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Antitumor activity of the sporoderm-broken germinating spores of *Ganoderma lucidum*

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Abstract

The inhibitory effects of the dormant spores, the germinating spores, the sporoderm-broken germinating spores (SBGS), and the lipids extracted from the germinating spores of *Ganoderma lucidum* on the growth of mouse hepatoma, sarcoma S-180, and reticulocyte sarcoma L-II cells were investigated, respectively. The dormant spores could be activated by germination, and thus the bioactivities of the spores might be enhanced. The sporoderm-broken spores could show much higher bioactivities than the whole spores. Both the lipids extracted from the germinating spores and the SBGS of *G. lucidum* had remarkable antitumor effects in a dose-dependent manner, and could significantly inhibit three tumors with an inhibition of 80–90%.

Keywords: Antitumor activity; *Ganoderma lucidum*; Germinating spores; Sporoderm-broken; Hepatoma; Sarcoma

1. Introduction

Ganoderma lucidum (Fr.) Karst. (Polyporaceae) is a species of basidiomycetes that belongs to Ganodermataceae of Aphyllophorales [1]. *G. lucidum* called 'Lingzhi' in China is a fungus widely used in the traditional Chinese medicine for the prevention and treatment of various kinds of diseases, such as hypertension, bronchitis, arthritis, neurasthenia, hepatopathy, chronic hepatitis, nephritis, gastric ulcer, tumorigenic diseases, hypercholesterolemia, immunological disorders, and scleroderma [1–7] in China and other countries of the Orient. Its antitumor and immune enhancing properties, along with no cytotoxicity, raise the possibility that it could be effective in preventing oxidative damage and resulting disease [8]. The potential medicinal value and wide acceptability of *G. lucidum* have attracted intense interest in the search for pharmacological compounds from these edible mushrooms [9,10]. *G. lucidum* appears to be very safe because oral administration of the extract does not display any toxicity [11,12], and merits investigation as a potential preventive agent in humans [8].

Though the fruit body of *G. lucidum* had been utilized as medicine for several thousand years in China, the spores of *G. lucidum* were realized and utilized only in the 20th century. The spores of *G. lucidum* also contain a large amount of bioactive substances like the fruit body of *G. lucidum*. The bioactivity of the spores may be higher than that of the fruit body of *G. lucidum* [13]. Recent studies on this fungus have demonstrated that the spores of *G.*

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lucidum show significant antitumor activity [14] and anti-human immunodeficiency virus-1 protease activity [13]. However, these effects are closely related to the status of the sporoderm. The breaking of the spores of *G. lucidum* can improve the release of activity, and no effect is observed when the sporoderm is not broken [14]. We have succeeded in breaking the sporoderm of the germinating spores on a large scale. The sporoderm-broken germinating spores (SBGS) have been used as health care food for the effectual prevention and treatment of immunological disorders, hypertension, hypercholesterolemia, diabetes mellitus, neurasthenia, hepatopathy, gastric ulcer, tumorigenic diseases etc. in China. The objective of the present work was to study the antitumor activity of the SBGS of *G. lucidum*. In this study, the effects of different status of spores on the mouse hepatoma, sarcoma S-180, and reticulocyte sarcoma L-II cell growths were investigated.

2. Materials and methods

2.1. Plants and spores

G. lucidum was cultivated at a base located in a 1000 m-high forested area in Fujian, China, which was established by Food Engineering Research Center of State Education Ministry, and Guangzhou Green-Enhan Bio-Engineering Co. Ltd. The spores of *G. lucidum* were collected and activated subsequently by germination. The sporoderm of the germinating spores was then broken and the broken rate could reach 99.8%. The bioactive substances were extracted from the SBGS of *G. lucidum* by supercritical carbon dioxide extraction. For the purpose of comparison, ganoderma spore powder (a dormant spore) was purchased from a local drugstore.

2.2. Animals

Seven-week-old NIH mice weighing between 20 and 22 g were purchased from the Guangdong Laboratory Animals Resource Center, Guangzhou, China. These animals were specifically pathogen free and housed under conventional conditions and fed standard laboratory chow pellets and water ad libitum.

2.3. Tumor cells and chemicals

The mouse hepatoma, mouse sarcoma S-180, and mouse reticulocyte sarcoma L-II cells were obtained from Cancer Institute, Sun Yat-Sen University of Medical Sciences. Cyclophosphamide (CTX) was purchased from Shanghai No. 12 Pharmaceutical Factory, Shanghai, China.

2.4. Animal experiments

The mouse hepatoma, sarcoma S-180, and reticulocyte sarcoma L-II cells were intraperitoneally transplanted into mice. The ascites fluid was collected on the 7th day after transplantation. The ascites tumor cells were diluted with normal saline to obtain 5×10^7 cells/ml suspension. For the experiment, 0.2 ml (1×10^7 cells) of a cell suspension was carefully inoculated subcutaneously (s.c.) into the right axilla of mice under light ether anesthesia.

Tumor-bearing mice were randomly assigned to one of eight treatment experimental groups (ten mice in each group). The mean weight per group was approximately the same. Twenty-four hours after the tumor inoculation the animals were orally treated through gastric tube for 7 days consecutively as follows: ten with 20 ml/kg per day of normal saline as a negative control (group 1), ten with 20 ml/kg per day of CTX as a positive control (group 2), ten with 8 g/kg per day in twice of the ganoderma spore powder (group 3), ten with 8 g/kg per day in twice of the germinating spores (group 4), ten with 2 g/kg per day of the SBGS (group 5), ten with 4 g/kg per day of SBGS (group 6), ten with 8 g/kg per day in twice of SBGS (group 7), ten with 5 g/kg per day of the lipids extracted from the SBGS of *G. lucidum* (group 8).

The animals were killed by cervical dislocation on the 8th day after tumor inoculation in experimental animals, i.e. the day after the last day of treatment. The tumors were rapidly removed and weighed. The antitumor activities were evaluated by the reduction in the weight of implanted tumor and inhibitory effect.

2.5. Statistical analysis

Differences between different treatment groups were analyzed for significance by multiple comparison using the analysis of variance. The P values of less than 0.05 were considered as the level of signifi-

cance for values obtained for treated groups, compared with the negative control (untreated saline control) group.

3. Results and discussion

A common feature of traditional Chinese medicine such as *G. lucidum* was the presence of multiple compounds, which could act either independently, or synergistically to elicit their pharmacological effects [10]. Therefore, the whole crude extracts from the germinating spores of *G. lucidum* were used in the present study. Since the traditional medicine generally had been used by oral administration, the inhibitory effects of the spores of *G. lucidum* on the carcinogenesis were also examined by the oral administration.

The mouse hepatoma, sarcoma S-180, and reticulocyte sarcoma L-II, which were three different pathological types of tumor cells, were often used for the investigation of the antitumor activity of medicines. The inhibitory effects of oral administration of the spores of *G. lucidum* given for 7 consecutive days starting from the day after tumor cell inoculation on the growth of the three different types of tumor cells were investigated. The tumor weights after the treatments were weighed and are shown in Figs. 1–3, respectively. The tumor weights of the negative control (group 1: untreated saline control) after the treatments for 7 days were 1.78 ± 0.13, 2.17 ± 0.16, and 2.21 ± 0.21 g for mouse hepatoma, sarcoma S-180, and reticulocyte sarcoma L-II, respectively.

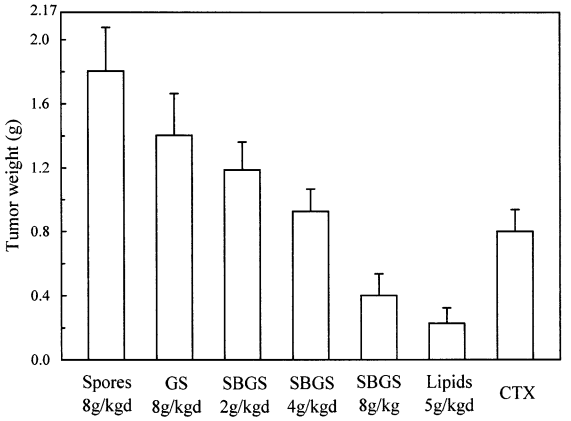

Fig. 2. The inhibitory effects of oral administration of the spores, the germinating spores (GS), the SBGS, the lipids extracted from the germinating spores of *G. lucidum*, and CTX on the growth of mouse sarcoma S-180 cell. Each column expresses the mean tumor weight ± SD of ten mice. A significant difference from the negative control (untreated saline control) group (2.17 ± 0.16 g) is indicated by $P < 0.001$.

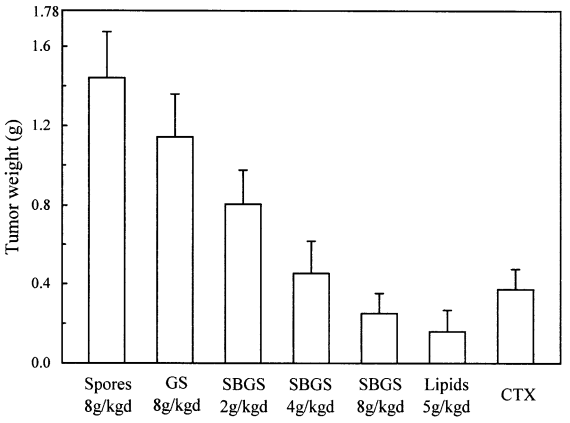

Fig. 1. The inhibitory effects of oral administration of the spores, the germinating spores (GS), the SBGS, the lipids extracted from the germinating spores of *G. lucidum*, and CTX on the growth of mouse hepatoma cell. Each column expresses the mean tumor weight ± SD of ten mice. A significant difference from the negative control (untreated saline control) group (1.78 ± 0.13 g) is indicated by $P < 0.001$.

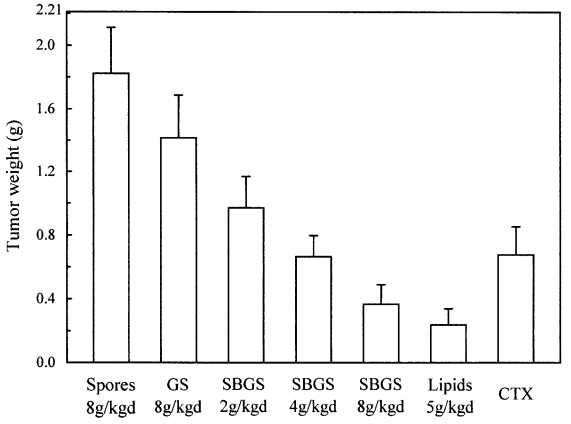

Fig. 3. The inhibitory effects of oral administration of the spores, the germinating spores (GS), the SBGS, the lipids extracted from the germinating spores of *G. lucidum*, and CTX on the growth of mouse reticulocyte sarcoma L-II cell. Each column expresses the mean tumor weight ± SD of ten mice. A significant difference from the negative control (untreated saline control) group (2.21 ± 0.21 g) is indicated by $P < 0.001$.

Figs. 1–3 indicate the inhibitory effects of the spores of *G. lucidum* on the growth of three tumors. The results showed that the spores of *G. lucidum* could markedly reduce the tumor weight in comparison with the control level. As shown in Figs. 1–3, the dormant spores (8 g/kg per day) reduced the tumor weights to 81.0–83.2% of the control levels, while the germinating spores (8 g/kg per day) could reduce the tumor weight to 64.1–64.7% of the control levels for three tumors, indicating that the germinating spores had a higher bioactivity than the dormant spores. Germination was the process by which a dormant spore was converted into a vegetative spore. Increased activities in the germinating spores might be accompanied by increase in the content of bioactive substances and the production of new active compounds while a dormant spore was activated.

Our results suggested that the SBGS had more obvious effect on the growth of tumor cells than the whole germinating spores (GS) in the same oral dose (8 g/kg per day) and could significantly inhibit cell growth in a dose-dependent manner. As shown in Figs. 1–3, the oral administration of SBGS (8 g/kg per day) significantly reduced the tumor weight to 14.1, 18.5, and 16.6% of the control levels, while germinating spores (8 g/kg per day) only reduced the tumor weight to 64.3, 64.7, and 64.1% of the control levels, respectively, for mouse hepatoma, sarcoma S-180, and reticulocyte sarcoma L-II cells, indicating that these effects were related to the status of the sporoderm. The results that whole spores had less inhibitory effect on the growth of tumor cells than sporoderm-broken spores indicated that the bioactive substances in the spores were not completely reduced and utilized in vivo when the sporoderm was not broken. The whole spores had been found in the dejecta of animals and humans, who took the whole spores during treatments, indicating that the sporoderm was indestructible in vivo. The previous study showed that no effect on HeLa cells in vitro was observed when the sporoderm was not broken [14]. However, our results showed that the whole spores had still less antitumor activity, suggesting that the whole spores, especially the germinating spores, could show some bioactivities in vivo.

To further study the antitumor activity of the germinating spores of *G. lucidum*, the lipids in SBGS were extracted by supercritical carbon dioxide extraction. Lipids (37.5 g), a mixture of bioactive substances, were obtained from 100 g of SBGS. After the mice bearing hepatoma, sarcoma S-180, and reticulocyte sarcoma L-II, respectively, were treated with 5 g/kg per day of the lipids from SBGS, 91.0, 89.5 and 89.3% decreases in cell proliferations were observed, indicating that the lipids in the germinating spores had much higher antitumor activity than CTX (Figs. 1–3), which would maximally inhibit tumor growth and metastasis and was used as a positive control. Fig. 4 shows the dose-dependent inhibition (%) of the lipids in the germinating spores on mouse hepatoma, sarcoma S-180, and reticulocyte sarcoma L-II cells, respectively. The dose of the lipids in SBGS was calculated by applying the lipid content of 37.5%. As shown in Fig. 4, higher doses of SBGS (13.4 g/kg per day) or the lipids (5 g/kg per day) could significantly inhibit three tumors with ~90% of inhibition, indicating that the lipids and SBGS of *G. lucidum* were very effective in antitumor activity.

No evident toxic or side effects were observed at the end of the treatments. The skin and hair texture, and behavioral pattern did not reflect any toxic reaction in host mice at this experimental dose. There were no significant differences in mean body weights of mice fed all the experimental diets (data not shown). Thus, it is likely that SBGS of *G. lucidum* may improve survival of patients with hepatocellular

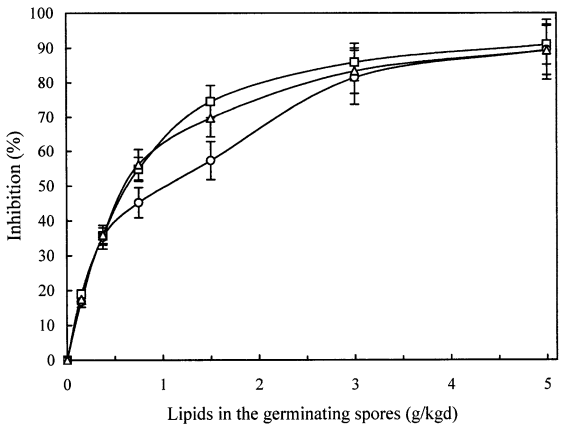

Fig. 4. The dose-dependent inhibitions (%) of the lipids within the germinating spores (0–3 g/kg per day) and the lipids extracted from the germinating spores (5 g/kg per day) of *G. lucidum* on mouse hepatoma (□), sarcoma S-180 (○), and reticulocyte sarcoma L-II (△) cells, respectively.

carcinoma and other cancers, and be therapeutically useful in clinical situations as a novel antitumor agent either when used alone or in combination with other anticancer drugs.

The mechanism of the antitumor effect of *G. lucidum* is still not well understood. Previous studies suggested that polysaccharides in *G. lucidum* had immunomodulating properties, including the enhancement of lymphocyte proliferation and antibody production [15] and produced both anti-genotoxic and antitumor promoting activities [16]. A polysaccharide isolated from the spores of *G. lucidum* was a complex glucan, in which the degree of substitution on the main chain and the length of side chains might be very important factors in determining the bioactivities [15]. An acidic protein bound polysaccharide isolated from *G. lucidum* had a direct virucidal effect on herpes simplex virus types 1 and 2, which were responsible for a broad range of human infectious diseases [6]. It had been reported that herpes simplex virus type 1 infections were recognized as a risk factor for human immunodeficiency virus infection, and type 2 was known as oncogenic virus, which had the ability to convert cells into tumor cells [17]. Polysaccharides and glycoproteins possessing hypoglycemic and immunostimulant activities had also been isolated [3]. Recent findings suggested that the antitumor effect of *Ganoderma* mediated by the polysaccharides was a consequence of the potentiation of cytokine production by activated macrophages and T lymphocytes [18]. However, when used at its equivalent dose in the crude extract, polysaccharides were not as effective as the whole extract in antitumor activity, suggesting that besides polysaccharides, other components might also contribute to the bioactivities of *G. lucidum*. The synergistic effect of polysaccharides and other bioactive components such as terpenes could not be neglected.

Terpenes, ganoderic acids A, B, C, and D, lucidenic acid B, and ganodermanontriol as major ingredients, were found to possess higher bioactivities compared with the others [19]. Several bioactive triterpenes and sterols were isolated from ganoderma and proved effective as cytotoxic, antiviral, and antiinflammatory agents [3]. Some intensely bitter components including lucidenic acids A, B, C, D, E, lucidone A, and ganoderic acids B and C found in *G. lucidum* had various bioactivities [20,21]. Ganoderic acid α, a highly oxygenated triterpene, as well as 12 other compounds, which were isolated from a methanol extract of *G. lucidum*, were found to be active as anti-HIV-1 agents [3]. Ganoderic acid, lucidumol B, ganodermanondiol, ganodermanontriol, and ganolucidic acid A showed significant anti-human immunodeficiency virus-1 protease activity [13]. Ganodermic acid S, a major oxygenated triterpenoid isolated from the fungus [22], was a bioactive lipid [23,24] and exhibited inhibitory effects on platelet responses to various aggregating agonists [2]. Ganoderenic acid A in *G. lucidum* was the potent inhibitor of β-glucuronidase and had a potent hepatoprotective effect against CCl_4-induced liver injury [25]. Ganoderic acids A, B, G, and H and compound C6 isolated from *G. lucidum* were active as the antinociceptive components [26]. Administration of hot water soluble extracts of *G. lucidum* decreased pain dramatically in two patients with postherpetic neuralgia recalcitrant to standard therapy and in two other patients with severe pain due to herpes tester infection [27]. Three terpenoids, lucidenic acid O, lucidenic lactone, and cerevisterol in *G. lucidum* could selectively inhibit eukaryotic DNA polymerase activities [28]. The bioactive components in the spores of *G. lucidum* might be inhibitors of the onset of DNA synthesis, and could also strongly reduce the level of intracellular calcium, which resulted in the inhibition of cell growth when the spores were used to treat human cervix uteri tumor HeLa cells [14]. *G. lucidum* could stimulate cytokine gene expression and proliferation in human T lymphocytes [29]. The antitumor effect of *G. lucidum* was mediated by cytokines released from activated T lymphocytes and macrophages. *G. lucidum* could potentiate the production of cytokine including interleukin-1, interleukin-6, tumor necrosis factor, and interferon, in which two antitumor cytokines, tumor necrosis factor and interferon, acted synergistically on the inhibition of leukemic-cell growth and markedly induced leukemic-cell apoptosis [18]. *G. lucidum* comprised a rich source of neuroactive compounds that could mediate the neuronal differentiation and neuroprotection of tumor cells [10]. Otherwise, the antioxidant and radical scavenging activities of *Ganoderma* might have an important role in the inhibition of lipids peroxidation in biological systems [7].

In conclusion, the dormant spores of *G. lucidum*

could be activated by germination, and thus the bioactivities of the spores might be enhanced. When the sporoderm was fully broken, the bioactive substances in the germinating spores might totally be released and assimilated in vivo. Therefore, the SBGS of *G. lucidum* could show a significant antitumor effect, especially, in the prevention of the recrudescence or metastasis of cancerous cells. It had been found that SBGS could remarkably mitigate the toxic and side effects of radiotherapy and chemotherapy in some patients. Therefore, radiotherapy or chemotherapy combined with SBGS of *G. lucidum* might be attended with good results. Research is in progress to further investigate the antitumor activity of SBGS.

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