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Novel antimutagenic factors derived from the edible mushroom *Agrocybe cylindracea*

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Abstract

Studies have shown that certain foods contain compounds with antigenotoxic activities. Here, we ask if dried powders and/or extracts from three edible mushrooms, *Agrocybe cylindracea*, *Lentinula edodes* and *Pleurotus ostreatus*, have a mitigating effect on genotoxicity. We used two in vivo assays: the Drosophila DNA repair test and the Drosophila wing spot test (also known as SMART) which measures somatic mutation and recombination. Eight carcinogens were tested with the mushroom powders: 2-AAF, aflatoxin B1, DMBA, IQ, MeIQx, MNU NDMA, and 4NQO. We found that *A. cylindracea* and *P. ostreatus* powders can suppress DNA damage induced by each of the mutagens we tested. In contrast, *L. edodes* has an inhibitory effect on DNA damage induced by only a sub-set of mutagens, namely aflatoxin B1, NDMA, MNU and 4NQO. In addition, *A. cylindracea* extracts were able to suppress somatic cell mutation induced by aflatoxin B1, MMC, MNU, NDMA, NMOR and 4NQO. These results suggest that Agrocybe genus mushrooms contain factors with antigenotoxic activity, including anti-recombinogenic activity. Furthermore, the antigenotoxic activity of *A. cylindracea* powder can be extracted in water but not in ethyl acetate or methanol, and is sensitive to heat treatment. The data suggest that there is a novel antigenotoxic factor(s) in *A. cylindracea*, possibly in the form of a peptide or protein.

Keywords: *Agrocybe*; Mushroom; Antigenotoxicity; Chemoprevention; Drosophila SMART; Ames test

Abbreviations: 2-AAF, 2-acetylaminofluorene; AFB_1, aflatoxin B_1; DMBA, 7,12-dimethylbenzo[*a*]anthracene; MeIQx, 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline; IQ, 3-amino-3-methylimidazo[4,5-*f*]quinoline; MMC, mitomycin C; MNU, *N*-methyl-*N*-nitrosourea; NDMA, *N*-nitrosodimethylamine; NMOR, *N*-nitrosomorpholine; 4NQO, 4-nitroquinoline *N*-oxide

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1. Introduction

Several food components, including chlorophyllin [1–3], anthraquinone pigments [4] and tea polyphenols [5,6], reduce the level of genotoxicity induced in Drosophila larvae when chemical carcinogens are administered. The identification of additional and more effective antigenotoxic compounds could aid efforts to develop nutritional supplements that prevent cancer in humans. Numerous kinds of mushrooms are utilized as foods and traditional medicines in many countries and there have been investigations of the biological activities of mushroom extracts. The activities of various mushroom extracts include anticarcinogenic effects [7–11], antimutagenic effects [12–16], and protection from blocks to gap junction-based intercellular communication [17].

At the molecular level, researchers have found that antigenotoxic factors in mushrooms include polysaccharides, such as beta- and alpha-glucan. In *Agrocybe cylindracea* (Yanagimatsutake), the anticarcinogenic substances detected in the mushroom have been identified as alpha-D-glucan-*O*-carboxy methylated derivatives [18]. Moreover, several biologically active ingredients have been identified in *A. cylindracea*: immunomodulative polysaccharides [19], polysaccharides that can cause hypoglycemia [20], and indole derivatives with free radical scavenging activity [21]. Ngai et al. reported that a ubiquitin-like peptide from *A. cylindracea* stimulates macrophages and shows ribonuclease activity [22]. Here, we show that in vivo Drosophila assays detect antigenotoxic activity in *A. cylindracea*, and we demonstrate that the active factor(s) are water-soluble, heat-labile, and have a large molecular weight. The active factor(s) appears to be novel biologically active molecules that are different from anticarcinogenic polysaccharides that have been reported in the literature [7–11,18].

2. Materials and methods

2.1. Materials

MeIQx (2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline; CAS 77500-04-0), IQ (3-amino-3-methylimidazo[4,5-*f*]quinoline; CAS 76180-96-6), 2-acetylaminofluorene (CAS 53-96-3), and 4-nitroquinoline *N*-oxide (CAS 56-57-5) were purchased from Wako Fine Chemicals (Osaka, Japan); 7,12-dimethylbenzo[*a*]anthracene (CAS 57-97-6), aflatoxin B_1 (CAS 1162-65-8), *N*-nitrosomorpholine (CAS 59-89-2) and mitomycin C (CAS 50-07-7) from Sigma Chemical (St. Louis, MO); *N*-methyl-*N*-nitrosourea (CAS 684-93-5) from Nacalai Tesque (Kyoto, Japan); and *N*-nitrosodimethylamine (CAS 62-75-9) from Tokyo Kasei Kogyo (Tokyo, Japan). Dried powders from three species of mushroom, *A. cylindracea* (Yanagimatsutake), *Lentinula edodes* (Shi-itake), and *Pleurotus ostreatus* (Hiratake), were provided by Mr. Masaharu Yabe (Yabe Kinoko-en, Okayama, Japan).

2.2. Extraction of active agents from mushroom powders

Dried powder (4 g) was stirred in distilled water (100 ml) for 30 min at room temperature. The water-soluble fraction was separated from insoluble residue by filtration and aspiration. The mixture was then lyophilized. The lyophilized powder was dissolved in 20 ml distilled water for use in the Drosophila assay. Methanol and ethyl acetate extract were prepared by stirring with methanol or ethyl acetate for 30 min at room temperature and the soluble fraction was collected by, allowed to evaporate, and then dissolved in water or ethanol. Residues were dried under vacuum and crushed to powders that were then mixed into the food given to the Drosophila larvae.

The water-soluble extract was dialyzed against distilled water at 4 °C overnight (dialysis membrane, size 27, Wako Fine Chemicals, Osaka, Japan). After removal of aggregates by centrifugation, the fraction inside the dialysis membrane was used in both the Drosophila assay and the Ames test. In order to examine the heat stability of antigenotoxic factor(s), the activity of the water-soluble extract was also tested after treatment at 37, 60, and 100 °C for 30 min.

2.3. Drosophila assay

The Drosophila assays were performed as previously described [1,23]. The Drosophila strains used in this study were provided by Dr. H. Ryo (Osaka University) and Dr. K. Fujikawa (Kinki University). Third instar larvae were fed a diet containing a mutagen, with