

Screening Systems for Medicinal Basidiomycetes Antitumor Extracts

Larissa Krasnopolskaya,¹ Igor Belitsky,¹ Anastasia Avtonomova,¹ N. Soboleva,¹
A. Usov,² Elena Isakova,¹ Alex Libenzon,¹ & Vladimir Bukhman¹

¹Gause Institute of New Antibiotics, B. Pirigovskaya St., 11, 119021 Moscow, Russia; ²Organic Chemistry Institute, Leninsky Prospect, 47, 117913 Moscow, Russia, email: info@gribomir.ru, Lkrasnopolskay@yandex.ru

Prevention and treatment of oncological diseases is one of the most important fields of possible practical application of preparations on the basis of medicinal Basidiomycetes. The present experimental data demonstrate the ability of mushrooms to synthesize metabolites, revealing anticarcinogenic effects, possessing antitumor activities due to cy-

tostatic or/and immunostimulating action, and inhibiting the process of metastasis. One of the most important current problems in our view is the development of screening systems for mushroom antitumor preparations, including their effectiveness and cost. The present investigation is the first step in this direction.

Strains of *Ganoderma lucidum* (W.Curt.: Fr.) Lloyd, *Hericius erinaceus* (Bull.: Fr.) Pers., *Lentinus edodes* (Berk.) Singer, *Trametes versicolor* (L.: Fr.) Pilát, having taxonomic proximity and affiliation with the group of xylosaprotrophes (causing white mould) due to their ability to use lignin, was studied.

The action of extracts of submerged mycelium, fruit bodies, and basidiospores from Russian and Chinese mushroom farms was investigated.

Submerged cultivation was carried out in the Gause Institute of New Antibiotics of Russian Academy of Medical Sciences. The strategy for developing methods of submerged cultivation of the species studied, which secures maximal shortage of cultivation costs owing to possibilities of strain diversity, reduction of process length 2–4 times, and increase of biomass yield was worked out. The methods of submerged culture were developed individually for each studied object and included the

mice, and in 9-month-old adult mice antitumor growth was not effected by itself but authentically strengthened the CF effect. A similar effect was observed in adult (5-month-old) mice after oral introduction of *G. lucidum* mycelium combined extract. Thus, the dependence of the antitumor effect of the mushroom extract on the age of the laboratory animal age was determined.

Some additives were selected to strengthen the antitumor effect of *G. lucidum* mycelium extracts. The highest self-activity was shown by orally introduced mycelium polysaccharide fractions with various ratios of monosaccharides.

Unlike basidiom extracts, submerged mycelium extracts of *Hericius erinaceus* showed self-antitumor activity on a solid lymphoma model, resulting in inhibition of tumor growth. This effect was distinctly increased by the single prior introduction of a low dose (50 mg/kg) of CF.

strain of certain mushroom species, liquid media composition, initial pH, aeration, lighting conditions, temperature rate, and process duration.

Various methods of extraction were tested on the mycelia and fruit bodies of the studied mushroom species. For a number of cases, fractionating of extracts was done and monosaccharide composition of extracted polysaccharides was analyzed.

Obtained materials were investigated *in vivo* on hybrid mice (C57Bl/6J × DBA/2) F1 (B6D2F1) with transplantable ascites lymphoma EL₄ as a model. Extracts were inoculated orally and intraperitoneally.

Some groups were treated with cyclophosphan (CF) (50 mg/kg) with the aim of inhibiting leukocyte suppressors. General host status of mice, body weight changes, tumor engraftment and its growth, and the survival rate of mice were observed during the experiment. The cause of mice death, if it occurred, was determined by analysis of observation data and dissection results.

Investigation of *Ganoderma lucidum* extract antitumor activity showed that the antitumor activity of submerged mycelium extract is much higher than the antitumor activity of Russian and Chinese basidioms and basidiospore extracts.

Ganoderma lucidum water extract (introduced intraperitoneally) inhibited tumor growth in young

A *Lentinus edodes* extract study with a solid lymphoma model did not reveal any differences between submerged mycelium and basidioms. Oral administration of these extracts substantially inhibited growth of subcutaneous lymphoma. This effect was also distinctly increased by the single prior introduction of a low dose (50 mg/kg) of CF. A new way to rapidly receive submerged mycelium extracts of *L. edodes* with marked antitumor activity was proposed.

Intraperitoneal administration of water extracts of submerged mycelium of *Trametes versicolor* results in the inhibition of ascites lymphoma growth. On day 27 the amount of mice in the experimental group was reliably (χ^2 $p=0.03$) bigger than in untreated group.

Summing up the obtained results, we should underline the principle conclusions. The selection of raw material for drugs, biologically active supplements, and other functional food products should be done individually for each basidiomycetes species. The chemical purification expediency of the active substances depends on the observed effects. Thus, the necessity of initial extract fractionating for obtaining well defined antitumor effects was shown for *Ganoderma lucidum*. At the same time, the results for *Lentinus edodes* gave proof for practical uses of crude extracts. We should keep in mind the dependence of the obtained results

on the laboratory model used and the direction of clinical studies. At the present time, the question of which is the preferable choice of Basidiomycetes species for definite oncological disease treatment

is discussed. The problem of specific individual medicinal Basidiomycetes species antitumor action in different patient age groups is also very important.