

Original Research Article

Effect of bioactive whey protein-supplemented paclitaxel on nutritional status and tumor growth in a mouse model of subcutaneously transplanted triple-negative breast cancer

Xi Chen¹, Min Zhang^{2*}

¹Department of Oncology, ²Department of Thoracic Surgery, The Third Affiliated Hospital of Chongqing Medical University, Chongqing 401120 China

*For correspondence: **Email:** cx870325@163.com

Sent for review: 12 December 2021

Revised accepted: 24 May 2022

Abstract

Purpose: To study the effect of bioactive whey protein-supplemented paclitaxel on nutritional status and tumor growth in a mouse model of subcutaneously transplanted triple negative breast cancer (TNBC).

Methods: Eighty (80) mice were equally assigned to chemotherapy and control groups. The chemotherapy-treated mice received bioactive whey protein-supplemented paclitaxel chemotherapy, while the control group received paclitaxel chemotherapy only. Differences in body weight, glutathione (GSH) contents in tumor tissue and blood, tumor volume and extent of tumor suppression, serum levels of CA153 and TSGF, and survival time were determined and compared between the two groups.

Results: After 4 weeks, tumor tissue GSH was markedly lower in chemotherapy-exposed mice than in control mice, while blood GSH content in the chemotherapy group was significantly higher than that of control group ($p < 0.05$). After drug administration, volume of transplanted tumor and tumor weight were significantly lower in chemotherapy mice than in control mice, while levels of CA153 and TSGF in serum were down-regulated in chemotherapy-exposed mice, relative to control mice. Moreover, the mean survival time of mice in chemotherapy group was significantly longer than that in control group ($p < 0.05$).

Conclusion: Bioactive whey protein-supplemented paclitaxel improves the nutritional status of mice, slows down weight loss, lowers the levels of tumor growth factors, suppresses tumor growth, prolongs survival period, and improves prognosis of subcutaneously transplanted triple negative breast cancer in mice. This therapeutic strategy can potentially be used for the management of breast cancer but this requires prior successful clinical trials

Keywords: Whey protein, Paclitaxel, Triple-negative breast cancer, Mouse subcutaneous graft tumor, Nutritional status, Tumor growth

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INTRODUCTION

Breast cancer, the most rampant malignant tumor in females, is characterized by

uncontrolled proliferation of breast epithelial cells under the influence of a variety of carcinogenic factors [1]. Moreover, TNBC is a subtype of breast cancer which accounts for 10 - 15 % of

mammary carcinomas. It refers to breast tumor patients with negative estrogen receptor (ER), negative progesterone receptor (PR) and negative human epidermal growth factor receptor 2 (HER-2). Breast tumors are divided into luminal type, HER-2 (+) type and basal-like type [2].

Research has shown that TNBC is highly invasive and highly prone to local recurrence and distant metastasis, as well as poor prognosis [3]. Therefore, early detection and early treatment are required to effectively improve prognosis of the disease. Surgical treatment in combination with chemotherapy is used in the early clinical stage of TNBC, with chemotherapy applied following surgery [4]. In patients with advanced and clinically inoperable cancer, the disease can only be controlled using chemotherapy, and the main clinical treatment regimen comprises anthracycline and taxane drugs [5].

It has been reported that body weight can be used as an index of nutritional status. Nutritional status also affects the outcome of TNBC, and it is a crucial indicator of the quality of life of patients, while tumor growth is an important indicator of treatment efficacy and prognosis [6]. Based on detailed studies on treatment strategies, neoadjuvant chemotherapy (NCT) has been applied in clinical practice. Indeed, NCT effectively reduces the degree of tumor deterioration, decreases the level of tumor growth factor, and inhibits tumor growth [7]. Bioactive whey protein-ABD is a high-quality multi-component protein extracted from milk at low temperature, thereby maintaining optimal glutathione (GSH) concentration which enhances immunity and improves antioxidant capacity [8]. Studies have shown that bioactive whey protein corrected malnutrition and improved the quality of life of tumor-bearing mouse model of pancreatic cancer [9].

However, not much work has been carried out on mouse model of subcutaneously transplanted TNBC. In this research, subcutaneously transplanted tumor model of mice with triple negative breast cancer was established and used to study the effects of bioactive whey protein-supplemented paclitaxel on nutritional status and tumor growth [10].

EXPERIMENTAL

Animals

Forty (40) female SPF BALB/ C mice aged 3 - 5 weeks were bred at temperature range of 21 - 25 °C, and humidity of 45 - 65 %, in the Animal

Experiment Center of Anhui Medical University. The study received approval from the Animal Ethics body of The Third Affiliated Hospital of Chongqing Medical University (approval no. 20210212), and was conducted in line with the "Principles of Laboratory Animal Care" (NIH paper) [11].

Cell lines

Human TNBC cell lines were cultured in complete medium and incubated in a constant temperature incubator at 36 °C in a humidified atmosphere containing 6 % carbon dioxide. The cells were passaged when the petri dish was 80 % full. A mouse model of subcutaneously transplanted triple-negative breast cancer was established using well-growing breast cancer cells at logarithmic growth stage. Sufficient number of cells were suspended in serum-free DMEM for adjustment of cell concentration, and 150 µL of the cell suspension was inoculated subcutaneously on the back of each mouse. On the 9th day, the tumors were palpated subcutaneously on the back of mice. The successfully established triple-negative breast cancer model mice were assigned to chemotherapy and control groups, each with 20 mice.

Study design and treatments

Mice in control group were given paclitaxel chemotherapy (6 mg/kg). The drug was solubilized in physiological saline and given intraperitoneally. The mice in the chemotherapy group were given bioactive whey protein-ABD-supplemented paclitaxel. A suspension of the bioactive whey protein was prepared in sterilized drinking water, and it was administered at a level of 130 mg/mL, plus the same paclitaxel chemotherapy in the control group. The whey protein was replaced once a day to ensure normal dietary protein intake of mice.

The diameter of the transplanted tumor was measured with vernier calipers, while mice were weighed with an electronic scale. All animal deaths were carefully noted, and the survival period of the mice in each group was recorded. After 28 days, all mice were sacrificed via decapitation under carbon dioxide anesthesia. Blood samples were collected *via* the retro-orbital plexus, and whole lumps were cut out, followed by measurement of tumor volumes.

Evaluation of parameters/indicators

Mice in each group were weighed using an accurate electronic scale.

GSH contents of blood and tumor tissues

Whole tumor tissue homogenate was prepared, and after centrifugation, the content of GSH in the supernatant was determined. The blood samples were centrifuged to obtain sera. The tumor tissue and serum samples were successively added to 96-well plate. Then, 150 μ L of total glutathione detection solution was added, followed by full mixing for 5 min, and addition of NADPH solution. The reaction was allowed to proceed for 20 min, after which absorbance was read at 410 nm. Total glutathione content was calculated from a GSH standard calibration curve.

Serum levels of CA153 and TSGF

Morning fasting venous blood (3 mL) was obtained from each mouse, and the serum was separated after centrifugation for 4 min. A portion of the serum was used for assay of CA153, while the other portion was frozen at -80°C and used for assay of TSGF.

Tumor volume

The volume of the excised transplanted tumor in mouse was measured using vernier calipers.

Survival of mice

The survival of mice in both groups of mice was recorded and compared.

Statistics

Data analysis was done with the SPSS 20.0 package. All measured data were consistent with normal distribution, and are presented as mean \pm SD. Independent sample *t*-test was applied for inter-group comparison, while paired sample *t*-test was used for comparison pre-and post-treatment. Statistical data are expressed as numbers and percentages {n (%)}, and chi square (χ^2) test was used for comparison between groups. Values of $p < 0.05$ were

considered indicative of statistically significant differences.

RESULTS

Mean body weight

On days 20 and 28, the mean body weights of mice were markedly higher in chemotherapy group than in control mice. These data are shown in Table 1.

Table 1: Body weight of the two groups of mice (mean \pm SD, n = 20)

Group	On day 20 (g)	On day 28 (g)
Chemotherapy	23.25 \pm 1.45	21.89 \pm 2.12
Control	19.12 \pm 1.32	16.23 \pm 1.25
<i>T</i>	9.419	10.285
<i>P</i> -value	<0.001	<0.001

GSH levels of tumor tissues and blood

After 4 weeks of the trial, the tumor content of GSH was markedly lower in chemotherapy group than in control group, while blood GSH content of the chemotherapy group was markedly higher than control values ($p < 0.05$; Table 2).

Table 2: GSH levels in tumor tissues and blood (mean \pm SD, n = 20)

Group	Content of GSH in tumor tissues ($\mu\text{mol/L}$)	Content of GSH in blood ($\mu\text{mol/L}$)
Chemotherapy	33.12 \pm 17.54	20.45 \pm 2.32
Control	52.45 \pm 10.23	15.26 \pm 6.38
<i>T</i>	4.257	3.419
<i>P</i> -value	0.0001	0.0015

Weight and volume of transplanted tumor

Before drug administration, volume of transplanted tumor was comparable in the two groups. However, after drug administration, the volume and weight of transplanted tumor were markedly lower in chemotherapy group than in control group ($p < 0.05$; Table 3).

Table 3: Comparison of weight and volume of transplanted tumor between the two groups (mean \pm SD, n = 20)

Group	Transplanted tumor volume (mm^3)		Mean tumor weight (g)
	Before administration	After administration	
Chemotherapy	88.12 \pm 62.35	212.23 \pm 104.25	0.50 \pm 0.58
Control	85.86 \pm 54.23	325.43 \pm 223.45	0.92 \pm 0.12
<i>T</i>	0.122	2.053	3.173
<i>P</i> -value	0.903	0.047	<0.001

Serum CA153 and TSGF levels

There were markedly down-regulated levels of CA153 and TSGF in chemotherapy group, relative to control mice, as depicted in Table 4.

Table 4: Comparison of serum levels of CA153 and TSGF levels between both groups (mean \pm SD, n = 20)

Group	Serum CA153 (U/mL)	Serum TSGF (U/mL)
Chemotherapy	30.45 \pm 8.63	55.25 \pm 2.56
Control	54.89 \pm 15.58	64.23 \pm 3.14
T	6.137	9.913
P-value	<0.001	<0.001

Survival time of mice

The average survival time of mice was markedly longer in chemotherapy group than in control mice ($p < 0.05$; Table 5).

Table 5: Survival times in the two groups of mice

Group	Mean survival time (days)
Chemotherapy	38.12 \pm 8.23
Control	22.45 \pm 5.36
T	7.135
P-value	<0.001

DISCUSSION

Malignant tumor is caused by uncontrolled cell growth and proliferation. Mammary carcinoma ranks amongst the most rampant malignancies in women. It is characterized by high incidence, high recurrence rate, high metastasis rate, drug resistance and poor prognosis. The condition of the patients may be stabilized through chemotherapy, endocrine therapy and targeted therapy. At present, chemotherapy is one of the most effective means of killing cancer cells and treating tumors. Clinically, paclitaxel drugs are the commonly used chemotherapy drugs [12].

Chemotherapy drugs are cytotoxic. Thus, they produce toxic and adverse side effects. Nausea and vomiting are amongst the most common adverse reactions to chemotherapy. The therapeutic effects of drugs are affected by dietary intake and weight loss, and nutritional status is closely related to prognosis [13]. Therefore, for triple-negative breast cancer chemotherapy patients, there is need for close monitoring of nutritional status. Timely nutritional intervention improves the treatment of patients with drug resistance, thereby enhancing prognosis.

Whey protein is extracted from milk. It is rich in β -lactoglobulin, alpha-lactalbumin, immunoglobulin, and lactoferrin. It is highly nutritious, and it is easily digested and absorbed. Bioactive whey protein (ABD active factor) is one of the high-quality protein supplements for humans. It is extracted from milk using low-temperature extraction technology, and it has higher nutritional value than whey protein, being easier to digest and absorb [14]. In recent years, studies have shown that the combination of bioactive whey protein with chemotherapy effectively slowed down weight loss in breast tumor-bearing mice, improved nutritional status, enhanced tolerance of chemotherapy, effectively inhibited tumor growth, and enhanced their standard of life [15].

In the present research the mean body weights of mice in the chemotherapy group on the 20th and 28th days were significantly higher than the corresponding mean weights of mice in the control group. These results indicate that bioactive whey protein-supplemented paclitaxel effectively slowed down the weight loss and improved the nutritional status of the mice. After drug administration, the volume and average weight of transplanted tumor were appreciably reduced in chemotherapy group, relative to the corresponding control values, suggesting that bioactive whey protein-supplemented paclitaxel effectively suppressed tumor growth.

Bioactive whey protein (ABD active factor) is a high-quality protein containing a variety of bioactive components which promote the biosynthesis of glutathione and improve its levels *in vivo* [16]. Glutathione (GSH) is a tripeptide composed of glutamic acid, cysteine and glycine. Glutathione exists in two forms: oxidized form (GSSG) and reduced form (GSH). The latter is an important antioxidant (reducing agent) which participates in REDOX reactions *in vivo*. It is involved in detoxification, biotransformation, and normal immune function by converting harmful substances into harmless products prior to their elimination from the body [17].

When tumors are treated with chemotherapy, oxygen free radicals are produced, causing oxidative damage. It has been reported that whey protein promotes the synthesis of GSH, inhibits chemotherapy-induced generation of oxygen free radicals, and reduces oxidative damage to the body. Moreover, it exerts an anti-tumor effect. Serum CA153, a glycoprotein molecule derived from peptide epithelial mucin, is a tumor-related protein. Serum level of CA153 is significantly increased in breast cancer patients. It is the most important specific marker

of breast cancer, and an important indicator of postoperative efficacy in breast cancer patients [18]. Serum TSGF, a malignant tumor-specific growth factor, is part of a group of malignant tumor-related proteins which are expressed, infiltrated and transferred by tumor cells, thereby enhancing vascular proliferation of malignant tumors [19].

In this study, after 3 weeks, tumor tissue GSH content in chemotherapy group was significantly lower than that in control group, and blood GSH content in model group was significantly higher than that of control group. The pattern of changes in GSH content shows that whey protein enhanced GSH formation in mice with transplanted tumor, thereby improving the nutritional status of the mice, leading to enhanced immunity and improved curative effect. Due to extra needs as a result of cell proliferation, the content of GSH in tumor tissues is usually higher than that in normal cells, resulting in rapid consumption of GSH in tumor cells. When the *in vivo* level of GSH is increased, the tumor tissue produces negative feedback inhibition and consumption, resulting in low GSH content in tumor cells, which can promote apoptosis of the tumor cells.

The serum levels of CA153 and TSGF in chemotherapy group were significantly lower than those in control group, indicating that bioactive whey protein-assisted paclitaxel effectively reduced the levels of these factors and inhibited the metastasis and growth of transplanted tumor. The mean survival time of animals was markedly longer in chemotherapy category than in controls, indicating that bioactive whey protein-supplemented paclitaxel improved the nutritional status, prolonged the survival time, and improved the quality of life of mice with subcutaneously transplanted triple-negative breast cancer.

CONCLUSION

The results obtained in this study suggest that paclitaxel-supplemented bioactive whey protein (ABD active factor) may enhance the nutritional status of women with triple-negative breast cancer during chemotherapy. It slows down weight loss, increases *in vivo* levels of GSH, regulates serum levels of CA153 and TSGFs, inhibits tumor growth, and improves quality of life. However, due to limited number of models of subcutaneously transplanted tumor, and the short time of the research, it is necessary to establish more models for further analysis of the long-term effect of this treatment on nutritional status, and its impact on tumor growth.

DECLARATIONS

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

We declare that this work was performed by the authors named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Min Zhang designed the study, supervised the data collection, and analyzed the data. Xi Chen interpreted the data and prepared the manuscript for publication. Xi Chen supervised the data collection, analyzed the data and reviewed the draft of the manuscript.

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