Original Article

Cod skin peptide reduces chemotherapy-induced toxicity in gastric cancer patients

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Background and Objectives: The present study was conducted to evaluate the effect of cod skin peptide (CSPE) on chemotherapy-induced toxicity in gastric cancer patients. Methods and Study Design: A cohort of 60 gastric cancer patients for chemotherapy was randomly divided into two groups (n=30 per group), who were orally treated with either supplemental CSPE or placebo apart from chemotherapy. The hematologic and gastrointestinal toxicities experienced by the patients, as well as their Karnofsky Performance Status (KPS) as an index of quality of life was evaluated. Results: Leukocyte counts and haemoglobin levels were significantly reduced in the group treated with peptide (p<0.05), while gastrointestinal toxicity was not affected (p>0.05). KPS consists of 11 categories of quality of life, and the score denoted in deciles ranges from 100 (asymptomatic, normal function) to 0 (death). The KPS score is used to evaluate a cancer patient’s ability to function at work and home, the severity of symptoms, and the patient’s need for personal and medical care. Treatment with CSPE significantly improved the quality of life of patients, as indicated by increased KPS scores (p<0.05). Conclusions: CSPE can potentially be considered as a food supplement that can be used to improve the quality of life of cancer patients.

Key Words: cod skin peptide, gastric cancer, chemotherapy, toxicity

INTRODUCTION

Gastric cancer is a common and highly fatal disease; the extremely low 5-year survival rate (<20%) is related to the fact that most patients present with advanced disease upon diagnosis.\(^1\) Worldwide, gastric cancer accounts for ~800,000 deaths annually, making it the second leading cause of cancer deaths; however, the highest incidences are reported in developing countries. In China, the incidence of gastric cancer has increased by 11.9% during the past decade.\(^2\)

Despite the tremendous progress made over the past decades in molecular targeted cancer therapies, chemotherapy remains the standard treatment for gastric cancer, and shows therapeutic efficacy when applied before or after surgery. The most commonly used chemotherapy regimens for gastric cancer employ a combination of independently efficacious drugs: The XELOX regimen consists of 3-week cycles of oral capecitabine (1,000 mg/m\(^2\) twice daily on days 1-14 of each cycle), plus intravenous oxaliplatin (130 mg/m\(^2\) on day 1 of each cycle). However, toxicity remains a significant disadvantage of all chemotherapy regimens, and limits the dosages that can be safely delivered. Side-effects of the various chemotherapy agents range from adverse impacts on life quality (such as nausea and hair loss) to life-threatening conditions (such as immunodeficiency); moreover, the extent of toxicity can depend on the dosages administered and duration of administration.\(^2\)

Reducing the incidence and severity of chemotherapy-induced toxicity is undoubtedly an important step towards improving the overall outcomes of cancer patients.

Peptide is the most abundantly expressed protein in vertebrates, and comprises ~33% of the total systemic protein content in human bodies.\(^2\) Peptide extracts from animal bones and hides, or fish scales are collectively identified as gelatin, and the hydrolysate derivative of gelatin is known as peptide. Peptides are commonly used as nutritional supplements, and recent studies have demonstrated its broad range of bioactivities, including enhancement of corneal moisture content and viscoelasticity, and promotion of hyaluronic acid-synthesizing enzymes’ expression in human skin.\(^3\) In addition, peptides derived from animal sources, such as cod skin, exert a bioactivity of particular benefit to cancer patients.\(^6,7\) While several clinical trials have been initiated to test the benefit of peptides as a nutritional supplements in patients with cancer (http://www.clinicaltrials.gov) and other diseases,\(^4\) the benefits of administering peptides to cancer patients remain unclear. CSPE has advantages of a small molecular weight, good safety, easy absorption and digestion, and being rich in amino acids required by the human

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body. Also, studies have indicated that it may help protect against oxidative damage to the liver in vitro and in vivo, suggesting it might be beneficial for cancer patients.

In this study, we attempted to analyze the effects of a peptide extract from obtained cod skin on chemotherapy-induced toxicities in gastric cancer patients. The results of our study represent a foundation upon which CSPE may be further developed as an adjunct therapeutic nutritional supplement to improve the life quality of cancer patients undergoing chemotherapy.

**MATERIALS AND METHODS**

**Patient eligibility**

Consecutive gastric cancer patients presenting for treatment at the ZhouShan Hospital and People’s Hospital of Jiangxi Province between January and August 2012 were recruited in this study. Study enrollment was based upon the following inclusion criteria: (1) pathological and radiological-based diagnosis of gastric cancer; (2) measurable tumors (sizes used in quantitative analysis); (3) treatment-naïve status, but suitable for radio- or chemotherapy; (4) previous chemotherapy with the XELOX regimen only; (The XELOX regimen consists of 3-week cycles of oral capecitabine 1,000 mg/m² twice daily on days 1-14 of each cycle plus intravenous oxaliplatin 130 mg/m² on day 1 of each cycle); (5) KPS: The KPS consists of 11 categories denoted in deciles ranging from 100 (asymptomatic, normal function) to 0 (death), and the score evaluates the patient’s ability to function at work and home, the severity of symptoms, and the need for personal and medical care. Karnofsky quality of life score >70 (Patients with a KPS >70 are able to perform normal activities, work without special care, and are acceptable candidates to continue receiving chemotherapy); (6) age ≥18 years; (7) adequate bone marrow (WBCs ≥4,000/L, platelets ≥100,000/L), liver function (serum bilirubin level ≤2.0 mg/dL and serum transaminase level ≤100 IU/L), and renal function (serum creatinine level ≤1.5 mg/dL); (8) no prohibitive disabilities regarding listening, speaking, reading, writing, or communicating; (9) must have provided written informed consent prior to enrollment in the study.

Patients were excluded from study enrollment if any of the following criteria were met: (1) presence of a gastrointestinal obstruction; (2) on-going or history of non-drug-induced intractable vomiting; (3) history of simultaneous treatment with chemotherapeutic and radiotherapy; (4) history of combined cardio cerebral and renal complications; (5) history of mental disorders or disturbance of consciousness; (6) unclear tumor diagnosis or staging.

The demographic and tumor-related characteristics of the sixty patients who met enrollment criteria are summarized in Table 1.

**Source of peptide**

The peptide used in this study was isolated from the skin of deep-sea cod, salmon, and other deep-sea fish. The isolated product contained pure collagen peptide in powdered form without starch. The average molecular weight of the peptide mixture was ~2 kDa, and the peptide was obtained via a standard process involving steps of isolation, extraction, enzyme digestion, and purification. The peptide mixture was pure white to pale yellow in color, soluble in water, and had a slight seafood taste when dissolved in water. The peptide showed a 94.2% composition of amino acids, which consisted primarily of threonine (15.3%), isoleucine (10.1%), alanine (9.77%), arginine (8.49%), valine (8.01%), leucine (6.12%), and phenylalanine (5.56%). The fish skin peptide also featured high amounts of essential amino acids. The peptide (batch # 110502, Zhejiang Hailisheng Biotechnology Co., Ltd., 88 Hailisheng Rd., Lincheng New Area, Zoushan, Zhejiang, China) used in this study is a commercially available product used as a nutritional supplement in China. It comprises 15 amino acids with a total MW of ~1,563 Da. The standard dosage prescribed for patients in the treatment group was 0.08 g/kg/d. The authors have no conflicts of interest regarding the use of this product in the current study.

**Study design**

All study participants received standard chemotherapy regimens, individualized according to their tumor histopathological classification and clinical stage. Tumor characteristics (type and stage) and pretreatment laboratory data (white blood cell count, haemoglobin, blood urea nitrogen, creatinine, albumin, and liver function tests) were recorded. The 60 patients recruited for the study were randomly
assigned to two groups. Thirty patients received chemotherapy alone and the other 30 patients received chemotherapy plus fish collagen. Sample size was calculated by comparing two means using the following equation.

\[ N = \frac{2 \left( \frac{u_{a} + u_{\beta}}{\delta / \sigma} \right) + 1}{4} \]

\( N \), sample size; \( u_{a} \), the critical value in the standard normal distribution at significant level \( \alpha \); \( u_{\beta} \), the critical value in the standard normal distribution when probability of type II error is \( \beta \); \( \delta \), the difference between the two overall means; \( \sigma \), the estimated value of the overall standard deviation.

During the first cycle of treatment, chemotherapy was administered for 15 days, which is in accordance with the standard National Cancer Guidelines of China. Each patient was followed from the beginning until the end of the chemotherapy course. Toxicities were monitored during the first cycles and monitoring lasted until one week after the completion of chemotherapy. Chemotherapy-induced toxicities were classified by using the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE), version 3.0.25.

All patients received standard care; i.e., psychological counseling, health education, venipuncture, monitoring of diet and gastrointestinal reactions, etc. Unless otherwise indicated, no additional treatment was allowed. Thirty of the study participants were randomly selected for inclusion in the experimental group, for receipt of supplemental treatment with fish peptide (2 g/dose, twice per day). Patients were treated with fish peptide for 28 days. Treatment was initiated seven days prior to chemotherapy and continued until initiation of the next chemotherapy cycle. The 30 remaining patients serving as the control group were given a placebo made of starch. The dosage and regimen for the placebo were the same as those for the fish peptide. All patients were monitored for approximately one month after the completion of chemotherapy. To improve patient compliance, peptide was distributed daily in the hospital, and its proper dosing was recorded by nurses. All patients received follow-up phone calls on a weekly basis after leaving the hospital. During treatment, all patients were encouraged to consume a diet consisting of foods and liquids that had high contents of important nutrients (vitamins, minerals, protein, carbohydrates, fat, and water) required by the body.

The study protocol was approved by the local ethics committee and academic committee of Zhoushan Hospital (Zhoushan, China) and People’s Hospital of Jiangxi Province (Nanchang, China). All patients provided written informed consent prior to study participation.

**Toxicity assessment**

Patients received chemotherapy for one week. Blood samples were drawn prior to and on day 22 after initiation of the second chemotherapy cycle. EDTA was used as an anticoagulant. Total leukocyte and haemoglobin counts were estimated using standard methods. Hematologic toxicity grade: leukocytes \((10^9/L)\): 0, ≥4.0; I, ~3.0; II, ~2.0; III, ~1.0; IV, <1.0. Haemoglobin (g/L): 0, ≥110; I, ~95; II ~80; III, ~65; IV, <65. Platelets \((10^9/L)\): 0 ≥100; I, ~75; II, ~50; III, ~25; IV, <25. The evaluation of gastrointestinal (GI) toxicity assessed the degree of patient nausea, and was graded from grade 0 to grade IV using the following criteria: grade 0: symptomless; grade I: nausea; grade II: temporary vomiting; grade III: vomiting and in need of treatment; grade IV: vomiting that was difficult to control.

**Statistical analysis**

Data were analyzed using SPSS for Windows, Version 13.0. Chicago, IL: SPSS Inc. Differences in gender and tumor stage between the two groups were analyzed using the Chi-Square test. The \( t \)-test was used for comparisons of age and haematological indicators between the two groups. The Mann-Whitney \( U \) test and Kolmogorov-Smirnov test were used to compare values for haematological parameters and KPS scores, respectively. \( p \) values <0.05 were considered statistically significant.

**RESULTS**

**Fish skin peptides attenuate chemotherapy-induced hematologic toxicity in gastric cancer patients**

Hematologic toxicity is the most frequently reported side-effect of chemotherapy. In this study, patients receiving chemotherapy exhibited severe decreases in their leukocyte and platelet counts, and haemoglobin levels, irrespective of the treatment regimen administered (Table 2). The leukocyte count of patients in the control group was decreased by 80.0%, which was significantly greater than the 66.7% decrease observed in patients given fish skin peptide \( [66.7\% (10/30) \text{ vs } 80\% (6/24), p<0.05] \) (Tables 2-4).

<table>
<thead>
<tr>
<th>Group</th>
<th>Leukocytes ((10^9/L))</th>
<th>Hemoglobin (g/L)</th>
<th>Platelets ((10^9/L))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0   I   II  III  IV</td>
<td>0   I  II  III  IV</td>
<td>0   I  II  III  IV</td>
</tr>
<tr>
<td>Experimental, n</td>
<td>10  18  2   0   0</td>
<td>19  11  0   0   0</td>
<td>22  8  0   0   0</td>
</tr>
<tr>
<td>Control, n</td>
<td>6   14  8   2   0</td>
<td>11  18  1   0   0</td>
<td>22  7  1   0   0</td>
</tr>
<tr>
<td>Kolmogorov-Smirnov (Z)</td>
<td>-3.82</td>
<td>-2.01</td>
<td>-0.61</td>
</tr>
<tr>
<td>( p ) value</td>
<td>&lt;0.0001</td>
<td>0.045</td>
<td>0.542</td>
</tr>
</tbody>
</table>

Hematologic toxicity grade:
Leukocytes \((10^9/L)\): 0, ≥4.0; I, ~3.0; II, ~2.0; III, ~1.0; IV, <1.0.
Haemoglobin (g/L): 0, ≥110; I, ~95; II ~80; III, ~65; IV, <65.
Platelets \((10^9/L)\): 0 ≥100; I, ~75; II, ~50; III, ~25; IV, <25.

Data represent numbers of patients.

\( p \) values <0.05 were considered statistically significant. The Kolmogorov-Smirnov test was used.
Table 3. Impact of fish skin collagen peptide on chemotherapy-induced leukocyte count (10^9/L)

<table>
<thead>
<tr>
<th>Group</th>
<th>Normal</th>
<th>Decreased</th>
<th>Total</th>
<th>Incidence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experimental, n</td>
<td>10</td>
<td>20</td>
<td>30</td>
<td>66.7</td>
</tr>
<tr>
<td>Control, n</td>
<td>6</td>
<td>24</td>
<td>30</td>
<td>80.0</td>
</tr>
</tbody>
</table>

χ² = 13.1, p value = 0.001

Data represent number of patients.

*p values <0.05 were considered statistically significant. The chi-square test was used.

Table 4. Impact of fish skin collagen peptide on chemotherapy-induced hematologic toxicity (±s)

<table>
<thead>
<tr>
<th>Items</th>
<th>Groups</th>
<th>Mean±SD</th>
<th>Z value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukocytes (10^9/L)</td>
<td>Experimental Control</td>
<td>3.26±1.53</td>
<td>5.18</td>
<td>0.009</td>
</tr>
<tr>
<td>Haemoglobin (g/L)</td>
<td>Experimental Control</td>
<td>105±4.00</td>
<td>2.08</td>
<td>0.038</td>
</tr>
<tr>
<td>Platelets (10^9/L)</td>
<td>Experimental Control</td>
<td>126±18.5</td>
<td>1.04</td>
<td>0.382</td>
</tr>
</tbody>
</table>

*p values <0.05 were considered statistically significant. The nonparametric Mann-Whitney U test was used.

Table 5. Impact of fish skin collagen peptide on chemotherapy-induced gastrointestinal toxicity

<table>
<thead>
<tr>
<th>Group</th>
<th>Gastrointestinal toxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Experimental, n</td>
<td>4</td>
</tr>
<tr>
<td>Control, n</td>
<td>5</td>
</tr>
</tbody>
</table>

| Kolmogorov-Smirnov (Z) | -0.869 |
| p value               | 0.385  |

Data represent number of patients.

*p values <0.05 were considered statistically significant. The Kolmogorov-Smirnov test was used.

Further assessments of haematologic toxicity showed that 11/30 patients who received fish skin peptide developed grade I toxicity for haemoglobin level, eight developed grade I toxicity for platelet count, and 18 showed grade I toxicity for leucocyte count (Table 2 and Table 4). The number of patients showing grade I toxicity for haemoglobin levels was lower in the group receiving the collagen peptide supplemental treatment. The mean value of toxicity for the patients receiving peptide was higher than that for patients in the control group. There were statistically significant differences in the values for leucocyte numbers and haemoglobin levels between the two groups (leucocytes: 3.26±1.53 for CSPE group vs 2.79±1.62 for control group, p<0.05; haemoglobin: 105±4.00 for CSPE group vs 95.1±3.43 for control group, p<0.05) (Table 3 and Table 4).

While the toxic effects regarding haemoglobin levels were reduced in the fish collagen group, the toxicities regarding platelet and leucocyte counts were not as dramatically affected (Table 2-4). These data suggest that peptides from fish skin can protect gastric cancer patients from haematological toxicity, and in particular, haemoglobin toxicity caused by chemotherapy.

Fish skin peptides decrease chemotherapy-induced gastrointestinal toxicities in gastric cancer patients

The gastrointestinal toxicities resulting from chemotherapy in both groups were assessed using a scoring system ranging from grade 0 to grade IV. The percentages of patients who developed grade 0, I, II, III, or IV gastrointestinal toxicity in both treatment groups are shown in Table 5. The two groups did not show significant differences regarding the development of gastrointestinal toxicities (Table 5).

Effects of fish skin peptides on quality of life for chemotherapy-treated gastric cancer patients

The Karnofsky Performance Index scores for patients in this study are summarized in Table 4. In the control group, the Karnofsky score decreased (from 79.1±4.7 to 68.9±6.1, p<0.001) over the course of the chemotherapy treatment, suggesting that the patients’ life quality was considerably affected by the treatment. For patients who received the supplemental peptide treatment, the Karnofsky score also decreased (from 79.9±5.3 to 71.1±5.9, p<0.05) over the course of treatment, but to a significantly lesser extent than in the control group, p<0.05, suggesting that the supplemental treatment may help to protect the patient from experiencing some of the chemotherapy-related detrimental impacts on life quality (Tables 6-7).

DISCUSSION

Adverse effects experienced from receiving chemotherapy have largely impeded the clinical success of this treatment modality, in spite of its undeniable therapeutic efficacy in most tumor types. Older adults, who represent a major proportion of the solid tumor patient population,
Table 6. Impact of fish skin collagen peptide on quality of life of gastric cancer patients treated with chemotherapy

<table>
<thead>
<tr>
<th>Group</th>
<th>KPS</th>
<th>Before treatment</th>
<th>After treatment</th>
<th>Z</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experimental, mean±SD</td>
<td>79.9±5.3</td>
<td>71.1±5.9</td>
<td>6.40</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>Control, mean±SD</td>
<td>79.1±4.7</td>
<td>68.9±6.1</td>
<td>5.68</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>Kolmogorov-Smirnov (Z)</td>
<td>2.08</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p value</td>
<td>0.037</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data represent number of patients.

As published in Am J Clin Oncol the Eastern Cooperative Oncology Group, Robert Comis M.D., Group Chair.

$p$ values <0.05 were considered statistically significant. The Kolmogorov-Smirnov test was used.

Table 7. Impact of fish skin collagen peptide on body weight and BMI (Mean±SD)

<table>
<thead>
<tr>
<th>Items</th>
<th>Groups</th>
<th>Mean±SD</th>
<th>Z value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>Experimental</td>
<td>61.3±7.1</td>
<td>2.60</td>
<td>0.021</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>59.9±6.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td>Experimental</td>
<td>21.9±1.5</td>
<td>2.08</td>
<td>0.037</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>20.1±1.2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$p$ values <0.05 were considered statistically significant. The nonparametric Mann-Whitney U test was used.

are particularly vulnerable to chemotherapy-induced toxicity, leaving them with very limited options for cancer therapy. 1,2 The identification of adjuvant therapies that can reduce therapy-related toxicities will increase the total population of patients capable of withstanding cancer therapy. While increased amounts of time and effort have been invested to identify appropriate adjuvant therapies, an effective adjuvant therapy suitable for clinical use has yet to be identified.

In this study, we demonstrated that fish skin peptide used in combination with chemotherapy significantly reduces hematologic toxicity and improves the quality of life of gastric cancer patients receiving chemotherapy. As a nutritional supplement, fish skin peptide has been shown to exhibit several bioactive properties, including antihypertensive and angiogenic activities. 3,4 Peptide from fish skin has also been shown to protect skin from UV damage and to modulate lipid profiles and bone development in animal models. 5,6 As a potential adjuvant therapy, as suggested by our study and others, 1,2,13 fish skin peptide features several advantages. First, the peptide is composed of 94.2% amino acids, including substantial percentages of essential amino acids (15.3% threonine, 10.1% isoleucine 8.49% arginine, 8.01% valine, 6.12% leucine, and 5.56% phenylalanine). These essential amino acids can provide comprehensive nutrition, and enhance patient immunity during chemotherapy. Second, the safety of fish skin peptide is well established through its widespread use as a dietary supplement; and its risk of toxicity is considered nearly negligible. Third, the widely distributed sources of fish skin ensure ready availability of this type of peptide. Fourth, from the standpoint of costs associated with treating chemotherapy-induced toxicities, addition of fish skin collagen to a chemotherapy treatment regimen may benefit a large number of cancer patients. Nevertheless, it must be mentioned that the current investigation was a pilot study, and more clinical evidence is needed to understand the possible benefits of fish skin peptides for cancer patients receiving chemotherapy.

The mechanism by which fish skin collagen reduces chemotherapy-induced toxicities, in particular hematologic toxicity, remains unclear. We speculate that its action may be mediated by a broad range of systemic and/or tissue/condition-specific molecules and signals, such as chemokines and other immune system components. These details should be investigated in future studies to aid in attempts to develop this supplemental treatment for clinical use.

ACKNOWLEDGMENTS

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AUTHOR DISCLOSURES

None of the authors had any conflicts of interest.

REFERENCES


Original Article

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鱼皮寡肽降低胃癌患者化疗副反应

背景和目的：探讨鱼皮寡肽（CSPE）在降低胃癌患者化疗副反应的作用研究。方法与研究设计：随机将60例胃癌化疗患者分为两组（n=30/组），两组患者除化疗外，分别口服CSPE或安慰剂，比较两组患者血液学、胃肠道毒性和生活质量Karnofsky（KPS）评分。结果：安慰剂组白细胞计数和血红蛋白水平显著低于肽治疗组（p<0.05），两组胃肠道毒性无差异（p>0.05），KPS评分主要用于癌症患者工作、家庭生活状况、症状严重程度以及患者对医疗的需求等11个类别的生活质量，得分范围从100（无症状，功能正常）到0（死亡）。CSPE组KPS评分显著高于安慰剂组（p<0.05），CSPE明显提高了患者的生活质量。结论：CSPE可作为食品补充剂改善癌症患者的生活质量。

关键词：鱼皮寡肽、胃癌、化疗、副反应