Biomedical Effects of Mushrooms with Emphasis on Pure Compounds

R. Russell M. Paterson, Nelson Lima

Medicinal mushrooms show great promise for disease treatments. They have been employed in the Orient and Occident for thousands of years, although the practice has persisted in the East. They remain highly valuable. Authentic human trials and pure compounds are emphasized in this review of the most current literature. Polysaccharides from the fungi appear effective in cancer treatments and low-molecular-weight compounds also attract much interest. However, reports of toxicity must be taken seriously. Prescriptions for mushrooms and preparations need to be given by qualified medical practitioners. The reason why these preparations are not more widely used in the West is related to problems of (A) intellectual property rights, (B) mass production, and (C) obtaining pure compounds that retain activity. Mushroom compounds require testing against infectious diseases such as those caused by bacteria, because the current antibiotics are failing from resistances. Overall, the future is assured for medicinal mushrooms. *(Biomed J 2014;37:357-368)*

Dr. R. Russell M. Paterson

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There are approximately 150,000 species of mushrooms.[1,2] Some have been used in medicine for thousands of years and are still employed. Unlike the fruiting bodies of *Cordyceps* [Figure 1A], mushrooms have the more classical macroscopic morphology [Figure 1B-F], wherein only 10-15% have been described scientifically, leaving a huge untapped resource of potentially active natural products. Approximately 60% of novel species are from the tropics, where 22-55% (in some cases 73%) remains undescribed.

Mushrooms have high value as food in many societies. Early civilizations had gained a practical knowledge of these to (A) eat and (B) avoid, for example, the poisonous or even psychotropic mushrooms. It is relevant to mention that much of mushroom carbohydrates consist of dietary fiber which cannot easily be digested by humans. The consumption of whole edible medicinal mushrooms or extracts (i.e. dietary supplements)[3] may offer ingredients beneficial to health as functional foods,[4] which have only quasi-scientific acceptance because the active ingredients are poorly defined. Nevertheless, reports on the medical benefits of mushrooms continue to reach important Western national newspapers,[5] although often considered as significant only in the Orient.

Fungi, in general, produce society-changing pharmaceuticals including penicillin, statins, ergot alkaloids, mycophenolic acid, and cyclosporine. However, there is much concern currently about resistance to antibiotics, which creates interest in discovering novel chemical leads. Some fungal compounds are toxic at low concentrations, such as the mycotoxins – aflatoxin, ochratoxin A and T2 toxins – all of which enter the food chain and have detrimental effects on humans.[6] This has some parallels for mushrooms where toxic examples are well known. The interest in discovering useful medicinal properties of mushrooms is understandable given these established biological activities from the fungal kingdom.

Surprisingly, whether mushrooms are effective medicines in modern Western terms remains debatable, although Chinese Pharmacopeias document the use of over 100 species for many ailments.[7] Mushroom-derived medicinal products are produced currently by major Japanese, Korean, Chinese, and increasingly USA companies, and are being used worldwide by holistically oriented physicians, chiropractors, herbalists,

From the Centre of Biological Engineering, Universidade do Minho, 4710-057 Braga, Portugal
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Correspondence to: Dr. R. Russell M. Paterson, Centre of Biological Engineering, Universidade do Minho, Portugal. 4710-057 Braga, Portugal. Tel: 351-253604423; Fax: 351-253678986; E-mail: russell.paterson@deb.uminho.pt

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Figure 1: (A) *Cordyceps* growing on a dead insect (adapted from Smith *et al*.[7]). (B) *Piptoporus betulinus* (adapted from Wikipedia): The birch fungus is known to have antibacterial properties and was likely used for medicinal purposes. This was found in the belongings of Ötzi the Iceman, Chalcolithic (Copper Age) man at about 3300 BC. (C) *Lentinus edodes* growing on wood for mass production (adapted from Smith *et al*.[7]). (D) *Pleurotus ostreatus* growing on sawdust media for commercial production (adapted from Smith *et al*.[7]). (E) *Grifola frondosa* growing on sawdust media (adapted from Smith *et al*.[7]). (F) *Phellinus linteus* growing on a tree in nature (adapted from Smith *et al*.[7]).

and naturopathic physicians in a clinical environment. Western medicine has made little use of these, in part, due to their complex structures and unacceptable pharmaceutical purity. Also, there may be problems in obtaining large amounts of active ingredients, due to the difficulty in growing mushrooms *in vitro* in large volumes, sufficient to make production highly profitable.[7] It is interesting that large bioreactors are available in the West for growing these organisms.[8]

Perhaps most importantly, suitably rigorous human trials were seldom performed on the mushroom preparations.[7] Throughout the literature, there is skepticism about whether traditional procedures in Oriental countries can be translated to Occidental concepts, and even today there are reports relating the effects to the Oriental concepts of "Ying and Yang" in non-Western papers for *Cordyceps* [Figure 1A] (some revised to *Ophiocordyceps*).[9]

Medicinal mushrooms have been employed for millennia in Asian countries, as discussed by Paterson about *Ganoderma* and *Cordyceps*[9,10] and in general by others.[11,12] However, they were also used thousands of years ago in the West, as exemplified by the discovery of the mummified body (the Ice Man) in the Val Senales glacier, Italy. The Ice Man had among his effects a fruiting body of *Piptoporus betulinus* [Figure 1B], which is suggested to have been used as a purgative, probably because it produces agaric acid and toxic resins known to have this effect. The mushroom produces oils that are toxic to metazoans and bacteria, for example, mycobacteria, according to Capasso.[13] The species could have had a spiritual significance,[14] perhaps related to hallucinogenic effects, which may have associated medicinal effects, such as treating depression. It is in the Orient where using mushrooms has continued. The reasons for the discontinued use in the West may be related to the greater adherence to (Western-based) scientific approaches to medicine, and/or the requirement for making sufficient profits by pharmaceutical companies.

The edible mushrooms which demonstrate useful activities include species belonging to *Lentinus* (*Lentinula*) [Figure 1C], *Auricularia, Hericium, Grifola, Flammulina, Pleurotus* [Figure 1D], and *Tremella*.[7] Others are known only for their medicinal properties, for example, *Ganoderma* and *Trametes*; these are inedible due to their coarse and hard texture or bitter taste. The historical preparation of the inedible and scarce, forest-obtained medicinal mushrooms would have been as hot water extracts, concentrates, liquors, or powders, as used in health tonics, tinctures, teas, soups, and herbal formulae.[7] However, almost all of the important medicinal mushrooms have now been subjected to large-scale artificial cultivation, thus avoiding scarcity and ensuring accuracy of identification, increased reliability, and consistency of medicinal products. Medicinal mushrooms are normally consumed as powdered concentrates or extracts in hot water. The extract is concentrated and used as a drink, freeze-dried or spray-dried to form granular powders

which allow easier handling, transportation, and consumption.[15] Regular intake of these concentrates is believed to enhance the immune responses of the human body, thereby increasing resistance to disease and, in some cases, causing regression.[16]

Modern medical practice relies on highly purified pharmaceutical compounds, the activity and toxicity of which show structure/function relationships. It is difficult for pharmaceutical companies to patent mushroom extracts containing various ingredients, as opposed to specific active compounds. Medicinal mushrooms may contain mixtures of compounds that have not undergone thorough chemical analysis and their mechanisms of action remain unknown.[2] However, a considerable effort has been made to rectify this situation with high-quality trials and pure compounds. Increasing evidence in well-respected journals is accumulating to support the activity of some of the treatments[17] [Tables 1 and 2],[18,19] although the society-changing cures for diseases such as penicillin remain elusive. The technical problems in making synthetic products from, for example, complex polysaccharides, will also be detrimental to these compounds becoming commercial.

Crucially, explanations of how compounds from mushrooms function in animal and human systems are appearing in peer-reviewed scientific and medical journals[19] (see subsequently). Many are being viewed as functional foods and a source of physiologically-beneficial and non-invasive medicines. Some compounds have promising immunomodulatory, antitumor, cardiovascular, antiviral, antibacterial, antiparasitic, hepatoprotective, and antidiabetic properties.

This current review will consider reports where (A) more relevant medicinal activities have been determined with purified preparations and (B) valid human trials have been performed.

Compounds of high molecular weights

Perhaps the most encouraging property of the mushroom-derived polysaccharides [Table 1] is the ability to significantly reduce the side-effects of radiotherapy and/or chemotherapy.[7] The extent to which the activities are specific to mushrooms, or are general to polysaccharides, is unknown. For example, polysaccharides from plants and other fungi also have medicinal effects.[17]

The predominant products submitted for clinical testing include (A) schizophyllan from *Schizophyllum commune* mycelium, (B) lentinan from *Lentinus edodes* mushrooms, (C) polysaccharide K (PSK) and polysaccharide-peptide (PSP) from the mycelia of *Trametes versicolor,* and (D) Grifron-D from the mushrooms of *Grifola frondosa* [Figure 1E], all of which passed Phase I, II, and III clinical trials, mainly in Japan and China.[7] However, in many cases, the standards of these trials may not meet the current Western regulatory requirements, although significant improvements in quality of life and survival of patients are reported. Several of these compounds are now used extensively in Japan, Korea, and China as adjuncts to standard radio- and chemotherapy. Some recent studies from Japan have revealed that mixtures of extracts from medicinal mushrooms have shown beneficial effects on the quality of life for some advanced cancer patients. Polysaccharides exert effects on cancers, immune system function, and inflammation;[20,21] others support (A) normal blood glucose and lipid levels and (B) bowel function, although much of the research was undertaken using injections rather than oral administration.[17] Effects observed *in vitro*, or by injection, may not occur, or have different results, compared to when medicines are taken orally.[22]

Immunomodulation

Diseases associated with immune dysfunction such as cancer, chronic fatigue syndrome, AIDS/HIV, hepatitis, and autoimmune conditions are at the forefront of society's disease concerns.[23] However, cancer treatment by conventional chemotherapy and radiotherapy is known to have adverse effects on the human immune system. Cancer immunology has become a rapidly growing field in basic cancer research. Methods are being sought to promote host antitumor, immune cell activity and to overcome the ability of the cancer cells to evade immune surveillance. Hence, immunostimulating agents could be useful adjuncts to conventional cancer treatments. These approaches are designed to cause the destruction of tumor cells, and to be much more tumor-specific and less harmful to normal cells. Many medicinal mushrooms function as immunomodulators. The physiological constitution of host defense mechanisms is improved, which restores homeostasis and enhances resistance to disease.

Polysaccharide-rich fungi have been employed for centuries by human cultures for their dietary and medicinal benefits.[24] Highly purified polysaccharide compounds derived from medicinal mushrooms are being used as pharmaceutical grade products, particularly in Japan,[7] which are able to modulate animal and human immune responses and inhibit certain tumors.[19] The majority of studies involving polysaccharides employed models investigating immune stimulation, and fewer studies explored their anti-inflammatory effects.[17] Animal studies reported effects on the immune system in the spleen, bone marrow, gut, liver, blood, thymus, lungs, and saliva. Evidence revealed the following: (A) Immune stimulation in the blood, (B) anti-inflammatory effects in nasal lavage fluid, and (C) improved survival in controlled studies of cancer patients. Nevertheless, the literature is insufficient to support broad structure/function generalizations, although recently

Table 1: Immunomodulatory polysaccharide mushroom extracts: Oral animal and human studies with valid controls

Fungus	Extract	Animal	Effect
Agaricus subrufescens (Agaricus blazei)	α-1,6 and α-1,4 glucans	Mice	↑ # of splenic T lymphocytes[15]
	Aqueous glucan	Mice	All doses ↑ serum IgG levels, CD3+ T cell populations, and phagocytic activity. Concentrations of 0.6 N and 3 N ↑ the levels of OVA-specific serum IgG 28 days post-immunization; all doses ↑ delayed-type hypersensitivity and TNF-α secreted from splenocytes at 10 weeks; 0.6 N ↑ splenocyte proliferation at 10 weeks[28] ↓ CFU in blood of mice with severe peritonitis and improved overall survival rate in all peritonitis groups[98] ↓ Tumor size and weight after 21 days treatment[38]
	Aqueous, acid glucan	Mice	500 μg/ml ↓ tumor weight 100 and 500 μg/ml ↓ #s metastatic tumors[39]
	Aqueous, with 200 ng/day β-glucan	Mice	↓ Tumor size and weight. ↑Cytotoxic T lymphocyte activity and spleen cell IFN-α protein[29]
	Heteroglycan: New polysaccharide protein complex (ATOM) (non-glucan)	Mice	Both doses ↓ Sarcoma-180 tumor size at 4 weeks and ↑ survival; 300 mg/kg ↑ peritoneal macrophage and C3-positive cells; 300 mg/kg ↓ Shionogi and Meth A tumor sizes at 4 weeks Both doses ↑ survival of Ehrlich ascites mice[54]
	Polysaccharide-rich powder from fruit bodies	Mice	32 and 64 mg ↑ liver mononuclear cell cytotoxicity[31]
	Oral glucans	Humans	↑ NK cell activity, ↓ chemotherapy side effects[65]
Ganoderma lucidum	Aqueous glucan	Mice	↓ Aberrant crypt foci per colon, tumor size, cell proliferation, nuclear staining of β-catenin.[41] ↓ In tumor weight was dose dependent: 27.7, 55.8, 66.7%, respectively[40] For mycelium: Two doses ↓ colonic adenocarcinoma incidence, 2.5% ↓total tumor incidence, two doses ↓ nuclear staining of β-catenin and cell proliferation[42]
Ganoderma tsugae	Aqueous glucan	Mice	In splenocytes, both doses of both extracts ↑ IL-2 and IL-2/ IL-4 ratios; 0.2% early extract and 0.66% later extract IL-4. In Mø, 0.66% later extract ↓ IL-1b, both doses of both extracts ↑ IL-6[36]
Grifola frondosa	Glucan – D fraction	Mice	↓ Tumor weight and tumor growth rate: 1) 58%, 2) 64%, and 3) 75%, respectively.[44] ↓ (62.5%) # of animals with tumors; ↑ H_2O_2 production by plasma Mø; ↑ cytotoxic T cell activity[45]
	Polysaccharide-rich powder	Mice	↓ #s of animals with bladder tumors; ↓ tumor weight; ↑ peritoneal Mø chemotactic activity, splenic lymphocyte blastogenic response and cytotoxic activity[43]
Lentinula edodes	Glucan extract	Mice	↓ Tumor size[48]
	β-glucans	Mice	250 μg dose ↑ spleen cell IL-2 secretion; ↓tumor weight[27]
	Lentinan (glucan)	Rats Mice	↑ T cell #s, helper cell #s and helper/suppressor ratio, ↓suppressor cell level at 4, but not 8 weeks[26] ↓ Tumor weight; ↑ tumor inhibition rate (94%); ↓ tumor weight, ↑ tumor inhibition rate (>90%)[50] ↑ Serum IFN-α and TNF-α, peak at 4 h and then normal at 24 h; ↑ IL-2 and IL-1α, peak at 2 h and normal at 24 h; ↑ CD3 + T, CD4 + T, CD8 + T, B lymphocytes[49]
	Glucomannan (KS-2) Non-glucan	Mice	↑ Survival; 1, 10, and 100 mg/kg doses ↑ survival[51]
	Polysaccharide-rich powder	Mice	↓ # of animals with bladder tumors; ↓ tumor weight; ↑ Mø chemotactic activity, splenic lymphocyte blastogenic response, cytotoxic activity[43] All three doses of one lineage and the 5% dose of two other lineages ↓ #s of micronucleated bone marrow polychromatic erythrocytes[47] All three doses ↓ Sarcoma-180 tumor weight; ↓ growth of MM-46, B-16, Lewis lung, and IMC tumors; ↑ lifespan in Lewis lung and MM-46 animals; ↓tumor weight and growth; ↑ spreading rate of activated Mø phagocytic activity[46]
Phellinus linteus	Aqueous, alcohol-precipitated glucan	Mice	↑ Production and secretion of IFN-γ by ConA stimulated T cells[33]
	Polysaccharide-rich powder	Mice	↓ Serum and splenocyte IgE production; ↑ proportion of splenic CD4 + T cells and splenocyte IFN-γ production[32]
Sclerotinia sclerotiorum	SSG (a β-glucan)	Mice	10 mg dose ↑ acid phosphatase activity of peritoneal Mø (day 14); 80 mg dose ↓ tumor weight[99] 2 mg ↓ # of some surface lung nodules at 2 weeks[100]

Contd..

Table 1: Contd...

Fungus	Extract	Animal	Effect
Sclerotium rolfsii	Glucan phosphate	Mice	↑ Systemic IL-6; ↑ Mø expression of Dectin-1 in GALT cells; ↑ *TLR2* expression in dendritic cells from Peyer's patches[101]
Trametes (Coriolus) versicolor	Polysaccharide-peptide (PSP)	Mice	↓ Tumor growth and vascular density[55]
	Oral glucans (PSK) PSP	Humans	Controlled: ↑ survival from cancer deaths; no difference in disease-free or overall survival[59] Controlled: ↑ survival in stage III patients; ↓ recurrence in stage II and III patients[60] Controlled: ↑ 5-year disease-free survival rate, overall 5-year survival[61] Controlled: 14 day treatment: ↑ peripheral blood NK cell activity, PBL cytotoxicity, proportion of PBL helper cells; ↓ proportion of PBL inducer cells; <14 day treatment: ↑ PBL response to PSK and ConA, proportion of regional node lymphocyte suppressor cells[64] Controlled: ↑ survival time[62] Randomized controlled: ↑ disease-free survival and overall survival[58] Randomized, double-blind, placebo-controlled: ↑ remission and survival rates[63] Randomized, double-blind, placebo-controlled: ↑ blood IgG and IgM, total leukocyte and neutrophil counts, % body fat; ↓ patient withdrawal due to disease progression[34]
Pholiota nameko	Heteroglycan (PNPS-1) non-glucan	Rats	↓ Granuloma growth positively correlated with dose: 11%, 18%, and 44%, respectively[37]
Lentinus lepideus	Heteroglycan (PG1) non-glucan	Mice	↑ Colony forming cells, granulocyte CFUs/Mø, erythroid burst-forming units, and myeloid progenitor cells in bone marrow; induced proliferation of granulocyte progenitor cells in bone marrow; ↑ serum levels of GM-CSF, IL-6, IL-1β[53]
Pleurotus ostreatus	Polysaccharide-rich powder	Mice	↓ #s of animals with bladder tumors; ↓ tumor weight; ↑ plasma Mø chemotactic activity, splenic lymphocyte blastogenic response, cytotoxic activity[43]

↑, increased effects; ↓, decreased effects; #, number. Abbreviations: CD: Cluster of differentiation; CFU: Colony forming unit; ConA: Concanavalin A; GALT: Gut-associated lymphoid tissue; GMCSF: Granulocyte macrophage colony-stimulating factor; IFN-γ: Interferon gamma; IgG: Immunoglobulin G; IL: Interleukin; IMC: Invasive micropapillary carcinoma; MM-46 carcinoma: Mouse mammary carcinoma; Mø: Macrophage; NK: Natural killer; PBL: Peripheral blood leukocytes; TNF-α: Tumor necrosis factor alpha

Table 2: Brief overview of the medical activity of low-molecular-weight compounds from mushrooms in various systems[18]

Evaluation	Compound	Mushroom	Effect
Human population or human cell lines	Ergosterol	Unspecified	Anti breast cancer[102]
	Hydroxylated triterpenes	Multiple species	Anti breast cancer[79]
	Clitocinet	*Leucopaxillus giganteus*	Anticancer (cervical, ovarian, endometrial)[83]
	Irofulven (cytotoxin)	*Omphalotus olearius* (inedible)	Anticancer (pancreatic advanced solid malignancy)[91]
	Hericenones C-H Erinacines A-I Dilinoleoyl phosphatidylethanolamine	*Hericium erinacium*	Brain health and cognition[103]
	Farnesyl hydroquinone, ganomycin I	*Ganoderma colossum*	Antiviral (HIV)[93]
	Ganomycin B	*Ganoderma colossum*	Antiviral (HIV)[93]
Animal models and animal cell lines	Cordycepin (30-deoxyadenosine)	*Cordyceps militaris*	Anticancer (bladder)[71]
	Triterpenoids	*Ganoderma lucidum*	Anticancer (liver)[84]
	Lucialdehydes A-C	*Ganoderma lucidum*	Anticancer (lung)[89]
	Blazein	*Agaricus blazei* Murill (Himematsutake)	Anticancer (lung and stomach)[90]

Abbreviation: HIV: Human immunodeficiency virus

structural details have been provided and the situation is improving.[19] Effects can be attributed unequivocally to polysaccharides in the limited number of studies that investigated well-characterized, isolated products: Most of these studies are related primarily to glucan products, although such associations are more tenuous without complete compositional analyses.

In most cases, the mechanisms by which the polysaccharides influence immunologic function are merely speculated, particularly when the complex environment of the gastrointestinal (GI) tract is considered. Fragments of polysaccharides partially hydrolyzed by GI bacteria may (A)

be absorbed into the bloodstream, with the potential to exert systemic effects, or (B) bind to gut epithelia and exert localized and/or systemic immune system influences.[25] Furthermore, current studies investigating the link between the (A) bioconversion of dietary polysaccharides, (B) bioavailability, and (C) downstream effects on the host metabolism and physiology, utilize metabolomic and metagenomic approaches that can detect and track diverse microbial metabolites from alterations of immunomodulatory polysaccharides. Of course, mushrooms offer an abundant source of such polysaccharides.

Certain polysaccharides appear to affect immune system function, which is revealed predominately by work based on *in vitro* studies or injected polysaccharides.[17] Effects of orally administrated polysaccahrides from various sources, including mushrooms, are more difficult to interpret, except for orally ingested heteroglycans, glucans, pectins, glucomannans, arabinogalactans, fucoidans, galactomannans, and mixed polysaccharide products in rodents. Some studies investigated the anti-inflammatory effects, although most involved the ability to stimulate the immune system [Table 1] and the polysaccharide products appear well tolerated. However, the literature for oral polysaccharides is inadequate currently for structure/function conclusions. Many dietary polysaccharides, particularly glucans, appear to elicit various immunomodulatory effects in numerous animal tissues (e.g. GI tract, blood, and spleen). Importantly, *T. versicolor* glucan extracts improved the immune function and survival in randomized control trials (RCTs) of human cancer patients.[17] In general, it is crucial to use well-characterized polysaccharides for future research on immune modulation.

Furthermore, studies in healthy animals demonstrated a number of immune-stimulating effects of various glucan products from *Lentinula edodes* (shiitake);[26,27] *Agaricus subrufescens* (*Agaricus blazei*),[17,28-31] and *Phellinus linteus* [Figure 1F] powder.[32,33] Importantly, *T. versicolor* PSP from fruit bodies increased IgG and IgM antibodies and total leukocyte and neutrophil counts in an RCT of humans with advanced stage lung cancer:[34] Fewer patients withdrew from the study due to disease progression.

Mushroom glucan–protein complexes may be cytotoxic, while glucans are predominantly non-cytotoxic, but they all potentiate innate (non-specific) and acquired (specific) immune responses.[7] The polysaccharides activate many immune cells important for the maintenance of homeostasis, such as host cells (e.g. cytotoxic macrophages, monocytes, neutrophils, natural killer (NK) cells, and dendritic cells) and chemical messengers (e.g. cytokines such as interleukins, interferons, and colony stimulating factors) that trigger complement and acute phase responses. Also, they are multi-cytokine inducers, able to induce gene expression of various immunomodulatory cytokines and cytokine receptors. Lymphocytes governing antibody production (β-cells) and cell-mediated cytotoxicity (T-cells) are also stimulated.[7] However, a complete comprehension of the exact mode of action has not been elucidated for most of the anticancer compounds, although even this has been addressed as discussed below:

In a breakthrough report, Liao *et al.*[19] demonstrated that the l-fucose (Fuc)-enriched Reishi polysaccharide fraction (FMS) exhibits unique immunogenicity, and that the mice immunized with the crude extract fraction of water-soluble and l-fucose (Fuc)-containing polysaccharides from *Ganoderma lucidum*, or FMS, could exert an effective antibody-mediated reaction against Globo H-expressing murine LLC1 cells. The most likely fucosyl glycan moieties are Fucα1-2Gal-R, Fucα1-3/4Man-R, Fucα1-4Xyl-R, and Fucα1-2Fuc-R. It is probable that some of them activate the antibody responses against tumor-specific glycan epitopes, allowing the development of complex carbohydrates for immunomodulation-based therapy. The host immune function which is enhanced by Reishi polysaccharides offers great promise for the immunotherapy of Globo H-positive lung cancer patients. The approach of high-throughput glycan microarray analysis and detailed structural analyses of carbohydrate antigens should be applicable to other medicinal mushroom polysaccharides which induce different antibody-mediated biological functions. (The well-researched website for *G. lucidum* is interesting to consult in relation to activities in general.[35])

Anti-inflammatory

Fewer studies have explored the anti-inflammatory effects.[17] In these studies, *A. subrufescens*[28] and *Ganoderma tsugae*[36] aqueous extracts have demonstrated anti-inflammatory/allergy effects in animals. These effects have also been demonstrated in animals fed a *Pholiota nameko* heteroglycan (PNPS-1).[37]

Anticancer (animals)

A wide range of polysaccharides demonstrate anti-tumorogenic effects in numerous animal models of cancer. Glucan products from *A. subrufescens* showed anticancer activities,[38,39] and included standardized levels of β-glucans.[29] In addition, anticancer effects have been reported following intake of various extracts and powders of *G. lucidum,*[40-42] *Gr. frondosa*,[43-45] *L. edodes,*[27,43,46-50] and *Pleurotus ostreatus.*[43] A glucomannan from *L. edodes* improved the survival of animals with cancer cell injections,[51] and heteroglycans from *Lentinus lepidus* and *A. subrufescens* were effective in animal cancer models.[52-54] Finally, animals with cancer cell implantations and administered *T. versicolor* glucans showed decreased tumor growth and vascular density.[55]

Human cancer effects

Hyperbranched β-glucan, extracted from *Pleurotus tuber-regium*,[56] inhibited the proliferation of HepG2 hu-

man hepatocellular carcinomas without having significant effects on normal human liver cell lines.[57] Furthermore, PSK glucans from *T. versicolor* mycelia increased the survival of advanced stage gastric, colon, and colorectal cancer patients in two RCTs and five controlled trials,[58-63] with one study showing increased immune parameters.[64] These are highly convincing data ranging in dates from 1990 to 2004, and therefore should have had substantial influence on the current practice. A randomized, controlled trial of ovarian or endometrial cancer patients consuming *A. subrufescens* glucans showed increased NK cell activity and fewer negative chemotherapy side effects.[65]

Safety

The safety criteria for the mushrooms have been exhaustively studied with some reports of toxicity, although mushroom polysaccharides demonstrated remarkably few adverse reactions in Phase I clinical trials.[7] Several purified mushroom polysaccharides have been in clinical use in Japan, Korea, China, and more recently, in the USA for several years, with no reports of any short-term or long-term toxicity. The safety of mushroom polysaccharides is largely based on No-Observed-Adverse-Effect-Level (NOAEL) acute and/or chronic toxicity testing in rodents.

However, three cases of hepatotoxicity and/or death following intake of an *A. subrufescens* aqueous extract[66] have been reported. Toxicity requires careful consideration and cannot simply be dismissed as possible contamination. It indicates the widespread problem of administration of these preparations, often by practitioners with limited medical experience. The paper states that patients can be reluctant to mention taking such medicinal mushrooms to their doctor when receiving conventional treatment for cancer.

Seven animal studies found no evidence of toxicity and reported positive immunologic effects of *A. subrufescens* extracts in healthy animals or animals with cancers.[17] Furthermore, 6 weeks of *A. subrufescens* glucans intake was safe for cancer patients, as was 4 months of 3 g/day intake by 24 healthy adults and 24 adults with liver disease. Liver toxicity was reported from taking a preparation of *G. lucidum* shortly after the subject changed the formulation from one that had not caused problems.[67] More work is required to determine the exact cause and if the toxicity was experienced by other users. Three animal studies reported no adverse effects following intake of *G. lucidum* aqueous extracts. However, 10 human adults consumed 4 g/day of *L. edodes* powder for 10 weeks and experienced adverse effects,[68] although animal studies reported no ill effects from *L. edodes* powder or extract.[17] Intake of 319 mg/kg of *P. ostreatus* extract by mice for 1 month caused hemorrhages, but there was no toxicity when consumed at 5% of diet for 9 months.[69] Furthermore, *T. versicolor* glucan products (1 g/day) were safely consumed by cancer patients for up to 10 years. The safety of the other polysaccharides discussed herein remains unknown.

Compounds of low weight compounds

These compounds are of particular interest as they are simpler chemically, more easily purified, and are often taxon specific. They may be more easily synthesized, modified, and patentable, and thus are of particular interest to pharmaceutical companies. As such, they are equivalent to existing fungal-based pharmaceuticals, for example, penicillin and cephalosporins. Information on the activity of low-molecular-weight compounds from mushrooms is provided in Table 2.

Cancers in general

Cordycepin from predominantly *Cordyceps militaris* has been under investigation as an anti-proliferative drug for nearly 50 years.[70,71] The instability of the molecule in the body has been problematic due to the presence of adenosine deaminases. A combination therapy with an adenosine deaminase inhibitor as a treatment for terminal deoxynucleotidyl transferase (TdT)-positive leukemia is currently in Phase I/II clinical trials (OncoVista, Inc.). Recently, more stable prodrugs have been synthesized, but their potential therapeutic effects remain unassessed.[72] Two aspects of one study indicate that cordycepin continues to be an interesting lead compound for cancer therapy and a potentially useful tool to identify therapeutic targets:[70] Cordycepin affects the poly (A) tails of specific mRNAs at low doses, and this correlates with a reduction in cell proliferation. Surprisingly, at higher doses, cordycepin also inhibits cell adhesion and virtually stops protein synthesis, probably through its effects on Akt and 4E binding protein 4E-BP phosphorylation. This is an example of how further development of a compound with proven activity is not guaranteed, as unforeseen hurdles may arise. This problem also implies that cordycepin may not be the active component of the whole fungus or extracts, as presumably it would be degraded in the body as mentioned, unless there is a natural inhibitor of the deaminase present in the fungus: Fungi are known to produce a wide range of enzyme inhibitors.[73,74]

Breast cancer

There is profound interest in effective treatments for breast cancer of women, and an additional decreased risk was observed from dietary intake of mushrooms and green tea in a case–controlled study;[75] a lesser decrease was found when using only mushrooms. Vitamin D2 could be one of the protective nutrients, as mushrooms are rich in ergosterol, which generates vitamin D2 when exposed to UV B light. Ergocalciferol becomes bioavailable and increases 25(OH)

vitamin D2 levels in human serum. However, the work did not involve direct intervention trials, and mushroom consumption was assessed by food frequency questionnaires, which can be affected by recall bias. Nevertheless, the work is promising. Inhibition of proliferation of human breast cancer cell lines was suggested to be mediated via downregulation of Akt/nuclear factor kappa B (NFκB) (transcription factor) signaling using several mushroom varieties.[76-78] The active components in mushrooms with these effects may be hydroxylated triterpenes,[79] which requires confirmation.

Leukemia

Agaritine (a naturally occurring phenylhydrazine derivative) and ergosterol (see the subsection "Breast Cancer") are present in *Agaricus* spp. Agaritine from *A. blazei* exerted antitumor activity against leukemic cells *in vitro* and this was distinct from that of β-glucan,[80] which indirectly suppresses proliferation of tumor cells. The mechanism appears to involve apoptosis.[81] The reported direct antitumor activity by agaritine against leukemic tumor cells *in vitro* contrasts with the carcinogenic activity previously described in animal studies carried out in the 1980s-1990s, the validity of which has been challenged. Animal models and human food safety studies have concluded that agaritine obtained from consumption of cultivated *Agaricus bisporus* mushrooms poses no known toxicological risk to healthy humans.[82] Ergosterol also inhibited proliferation of leukemic cells without effects on normal lymphatic cells and this activity was distinct from that of β-glucan.[80] However, ergosterol is common to all fungi and is of less interest than the more taxonomically restricted compounds.

Cervical cancer

Ren *et al*.[83] reported the anti-proliferative effect of human cervical cancer HeLa cells by clitocine from *Leucopaxillus giganteus* which works via induction of apoptosis.

Bladder cancer

Cordycepin from *C. militaris* had antitumor effects in two bladder cancer cell lines.[9] The compound resulted in a significant and dose-dependent growth inhibition, which was largely due to G2/M-phase arrest during cell-cycle progression.[71]

Liver cancer

Lucidenic acids (triterpenoids) isolated from *G. lucidum,*[84] extracts from *Cordyceps sinensis*[85] and Chaga (*Inonotus obliquus*) mushrooms[86] inhibited the proliferation of HepG2 human hepatocellular carcinomas. *I. obliquus* appeared to cause oxalate nephropathy and subsequent death of a patient during the use of the fungus as a cancer treatment; also, the mushroom contains very high concentrations of oxalate, and "this might be one to avoid!" as an editor stated when referring to the fungus,[87] although this could be remedied if a pure preparation of the active ingredient(s) was obtained which removed the oxalate.

As reported above for human leukemia cell lines, such extracts appear to have tumor-selective cytotoxicity, without significant effects on normal human liver cell lines.[57] Finally, on 25 May 2005, MGI Pharma announced that Phase II trials of irofulven (see Table 2 and below) were ongoing for inoperable hepatocellular carcinoma.[88]

Lung cancer

In vitro studies have shown that three triterpene aldehydes, lucialdehydes A-C, from the fruiting bodies of *G. lucidum*, possess cytotoxicity against murine and human tumor cells.[89] Furthermore, blazein, a steroid isolated from *A. blazei*, was reported to induce cell death and morphological change indicative of apoptotic chromatin condensation in human lung cancer cells (see the subsection "Stomach Cancer").[90]

Stomach cancer

Blazein had the same effect on stomach cancer cells, as it had on those of lung cancer.[90]

Pancreatic cancer/solid malignancies

A Phase I trial and pharmacokinetic study of irofulven, from the inedible *Omphalotus olearius*, was carried out in 46 patients with advanced solid pancreatic cancer malignancies.[91] A lower dose, as an intravenous infusion daily for 5 days every 4 weeks, was recommended; the highest dose was poorly tolerated, causing neutropenia and renal toxicity. Further trials on irofulven were indicated from the preliminary antitumor activity documented in a patient with advanced pancreatic cancer and positive pre-clinical antitumor effects were observed.[91] However, clinical trials on irofulven were discontinued apparently by MGI Pharma, USA for pancreatic cancer (R and D Focus Drug News, July 25, 2005). MGI Pharma had been conducting a Phase III trial of irofulven for the treatment of gemcitabine-refractory pancreatic cancer, but discontinued it in April 2002 because the compound did not demonstrate significant efficacy.

Others

On 25 May 2005, MGI Pharma announced that Phase II trials of irofulven were ongoing for the treatment of refractory/recurrent ovarian cancer, hormone-refractory prostate cancer, and recurrent malignant glioma.[92]

Antiviral

Ganomycin I, a farnesyl hydroquinone, and ganomycin B from Vietnamese *Ganoderma colossum* inhibited HIV-1

protease with IC50 values of 7.5 and 1.0 l μg/ml, respectively.[93] Ganomycin B competitively inhibited the active site of the enzyme, with both compounds docking with the HIV-1 protease crystal structure.

DNA damage

It was suggested that synthetic agaritine is quickly metabolized in mice and disappears in the plasma, whereas DNA damage occurring after a single administration of synthetic agaritine persists longer.[94] However, the authors made these comments based on results with one particular marker only and it may not be more generally valid. The mutagenic qualities of fungal secondary metabolites, including some from mushrooms, have been reported frequently.[95-97] Hence, DNA damage is possible with the compounds under discussion herein.

Conclusions

There is now excellent evidence to suggest a scientific basis for some of the effects of medicinal mushrooms, but earlier there was considerable doubt. The reasons why they are not used in the West may be related to economics and IP rights. There is no better time for products to emerge as disease treatments, because of the new resistances of bacteria developing to current antibiotics. However, there have been few studies on antibacterial compounds from the mushrooms described and there is a case for screening previously untested mushrooms for novel activities. No doubt, the mushrooms will continue to be employed in the Orient. Whereas pure compounds will always be preferred, it is possible that other components in the whole mushroom or crude extracts could enhance and/or protect activity. The toxicity of mushrooms requires monitoring. Finally, these fungi appear active particularly against cancers and may be of considerable utility in the near future in Western medicine.

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