Cytotoxicity Effect of Selected Medicinal Mushrooms on BV2 Microglial Cells

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Abstract: Microglial cells are the resident macrophages of the brain and spinal cord, and thus act as the first and main line of active defence in the central nervous system against assaults including inflammation. In the search for safe anti-inflammatory agents, mushrooms are being actively sought. This study was conducted to determine the cytotoxic effects of various solvent extracts of three culinary and medicinal mushrooms--Lignonous rhinoceratus (Cooke) Ryvarden, Cordyceps militaris (L.) Link and Hericium erinaceus (Bull. : Fr.) Pers-to BV2 microglial cells. Cytotoxic effects were assessed by MTT assay. All fractions tested were not toxic to the BV2 cells at the concentrations tested. Ethanol extracts (100% - 191.77%) showed 35%-90% higher proliferation of BV2 cells when compared to the aqueous extract (19.38% - 98.22%). The extract concentrations tested were 0.1μg/ml and 100μg/ml. The ethanol extracts were further fractioned to hexane, ethyl acetate and aqueous fraction. These fractions were also tested for cytotoxic effects to the BV2 cells. The hexane fractions of the three mushrooms tested showed the highest proliferation (ranging 100% - 200.16%) effects on the microglial cell line, followed by ethyl acetate fraction (92.06 % - 167.41%). However, the aqueous fraction (2.00% - 93.21%) showed toxic effects to the cells in a dose dependent manner.

Keywords: microglial cells, medicinal mushrooms, MTT, culinary mushrooms, solvent fractions

1 Introduction

Microglia is the resident macrophages immune cells of the brain and spinal cord in the central nervous system (CNS)3. They are distributed in large non-overlapping regions throughout the brain and spinal cord2-3. Microglia is constantly scavenging the CNS for damaged neurons, plaques, and infectious agents and plays an active role in host defence and tissue repair in the brain3. In response to stress or injury, they assume a pro-inflammatory phenotype that can lead to profound neuronal damage if uncontrolled or dysregulated2. Since this process must be done quickly to prevent potentially fatal damage, microglia is extremely sensitive to even small pathological changes in the CNS4.

The greatest attribute of mushrooms, besides their taste, is their peculiar healing properties. Medicinal mushrooms have been appreciated and used since ancient times by the Chinese and Egyptians. These mushrooms were related with longevity and immortality9. Today, there are over 270 identified fungal species with known therapeutic properties. These include anti-oxidants, hypotensives, hypocholesterolemic, liver protectants, immune suppressants, anti-fibrotics, as well as anti-inflammatory, anti-diabetic, anti-viral and anti-microbial properties7. They also contain
Investigation of the Role of Culinary and Medicinal Mushrooms in Neurodevelopment by Using Differentiating Neuroblastoma-2a Cells

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Abstract: Neurite outgrowth is an essential part for establishment of synaptic connections during early development. Accumulated evidences suggested that culinary-medicinal mushrooms play a pivotal role in the prevention of many age-associated neurological dysfunctions. We had shown that *Hericium erinaceus* (Bull.: Fr.) Pers. (lion’s mane mushroom, *Yamabushitake*) grown in tropical areas, *Lignosus rhinocerotis* (Cook) Ryvarden (tiger’s milk mushroom), and *Pleurotus giganteus* (Berk.) Karunarathna & K.D. Hyde (cow’s stomach mushroom) exhibited stimulatory activity of neurite outgrowth using NG108-15 and PC12 cell lines. This observation raised a question with respect to the neurodevelopmental effects, if any, of culinary-medicinal mushrooms. Thus, we aimed to screen and evaluate neurite outgrowth stimulatory effects of selected culinary-medicinal mushrooms using neuroblastoma-2a (N2a) cells (ATCC CCL-131). *In vitro* embryotoxicity or neurotoxicity was investigated using mouse embryonic fibroblast (BALB/3T3) and N2a cells respectively. The five mushrooms used in this study were *Ganoderma lucidum* (M.A. Curtis: Fr.) P. Karst (Ling Zhi), *L. rhinocerotis*, *P. giganteus*, *Grifola frondosa* (Dicks. Fr.) S.F. Gray (Maitake) and *Cordyceps militaris* (L.: Fr.) Link (Dong Cong Xia Cao). Neurite length was measured using Image-Pro Insight processor system. After 24h of N2a and BALB/3T3 cells was exposed to mushroom extracts, no toxic effect was observed by MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay. The positive control, nerve growth factor (NGF) induced 26.4 ± 3.6% in neurite-bearing cells. Aqueous extracts of *G. lucidum*, *L. rhinocerotis*, *P. giganteus* and *G. frondosa* as well as ethanol extract of *C. militaris* significantly (p< 0.05) promoted the neurite outgrowth in N2a cells. The average neurite length of NGF-stimulated cells was 78.58 ± 18.6 μm, which was approximately 4.5 longer than the control. Cells treated with *G. lucidum* were found to develop the longest neurite length, followed by *P. giganteus*. As a conclusion, our results showed that the mushrooms had the potential in promoting neurite outgrowth during neurodevelopment. The synergism of the various active entities in these mushroom extracts may be responsible for the activity. The signalling pathways involved is yet to be elucidated, but based on our previous results, phosphorylation and activation of the ERK and Akt may be involved.

Keywords: embryotoxicity, neurite outgrowth, mushrooms, neurotoxicity, hot aqueous extracts

1 Introduction

Neurite outgrowth is important in neuronal path finding, synaptic connections, neuronal plasticity, and neurodegenerative conditions such as Alzheimer and Parkinson diseases [1]. Prevention of age-related neuronal diseases aims at neurite outgrowth promotion and preservation of the neuronal network and synaptic connections. Over the last few years, the culinary-medicinal mushrooms using in alternative and complementary medicine has been validated. Previous research in our lab showed that *Hericium erinaceus* (Bull.: Fr.) Pers. (monkey’s head mushroom, lion’s mane mushroom and *Yamabushitake*)[1], *Lignosus rhinocerotis* (Cook) Ryvarden (tiger’s milk mushroom)[2], and *Pleurotus giganteus* (Berk.) Karunarathna & K.D. Hyde (morning glory mushroom, cow’s stomach mushroom)[3] exhibited neuroregenerative effects by using NG108-15 and PC12 cell lines. This observation raised a question with respect to the neurodevelopmental effects of culinary-medicinal mushrooms. Thus, the aim of the present study was to evaluate stimulatory effects of culinary-medicinal mushrooms on neurite outgrowth by using mouse neuroblastoma-2a (N2a) cells. We also assessed the neuro- and embryotoxicity of the mushroom extracts using N2a cells and mouse embryonic fibroblast cells (3T3 fibroblasts).
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Lignosus rhinocerotis (Cooke) Ryvarden Induces Neuritogenesis via Extracellular Signal-Regulated Kinase (ERK) Pathway in Rat Pheochromocytoma Cells

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Neuritogenic activity has become one of the focuses of study in search of preventive and therapeutic agents for neurodegenerative diseases. As one factor in the neurotrophin family, nerve growth factor (NGF) has been shown to promote various biological activities, e.g. promoting neuronal survival, proliferation, neuritogenesis. It is expected to be useful in the treatment of neurodegenerative diseases. Among the natural sources explored for NGF mimics, the traditional use of culinary and medicinal mushrooms has attracted intense interest. Traditional application of the mushroom Lignosus rhinocerotis by indigenous folk as remedy and tonic to treat variety of ailments has been documented in Malaysia. This study aimed to investigate the potential of NGF-like activity of L. rhinocerotis in rat pheochromocytoma (PC-12) cells, used as a positive control. The aqueous extract of L. rhinocerotis sclerotium promoted neuritogenesis in PC-12 cells significantly (p<0.05) at a low concentration (25 μg/ml). The involvement of the mitogen-activated protein kinase/extracellular signal-regulated kinase (MEK/ERK1/2) signaling pathway in aqueous extract-induced neuritogenesis was investigated using two specific inhibitors of the MEK/ERK1/2 pathway (U0126 and PD98059). The neuritogenic activity of aqueous extract was blocked by these inhibitors significantly (p<0.001). Furthermore, the protein expression of total p44/42 MAPK (Erk1/2) and phosphor-p44/42 MAPK was determined. The data revealed the first evidence of the involvement of MEK/ERK1/2 signalling pathway in aqueous extract of L. rhinocerotis sclerotium-induced neuritogenesis in PC-12 cells.

Keywords: Lignosus rhinocerotis, Nerve growth factor, MEK/ERK1/2 signaling pathway, Neuritogenesis, neuronal degenerative diseases.

1 Introduction

Lignosus rhinocerotis (Cooke) Ryvarden is also known as the ‘tiger’s milk mushroom’ in English or 'mamam ussurinai' in Malay. In Malaysia, Lignosus rhinocerotis is the most popular medicinal mushroom used by indigenous communities of Peninsula Malaysia[1]. The sclerotium used to be known as a decoction by indigenous communities as general tonic and also to treat ailments, including fever, cough, asthma, chronic hepatitis, gastric ulcer, cancer and food poisoning[1][2]. The medicinal polysaccharides of L. rhinocerotis demonstrated very high anti-inflammatory, antioxidant, anti-poliferative[3] and immunomodulating effects[4]. Recent findings disclosed that the aqueous extract of L. rhinocerotis sclerotium might possess NGF-like bioactive compound(s) that induced neuritogenesis in PC-12 cells[5]. The present study aimed to further investigate the involvement of MEK/ERK1/2 signalling pathway in aqueous extract of L. rhinocerotis sclerotium-induced neuritogenesis.

2 Materials and Methods

Preparation of aqueous extracts

Lignosus rhinocerotis sclerotium (LiGNO™ TM02) freeze-dried powder was purchased from Ligno Biotech Sdn. Bhd., Malaysia. The aqueous extracts were prepared according to Elker[5].