzed by Chi square test using SPSS version 18. $P < 0.05$ was taken as indicative of statistically significant difference.

RESULTS

Levels of T-lymphocyte subsets in patients

The levels of CD3 + and CD4 + in the liver cancer patients were clearly lower than those of the control group, while CD8 + was higher ($p < 0.05$), which indicated that the cellular immunity of patients with pre-operative liver cancer (PLC) was lower than that of the control group (Table 1).

Table 1: T- cell subsets in pre-operative (PLC) patients

Group	N	CD3 +	CD4 +	CD8 +
PLC	75	52.29 ± 6.84	32.12 ± 4.66	36.15 ± 5.43
Control	20	70.13 ± 9.20	46.59 ± 5.07	27.14 ± 3.64
t value		-11.66	13.88	-7.42
p value		<0.01	<0.01	<0.01

Note: Values of T-lymphocyte sub-types are expressed as mean ± SD

Levels of T-cell sub-types in patients after surgery

The levels of CD3 +, CD4 + and CD8 + in group A were of no great difference from their corresponding values prior to surgery ($p > 0.05$), but significantly changed after the operation in the other groups ($p < 0.05$), which indicates that TPN and immune nutrient preparation effectively improved patients' immunity. The CD3 + and CD4 + levels in group E were significantly higher than those in group B, C, D (Table 2), showing that the effect by mixing ω-3 unsaturated fatty acids, Arg and glutamine, was better than that by using these components separately.

Table 2: Number of T-cell subs-types in patients post-surgery

Group	CD3 +	CD4 +	CD8 +
Pre-surgery	52.29 ± 6.84	32.12 ± 4.66	36.15 ± 5.43
A	53.73 ± 5.86$^{\&}$	31.91 ± 3.94	35.67 ± 5.18
B	58.60 ± 5.06$^{*\#\&}$	36.89 ± 3.59$^{*\#\&}$	30.17 ± 4.42$^{*\#\&}$
C	59.11 ± 4.97$^{*\#\&}$	35.62 ± 4.01$^{*\#\&}$	30.28 ± 3.60$^{*\#\&}$
D	59.83 ± 4.70$^{*\#\&}$	36.02 ± 4.25$^{*\#\&}$	31.09 ± 2.88$^{*\#\&}$
E	69.82 ± 5.35$^{*\#}$	43.58 ± 3.73$^{*\#}$	26.00 ± 3.64$^{*\#}$

Note: Values are mean ± SD. * $p < 0.05$ compared with pre-operative group; $^{\#}$ $p < 0.05$ compared with group, $^{\&}p < 0.05$ compared with group E. A: Patients received TPN. B: Patients received TPN + fish oil. C: Patients received TPN + Arg. D: Patients received TPN + glutamine. E: Patients received TPN + ω-3 PUFA + Arg + glutamine

Levels of cellular inflammatory factors in patients post-operation

No significant difference was in the levels of IL-6 in B, C, and D relative to group A ($p > 0.05$), but IL-6 in group E was obviously lower ($p < 0.05$). Higher amounts of IL-10 were seen in group B, C and D when compared to group A ($p < 0.05$), the level of which in E group was significantly higher ($p < 0.05$). Besides, significantly lower levels of TNF-a were obtained in group B and C than in group A ($p < 0.05$). The level of TNF-a in group D was significantly higher than that in group E.

Table 3: Cytokine levels of patients after operation

Group	IL-6 (pg/L)	IL-10 (pg/L)	TNF-a (pg/L)
Before operation	1590.18 ± 266.02	1971.92 ± 279.51	3558.72 ± 354.11
A	1593.57 ± 285.43$^{\&}$	1980.47 ± 323.71$^{\&}$	3591.42 ± 436.58$^{\&}$
B	1560.25 ± 271.19$^{\&}$	2038.92 ± 355.64$^{*\#\&}$	3278.47 ± 441.50$^{*\#}$
C	1574.73 ± 354.63$^{\&}$	2057.13 ± 302.78$^{*\#\&}$	3206.28 ± 378.61$^{*\#}$
D	1565.24 ± 297.51$^{\&}$	2069.52 ± 314.53$^{*\#\&}$	3415.09 ± 412.84$^{\&}$
E	1510.36 ± 277.35$^{*\#}$	2292.2 ± 386.55$^{*\#\&}$	3129.60 ± 386.85$^{*\#}$

Note: Values are mean ± SD. * $p < 0.05$ compared with preoperative group; $^{\#}$ $p < 0.05$ compared with group A; $^{\&}p < 0.05$ compared with group E. A: Patients received TPN. B: Patients received TPN + fish oil. C: Patients received TPN + Arg. D: Patients received TPN + glutamine. E: Patients received TPN + ω-3 PUFA + Arg + glutamine

Immunoglobulin content of patients after operation

Group E had significantly higher IgA than the pre-operative group as well as any of the other four groups ($p < 0.05$. The IgA content had no significant difference between group A and group B, C and D ($p > 0.05$). The content of IgM in group E was clearly lower than that in group A, B and D ($p < 0.05$). These results are shown in Table 4.

Table 4: Immunoglobulin levels in the 5 groups after operation

Group	IgA (g/L)	IgM (g/L)
Before operation	2.25 ± 0.58	1.40 ± 0.30
A	2.20 ± 0.67$^{\&}$	1.38 ± 0.44$^{\&}$
B	2.53 ± 0.73$^{\&}$	1.23 ± 0.28$^{\&}$
C	2.44 ± 0.86$^{\&}$	1.03 ± 0.26$^{*\#}$
D	2.56 ± 0.82$^{\&}$	1.24 ± 0.22$^{\&}$
E	2.94 ± 0.71$^{*\#}$	1.08 ± 0.23$^{*\#}$

Note: Values are mean ± SD; * $p < 0.05$ in comparison with pre-operative group; $^{\#}p < 0.05$ compared with group A; $^{\&}p < 0.05$ in comparison with group E. A: Patients received TPN; B: Patients received TPN + fish oil; C: Patients received TPN + Arg; D: Patients received TPN + glutamine; E: Patients received TPN + ω-3 PUFA + Arg + glutamine

DISCUSSION

The normal immune function of the human body is maintained by cellular immunity and humoral immunity. The immune system of post-operative

patients with malignant tumor can be easily affected by such factors as surgical trauma, drugs and malnutrition, although the suppressed immune function is gradually restored to normal 1 month after surgery [5]. What's the problem is that the reduced immunity affects the healing of surgical wounds, may causing post-operative infection and complications. Thus, it is of crucial to provide adequate immune and nutritional support for patients during the post-operative stage, owing to the fact that nutritional support is conducive to patients' recovery, status improvement and further treatment. Studies have demonstrated that T-lymphocyte sub-types and serum cytokines are important indicators of the immune system [6]. This study investigated the effect of added immune nutrients on cellular immunity.

Deep sea fish oil is rich in ω-3 unsaturated fatty acids which can enhance patients' humoral and cellular immune functions, and effectively resist inflammatory reactions. Unsaturated fatty acids as precursors of prostaglandins, are important components of cell membranes, which maintain the fluidity of cell membranes, and participate in the regulation of cellular and biochemical responses [7]. Fish oil/kg body weight/day (0.2 g) in addition to TPN has been recommended to be taken by gastrointestinal tumor patients after surgery, for reduction of inflammatory response syndrome and post-operative recovery, on the basis of the fact that ω-3 unsaturated fatty acids inhibit the formation of pro-inflammatory factors (IL-6 and TNF-a), increase the synthesis of anti-inflammatory factor IL-10, improve immune function and eliminate excessive inflammation [8,9].

As a major component of the mucosal defense system and an important protective barrier, IgA slows down viral proliferation. Appearing in the first humoral immune response, IgM is a marker of infection. T-lymphocytes are the most important immune cells in the human body; CD3 + represents mature T cells and the overall immune level, while CD4 + stimulates T-lymphocytes directly involved in the immune process, enhancing immunity. On the other hand, CD8 + is a scavenger in the immune process: which removes infected cells, and of which the main role is to suppress immunity. These three immune cells oppose one another, and together balance the immune system [10].
Studies have shown that glutamine supplementation in tumor patients after operation can inhibit the expression of TH-related cytokines, decrease endotoxin translocation and the incidence of post-operative infection, increase glutathione synthesis, and shorten recovery period [11]. In the current study, glutamine supplementation in TPN significantly increased CD3 + and CD4 + levels, and decreased CD8 + content, compared with their corresponding values before surgery. Participates in the regulation of immunity, Arginine (Arg), an essential amino acid and a source of citrulline and nitric oxide in the body, promotes the production of Il-6 and TNF-a by T-lymphocytes. Nitric oxide is a messenger molecule involved in mediating many immune responses [12,13].

Studies have also shown that animals infected with bacteria can improve lymphocyte immune function and survival rate after Arg supplementation [14]. In this study, it was found that addition of Arg to TPN significantly increased the production of anti-inflammatory factor IL-10and the synthesis of CD3 + and CD4 +; but decreased the levels of bacterial killer CD8 +. The levels of CD3 + and CD4 + in the liver cancer patients were significantly lower, but the level of CD8 + was higher, than those of the control group, which clearly indicates that the cellular immune level of the pre-operative liver cancer patients was lower than that of the control one.

The pattern of changes in the levels of anti-inflammatory factors, cytokines and immunoglobulins in the five groups relative to the pre-operative group clearly signifies an improvement in cellular immunity of the post-operative liver cancer patients by incorporation of ω-3 PUFA, Arg and glutamine into TPN.

Limitations of the study

Few participants were included in this research. What's more, this is a single-center study. Therefore, caution should be exercised in generalizing the findings.

CONCLUSION

The findings of this investigation show that the combined use of TPN, ω-3 polyunsaturated fatty acids, arginine and glutamine is more effective than the individual compounds in improving cellular immunity levels and reducing inflammatory responses in postoperative liver cancer patients.

DECLARATIONS

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

We declare that this work was done by named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. All authors read and approved the manuscript for publication. Ma Xiaoyong conceived and designed the study, Ge Changqing, Han Bing, Qie Zengwang, Kang Yanjie, Liu Bo, Zhu Ya, Wang Weihong, Tian Liqing, Feng Xin collected and analysed the data, Guo Zhixue wrote the manuscript.

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