Competence of immunotherapeutic agents from natural products on the management of chronic diseases

Accepted 18th August, 2015

ABSTRACT

Natural products with health benefits are currently generating keen interest, particularly in the prevention and treatment of several chronic diseases. The mode of action of most products from plants, bacterial and fungal metabolites especially mushrooms are largely due to polysaccharides or polysaccharide–protein complexes which are able to stimulate the non-specific immune system and to exert antitumor activity through the stimulation of the host's defense mechanism. Natural products have therefore demonstrated profound therapeutic effects which are of immense help in the modern treatment of hematologic and solid malignancies as well as some other chronic diseases. This review is to provide current knowledge on the competence of immunotherapeutic agents from some natural products especially from edible mushrooms and from protein A obtained from Staphylococcus aureus.

Key words: Cancer, immunotherapy, mushrooms, natural products, Staphylococcus aureus.

INTRODUCTION

The impact of natural products on human health has been enormous, and the study of natural products continues to influence research in the fields such as biotechnology, pharmaceutical sciences, chemistry, biology, and ecology. Historically, majority of our medicines originate from natural products and their synthetic derivatives, many of which have taught us valuable lessons about biology (Kulka, 2009). Natural products of plant and fungal origins have served and shall continue to provide helpful nutritional source for human growth development and sustenance (Phillipson, 2001).

The term 'mushroom' used in this review is according to the definition of Chang and Miles as ‘a macrofungus with a distinctive fruiting body, which can be either hypogeous or epigeous, large enough to be seen with the naked eye and to be picked by hand’ (Chang and Miles, 1992). From a taxonomic point of view, mainly basidiomycetes but also some species of ascomycetes belong to mushrooms. Mushrooms constitute at least 14 000 and perhaps as many as 22 000 known species. The number of mushroom species on the earth is estimated to be 140 000, suggesting that only 10% are known. Assuming that the proportion of useful mushrooms among the undiscovered and unexamined mushrooms will be only 5%, which implies 7000 yet undiscovered species will be of possible benefit to mankind (Hawksworth, 2001). Even among the known species the proportion of well investigated mushrooms is very low. This fact together with the knowledge about the great potential of microscopic fungi for production of bioactive metabolites [e.g. Penicillium, Aspergillus, Tolypocladium inflatum W. Gams, Claviceps purpurea(Fr.), the experience in ethno medicinal use of mushrooms, the ecologic need for fungi to produce bioactive secondary metabolites and the improved possibilities for genetic, pharmacological and chemical analysis let us assume that mushrooms have a great potential for successful bioprospecting.

Mushrooms comprise a vast and yet largely untapped source of powerful new pharmaceutical products. In particular, and most importantly for modern medicine, they...
represent an unlimited source of polysaccharides with antitumor and immunostimulating properties. This review is to provide current knowledge on the competence of immunotherapeutic agents from natural products especially from mushrooms and protein A obtained from Staphylococcus aureus.

The active components of mushrooms have been known to be polysaccharides or polysaccharide–protein complexes which are able to stimulate the non-specific immune system and to exert antitumor activity through the stimulation of the host’s defense mechanism (Chihara et al., 1969; Mizuno, 1999; Wasser and Weis, 1999; Reshetnikov et al., 2001). The drugs activate effector cells like macrophages, T lymphocytes and NK cells to secrete cytokines like TNF-α, IFN-γ, IL-1β, etc which are antiproliferative and induce apoptosis and differentiation in tumor cells.

**Immunocompetent agents from edible mushrooms**

According to Akramienë et al (2007), beta glucans which are the active component of mushrooms having the healing and immunostimulating properties have been known for thousands of years in the Eastern countries. These mushrooms contain biologically active polysaccharides that mostly belong to group of b-glucans. These substances increase host immune defense by activating complement system, enhancing macrophages and natural killer cell function. The induction of cellular responses by mushroom and other b -glucans is likely to involve their specific interaction with several cell surface receptors, as complement receptor 3 (CR3; CD11b/CD18), lactosylceramide, selected scavenger receptors, and dectin-1 (b GR). Beta-Glucans also show anti-carcinogenic activity. They can prevent oncogenesis due to the protective effect against potent genotoxic carcinogens. As immunostimulating agent, which acts through the activation of macro- phages and NK cell cytotoxicity, b -glucan can inhibit tumor growth in promotion stage too. In allaying the concern of consumption of edible mushrooms in order to benefit maximally from their immune boosting capacities, a study on the chromosomal damaging potential screening reveals that the Pleurotus ostreatus and Pleurotus pulmonarius metabolites like many edible mushrooms are not clastogenic (genotoxic) that is, unlikely to cause cancer producing mutations, but rather enhanced erythropoiesis. They could therefore be useful anticancer agents when the potentials are fully explored (Akanni et al., 2010).

Many researchers have shown that extracts from medicinal mushrooms and plants have ability to stimulate or modulate the host immune responses. Numerous bioactive polysaccharides or polysaccharide-protein complexes from medicinal mushrooms are known to enhance innate and cell-mediated immune responses, and exhibit antitumour activities in animals and humans.

Stimulation of the host immune defense systems by bioactive polymers from medicinal mushrooms and plants has significant effects on the maturation, differentiation and proliferation of many kinds of immune cells in the host. Medicinal mushroom research has focused on discovering compounds that can modulate positively or negatively the biological response of immune cells. Those compounds which appear to stimulate the human immune response are being sought for the treatment of cancer, immunodeficiency diseases, or for generalized immunosuppression following drug treatment; for combinational therapy with antibiotics; and as adjuncts for vaccines (Jong et al., 1983). Several classes of compounds, such as proteins, peptides, lipopolysaccharides, glycoproteins, and lipid derivatives, have all been classified as molecules that have potent effects on the immune system. Whilst polysaccharides are generally considered to be classic T-lymphocyte-dependent antigens that do not elicit cell-mediated immune responses (host defense that are mediated by antigen-specific T lymphocyte cells and various non-specific cells of the immune system), certain polymers have recently been shown to act as potent immunomodulating agents (Tzianabos, 2000).

In China and many other Asian countries, Ganoderma lucidum has been used extensively as “mushrooms of immortality” for over 2000 years (Shiao, 2007). Several major substances with potent immunomodulating action have been isolated from this mushroom, including polysaccharides (in particular β-D-glucan), proteins (e.g. Ling Zhi-B) and triterperoids (Jong and Birmingham, 1992; Zhou and Gao, 2002). Other components such as steroids and organic gemanium also play an important role in the immunomodulating activity of G. lucidum. The major immunomodulating effects of these active substances derived from G. lucidum include mitogenicity and activation of immune effectors cells such as macrophages, NK and T cells. Stimulation of these immune effectors cells results in the production of cytokines such as interferon (INF), interleukins (IL) and tumour necrosis factor (TNF)-α (Zhou and Gao, 2002).

Protein bound polysaccharides PSK (Krestin) and PSP have been isolated from the mushroom Trametes versicolor. The polysaccharides were reported as potent immunostimulators with specific activity for T-cells and for antigen-presenting cells such as monocytes and macrophages. The biologic activity is characterized by their ability to increase white blood cell counts, IFN-γ and IL-2 production and delayed type hypersensitivity reactions (Tzianabos, 2000). Numerous reports have documented the ability of PSK and PSP to activate cellular and humoral components of the host immune system. In addition, these polysaccharides have been shown to inhibit the growth of tumour cell lines and to have in vivo anti-tumour activity (Tzianabos, 2000).

It has been reported that PSK induces gene expression of some cytokines such as TNF-α, IL-1, IL-8, and IL-6, in vivo
and in vitro (Kato et al., 1995; Liu et al., 1996). These cytokines, produced by monocytes, macrophages, and various other cell types, mediate multiple biological effects by direct stimulation of cytotoxic T cells against tumours, enhancement of antibody production by B lymphocytes and induction of IL-2 receptor expression on T lymphocytes. The induction of TNF-α by PSK would contribute, in part, to potent tumouricidal effects of this agent, as the administration of neutralizing antibody against TNF-α significantly attenuates the anti-tumour activity of PSK in the murine model (Kato et al., 1995). Interestingly, recent studies indicate that PSK exerts tumouricidal activity by inducing T cells that recognize PSK as an antigen and kill tumour cells in an antigen-specific manner (Okazaki, 1995).

Furthermore, PSP has been described to enhance immunity in diverse manner; by enhancing B cell function (humoral immunity) in normal and tumour bearing mice that had been challenged with foreign antigen (i.e., sheep red blood cells). PSP increased the levels of haemolysin (antibody) in treated mice, this response was particularly pronounced in tumour bearing mice (Zhou, 1988) by promoting the proliferation of T-lymphocytes both in human peripheral blood and mouse splenocytes (Li et al., 2000) augmenting T-helper cell (CD4+) activation, and also increased the ratio of CD4+/T suppressor (CD8+) production (Wang et al., 1997), stimulating lymphokine-activated-killer (LAK) cell proliferation, and reducing the concentration of IL-2 needed to produce a cytotoxic response (Jiang et al., 1993).

**Antitumour activity of edible mushrooms**

In Nigeria, researchers had investigated antitumor potentials of metabolites of some edible mushrooms including locally cultivated *P. pulmonarius* and the exotic species *P. ostreatus* attributed to the polysaccharide−protein complexes which are able to stimulate the non-specific immune system and to exert antitumor activity through the stimulation of the host’s defence mechanism as well as ethanol extract from *Moringa oleifera* plant. The aqueous solution of the mushroom metabolites was orally administered (0.2 ml of 20 mg/ml) pre, during and post tumour induction. The tumour burden was estimated and scored with the antitumour effects of the metabolites evaluated by assessing changes in tumour size, number and some haematological parameters. The metabolites exhibited a good anticancer potential as they exhibited tumour suppressor and therapeutic potentials to animals on oral administration of metabolites in various experimental groups (Chi square (χ²) = 19.20, "P<0.05"). Metabolites of *P. pulmonarius* were observed to demonstrate a better performance than *P. ostreatus* metabolites although not statistically significant "P> 0.05". The non-toxic bioactive compounds studied have demonstrated important immunotherapeutic antitumour activities in the tumour bearing rats (Akanni et al., 2010).

Schizophyllan, from *Schizophyllum commune* is another mushroom polysaccharide-bound protein complex which has been shown to exert immunomodulating activities in vivo and in vitro with a mechanism similar to that of Lentinan (Tzianabos, 2000). The antitumour activity of Schizophyllan is mainly due to host-mediated immune responses and Schizophyllan has also been reported to be a T-cell oriented immunopotentiators which requires a functional T cell component for its biological activity (Nemoto et al., 1993; Fulzele et al., 2003).

Several other types of mushroom have been shown to demonstrate immunomodulatory activity. *Haridradi Ghrita* (HG) shows preferential stimulation of the components of cell-mediated immunity and shows no effect on the humoral immunity (Fulzele et al., 2003), *Agaricus brasiliensis* KA21 (i.e. *Agaricus blazei* (Ying et al., 2008). The white button (WB) extracts readily stimulated macrophage production of TNF-α. The crimini, Maitake, oyster and shitake extracts also stimulated TNF-α production in macrophage but the levels were lower than from WB stimulation.

The immunological potentials of bioactive compounds from different extracted fractions of various types of mushrooms from diverse locations around the world have been explored by many scientists in the field. Lentinan, a component of edible mushroom which is often given as part of a combination therapy for cancer in addition to conventional cytotoxic drugs was assessed. A case of advanced gastric cancer with multiple liver metastases successfully treated with TS-1/low-dose CDDP/lentinan combination chemotherapy and S-1 combined with lentinan in patients with unrespectable or recurrent gastric cancer (1.5 g) increased the levels of helper T cells in healthy volunteers. In those taking placebo, the levels were unchanged (Jones, 1998).

In 2002, the immunologic potential of edible mushrooms was reported (Wasser, 2002). According to the report, mushroom polysaccharides prevent onogenesis, show direct antitumor activity against various allogeneic and syngeneic tumors, and prevent tumor metastasis. However, polysaccharides from mushrooms do not attack cancer cells directly, but produce their antitumor effects by activating different immune responses in the host. The antitumor action of polysaccharides requires an intact T-cell component; their activity is mediated through a thymus-dependent immune mechanism. Practical application is dependent not only on biological properties, but also on biotechnological availability.

**Antileukemia and chemopreventive potential of natural products**

Antileukemia and immunomodulatory effects of fungal metabolites of *P. pulmonarius* and *P. ostreatus* on benzene-
induced leukemia in Wister rats were reported in Nigeria (Akanni et al., 2012). Leukemia was successfully induced in Wister rats by intravenous injection (0.2 ml) of a benzene solution every 2 days for 3 consecutive weeks. The aqueous solution of fungal metabolites (20 mg/ml) produced by submerged fermentation was orally administered (0.2 ml) before, during, and after leukemia induction. Leukemia burden was assessed by comparing the hematological parameters at baseline and after leukemia induction. The immunomodulatory potential of the metabolites was assessed by using a phagocytic assay (carbon clearance method). The ability to enhance leukopoiesis was assessed by using the total leukocyte count. Leukemia induction resulted in significant anemia indices and leukocytosis ($P<0.05$) in the experimental rats. Both metabolites equally enhanced leukopoiesis and demonstrated phagocytic actions; P. ostreatus activity was significantly higher than that of P. pulmonarius ($P<0.05$). The metabolites exhibited profound anti-leukemic potential by suppressing leukemia and demonstrating immunotherapeutic activities on animals after oral administration in various experimental groups (Table 1 and Figure 1).

The chemopreventive and antileukemic activities of ethanol extracts of M. oleifera leaves on benzene induced leukemia bearing rats were also investigated. Leukemia was induced by intravenous injection of 0.2 ml benzene solution 48 hourly for 4 weeks in appropriate rat groups. Ethanol extract of M. oleifera (EMO) leaves was administered at 0.2 ml of 100 mg/ml to respective treatment rat groups. A standard antileukemic drug (cyclophosphamide) was also used to treat appropriate rat groups. Clinical examination of liver and spleen with hematological parameters were employed to assess the leukemia burden following analysis of the rat blood samples on Sysmex KX-21N automated instrument. Leukemia induction reflected in severe anemia and a marked leukocytosis over the control/baseline group. Liver and spleen enlargements were also observed in group exposed to benzene carcinogen. The in vivo antioxidative potential of EMO was evaluated using Malondialdehyde (MDA) and reduced glutathione (GSH) levels. The liver MDA and GSH levels obtained in benzene induced leukemic rats treated with EMO compared favorably with those obtained in similar treatments with the standard drug ($p<0.05$). The extract demonstrated chemopreventive and anti-leukemic activities as much as the standard anti-leukemic drug ($p>0.05$) by ameliorating the induced leukemic condition in the affected rat groups owing to its bioactive constituents. This study reveals that the extract might be an active, natural and non-toxic anticancer drug lead (Akanni et al., 2014).

Further to the immunotherapeutic activity demonstrated by edible mushrooms being natural products on the management of chronic diseases such as cancer, diabetes mellitus, cardiovascular diseases, renal diseases etc metabolites of P. pulmonarius and P. ostreatus in a study on haematological characterization in n-nitroso-n ethylurea induced tumour bearing rats on oral administration of P. pulmonarius and P. ostreatus metabolites also ameliorated anaemia in cancerous states of the tumour bearing rats. However, metabolites of P. pulmonarius were observed to demonstrate a better cancer chemoprevention potential than P. ostreatus metabolites as evidenced by the results of the parameters studied although the difference is not statistically significant "$P>0.05$" (Akanni et al., 2010).

A study of the use of Trinity immunobooster (Trino IB) in HIV sero-positive persons without aids was carried out in Nigeria using a natural product formulation in the treatment and management of persons living with Human immunodeficiency virus. The product was found to boost immune status of the patients enrolled in the study using indices such viral load and CD4+ counts to measure their immune response to the natural product's treatment (Oke and Oloke, 2009).

**Immunocompetent agents from Protein A**

Protein A obtained from a local isolate of S. aureus was reported to protect rats against infection by pathogenic organisms. A good amount (5000 µg) of Protein A was extracted from a small quantity (approximately 40 g) of S. aureus culture using lysostaphin technique. This extract was found to have protective property against pathogenic Escherichia coli and Pseudomonas aeruginosa in rats even at a low concentration of 50 µg. The crude Protein A extract also compared favourably with imported standard Protein A in the study (Kobayashi and DeLeo, 2013).

Methicillin-resistant S. aureus (MRSA), is endemic in hospitals and is the most frequent cause of community-associated bacterial infections in the United States. Inasmuch as treatment options for severe MRSA infections are limited, there is need for a vaccine that protects against such infections. However, recent efforts to generate a staphylococcal vaccine have met with little success in human clinical trials (Falugi et al., 2013). A recent study, showed that vaccination with spa mutant S. aureus strains lacking antibody Fc- and/or Fab-binding capacity protects against subsequent challenge with the USA300 epidemic strain. The findings provide strong support for the idea that SpA promotes S. aureus immune evasion in vivo and form the foundation for a new approach in our efforts to develop a vaccine that prevents severe S. aureus infections (Gbadero and Oloke, 2009).

**Conclusions**

Natural products have demonstrated profound immunomodulatory activity as their mode of action in the prevention, treatment and management of both
Table 1. Comparison of phagocytic activity of *P. ostreatus* and *P. pulmonarius* metabolites using the carbon clearance assay.

<table>
<thead>
<tr>
<th>Group (concentration of metabolites)</th>
<th>Time (mins)</th>
<th><em>Pleurotus ostreatus</em></th>
<th><em>Pleurotus pulmonarius</em></th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3</td>
<td>50.13±0.85</td>
<td>70.13±0.85</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>2 (20 mg/ml)</td>
<td>6</td>
<td>55.69±0.93</td>
<td>75.65±0.94</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>59.9±1.41</td>
<td>79.99±1.41</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>61.34±0.69</td>
<td>81.34±0.69</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>44.85±1.20</td>
<td>64.85±1.20</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>3 (40 mg/ml)</td>
<td>6</td>
<td>46.65±0.71</td>
<td>66.65±0.71</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>50.15±0.82</td>
<td>70.15±0.82</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>53.85±0.75</td>
<td>73.85±0.75</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>43.40±0.29</td>
<td>63.40±0.29</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>4 (80 mg/ml)</td>
<td>6</td>
<td>44.50±0.77</td>
<td>64.50±0.77</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>47.63±0.45</td>
<td>67.63±0.45</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>52.75±0.59</td>
<td>72.75±0.59</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Figure 1. Effect of *Pleurotus pulmonarius* and *P. ostreatus* metabolites on leukocyte production.

haematological malignancies and variety of solid tumours. Further investigation of immunocompetence of natural products is still in progress as well as synergistic effect of combination therapy involving two or more products.

REFERENCES


Akanni EO, Oloke JK, Adebayo EA, Ola IO (2010). Antitumour activities of...


Cite this article as:


Submit your manuscript at http://www.academiapublishing.org/ajb