

Ganoderma lucidum spore oil enhances the effect of paclitaxel, improves the tolerance to paclitaxel and prolongs the survival in Lewis tumor-bearing mice

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Author contributions

Hongfei Cai, Zhaojian Jiang, and Qin Wang designed the study. Zhaojian Jiang and Lin Cao wrote the manuscript. Cheng Yuan, Yaming Han, Qin Wang, Jing Li, Wendong Xu, and Juyan Liu revised the manuscript. Hongfei Cai, Zhaojian Jiang, Qin Zhang, and Qin Wang carried out the experiments. Hongfei Cai, Zhaojian Jiang, and Lin Cao analyzed the data. All authors approved the final version of the paper.

Competing interests

The authors declare no conflicts of interest.

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Abbreviations

GLSO, *Ganoderma lucidum* spore oil; PTX, paclitaxel; BMN, bone marrow nucleated cell count; CCK-8, cell counting kit-8; NK cells, natural killer cells; LDH, lactate dehydrogenase; NSCLC, non-small cell lung cancer; WBC, white blood cells; RBC, red blood cells; HGB, haemoglobin; PLT, platelets; RET, reticulocytes; IFN- γ , interferon-gamma; ConA, concanavalin A; MST, median survival time; PBS, phosphate buffered saline; NS, normal saline; SD, standard deviation; ANOVA, analysis of variance.

Citation

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Abstract

Purpose: This study aims to investigate whether *Ganoderma lucidum* spore oil (GLSO) could enhance the effect of paclitaxel (PTX), improve the tolerance to PTX and prolong the overall survival of Lewis tumor-bearing mice, which has never been reported before. **Methods:** The tumor, spleen, and thymus were weighed at the end of the experiment. Whole blood was collected for hematological index analysis, and the intact femur was removed to determine the bone marrow nucleated cell count (BMN). The percentage of lymphocytes in the spleen of mice was detected by flow cytometry, the activity of NK cells was detected by LDH assay, and the proliferation index of lymphocytes was determined by CCK-8 assay. The overall and mean survival time and life extension rate were calculated using SPSS software. **Results:** Our data showed that GLSO could enhance the anti-tumor effect of PTX and prolong the survival of mice. The underlying mechanisms of the above effects might be related to the toxic reduction effect of GLSO by relieving hematotoxicity, myelosuppression and immunosuppression. Specifically, GLSO could increase the number of blood cells and bone marrow cells, alleviate the thymic index, and elevate the number and activity of NK cells in mice treated with PTX. **Conclusion:** GLSO may enhance the efficacy of PTX by boosting the activity of immune NK cells and prolong survival by counteracting PTX-induced bone marrow alterations and improving hematopoiesis. These findings suggested the promising role of GLSO in combination with PTX to extend the survival and increase the tolerance of patients in clinical chemotherapy of lung cancer.

Keywords: *Ganoderma lucidum* spore oil; Traditional Chinese Medicine; lung cancer; paclitaxel; tolerance; survival

Introduction

Lung cancer remains the leading cause of cancer incidence and mortality worldwide [1]. Paclitaxel (PTX) is one of the most substantial chemotherapeutic agents of the 20th century [2]. It was approved in 1998 for treating non-small cell lung cancer (NSCLC), which forms 80–85% of lung cancer cases. Since then, taxanes have been an essential component of chemotherapy which remains a standard option in treating NSCLC, despite the introduction of multiple targeted therapies and immunotherapy agents [3, 4]. Nevertheless, the same barriers handicapping chemotherapies were also encountered with PTX, including severe toxicity and narrow therapeutic index [5]. The adverse effects of PTX mainly include myelosuppression, neurotoxicity, cardiotoxicity and allergic reactions. How to better utilize the efficacy of PTX, reduce its adverse effects and prolong survival remain a concern and a problem that needs to be addressed in the clinical application of PTX-based chemotherapeutic drugs for lung cancer treatment.

The use of traditional Chinese medicines in conjunction with chemotherapy is common in China to minimize side effects and prolong patient survival [6]. *Ganoderma lucidum* (Curtis) P. Karst., known as “Lingzhi” in China or “Reishi” in Japan, is a well-known medicinal mushroom and traditional Chinese medicine [7]. Traditionally, it was believed that *Ganoderma lucidum* (*G. lucidum*) could extend life and promote health [8]. Nowadays, a great number of modern medical research has revealed that *G. lucidum* possesses multiple pharmacological properties, such as prolonging sleeping time [9], anti-diabetic effect [10], immunomodulatory effect [11] as well as anti-cancer effect [12].

In recent years, much research has been focused on the medicinal value of *Ganoderma lucidum* spores, especially its lipid-soluble active part *Ganoderma lucidum* spore oil (GLSO) extracted from sporoderm-broken spores using supercritical fluid CO₂. It was reported that GLSO mainly included five constituents: oleic acid (65.63%), palmitic acid (15.69%), linoleic acid (12.90%), stearic acid (4.05%), and hexadecenoic acid (1.73%) [13]. GLSO has been discovered possessing certain tumor-inhibiting effect, therapy-potentiating effects and toxicity-reducing effect [14]. Our previous study has discovered that GLSO could enhance the host immune function and increase the IFN- γ level in the serum significantly [15], and extend the average life span in *Drosophila melanogaster* by exerting antioxidant effect [16]. However, the effect of GLSO on the tumor growth and survival of the Lewis tumor-bearing mice treated with PTX remains unknown.

This study was conducted to investigate whether GLSO could influence the efficacy of PTX, the tolerance to PTX and the survival time in the Lewis tumor-bearing mice model. It's found that GLSO could enhance the anti-tumor effect of PTX and prolong the survival of mice, of which the underlying mechanisms might be related to the toxic reduction effect via relieving myelosuppression and hematotoxicity, and the immune system regulatory effect via alleviating the reduced thymus index and the number and function of splenic lymphocytes in mice, especially increasing the proportion and activity of NK cells. Together, GLSO may enhance the efficacy of PTX by boosting the activity of immune NK cells and prolong survival by counteracting PTX-induced bone marrow alterations and improving hematopoiesis. These findings suggested the promising role of GLSO in combination with PTX to extend the survival and increase the tolerance of patients in clinical chemotherapy of lung cancer.

Materials and Methods

Materials and chemicals

Ganoderma lucidum spore oil (GLSO) was manufactured by Guangzhou Hanfang Pharmaceutical Co., Ltd., Guangzhou, China. The components in GLSO have been reported before [18]. Paclitaxel was purchased from Cisen Pharmaceutical Co (China). ConA was purchased from Sigma-Aldrich (Germany).

Cell line and animals

The mouse Lewis lung cancer cells were purchased from the Laboratory Animal Center of Nanchang Research Institute of Sun Yat-sen University (Nanchang, China). Male C57BL/6 mice were purchased from Hunan SJA Laboratory Animal Co., Ltd (Changsha, China). All mice were housed in the Laboratory Animal Center of Nanchang Research Institute of Sun Yat-sen University in isolator cages within a pathogen-free environment (20–26°C, 40–70% humidity, 12/12 h day/night light cycles). Mice were given free access to regular food and water. The protocols were approved by the Animal Ethical and Welfare Committee of Sun Yat-sen University Nanchang Research Institute (Approval No. SYSUNC-IACUC-2023-0003), and conformed to the National Institutes of Health Guide for the Care and Use of Laboratory Animals (National Institutes of Health, 8th edition, 2011).

Establishment of Lewis tumor-bearing mouse animal model

Lewis lung cancer cells were subcutaneously injected into the right armpit of mice at a density of 2 million per mouse at 0.2 ml. On the 5th day after inoculation, the mice were randomly grouped according to their body weight using SPSS software as follows, GLSO (H, M, and L) + PTX those three groups contained 9 mice each and the rest groups contained 8 mice each: (1) Blank control group: mice were given normal saline (NS); (2) Solvent control group: mice were given solvent corn oil; (3) PTX group: mice were given 20 mg/kg PTX; (4) GLSO-L+PTX: mice were given 0.312 g/kg GLSO and 20 mg/kg PTX; (5) GLSO-M+PTX: mice were given 1.56 g/kg GLSO and 20 mg/kg PTX; (6) GLSO-H+PTX: mice were given 3.12 g/kg GLSO and 20 mg/kg PTX. After grouping, each group of mice was weighed daily for dosing and observed for health status. The mice were orally administrated daily with GLSO-L, GLSO-M, and GLSO-H. PTX was injected to mice intraperitoneally twice a week. After the last dose, the mice were fasted and weighed 24 h later, and the tumor, spleen and thymus were dissected and weighed to calculate the tumor suppression rate, spleen index and thymus index for each group. Blood was collected and the peripheral blood count of white blood cells (WBC), red blood cells (RBC), haemoglobin (HGB), platelets (PLT) and reticulocytes (RET) was measured.

Survival assay

Lewis lung cancer cells were subcutaneously injected into the right armpit of mice as mentioned above. On the 5th day after inoculation, the mice were randomly grouped according to their body weight using SPSS software as follows, each group contained 22 mice: (1) Solvent control group: mice were given solvent corn oil; (2) PTX group: mice were given 20 mg/kg PTX; (3) GLSO-L+PTX: mice were given 0.312 g/kg GLSO and 20 mg/kg PTX; (4) GLSO-H+PTX: mice were given 3.12 g/kg GLSO and 20 mg/kg PTX. The mice were orally administrated daily with GLSO-L and GLSO-H. PTX was injected to mice intraperitoneally twice a week. SPSS software was used to process the data in the survival experiment. Log Rank (Mantel-Cox) was used to compare the difference in median survival time (MST) between the two groups.

Measurement of Bone Marrow Number (BMN)

The intact femur of mouse was removed and the bone marrow cells were obtained using erythrocyte lysate (Sangon Biotech, China). A single cell suspension was made by filtration and 8 ml NS was added and centrifuged at 1,400 rpm for 5 min. The supernatant was discarded and the cells were resuspend with 1 ml NS, and 20 μ l of suspension were taken for counting.

Detection of spleen lymphocytes

After mice were sacrificed, the spleen was ground and filtered through a 200-mesh strainer. 1 ml erythrocyte lysate was added and terminated by 9 ml NS, and the concentration of cells was adjusted to $0.5\text{--}1.0 \times 10^8$ per ml. Cells were incubated with primary antibodies against CD3 ϵ , CD45, CD8a, CD4, CD19 and NK-1.1 (Biolegend, USA) for 30 min at 4°C. After centrifuged at 1,500 rpm for 5 min, the

supernatant was discarded and 400 μ l PBS containing 0.5% fetal bovine serum was added to resuspend the cells, and the changes in the proportions of Th cells (CD45+CD3+CD4+), Tc cells (CD45+CD3+CD8+), B cells (CD45+CD3-CD19+) and NK cells (CD45+CD3-NK1.1+) in the spleen were detected by flow cytometry.

LDH assay for mouse NK cell killing activity

The target cells YAC-L were cultured for 24 h before the experiment, and the cell concentration was diluted to 1×10^5 per ml. The lymphocytes were collected as mentioned above. The killing activity of NK cells was determined according to manufacturer's instructions using LDH assay kit (Roche, Swiss).

Lymphocyte transformation reaction induced by ConA

The lymphocytes were collected as mentioned above. The cells were suspended by RPMI 1640 medium (Gibco, USA) and divided into two groups. 5 μ g/ml ConA was added and cells were cultured for 48 h. 10 μ l CCK-8 was added to each well and cultured for 1 h. The proliferation index of lymphocytes was determined according to manufacturer's instructions using CCK-8 assay kit (Dojindo, Japan).

Statistical analysis

Data were expressed as mean \pm standard deviation (SD). Comparison of the difference among multiple groups was performed using one-way ANOVA. Two-tailed Student's t-test was used to compare the difference between two groups. SPSS 22.0 software was used to process the data. *P* values < 0.05 were considered as statistically significant.

Results

GLSO enhances the effect of PTX and prolongs the survival of Lewis tumor-bearing mice treated with PTX

To investigate the effect of GLSO on the growth of Lewis tumor treated with PTX *in vivo*, we measured the weight of tumors after following 21 days' treatment. The results of Figure 1A showed that PTX could dramatically decrease the weight of tumor, while the combination of high-dose GLSO and PTX could further inhibit the tumor growth. The tumor weight of blank control group, solvent control group, PTX group, GLSO-L+PTX group, GLSO-M+PTX group, and GLSO-H+PTX group were 7.41 ± 1.36 g, 6.27 ± 1.35 g, 3.23 ± 0.92 g, 2.75 ± 0.78

g, 2.66 ± 0.67 g, and 2.24 ± 0.50 g, respectively. The tumor inhibitory rate of PTX group, GLSO-L+PTX group, GLSO-M+PTX group, and GLSO-H+PTX group were 56.45%, 62.94%, 64.04%, and 69.75%, as shown in Figure 1B. These data indicated that the Lewis tumor-bearing mice models were successfully established, and high-dose GLSO could significantly enhance the effect of PTX in Lewis tumor-bearing mice.

The toxic effect of chemotherapy is closely related to poor life quality and survival, therefore, we conducted survival analysis to further explore the effect of GLSO on the survival of Lewis tumor-bearing mice treated with PTX. As shown in Figure 1C, PTX could drastically diminish the survival of mice, while both the combination of low-dose and high-dose GLSO could efficiently extend the life span of mice treated with PTX. The average survival time of solvent control group, PTX group, GLSO-H+PTX group, and GLSO-L+PTX group were 22.09 ± 1.22 days, 17.68 ± 1.42 days, 22.05 ± 1.49 days, and 22.55 ± 1.43 days, respectively. Furthermore, we also calculated the median survival time (MST) in each group, and the MST of solvent control group, PTX group, GLSO-H+PTX group, and GLSO-L+PTX group were 23 ± 1.85 days, 19 ± 2.93 days, 24 ± 1.76 days, and 25 ± 0.67 days, respectively. The results all indicated that GLSO could significantly increase the survival of Lewis tumor-bearing mice treated with PTX.

GLSO relieves PTX-induced hematotoxicity and myelosuppression in Lewis tumor-bearing mice

The severe side effects of PTX including hematotoxicity and myelosuppression have greatly obstructed its further application, therefore, we investigated whether the combination of GLSO could ameliorate the toxicity of PTX towards the blood and bone marrow cells. As shown in Figure 2A–2E, PTX could drastically decrease the levels of WBC, RBC, HGB, and RET in blood, meanwhile increase the level of PLT. After the combination administration of different doses of GLSO, the levels of WBC, RBC, HGB, and RET all had significantly recovered to some degree. Furthermore, we measured the BMN in the femur of mice to explore the effect of GLSO. As shown in Figure 2F, PTX could apparently reduce the level of BMN in Lewis tumor-bearing mice, while the combination of high-dose GLSO could relieve the reduction. These phenomena implied the effect of GLSO in relieving PTX-induced hematotoxicity and myelosuppression.

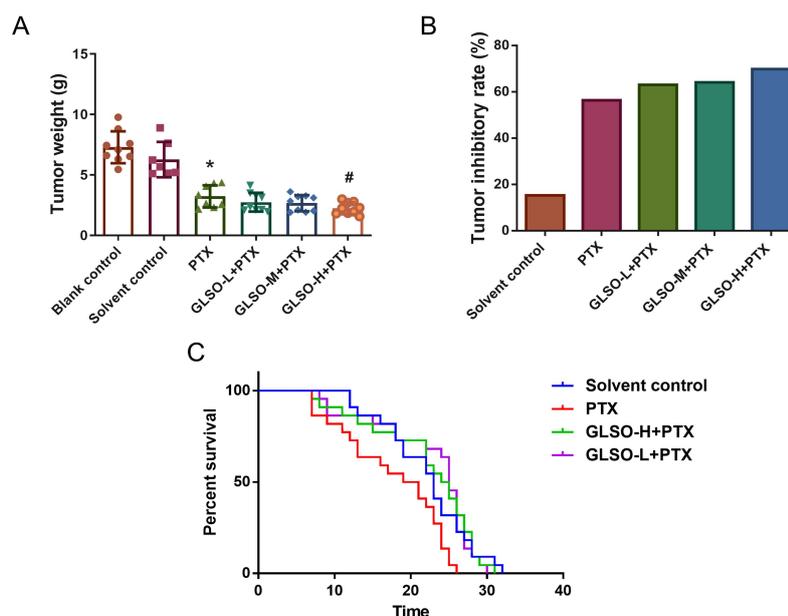


Figure 1 The effect of GLSO on the efficacy of PTX and the survival of Lewis tumor-bearing mice treated with PTX. (A) The weight and (B) inhibitory rate of tumors in each group. (C) The survival of mice in each group. Kaplan-Meier survival analysis was employed to compare the difference of survival rates between groups. Data are presented as the means \pm SD. **P* < 0.05 vs Solvent control group, #*P* < 0.05 vs PTX group.

GLSO relieves PTX-induced immunosuppression in Lewis tumor-bearing mice

Immunosuppression is another severe side effect in clinical treatment of PTX, therefore, we collected the spleen and thymus of mice and calculated organ indices. The results in [Figure 3A](#) and [Figure 3B](#) showed that, though PTX exhibited little toxic effect on the weight of spleen, the index of thymus had greatly diminished after PTX treatment. Interestingly, different doses of GLSO could relieve the inhibitory effect of PTX on thymus to some extent. Moreover, we measured the activity of NK cells and the proliferation of lymphocytes, to further elucidate the effect of PTX and GLSO on immune system. As shown in [Figure 3C](#) and [Figure 3D](#), PTX could inhibit the proliferation

of lymphocytes in spleen, while the activity of NK cells remained unchanged. Compared with PTX group, the results showed that GLSO could significantly increase the activity of NK cells in a dose-dependent manner. Moreover, the combination of medium-dose and high-dose GLSO could also elevate the proliferation index of lymphocytes in spleen. Based on the results above, the proportions of Th cells, Tc cells, B cells and NK cells were further detected by flow cytometry. As shown in [Figure 3E–3H](#), though the administration of PTX and GLSO did not alter the proportions of Th cells, Tc cells, and B cells in spleen. However, the combination of medium-dose and high-dose GLSO could significantly increase NK cells proportion, which was in consistent with our previous results of NK cells activity.

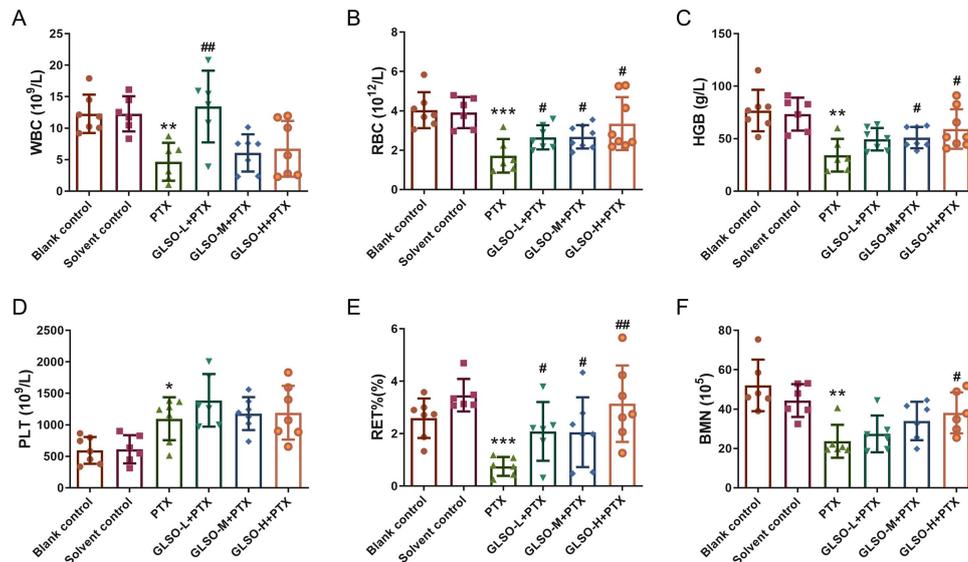


Figure 2 The effect of GLSO on PTX-induced hematotoxicity and myelosuppression. The levels of (A) WBC, (B) RBC, (C) HGB, (D) PLT, (E) RET, and (F) BMN in each group. Data are presented as the means \pm SD. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs Solvent control group, # $P < 0.05$, ## $P < 0.01$ vs PTX group.

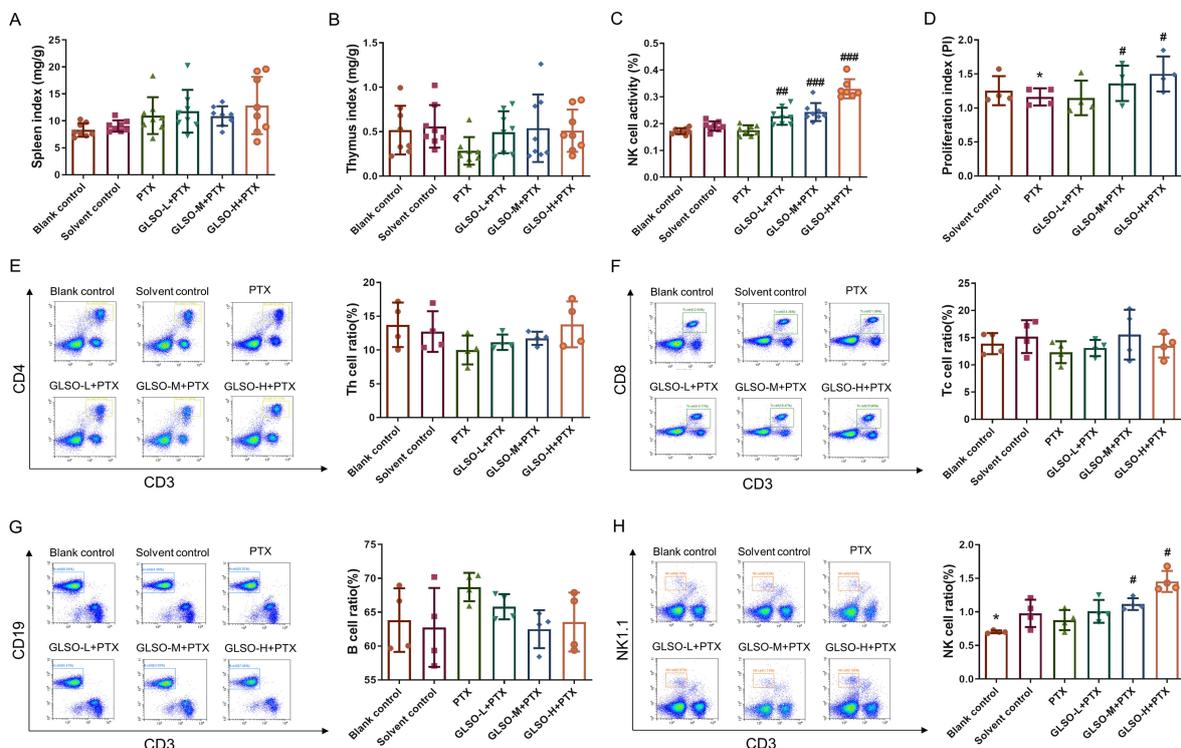


Figure 3 The effect of GLSO on PTX-induced immunosuppression. The organ indices of (A) spleen and (B) thymus in each group. (C) The level of NK cells activity in each group. (D) The proliferation index of lymphocytes in each group. The proportions of (E) Th cells, (F) Tc cells, (G) B cells, and (H) NK cells in each group. Data are presented as the means \pm SD. * $P < 0.05$ vs Solvent control group, # $P < 0.05$, ## $P < 0.01$, ### $P < 0.001$ vs PTX group.

Discussion

As the leading cause of cancer-related death worldwide, lung cancer poses a serious threat to human health. Although there are increasing new approaches to treating lung cancer, many chemotherapeutic agents remain the first line of oncotherapy, such as PTX. The current clinical use of anti-tumor chemotherapeutic drugs lack ideal selection of tumor cells with normal cells. In the process of killing malignant tumor cells, some normal tissues are also damaged to a certain extent, resulting in patients unable to tolerate chemotherapeutic drugs, which affects both the implementation of chemotherapy and the quality of life. Almost all chemotherapeutic drugs have a wide range of adverse effects, such as bone marrow suppression, gastrointestinal reaction, liver and kidney damage, cardiotoxicity, pulmonary toxicity, skin toxicity, and neurotoxicity. The same barriers in chemotherapies are also encountered with PTX, including severe toxicity and narrow therapeutic index [5], and greatly limited its further application. In addition, in the prevention and treatment of malignant tumors, it has been recognised that the treatment should not simply pursue the killing efficacy of the tumor, but focus on the improvement of the patient's quality of life and the extension of survival time.

In China, the treatment of traditional Chinese medicine combined with chemotherapy for cancer has been widely applied [17, 18]. Traditional Chinese medicine has been shown to benefit cancer patients with its ability to delay the progression of cancer and reduce complications and adverse effects from chemotherapy [19–21]. *Ganoderma lucidum* spore oil (GLSO) has been reported to have immunomodulatory [15, 22–24] and anti-cancer [25, 26] effects. It was reported that GLSO had significant immunoenhancing ability in mice, while the underlying mechanism was in correlation with ameliorated macrophage phagocytosis and NK cell cytotoxicity [27]. Therefore, we proposed whether GLSO could enhance the efficacy of PTX and increase the tolerance of body to PTX.

In this study, we investigated the effect of GLSO on Lewis tumor-bearing mice treated with PTX. Our data showed that GLSO could enhance the anti-tumor effect of PTX and prolong the survival of mice. The underlying mechanisms of GLSO might be related to its toxic reduction effect via relieving myelosuppression and improving hematological indicators and its immune system regulatory effect via alleviating the effect of paclitaxel in suppressing thymic index and the number and function of splenic lymphocytes (especially increasing the proportion and activity of NK cells) in mice. This is consistent with the currently available mechanism of *Ganoderma* spore oil. It implied that GLSO might improve hematological indicators and bone marrow hematopoiesis to help tolerance to PTX and better quality of life, which contribute to successful completion of chemotherapy, and even prolong survival. Its significance is obviously.

Natural killer cells (NK) are the first line of defence against tumors and a critical member of the body's intrinsic immunity. NK cell plays a vital role in tumor immunity. The number of NK cells in the tumor microenvironment and their function correlate with overall patient survival, progression-free survival and risk of recurrence [28]. Both mice and humans have similar NK cell localizations in the immune microenvironment of lung cancer [29, 30]. Increasing NK cell numbers and upregulating NK cell function are key to NK cell-based immunotherapy for lung cancer [31].

So our study suggested that *Ganoderma lucidum* spore oil may increase the efficacy of paclitaxel by enhancing the proportion and activity of immune NK cells and prolong survival by countering PTX-induced bone marrow alterations and enhancing hematopoiesis, which has never been reported before.

Conclusion

In summary, our study discovered that GLSO could enhance the anti-tumor effect of PTX and prolong the survival of Lewis tumor-bearing mice treated with PTX. The underlying mechanism of GLSO might be related to its toxic reduction effect and immune system regulatory effect in mice, especially increasing the proportion and

activity of NK cells. The results suggested the promising role of GLSO in combination with PTX to extend the survival and increase the tolerance of patients in clinical chemotherapy of advanced lung cancer.

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