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NOTE

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The effect of strain, growth stage, and cultivating condition of *Ganoderma lucidum* on 5α-reductase inhibition

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Abstract The inhibitory effects of 102 methanol extracts of 40 mycelia, 9 culture fluids, and 53 fruiting bodies of 40 strains of *Ganoderma lucidum* on 5α-reductase were investigated. The methanol extract of the fruiting body of each strain was found to show the strongest 5α-reductase inhibitory activity among the extracts tested.

Key words 5α-Reductase · *Ganoderma lucidum* · Antiandrogen activities · Benign prostatic hyperplasia (BPH)

Introduction

Androgen-mediated diseases such as prostate cancer, hirsutism, acne, andorogenic alopecia, and benign prostatic hyperplasia (BPH) are serious problems.[1,2] BPH is one of the most common symptoms seen in older men, and 40% of men aged 50 to 60 years and 90% of men aged 80 to 90 years were diagnosed with BPH. The principal prostatic androgen is dihydrotestosterone (DHT) which is formed by the catalytic action of the steroid enzyme 5α-reductase from its substrate testosterone.[3] Because the weight of seminal vesicles depends on the 5α-reduced androgens, it is important to regulate an adequate level of DHT. Two isoforms of 5α-reductase have been cloned, expressed, and characterized (type 1 and type 2), which display different tissue-expression patterns, enzyme kinetic parameters, and chromosomal localization.[4] Both isozymes are over expressed in BPH tissue.[5] For BPH therapy, because it could reduce DHT by blocking its conversion from testosterone, 5α-reductase inhibitors could be useful as a treatment.[6] A number of compounds have been identified for this purpose, including both a steroidal and a nonsteroidal inhibitor. However, it is reported that these inhibitors may cause adverse effects such as gynecomastia, impairment of muscle growth, and severe myopathy.[7] Therefore, the emergence of therapeutic materials that have fewer side effects, preferably from edible natural products, is desirable for safety to be guaranteed.

For thousands of years, mushrooms have been known as a source of medicine. They are widely sold as nutritional supplements and are touted as being beneficial to health. Therefore, we have focused on edible and medicinal mushrooms as potential sources of 5α-reductase inhibitors.

In our preliminary screening of 19 edible and medicinal mushrooms (*Ganoderma lucidum, Pleurotus ostreatus, Lentinula edodes, Lyophyllum decastes, Hericium ramosum, Agaricus blazei, Hypsizygus marmoreus, Panellus serotinus, Naematoloma sublateritium, Grifora frondosa, Pleurotus abalonus, Pleurotus eringii, Flammulina velutipes, Pholiota nameko, Pholiota adiposa, Pleurotus cornucopiae, Agrosybe cylindracea, Pleurotus pulmonarius, Agaricus bisporus*), we discovered that the methanol extract of *G. lucidum* (Polyporaceae) showed the strongest 5α-reductase inhibitory activity among them.

The fungi *G. lucidum* has been used for centuries in East Asia. Its fruiting body is called "Reishi" in Japan and "Lingzhi" in China. In these areas, *G. lucidum* has been a popular folk or oriental medicine to cure various human diseases such as hepatitis, hypertension, hypercholesterolemia, and gastric cancer.[1,8] However, 5α-redutase inhibitory activity of *G. lucidum* has never been reported.

In this study, we have extensively investigated the effects of growth stage and cultivation conditions on 5α-reductase inhibitory activity by using 102 methanol extracts of 40

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mycelia, 9 culture fluids, and 53 fruiting bodies of 40 strains of *G. lucidum*.

Material and methods

Sample preparations. The strains of *Ganoderma lucidum* used in this study were provided by several institutions or individuals as listed in Table 1. Cultures were maintained on SMY (sucrose 1%, malt extract 1%, yeast extract 0.4%) liquid medium or potato dextrose agar (PDA) medium. Incubation with SMY medium was carried out at 23°C for 18 days, and on PDA medium at 25°C for 7 days until mycelium grew well, after which the cultures were filtered through filter paper and separated into mycelium and liquid medium. The fruiting bodies grown on substrates containing *Fagus crenata* or *Cryptomeria japonica* sawdust or commercially available fruiting body grown on wood were obtained. The mycelium, liquid medium, and fruiting body were lyophilized. Each ground sample (0.5 g) was extracted with methanol (50 ml) at room temperature overnight, and the extract concentrated to dryness. The methanol extracts were stored in a dessicator prior to 5α-reductase inhibitor assay.

Preparation of rat microsomes. Rat (crj :CD (SD) IGS, Charles River Laboratories) liver microsomes from female Sprague-Dawley (SD) rats (7 weeks of age) and prostate microsomes from male SD rats (13 weeks of age) were prepared by a method previously reported by Shimizu et al.[9] with some modifications. Two mature female SD rats were killed. The livers were removed and minced tissue was then homogenized in four tissue volumes of medium A (0.32 M sucrose, 1 mM dithiothreitol, and 20 mM sodium phosphate, pH 6.5). Three mature male SD rats were also killed. The prostates were removed and minced tissues were then

Table 1. Inhibitory effects of the methanol extracts of *Ganoderma lucidum* on 5α-reductase

Fungus no.	Collection site	Mycelial (cultured by PDA)	Mycelial (cultured by SMY)	Culture fluid	Fruiting body
Gal-01	TFFPRC	–	17	–	53
Gal-02	TFFPRC	–	16	–	54
Gal-03	TFFPRC	–	18	–	47
Gal-04	TFFPRC	–	–	–	48
Gal-05	SFES	30	36	1	62
Gal-06	SFES	–	14	–	48
Gal-07	SFES	–	–	–	55
Gal-08	SFES	30	17	–	60
Gal-09	SFES	–	13	–	60
Gal-010	SFES	22	24	4	49
Gal-011	SFES	18	26	–	45
Gal-012	SFES	22	23	3	43
Gal-013	SFES	–	13	–	48
Gal-014	SFES	24	24	5	50
Gal-015	SFES	–	14	–	53
Gal-016	SFES	–	19	–	55
Gal-017	TFFPRC	–	–	–	51
Gal-018	TFFPRC	–	30	–	49
Gal-019	TFFPRC	–	–	–	50
Gal-020	TFFPRC	–	28	–	–
Gal-021	FPFREC	28	30	0	64
Gal-022	FPFREC	–	30	1	63
Gal-023	FPFREC	17	23	2	58
Gal-024	FPFREC	–	–	–	59
Gal-025	FPFREC	19	19	–	57
Gal-026	FPFREC	17	19	4	60
Gal-027	SFES	27	25	3	47
Gal-028	OMRI	–	20	–	–
Gal-029	OMRI	–	21	–	54
Gal-030	OMRI	–	22	–	49
Gal-031	OMRI	–	19	–	50
Gal-032	OMRI	–	20	–	49
Gal-033	OMRI	–	10	–	–
Gal-034	Okuti city	–	–	–	74
Gal-035	Ebino city	–	–	–	65
Gal-036	Shandong Province, China	–	–	–	62
Gal-037	Shandong Province, China	–	–	–	42
Gal-038	Bisoken	–	–	–	61
Gal-039	Bisoken	–	–	–	69
Gal-040	Bisoken	–	–	–	76

Extract concentration: 200 ppm. Inhibitory activity given as percentage
PDA, Potato dextrose agar, SMY, sucrose 1%/malt extract 1%/yeast extract 0.4%; TFFPRC, Toyama Forestry and Forest Products Research Center; SFES, Saga Forest Experiment Station; FPFREC, Fukuoka Prefecture Forest Research and Extension Center; OMRI, Oita Mushroom Research Institute

homogenized in four tissue volumes of medium A. The homogenate was then centrifuged at 10000*g* for 10 min. The resulting supernatant from the centrifugations was centrifuged a further two times at 105000*g* for 1 h. The washed microsomes were suspended in a one-pellet volume of medium A, and the dispersion of microsomes was achieved using a syringe with 18 G, 23 G, and 26 G needles in succession. The microsome suspension was stored at −70°C before use.

Measurement of 5α-reductase inhibitory activity. A complete reaction mixture contained 1 mM dithiothreitol, 20 mM phosphate buffer (pH 6.5 for 5α-R1 or pH 4.5 for 5α-R2), 1.9nCi [4-^{14}C] testosterone, 150μM testosterone, 167μM NADPH, and the enzyme preparation (1.54 mg of protein) in a final volume of 0.3 ml. The concentration of testosterone contributed by [4-^{14}C] testosterone was negligible. The incubation was carried out for 10 min at 37°C. The incubation was started with the addition of 10μl of microsomes to the preheated reaction solution in a tube. After 10 min, the incubation was terminated by adding 10μl of 3 M NaOH solution. To extract metabolites, 1 ml of diethyl ether was added, and the tubes were capped and shaken. The organic phase (200μl) was applied to a silica plate (Kieselgel 60 F_{254}). The plate was developed in ethyl acetate–*n*-hexane (7:3) at room temperature. The radioactivity profile was determined with an imaging analyzer (FLA-5000 RF, Fuji Film, Tokyo, Japan). The 5α-reductase activity was calculated from the percentage of the extent of the conversion of [4-^{14}C] testosterone to [4-^{14}C] dihydrotestosterone.

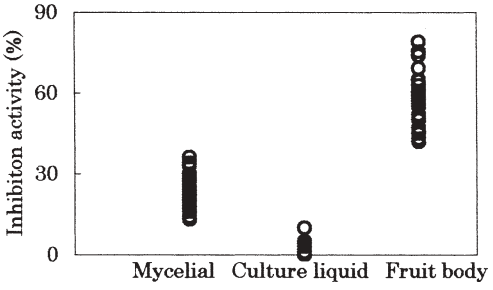

Fig. 1. The 5α-reductase inhibitory activity of the methanol extract of mycelial, culture liquid, and fruiting body of *Ganoderma lucidum*. Sample concentration: 200 ppm

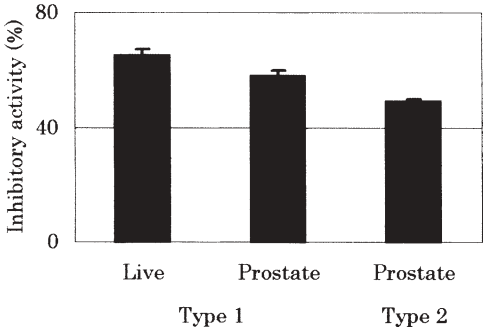

Fig. 2. The inhibitory activity of methanol extracts of fruiting body of *G. lucidum* (Gal-034 strain) on type 1 and type 2 5α-reductase prepared from prostate or type 1 5α-reductase from live. Each column represents the mean ± standard deviation, $n = 3$. Sample concentration: 200 ppm

Results and discussion

Nineteen species of edible and medicinal mushrooms were extracted overnight with methanol at room temperature. 5α-Reductase inhibitory activity was assayed against each of the methanol extracts at a concentration of 200 ppm. The different species showed different 5α-reductase inhibitory activities ranging from 3% to 60%. In our screening exercise, *Ganoderma lucidum* inhibited 74% of 5α-reductase activity at the same concentration of 200 ppm. Such a high 5α-reductase inhibitory activity made us focus our study on the fruiting body of *G. lucidum*.

40 mycelia, 9 culture liquids, and 53 fruiting bodies of 40 strains of *G. lucidum* were extracted overnight with methanol at room temperature. 5α-Reductase inhibitory activity was assayed in each of the methanol extracts at a concentration of 200 ppm. The microsome portion prepared from rat liver was used as type 1 isozyme source because it is more easily available than that of the prostate. In this screening assay for the methanol extracts of *G. lucidum*, the methanol extracts of the fruiting body showed the highest inhibitory activity (Fig. 1). The sequence of the 5α-reductase inhibitory activity is fruiting body > mycelium > culture liquid. These results suggested that the 5α-reductase inhibitory components are accumulated in the fruiting body. It should be noted that finasteride, which is known as a potent steroidal inhibitor, showed an IC_{50} of 0.73μM in our assay system.

Two 5α-reductase isozymes have been identified in rats and humans.[3] Both isozymes are over expressed in BPH tissue.[5] Coded by two different genes,[10] they display a maximal activity at different pH (6.5 for type 1 and 4.5 for type 2), and have different biochemical characteristics. In rats, the type 1 isozyme predominates in tissues such as liver, kidney, brain, lung, and skin but also exists in the prostate, whereas the type 2 isozyme is more abundant in genital tissues such as the prostate. Therefore, we examined the inhibitory effect of the extract of *G. lucidum* against both isozymes prepared from prostate. As shown in Fig. 2, the methanol extract of the fruiting body of *G. lucidum* (Gal-034 strain) inhibited both isozymes (Fig. 2). It should be noted that the inhibitory activity of this extract was 65% inhibition against type 1 5α-reductase prepared from liver at the concentration of 200 ppm.

The fungi *G. lucidum* (Reishi, Mannentake, or Lingzhi) has been used for centuries in East Asia to treat various human diseases such as hepatitis, hepatopathy, hypertension, nephritis, bronchitis, and cancers.[1,8] Its dried powder was especially popular as an anticancer agent in the Imperial Court of ancient China.[11] Some of the triterpenes, such as ganoderic and lucidic acids, recently isolated from *G. lucidum* demonstrated cytotoxicity against mouse sarcoma and mouse lung carcinoma cells in vitro.[12]

In this study, we found a new facet of the biological activities of *G. lucidum*, namely 5α-reductase inhibitory activity. The extracts of the fruiting body of *G. lucidum* inhibited both types of 5α-reductase, showing so-called dual inhibition. Therefore, this source might be advantageous for therapy against BPH, because it has been shown that the dual inhibitor dutasteride is more powerful in reducing the DHT plasma concentration than selective type 1 or type 2 inhibitors.[13] In the future, herbal therapies using mushrooms such as *G. lucidum* will become important and wide spread treatments for many diseases.[7] Antiandrogenic activity of the fruiting body of *G. lucidum* is an important biological activity that may be available for patients of androgen-related disease such as BPH. At this time, the clinical implications of this activity are unknown, so further research is needed before the fruiting body of *G. lucidum* can be made available for treatment.

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