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Original Research

Effect of glutamine on the immune function of paclitaxel intervention in ovarian cancer mice

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Abstract: To analyze the effect of amide Valley on the immune function of ovarian cancer mice treated with paclitaxel. Fifty SPF female BALB/c mice were selected as experimental subjects, and the mice were divided into five groups, namely normal control group, tumor control group, paclitaxel group, glutamine group and combined intervention group, with 10 mice in each group. Except for the normal control group, the other 40 mice were all subjected to ovarian cancer modeling. After modeling, the tumor control group mice were injected with saline at a dose of 25 mg/kg; the mice in the paclitaxel group were given a dose of 25 mg/kg. Intraperitoneal injection of paclitaxel; mice in the glutamine group were intraperitoneally injected with glutamine at a dose of 25 mg/kg; mice in the joint intervention group were intraperitoneally injected with glutamine and paclitaxel at a dose of 25 mg/kg once daily. The normal control mice did not receive any treatment. Observe and compare the status of the five groups of mice and regularly measure the quality of the mice; after 2 weeks of treatment, the CD3+, CD4, CD4+, CD8, CD8+ of the T lymphocyte subsets of each group of mice were detected by flow cytometry calculate the ratio of CD4 / CD8. The tumor formation rate of mouse ovarian cancer was 95% (38/40). The hair color, diet, excretion and activity of mice in the normal control group were normal, while the hair of the remaining four groups of ovarian cancer mice was sparse and dull compared with those in the normal control group, and the diet was decreased and the action was retarded. By measuring the body mass of mice at a regular time, we found that before treatment, the body mass of the other four groups of ovarian cancer mice increased significantly ($P< 0.05$) because of the tumor body compared with the normal control group mice; after treatment, compared with the other ovarian cancer mice group, the body mass of the combined intervention group mice decreased significantly, the difference was statistically significant ($P< 0.05$), suggesting that the tumor body was reduced. Compare with normal control mice, CD3+, CD4, and CD4 / CD8 of other ovarian cancer mice groups were significantly decreased, and CD8+ was significantly increased, the differences were statistically significant ($P< 0.05$); There was no significant difference in the levels of CD3+, CD4+, CD4 / CD8, and CD8+ between the tumor control group and the paclitaxel group ($P> 0.05$). Paclitaxel does not improve the immunity of patients during the treatment of ovarian cancer. Glutamine is effective immunomodulation. By regulating paclitaxel-treated ovarian cancer mice, it can simultaneously treat ovarian cancer, significantly improve the immune function of ovarian cancer mice, thereby improving the anti-tumor ability, and provide the possibility of significantly improving the body's immunity of ovarian cancer patients. Clinical research and long-term prognosis still need to be confirmed by further studies.

Key words: Glutamine; Paclitaxel; Ovarian cancer; Immune function.

Introduction

Ovarian cancer is a relatively common malignant tumor in the female reproductive system, with a higher incidence, second only to uterine body cancer and cervical cancer. The early onset of ovarian cancer is concealed, there are no obvious clinical symptoms and signs, and the diagnosis is difficult. A large number of patients have developed to an advanced stage when diagnosed with ovarian cancer, and tumor invasion and metastasis have appeared. Paclitaxel is currently the most excellent natural anti-cancer drug and is widely used in the treatment of ovarian cancer. Many studies (1, 2) have found that paclitaxel has a significant effect on ovarian cancer and promotes improvement in the patient's condition. However, due to the patient's weak immune system and poor drug resistance, there are obvious adverse reactions that seriously affect the prognosis of patients (3-8). As immunomodulation, glutamine is the most abundant non-essential amino acid in the human body, and it has a significant effect on improving the immune ability of patients with infections, burns, and tumors (9-19). In order to improve the patient's body immunity and reduce the adverse reactions against the treatment of patients with ovarian cancer, this experiment selected 50 SPF female BALB/c mice as the test object to analyze and discuss glutamine The effect of amide on the immune function of ovarian cancer mice treated with paclitaxel is now reported as follows.

Materials and Methods

Materials

Fifty SPF female BALB/c mice, weighing about 22 g, were purchased from Jiangsu Ailing Biotechnology Co., Ltd.; human ovarian cancer cell line A2780 was purchased from Shanghai Huiying Biotechnology Co.,

Ltd.

Equipment

Fetal bovine serum was purchased from Jiangsu Elisa Biotechnology Co., Ltd .; Aosu (paclitaxel injection) was purchased from Jiangsu Oxicon Pharmaceutical Co., Ltd .; RPMI 1640 medium was purchased from Thermo Fisher Scientific (China) Co., Ltd .; KL05A centrifuge was purchased from Hunan Kaida Scientific Instrument Co., Ltd.; and CytoFLEX flow cytometer was purchased from Beckman Kurt Co., Ltd., USA.

Experimental grouping

50 female BALB/c mice of SPF grade was selected as the experimental objects, and the mice were divided into five groups: normal control group, tumor control group, paclitaxel group, glutamine group, and joint intervention group, with 10 mice in each group Mice. Except for the normal control group without treatment, the remaining 40 mice were all modeled on ovarian cancer.

Ovarian cancer modeling

The human ovarian cancer cell line A2780 was cultured in RPMI 1640 medium under the conditions of 5% CO_2 and 37 °C using 10% concentration of fetal bovine serum, and passage every 2 days. Take tumor cells in the logarithmic growth phase, digest with 2.5 g/L trypsin, wash twice with phosphate buffer saline (PBS), then centrifuge at 1500 r/min for 5min, Use RPMI 1640 culture medium (without fetal bovine serum) for suspension, and adjust the cell density of 1 × 109 / ml. The left armpits of 40 BALB/c mice to be treated were inoculated subcutaneously with 0.1 mL of cell suspension. After the tumors in the mice had grown to 50 mm^2, the mouse model of ovarian cancer was successfully established.

Experimental methods

Mice in the normal control group received no treatment; mice in the tumor control group received an intraperitoneal injection of saline at a dose of 25 mg/kg; mice in the paclitaxel group received an intraperitoneal injection of paclitaxel at a dose of 25 mg/kg; mice in the glutamine group received an intraperitoneal injection of glutamine at a dose of 25 mg/kg; mice in the combined intervention group received an intraperitoneal injection of glutamine and paclitaxel at a dose of 25 mg/kg, each Once a day for 5 days.

Detection methods

The immune index of mice was detected by T lymphocyte subsets in the spleen. The mice that had been inoculated for 20 days were sacrificed by cervical dislocation, and their spleens were taken and prepared as cell suspensions. After culture sampling, freeze centrifugation and other operations, etc. add anti-CD3 antibody (PE-Cy7 label), anti-CD4 antibody (FITC label), anti-CD8 antibody (PE label) under dark conditions according to the instructions. Shaking and mixing uniformly, standing for 30 min under low temperature and light-proof conditions, and wash with Buffer solution. CytoFLEX flows cytometry was used to detect the ratio of CD3+, CD4+, CD8+, and CD4 / CD8.

Observation indicators

After the successful modeling of ovarian cancer mice, observe the activities of mice, hair color gloss, diet excretion and other aspects. The body weight of mice was measured every 3 days.

Statistical methods

The data were statistically analyzed and processed by SPSS18.0 software, and the measurement data were expressed by the t-test, mean standard deviation, and multi-component mean was analyzed by variance analysis and Q test. When $P< 0.05$, the difference was statistically significant.

Results

Comparison of signs and body mass of mice in each group

The normal control mice had normal signs of hair color, activity, and diet excretion, with no significant changes in body size. On the 10th day after modeling, 38 mice had a certain volume of tumor nodules in the body, and the ovarian cancer modeling rate was 95% (38/40). The 2 mice that failed tumor formation was the paclitaxel group One, one in the glutamine group. Compared with normal control mice, the other four groups of ovarian cancer mice showed signs of sparse and dull hair, decreased diet, and slow movement.

Through regular measurement and comparison of the body weight of the mice, it was found that the body weight of the other four groups of ovarian cancer mice increased significantly due to the tumor before treatment ($P< 0.05$); After treatment, the body mass of the combined intervention group mice gradually decreased after 3, 6, and 9 days of treatment, which was significantly different from that of the other ovarian cancer groups mice, with statistical significance ($P< 0.05$), suggesting that the tumor body in the mouse was reduced (Figure 1). Figure 1 shows that the body mass of mice in the tumor control group, glutamine group and paclitaxel group increased with time, and the tumor control group increased the most; the body mass of mice in the combined intervention group decreased significantly, while the body mass of mice in the normal control group did not change significantly.

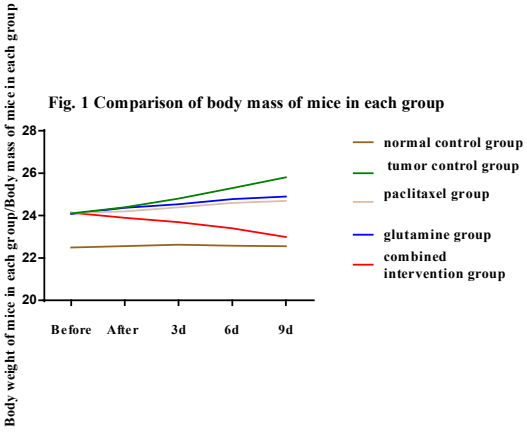

Figure 1. The abscissa is time and the ordinate is mouse body mass.

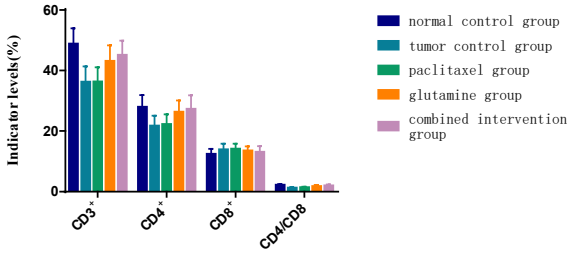

Figure 2. The abscissa is the relevant index of mouse T lymphocytes, and the ordinate is the index level.

Comparison of T lymphocyte indices in mice of each group

Compared with normal control mice, CD3+, CD4+ and CD4/CD8 in other ovarian cancer mice groups were significantly decreased, and CD8+ was significantly increased, the difference was statistically significant (P< 0.05), suggesting that all mice in the ovarian cancer group Immunity decline; there was no significant difference in the levels of CD3+, CD4+, CD4/CD8, and CD8+ (P> 0.05) between the tumor control group and the paclitaxel group (P> 0.05), suggesting that paclitaxel had no immunomodulatory effect; CD4/CD8 were significantly higher than other ovarian cancer mouse groups, CD8+ was significantly lower than other ovarian cancer mouse groups, the difference was statistically significant (P< 0.05), suggesting that glutamine has a significant effect on improving immunity in ovarian cancer mice (Figure 2). As shown in Figure 2, CD3+, CD4+, and CD4/CD8 were significantly decreased and CD8+ was significantly increased in other ovarian cancer mice compared with normal control mice. The levels of CD3+, CD4+, CD4/CD8, CD8+ in the combined intervention group were closest to those in normal mice.

Discussion

Ovarian cancer is a common malignant tumor in the female reproductive system in clinical practice, with a higher incidence, second only to uterine body cancer and cervical cancer. The early onset of ovarian cancer is concealed, there are no obvious clinical symptoms and signs, and the diagnosis is difficult. A large number of patients have developed into the middle and advanced stage when ovarian cancer is diagnosed, and most of them have tumor invasion and metastasis, which poses a serious threat to the life safety of patients (20). Seeking a low side effect, an effective and stable treatment method is the main problem that needs to be solved urgently at present.

Through continuous research on tumors, the study found that paclitaxel can promote the induction to tubulin polymerization and inhibit depolymerization by dynamically imbalance tubulin and its dimers related to cell mitosis, thereby inhibiting the mitosis of cancer cells, thereby starting to the role of anti-cancer, it is the best natural anti-cancer drug at this stage, widely used in the treatment of ovarian cancer, breast cancer and lung cancer, and some head and neck cancers (21). However, due to the rapid decline of immunity and poor physical fitness of most cancer patients, it is easy to have obvious adverse reactions in the course of treatment, so improving the immunity of patients is a major problem in clinical treatment. According to the research, glutamine is the most abundant non-essential amino acid in the human body and can be used as immunomodulation, which has a significant effect on improving the immune capacity of patients after infection, burns, tumors and other operations, and greatly reduces the adverse reactions to the treatment of patients (22).

T cells are the most important regulatory part of immune cells, fulfilling the function of the immune response to up-regulation and down-regulation. CD3+, CD4+, and CD8+, as the three major subpopulations of T cells, are important cellular immune effector cells. Therefore, by measuring the CD3+, CD4+, CD8+, and CD4/CD8 ratios of the body as indicators to evaluate the immune status of the body cells. Some studies have found that CD4+ can effectively regulate the immune response activity, by promoting the secretion of B cells to release antibodies, and then play a role in tumor suppression. If the dynamic imbalance of T lymphocyte subsets occurs, the body's immunity will be significantly reduced and the problem of promoting tumor growth will occur (23).

In order to improve the clinical effect of treating ovarian cancer, improve the body immunity of ovarian cancer patients, and enhance the tolerance of ovarian cancer patients to treatment, this experiment selected 50 SPF female BALB/c mice as the experimental objects. To investigate the effect of glutamine on the immune function of ovarian cancer mice treated with paclitaxel. The results of the study found that the modeling rate of ovarian cancer in mice was 95% (38/40). The mice in the normal control group had normal coat color, diet, excretion, and activity. The remaining four groups of ovarian cancer mice had thinner and fuller hair than the normal control group. By measuring the body weight of mice regularly, it was found that before treatment, compared with normal control mice, the body weight of the other four groups of ovarian cancer mice increased significantly due to tumor tumors (P< 0.05); after treatment, compared with the other ovarian cancer mice group, the body mass of the combined intervention group mice decreased significantly, the difference was statistically significant (P< 0.05), suggesting that the tumor body shrinkage. Compare with normal control mice, CD3 +, CD4 + and CD4/CD8 of other ovarian cancer mice groups were significantly decreased, and CD8 + was significantly increased, the differences were statistically significant (P< 0.05); the combined intervention group and glutamine group The CD3 +, CD4 + and CD4/CD8 of mice were significantly higher than those of other ovarian cancer mice, and CD8 + were significantly lower than that of other ovarian cancer mice. The difference was statistically significant (P< 0.05). There was no significant difference in the levels of CD3 +, CD4 +, CD4/CD8, and CD8 + between the tumor control group and the paclitaxel group (P> 0.05). The results showed that paclitaxel did not improve immunity in the treatment of ovarian cancer, and glutamine could improve the immunity of ovarian cancer mice with paclitaxel intervention, effectively improve the therapeutic effect of paclitaxel, and play a multiplier effect with half the effort.

The experimental results are consistent with those of

DeBerardinis and Cheng (19) that reviewed the protean roles of glutamine in cancer, both in the direct support of tumor growth and in mediating some of the complex effects on whole-body metabolism that are characteristic of tumor progression. Compared with the control group, the levels of CD3+ and CD4+ in the observation group increased significantly, while the levels of CD8+ decreased significantly, and the difference was statistically significant ($P< 0.05$). The difference was statistically significant ($P< 0.05$)." It is fully demonstrated that glutamine can effectively improve the body immunity of cancer patients, significantly reduce the occurrence of adverse treatment reactions to patients, and can be used as immunomodulation for the clinical treatment of cancer patients (24).

A large number of literatures (25-32) reported that glutamine has an effective effect on improving the immunity of patients with various tumors, such as gastric cancer, lung cancer, intestinal cancer, burns, sepsis and other critical diseases. Improve the prognosis and nutritional status of patients. Foreign studies (33-39) have found that glutamine can directly act on immune system cells by taking human cells, mice and so on as the research object, thus improving the immunity of the body. The indirect mechanism is to maintain the intestinal barrier function or maintain the antioxidant glutathione to affect the immune system of the body.

In summary, paclitaxel does not improve the immunity of patients during the treatment of ovarian cancer. Glutamine, as effective immunomodulation, can be used in the treatment of ovarian cancer by regulating paclitaxel-treated ovarian cancer patients. At the same time, significantly improve the immune function of patients with ovarian cancer, thereby improving anti-tumor ability and improving the prognosis of patients.

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