

Anti-androgenic activities of *Ganoderma lucidum*

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Abstract

The inhibitory effects of methanol extracts of 19 edible and medicinal mushrooms on 5 α -reductase activity were examined. The extract of *Ganoderma lucidum* Fr. Krast (Ganodermataceae) showed the strongest 5 α -reductase inhibitory activity. The treatment of the fruit body of *Ganoderma lucidum* or the extract prepared from it significantly inhibited the testosterone-induced growth of the ventral prostate in castrated rats. These results showed that *Ganoderma lucidum* might be a useful ingredient for the treatment of benign prostatic hyperplasia (BPH).

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1. Introduction

Nowadays, androgen-mediated diseases such as prostate cancer, hirsutism, acne, androgenic alopecia and benign prostatic hyperplasia (BPH) have become serious problems (Barrtsch et al., 2002). Above all, BPH is one of the most common ailments seen in older men; 40% of men 50–60 years of age and 90% of men 80–90 years of age have been diagnosed with BPH. The principal prostatic androgen is dihydrotestosterone (DHT), which is formed by the steroid enzyme 5 α -reductase from its substrate testosterone (Russell and Wilson, 1994). 5 α -Reductase is a membrane-bound NADPH-dependent enzyme that catalyzes the reduction of testosterone to the more potent androgen DHT. The effect of DHT is purely androgenic in that, unlike T, it cannot be transformed into estrogen. Since the weight of the seminal vesicles depends on the 5 α -reduced androgens,

it is important to regulate the level of the DHT. Therefore, 5 α -reductase inhibitory ingredients should be useful in the treatment of BPH (Barrtsch et al., 2000).

Two isoforms of 5 α -reductase (types 1 and 2) have been cloned, expressed and characterized; they display different tissue expression patterns, enzyme kinetic parameters and chromosomal localization (Jenkins et al., 1991). These two 5 α -reductase isozymes have been identified in both rats and humans, and both isozymes are over-expressed in BPH tissue (Iehle et al., 1999). Coded by two different genes (Andersson and Russell, 1990), they display a maximal activity at different pH (6.5 for type 1 and 4.5 for type 2); overall, they 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. A number of synthesized 5 α -reductase inhibitors with steroidal moiety have been reported. However, it should be noted that these inhibitors have the potential to cause adverse effects such as those reported for finasteride

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(Uygun et al., 1998)—i.e., gynecomastia, impairment of muscle growth, and severe myopathy—due to their structural similarity to steroidal hormones. Hence, the emergence of therapeutic materials having fewer side effects—preferably, edible natural products—would be highly desirable if their safety could be guaranteed.

Although there is no clear evidence that patients who develop BPH will ultimately have prostate cancer, androgens do influence the development of prostate cancer (Ross et al., 1992; Giovannucci et al., 1997; Hsing et al., 2002). The use of finasteride, the 5 α -reductase inhibitor, can lower the androgen levels in the prostate and reduce the risk of prostate cancer (Thompson et al., 2003).

For thousands of years, mushrooms have been known as a source of medicine. In East Asia, the fruiting body of the fungus *Ganoderma lucidum* has been used for centuries. It has long been used as a folk medicine to treat various human diseases such as cancer, hypertension, hepatitis, nephritis and so on (Mizuno et al., 1995). Although the inhibitory effects on the proliferation and migration of prostate cancer cells by *Ganoderma lucidum* (Jian et al., 2004) has been reported, 5 α -reductase inhibition and suppression of androgen-induced prostate cell growth by *Ganoderma lucidum* have never been reported. In this paper, we have demonstrated the in vitro and in vivo anti-androgenic activity of *Ganoderma lucidum* for the first time.

2. Materials and methods

This research was conducted in accordance with internationally accepted principles for laboratory animal use and care as found in, for example, the European Community guidelines.

2.1. Materials

Ganoderma lucidum was obtained from Bisoken Inc. (Fukuoka, Japan). Fungal samples other than *Ganoderma lucidum* were provided by Fukuoka Prefecture Forest Research & Extension Center (Fukuoka, Japan) and were identified by Mr. Shuhei Kaneko. They included: *Pleurotus ostreatus* Fr. Kummer (Pleurotaceae), *Lentinula edodes* Pegler (Tricholomataceae), *Lyophyllum decastes* Fr. Sing (Tricholomataceae), *Hericium erinaceum* Fr. Pers. (Hericaceae), *Agaricus blazei* Murr. (Agaricaceae), *Hypholoma sublateritium* Fr. Quel (Strophariaceae), *Panellus serotinus* Fr. Kuhn. (Tricholomataceae), *Hypsizygus marmoreus* Bigelow (Tricholomataceae), *Grifora frondosa* S.F. Gray (Polyporaceae), *Pleurotus abalonus* Han, K.M. Chen et S Cheng (Pleurotaceae), *Pleurotus eryngii* Fr. Quel (Pleurotaceae), *Flammulina velutipes* Fr. Sing (Tricholomataceae), *Pholiota nameko* S. Ito et Imai in Imai (Strophariaceae), *Pholiota adiposa* Fr. Kummer (Strophariaceae), *Pleurotus cornucopiae* Rolland var. *citrinopileatus* (Sing.) Ohira (Pleurotaceae), *Agaricus bisporus* Imbach (Agaricaceae),

Pleurotus pulmonarius Fr. Kummer (Pleurotaceae), and *Agrocybe cylindracea* Fr. Maire (Bolbitiaceae). The fruiting bodies were dried and ground to powder before use. Unless otherwise specified, chemicals were obtained from Sigma Aldrich Japan Co. Ltd. (Tokyo, Japan). Organic solvents were purchased from Wako Pure Chemical Industries Co. (Osaka, Japan). [4-¹⁴C] Testosterone was obtained from PerkinElmer Japan Co. Ltd. (Kanagawa, Japan). Sprague–Dawley (SD) rats were obtained from Charles River Japan Inc. (Osaka, Japan).

2.2. Methanol extracts of edible mushrooms

Each dried and milled fungal sample (1 g) was extracted with methanol at room temperature for 24 h. The extracts were filtered through ADVANTEC No. 2 filter paper, concentrated under a vacuum, and then freeze-dried. The methanol extracts were stored in the dessicator before assay.

2.3. Ethanol extracts of *Ganoderma lucidum*

Dried and chipped *Ganoderma lucidum* (15 kg) was extracted with 99.5% ethanol (126 l) at room temperature for 24 h using a blender. The extracts were filtered through ADVANTEC No. 2 filter paper, concentrated under a vacuum, and then freeze-dried. The extracts (571.1 g) were stored at -20°C before assay.

2.4. Preparation of rat microsomes

Rat liver and prostate microsomes from female (7 weeks age) and male (13 weeks age) SD rats, respectively, were prepared by a method previously reported by Shimizu et al. (2000) with some modifications. Two mature SD female rats were killed. Their livers were removed and minced tissue was homogenized in four tissue volumes of medium A (0.32 M sucrose, 1 mM dithiothreitol, and 20 mM sodium phosphate, pH 6.5). Also, three mature male SD rats were killed. Their prostates were removed and minced tissues were homogenized in four tissue volumes of medium A. The homogenate was then centrifuged at $10,000 \times g$ for 10 min. The resulting supernatant from the centrifugations was further centrifuged at $105,000 \times g$ for 1 h twice. The washed microsomes were suspended in one pellet volume of medium A, and the dispersion of microsomes was achieved using a syringe with 18G, 23G, and 26G needles in succession. The microsome suspension was stored at -80°C just before use.

2.5. Measurement of 5 α -reductase inhibitory activity

A complete reaction mixture included 1 mM dithiothreitol, 20 mM phosphate buffer (pH 6.5 for 5 α -R1 or pH 5.0 for 5 α -R2), 1.9 nCi [4-¹⁴C] testosterone, 150 μM testosterone, 167 μM NADPH, and the enzyme preparation (1.54 mg of protein) in a final volume of 0.3 ml. The concentration of testosterone contributed by [4-¹⁴C] testosterone was

negligible. Edible and medicinal mushrooms of 19 species, extracted with methanol overnight at room temperature, were added to the solution at a concentration of 200 ppm. The incubation of these samples was carried out for 10 min at 37 °C and was started by the addition of 10 μ l microsomes to the pre-heated reaction solution in a tube. After 10 min, the incubation was terminated by adding 10 μ l of 3 M NaOH. To extract the metabolites, 1 ml of diethyl ether was added, and the tubes were capped and shaken. The organic phase was applied to a silica plate (Kieselgel 60 F₂₅₄). 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 Co. Ltd., Tokyo, Japan). The 5 α -reductase activity was calculated from the percentage conversion of [4-¹⁴C] testosterone to [4-¹⁴C] dihydrotestosterone.

2.6. Growth suppression of the rat prostate by *Ganoderma lucidum*

The assay for growth suppression of the rat prostate was performed as described by Fukuta et al. (1999). The testes of SD rats were removed at 4 weeks of age under light anesthesia with pentobarbital. After 4 days, testosterone (100 μ g/body) was injected s.c. into the rats once daily for 7 days. Some animals were administered the indicated amount (0.3%) of milled fruiting body of *Ganoderma lucidum* with CE-2 diet food (CLEA Japan Inc.) at the same time. Ethanol extracts of *Ganoderma lucidum* suspended in 0.5% methylcellulose were orally administered at concentrations of 1.5 or 15 mg/kg of body weight once daily for 7 days. Flutamide (10 mg/kg body weight) was used as the positive control and was suspended in 0.5% methylcellulose and orally administered once daily for 7 days. After 7 days, rats were deprived of food and water for 18 h and sacrificed by pentobarbital. Then, their prostates were removed and their weights determined.

2.7. Statistics

Results were expressed as mean \pm S.E.M. or S.D. Statistical significance was determined by ANOVA and Bonferroni-type multiple *t*-test.

3. Results

3.1. 5 α -Reductase inhibitory activity of the extract of *Ganoderma lucidum*

The microsome portion prepared from rat liver was used as the type 1 isozyme source because it was more easily available than that of the prostate. In this screening assay, the methanol extracts of *Ganoderma lucidum* showed the highest inhibitory activity (Fig. 1) among the 19 species edible and medicinal mushrooms. The extract of *Ganoderma lucidum* showed 5 α -reductase inhibitory activity at dose dependently

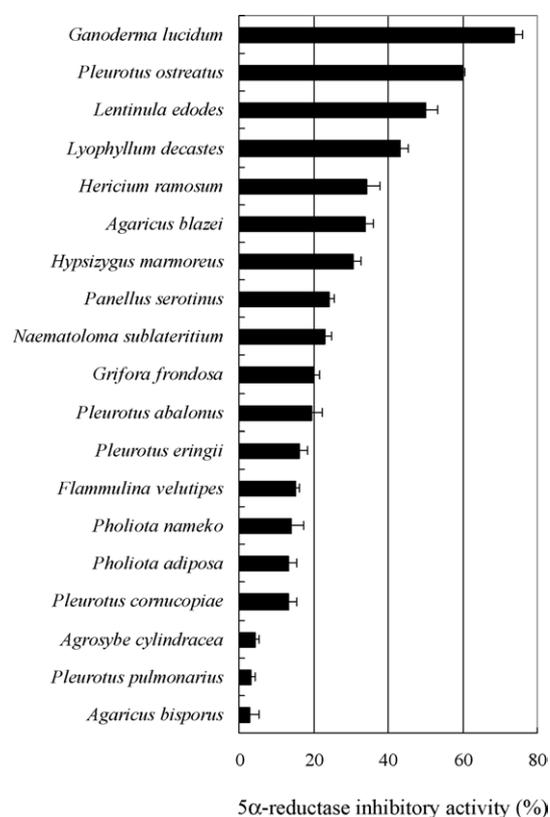


Fig. 1. Inhibitory effects of 19 methanol extracts prepared from the edible fungi on 5 α -reductase activities. Each column represents the mean \pm S.D., *n* = 3. Sample concentration is 200 ppm.

(IC₅₀ = 93 ppm). It should be noted that finasteride, which is known as a potent steroidal inhibitor, showed an IC₅₀ of 0.73 μ M in our assay system.

Generally, 5 α -reductase type 2 is thought to play a major role in the prostate because it is predominantly expressed in this tissue. However, some evidence shows that, in the human prostate, 5 α -reductase type 1 is expressed mainly in the epithelial cells, whereas 5 α -reductase type 2 is localized mainly in the stromal compartment (Thigpen et al., 1993; Bonkhoff et al., 1996). Because both isozymes are over-expressed in BPH tissue, we examined the inhibitory effect of the ethanol extract of *Ganoderma lucidum* against both isozymes prepared from the prostate. As shown in Fig. 2, the ethanol extract of *Ganoderma lucidum* inhibited both isozymes. It should be noted that the 5 α -reductase inhibitory activity of the ethanol extract was almost the same as that of the methanol extract. It is clear from the results that extracts of *Ganoderma lucidum* can inhibit both the types 1 and 2 isozymes of the rat.

3.2. Growth suppression of the rat prostate with administration of the feed (diet) containing milled *Ganoderma lucidum*

In this experiment, we used the anti-androgen receptor blockade flutamide not but 5 α -reductase inhibitor such

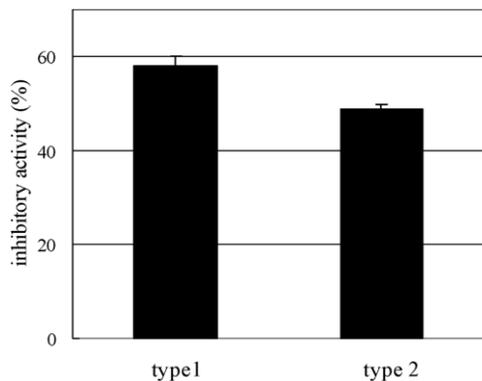


Fig. 2. The inhibitory activity of ethanol extracts of *Ganoderma lucidum* on types 1 and 2 5α -reductase. Each column represents the mean \pm S.D., $n = 3$. Sample concentration is 200 ppm.

as finasteride as the positive control. The reason is that it is still difficult to get enough amount of finasteride in Japan. It has been reported that the prostate size of animals treated with finasteride at 25 and 50 mg/kg/day significantly decreased, but flutamide-treated animals exhibited complete feminization of the genitalia at 24 mg/kg/day (Imperato-McGinley et al., 1992). Therefore we used a dose of 10 mg/kg/day of flutamide to inhibit the growth of the prostate. Four days after castration, the weights of the rat prostates were markedly reduced from 83.8 ± 9.71 to 6.02 ± 1.74 mg/100 g of body weight. The prostate weights recovered by s.c. injection of testosterone, but not completely. In the rats that received testosterone only, the prostate weight was 39.72 ± 10.76 mg/100 g of the body weight. In the rats that received testosterone and simultaneous administration of minced *Ganoderma lucidum*, this increase was reduced: the prostate weights were 26.62 ± 4.57 mg/100 g of the body weight (Fig. 3). Flutamide also limited the testosterone-induced increase in prostate weights to 16.87 ± 2.63 mg/100 g of the body weight (Fig. 3).

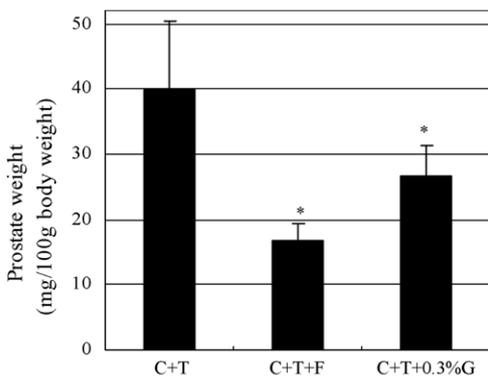


Fig. 3. Effects of *Ganoderma lucidum* and flutamide on testosterone-induced regrowth of the castrated rat prostate. Each column represents the mean \pm S.E.M., $n = 4$. C: castrated rat, T: testosterone, F: flutamide, G: food containing 0.3% milled *Ganoderma lucidum* mushroom. * $p < 0.05$ against C + T.

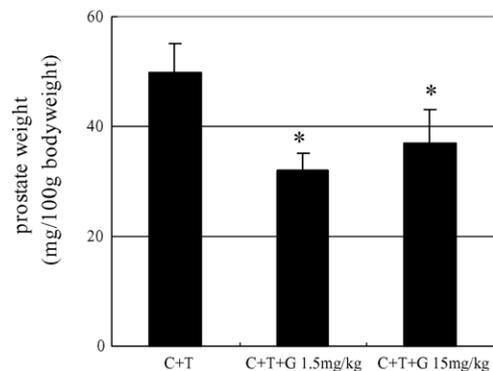


Fig. 4. Effects of ethanol extracts of *Ganoderma lucidum* on testosterone-induced regrowth of the castrated rat prostate. Each column represents the mean \pm S.E.M., $n = 6$. C: castrated rat, T: testosterone, G: ethanol extracts of *Ganoderma lucidum* * $p < 0.01$ against C + T.

3.3. Growth suppression of rat prostate with administration of ethanol extracts of *Ganoderma lucidum*

In the rats that received testosterone, administration of ethanol extracts of *Ganoderma lucidum* reduced the increased weight of the ventral prostate (Fig. 4). Interestingly, administration of ethanol extracts at the concentration of 1.5 mg/kg showed higher suppression effects on the prostate than that of 15 mg/kg. It should be noted that the body weights were almost the same in both groups.

4. Discussion and conclusions

Prostatic enlargement is dependent on tissue androgen, namely DHT, which is converted from testosterone by steroid 5α -reductase. In this study, we investigated the effects of *Ganoderma lucidum* on steroid 5α -reductase activity and on the testosterone-induced growth of the prostate in castrated rats. The extracts of *Ganoderma lucidum* inhibited both types of 5α -reductase, a so-called dual inhibition that might be advantageous for the therapy of BPH, since 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 (Graul et al., 1999). In addition, the treatment of *Ganoderma lucidum* itself or its extract significantly inhibited the testosterone-induced growth of the ventral prostate in castrated rats. These results suggest that the suppression effect of prostatic growth by *Ganoderma lucidum* might come in part from its ability to act as an inhibitor of 5α -reductase.

The fungi *Ganoderma lucidum* (Reishi, Mannentake, or Lingzhi) has been used for centuries in East Asia to cure various human diseases such as hepatitis, hepatopathy, hypertension, nephritis, bronchitis, and cancers (Wasser and Weis, 1999; Yun, 1999). Its dried powder was especially popular as a cancer chemotherapy agent in the Imperial

Court of ancient China (Mizushina et al., 1998). Some of the triterpenes recently isolated from *Ganoderma* such as ganoderic and lucidic acids have demonstrated cytotoxicity against mouse sarcoma and mouse lung carcinoma cells in vitro (Min et al., 2000).

In the last few years, the use of herbal therapies in alternative medicine has been increasing, and although the number of cancer patients using herbal dietary supplements is not exactly known, there is evidence of the increasing use of dietary supplements in cancer treatment (Eisenberg et al., 1998). *Ganoderma lucidum* is one of the herbs in the herbal mixture PC-SPES, which showed activity against hormone-refractory disease in two prostate cancer patients (de la Taille et al., 2000). Extracts of PC-SPES demonstrated estrogenic effects (DiPaola et al., 1998) and decreased growth of hormone-sensitive as well hormone-insensitive prostate cancer cells. Considering our results, these effects might be related not only to the anti-cancer effects of *Ganoderma lucidum* but also to its anti-androgen effects.

In this study, we found a new facet to the biological activity of *Ganoderma lucidum*: anti-androgenic activities on in vitro 5 α -reductase and in vivo growth suppression of the rat prostate. In the future, herbal therapies will become more widely used for treatment of diseases. The anti-androgenic activity of *Ganoderma lucidum* is an important biological activity for use with BHP patients. At this time, the clinical implications of this activity are unknown, so further research is needed before *Ganoderma lucidum* is used to treat BPH.

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