J Wood Sci (2008) 54:502–508
DOI 10.1007/s10086-008-0978-0
© The Japan Wood Research Society 2008

ORIGINAL ARTICLE

Kei Kumakura · Hiroyasu Kumakura · Michiro Ogura
Fumio Eguchi

Pharmacological effects of *Ganoderma lucidum* collected from ume (Japanese apricot) trees

Received: November 12, 2007 / Accepted: July 2, 2008 / Published online: October 10, 2008

Abstract We performed functional evaluation of the fruiting bodies of *Ganoderma lucidum* growing on ume trees (Japanese apricot, *Prunus mume*), and determined the suitability of pruned ume branches as a basic component of culture medium for this mushroom. We observed that all tested functional activities of the fruiting bodies of *G. lucidum* collected from ume trees were higher than those collected from other broadleaf trees or cultured artificially; the functional tests were angiotensin I-converting enzyme inhibitory activity, a platelet aggregation inhibition test, and an interleukin-8 (IL-8) gene expression inhibition test. When extracts from fruiting bodies of *G. lucidum* were orally administered to spontaneous hypertensive rats, hypotensive effects were found. Freeze drying was the most suitable procedure for preservation of the extracts, and the activities of 30% ethanol extracts and 30% methanol extracts were higher than those of hot-water extracts. The highest functional activities for extracts from *G. lucidum* mycelia cultured on sawdust media were for sawdusts based on ume wood.

Key words *Ganoderma lucidum* · Ume tree · Pharmacological effect

K. Kumakura
Department of Research and Development, Mush-Tec Co., Ltd., Takasaki, Gunma 370-0033, Japan

H. Kumakura
A Common Benefit Corporation NPO Gunma, Takasaki, Gunma 370-0831, Japan

M. Ogura
Ogura Clinic, Takasaki, Gunma 370-0035, Japan

F. Eguchi (✉)
Laboratory of Application Food Science (Mushroom Science), Department of Health and Nutrition, Faculty of Health and Welfare, Takasaki University of Health and Welfare, 37-1 Nakaorui, Takasaki, Gunma 370-0033, Japan
Tel. +81-27-352-1290; Fax +81-27-353-2055
e-mail: eguchi@takasaki-u.ac.jp

Part of this study was presented at the 56th Annual Meeting of the Japan Wood Research Society, Akita, Japan, August 2006

Introduction

Mushrooms have various pharmacological actions such as antitumor effects.[1] In particular, *Ganoderma lucidum* (Curtis) P. Karst. has been used as a traditional herbal medicine through observation of the pharmacological effectiveness of its fruiting body. *Ganoderma lucidum* is called *reishi* in Japanese and *lingzhi* in Chinese. Recent studies of *G. lucidum* have shown that the mushroom has antitumor effects,[2–4] anti-HIV effects,[5] hypertension-suppressive effects,[6,7] anti-inflammatory effects,[8,9] liver-function-improving action,[10,11] and suppressive effects on prostate hypertrophy and osteoporosis.[12,13] Moreover, various compounds, such as polysaccharides, triterpenoids, and proteins, have been identified as the respective active components against these diseases.

The ingredients and morphological properties of *G. lucidum* vary considerably depending on the growing conditions and the kind of trees used as culture medium, as well as the strains of *G. lucidum*. *Ganoderma lucidum* growing on old ume trees (*Prunus mume*) is called old-ume *reishi* or *umetake* and functional activities of this strain are higher than those of *reishi* growing on other trees.[14] However, scientific evidence to explain this remains unclear.

Recently, some reports have described pharmacological effects of *G. lucidum* that depended on the culturing conditions. Acetone extracts from fruiting bodies indicated that the content of total phenols varied by the type of tree used as culture medium, resulting in some differences in antioxidative effects.[15] Methanol extracts from mycelium cultivated under different conditions demonstrated variation in inhibitory activity on 5α-reductase.[16]

There are some reports describing that pharmacological effects and morphological properties of other mushrooms as well as *G. lucidum* differ depending on culture conditions.[17] The color and shape of *Grifola frondosa* changes depending on the combination of medium components.[18] Pharmacological activities of the fruiting body of *Agaricus blazei* cultured in a medium containing stem and leaves of sugarcane are very high.[19] Based on these results, it was

concluded that the relationship between the mushroom and the kind of tree used as culture medium affects the functional activities of the fruiting body. However, there are few reports on any relationship between the functional properties of *G. lucidum* and the kind of trees used in culture media. In this study, we focused on antihypertension effects based on the results of an angiotensin I-converting enzyme (ACE) assay and blood pressure changes in spontaneous hypertension rats (SHR) after single-dose administration of a test sample. We also included functional tests of blood coagulation (inhibition of platelet aggregation) and inflammation [interleukin-8 (IL-8) gene expression].

Given that the ume tree typically has highly spread branches, the number of branches that must be pruned for maintenance of an ume garden is very large. Field burning of the branches has become difficult recently because of the associated risk of forest fires spreading from cinders. As a result, pruned branches of ume have become troublesome biomass to remove. Therefore, we attempted to utilize such pruned branches as recyclable biomass resources. If we succeed in stable production of *G. lucidum* using pruned branches of ume as the culture medium, it could allow cultivation of the mushroom for its expected pharmacological effects. In addition, it offers the potential to elucidate the mechanism generating these functional activities and allow development of new strains with even higher activities. In this study, we performed our functional evaluation tests on mycelia of *G. lucidum* cultured on sawdust medium containing ume trees.

Materials and methods

Functional tests of 30% methanol extracts of *Ganoderma lucidum* collected from various trees

The dried fruiting bodies of 18 strains of *Ganoderma lucidum* collected in seven prefectures and in Tokyo and were evaluated functionally. These strains included 5 collected from ume trees, 10 from other broadleaf trees, and 3 were cultured strains on the market. The fruiting bodies of *G. lucidum* grown on ume trees or other broadleaf trees were dried by ventilation for 24 h in a shelf-type variable-temperature drier at 40° to 60°C and then broken into pieces using a Waring blender. In contrast, the *G. lucidum* strains on the market were broken into pieces without drying. Extraction was carried out on 5 g of the fruiting bodies using 100 ml of 30% methanol for 12 h at room temperature. After filtration (Advantec No. 2 filter paper), the filtrate was concentrated using an evaporator to obtain the final extract.

Functional tests of 30% methanol extracts of dried *G. lucidum* grown on the ume tree

The dried fruiting bodies of identical strains collected from one of the ume trees were tested for differences in functional activities among the drying methods. These were two hot-air drying methods, at 40°–60°C or 80°–90°C, and a freeze-drying method. Hot-air drying methods were carried out for 24 h. Dried fruiting bodies were broken into pieces using a Waring blender. Extraction was carried out using 5 g of the fruiting bodies and 100 ml of 30% methanol as solvent for 12 h at room temperature. After filtration, the filtrate was concentrated to obtain the final extract.

Functional tests of extracts of *G. lucidum* obtained by various methods

Dried fruiting bodies of the same strain collected from a single ume tree were tested for differences in functional activities among different extraction methods. The fruiting bodies were dried for 24 h by ventilation in a shelf-type variable-temperature drier at 40° to 60°C and broken into pieces using a Waring blender. Extraction was carried out as follows: 5 g of the fruiting bodies was extracted with 100 ml of 30% methanol or 30% ethanol for 12 h at room temperature, or with hot water (80°C) for 2 h. After filtration, the filtrate was concentrated to obtain the final extract. The extraction ratios were 5.9% (30% methanol), 5.6% (30% ethanol), and 13.2% (hot water).

Functional tests of extracts from mycelia of *G. lucidum* cultured on sawdust media

Functional activity tests were performed on 30% methanol extracts of mycelia of *G. lucidum* cultured on sawdust media. Mycelia isolated from the fruiting body of *G. lucidum* collected from a single ume tree were used for inoculation. Wood powders of ume (*Prunus mume*), which were pruned branches and stems, and of beech (*Fagus crenata*), oak (*Quercus serrata*), cherry (*Prunus* spp.), and cedar (*Cryptomeria japonica*) were used as the substrates in the culture medium. The wood powder of these trees was mixed with wheat bran as a nutritive factor. The ratio of the mixture was tree powder:wheat bran = 5:1, and the water content of the medium was adjusted to 65% ± 2%. The medium was put in a glass petri dish. After mycelia had fully grown on the culture medium, the cultures were further incubated for 1 week. Only mycelia were sampled from the sawdust medium. The drying method used for the samples was hot-air drying carried out at 40°–60°C for 24 h. Dried mycelia were broken into pieces using a Waring blender. Extraction was carried out on 5 g of mycelia with 100 ml of 30% methanol for 12 h at room temperature. After filtration, the filtrate was concentrated under evaporation to obtain the final extract.

Inhibition of platelet aggregation

Inhibitory effects on platelet aggregation induced with arachidonic acid and platelet-activating factor (PAF) released from phospholipids of cell membranes were evaluated. Blood samples were taken from the cubital mid-vein of

healthy adults who received no drugs in a period of 2 weeks before the study and these samples were centrifuged at 1100 rpm for 20 min at room temperature. After separating the upper layer as a fraction of platelet-rich plasma (PRP), the lower layer was further centrifuged at 3000 rpm for 5 min at room temperature. The supernatant was used as a fraction of platelet-poor plasma (PPP). Each 223 µl of PRP and PPP fractions was preincubated at 37°C, then added to 2 µl of *G. lucidum* extract (at a final concentration of 160 µg/ml) dissolved in dimethylsulfoxide (DMSO). The mixture was incubated for a further 3 min at 37°C. Then platelet aggregation was induced by adding 25 µl of PAF solution or 500 nM sodium arachidonate solution. Ion-exchange-purified water was used as the control solution. Measurement of induced platelet aggregation was carried out using an aggregometer (MCM hematracer 313M, MC Medical) and the maximum aggregation of each test sample (the peak value on the aggregation curve expressed as a percentage of the value of PPP) was compared with that of the control.

Inhibition of IL-8 gene expression

Inhibition of gene expression of IL-8, a CXC chemokine mainly acting on neutrophils, was assayed as follows: human dermal fibroblasts were cultured using Dulbecco's modified Eagle's medium (DMEM) containing 10% bovine fetal serum and *G. lucidum* extract, dissolved in dimethylsulfoxide (DMSO), was added to the culture. The final concentrations of the extract were 100 µg ml^{-1}. Moreover, tumor necrotic factor (TNF-α) at 1 ng ml^{-1} was added to stimulate IL-8 gene expression with culturing continued for 6 h at 37°C. After synthesizing cDNA using mRNA isolated from these cells by a conventional method, the level of IL-8 expression was determined by a quantitative polymerase chain reaction (PCR) method (TaqMan PCR method). Data correction was performed using the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene as an internal standard.

Assay of ACE inhibitory activity

ACE inhibitory activity was assayed by a modification of the method of Cushman and Cheung.[20] A mixture (280 µl) of sodium borate buffer (pH 8.3), NaCl (final concentration 400 mM), Hip (hippuric acid)–His–Leu (final concentration 5 mM), and the sample (final concentration 3.16 mg ml^{-1}) was preincubated for 5 min at 37°C. The reaction was started by adding 100 µl ACE (final concentration 1.58 mU) from rabbit lung, and terminated after incubation (30 min at 37°C) by adding 250 µl of 1 M HCl. The hippuric acid liberated was extracted with 1.5 ml of ethyl acetate, and 0.5 ml of the extract was evaporated using a Speed Vac concentrator. The residue was then dissolved in 1.5 ml of sodium borate buffer. The absorbance at 228 nm was measured to estimate ACE inhibitory activity.

Single-dose administration test with SHR

To clarify the effects of the fruiting body extract of *G. lucidum* on essential hypertension, blood pressure changes of SHR (SHR/NCr1Crlj, Japan Charles River) were monitored after single-dose administration of the extract using a feeding needle. Housing conditions for the SHR were 22° ± 1°C, humidity 60% ± 10%, light phase (7:00–19:00), dark phase (19:00–7:00). All of the animals enrolled in this study were given commercially available food (MF powder, Oriental Yeast) and tap water from the age of 5 weeks as a preliminary habituation to the housing conditions. After preliminary housing, MF pellets with 8% sodium chloride were given until the age of 15 weeks to enhance the hypertensive condition. Before starting the study, the rats were divided into groups with mean systolic pressures that were almost the same.

Fruiting bodies (4 g) dried at 40°–60°C using a hot-air drier were extracted with 200 ml of 30% methanol or 30% ethanol for 12 h at room temperature or with 200 ml of hot water (80°–90°C) for 1 h. After freeze drying, the powder was dissolved in tap water and 1 ml of the solution (0.4 g/100 ml) was given orally by force using a feeding needle. After the rats were habituated in an incubator at 38°C for several minutes, blood pressure was determined using a noninvasive automatic blood pressure recorder (BP-98A, Softron). Blood pressure was determined three times at every point of measurement and the mean value was recorded. Measurement of blood pressure was carried out in a room with constant temperature and humidity.

Statistical analysis

Data of functional evaluation tests were subjected to one-way analysis of variance (ANOVA) with Dunnett's multiple comparison post hoc test (Fig. 1 and Fig. 2). Data from the single-dose administration test with SHR were assessed by analysis of group differences and were considered statistically significant at $P < 0.05$, by Tukey's test (Fig. 3).

Results and discussion

Differences in functional activities among extracts of *Ganoderma lucidum* grown on various trees

The functional activities of extracts from the fruiting bodies of *Ganoderma lucidum* collected from ume or other broadleaf trees or from artificially cultured *G. lucidum* fruiting bodies were compared (Fig. 1). The extracts from fruiting bodies grown on ume trees showed greater effects than those grown on other broadleaf trees. The inhibitory effects were significant on platelet aggregation induced with PAF ($P < 0.05$), ACE inhibition ($P < 0.005$), and inhibition of IL-8 expression ($P < 0.05$). This is scientific evidence for the traditional ideas about *G. lucidum*, suggesting that some pharmacologically effective and ume-specific components or some new biofunctional component is accumulated more

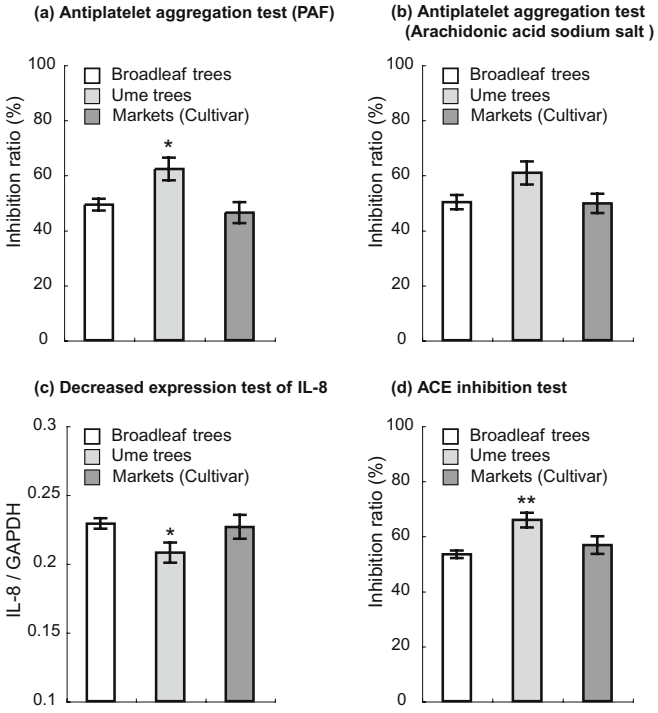

Fig. 1a–d. Functional tests of 30% methanol extracts from the fruiting bodies of *Ganoderma lucidum* grown on various trees. The number of samples from ume trees was 5 strains (n = 5); from other broadleaf trees, 10 strains (n = 10); and obtained from markets (cultivar), 3 strains (n = 3). **a, b** Inhibition test for platelet aggregation: inhibitory effects on platelet aggregation induced with platelet-activating factor (PAF) and arachidonic acid. **c** Inhibition of interleukin-8 (IL-8) gene expression. **d** Angiotensin I-converting enzyme (ACE) inhibition assay. *Bars* indicate standard error (SE). *Asterisk*, significant difference from broad-leaved trees at $P < 0.05$; *double asterisk*, significant difference at $P < 0.005$

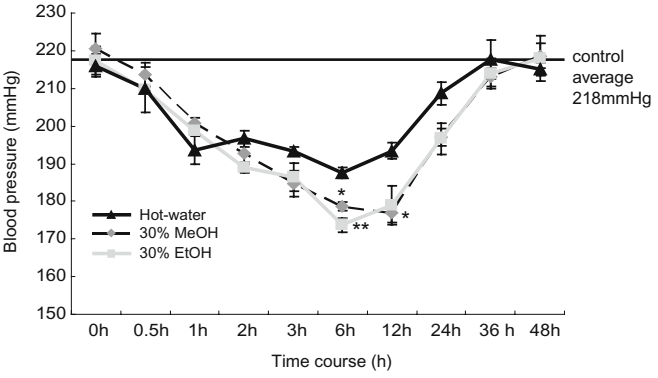

Fig. 3. Effect of orally administered extracts of *Ganoderma lucidum* on blood pressure in spontaneous hypertension rats (SHR). Effects of *umetake* fruit extracts on hypertension were examined. Fruiting bodies were hot-air dried (40°–60°C). Each sample (4 g) was extracted with 200 ml of 30% methanol or 30% ethanol for 12 h at room temperature or with 200 ml of hot water (80°–90°C) for 1 h. The extracts were concentrated to dryness. The concentrated extracts (4 mg) were dissolved in distilled water (1 ml). The extracts were used to treat 15-week-old SHR. Blood pressure in untreated SHR is shown as the control average (218 mmHg). *Bars* indicate SE (n = 3). *Asterisk*, significant difference from the hot-water group at $P < 0.05$; *double asterisk*, significant difference at $P < 0.005$ by Tukey's test

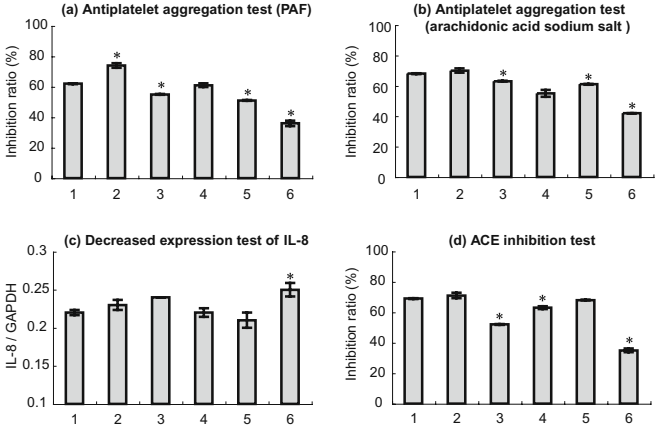

Fig. 2a–d. Functional tests of extracts of mycelia of *Ganoderma lucidum* cultured on sawdust media. **a, b** Inhibition test for platelet aggregation: inhibitory effects on platelet aggregation induced with PAF and arachidonic acid. **c** Inhibition of IL-8 gene expression. **d** ACE inhibition assay. Mycelia were cultured on sawdust medium containing: *1*, ume (pruned branches); *2*, ume (stems); *3*, beech; *4*, oak; *5*, cherry; and *6*, Japanese cedar. The drying method for the mycelia was hot-air drying at 40°–60°C for 24 h. Extraction was carried on 5 g of the mycelia using 100 ml of 30% methanol for 12 h at room temperature. After filtration, the filtrate was concentrated under evaporation to obtain the extracts. *Bars* indicate SE (n = 3). *Asterisk*, significantly different from ume pruned branch group at $P < 0.05$

abundantly in *G. lucidum* grown on ume but not on other trees. However, it is not possible to be certain that all *G. lucidum* growing on ume trees will have a higher effect in these functional evaluation tests than fruiting bodies grown on other broadleaf trees, because the fruiting bodies of *G. lucidum* examined in this study contain natural products. Formation of natural products depends on growth conditions, strains, and so on. It is necessary to examine these functional evaluation tests using identical strains grown under the same conditions.

Platelet aggregation induced with arachidonic acid is activated by thromboxane A2 (TXA2) generated by cyclooxygenase (COX) in the arachidonic acid cascade, whereas PAF-induced platelet aggregation occurs through binding of blood platelets to the PAF receptor. Given that this reaction was unaffected by an ADP scavenger, a thrombin inhibitor, or a COX inhibitor, it appears that there is a pathway different from COX inhibition. However, arachidonic acid and the precursor of PAF (lyso-PAF) are metabolites released from 1-alkyl-phosphatidylcholine by phospholipase A2 (PLA2). Based on these findings, the extracts of *G. lucidum* grown on ume trees inhibited platelet aggregation induced by either arachidonic acid or PAF. This observation suggested that the extract mediates the inhibition of PLA2 upstream of the two aggregation mechanisms or contains some unknown compound having inhibitory effects on both mechanisms. Some compounds that inhibit platelet aggregation, such as adenosine, were isolated from *G. lucidum* extracts in a previous study.[21] Therefore, there is a possibility that mushrooms grown on ume trees might accumulate such inhibitory compounds at a high level.

PAF acts as a contraction factor for smooth muscle, a vascular activation factor, a neutrophil activation factor, and a renal hypotensive factor as well as a platelet activating factor. Furthermore, it has been pointed out that

PAF is involved in improvement of platelet aggregation-associated vascular endothelial diseases, and also in improvement of anaphylaxis and asthma through its anti-inflammatory and hypotensive actions. The functional activities of *G. lucidum* grown on ume trees were higher in the platelet aggregation inhibition test than those of the mushroom grown on other kinds of trees, suggesting that the hypotensive effects of *G. lucidum* grown on ume trees might be more marked than those of *G. lucidum* grown on other kinds of trees.

IL-8 expression is induced by activation of intracellular nuclear factor NF-κB via binding of TNF-α to the TNF-α receptor on the surface of the cell membrane and the action of NF-κB is suppressed by IκB, a regulatory factor of activation. The phosphorylation of IκB occurs by activation of IκB-phosphokinase (IκB-kinase) via binding of TNF-α to its receptor. As a result, NF-κB is activated. The activated transcription regulatory factor, NF-κB, is transferred into the nucleus and acts on the promoter of target genes like that of IL-8, resulting in expression of the gene. Therefore, ingredients in *G. lucidum* extracts may interact with some region of TNF-α upstream of the signaling pathway for NF-κB, with a transcription regulation factor downstream, or with the primary product of IL-8 transcription by TNF-α.

In ACE inhibition tests, ACE is the enzyme that converts inactive angiotensin I to angiotensin II, which has hypertensive effects. A previous study demonstrated that the triterpenoids contained in *G. lucidum* have ACE inhibitory activity.[22] Therefore, it was assumed that *G. lucidum* might accumulate high levels of triterpenoids as the effective component of ACE inhibition or might contain a new constituent. Given that 30% methanol was used for extraction in this study, water-soluble polysaccharides, proteins, or peptides would have been present in the extract. Many kinds of food-derived peptides have been reported to inhibit ACE and some are obtained from mushrooms.[23,24] Orally administered hot-water extracts of *Mycoleptodonoides aitchisonii* exhibit hypotensive effects on SHR, and ACE inhibitory activity was related to the hypotensive mechanism,[25] which suggests that polysaccharides, proteins, or peptides in *G. lucidum* may be involved in ACE inhibition.

Differences in functional activities due to drying method

Functional activity differences due to drying method were evaluated with fruiting bodies of *G. lucidum* grown on a single ume tree (Fig. 4). Two hot-air drying methods, one at 40°–60°C and the other at 80°–90°C, and a freeze-drying method were tested. In all functional activity tests in this study, hot-air drying at 40°–60°C and freeze drying led to higher activities than hot-air drying at 80°–90°C. Hot-air drying at a low temperature is regarded as most suitable for commercial utilization. The observation that the functional activities of the extract were markedly decreased by hot-air drying at 80°–90°C would have been due to inactivation of the effective components through destruction of their tertiary structure. These findings suggest that the effective components are heat-sensitive compounds such as glycoproteins.

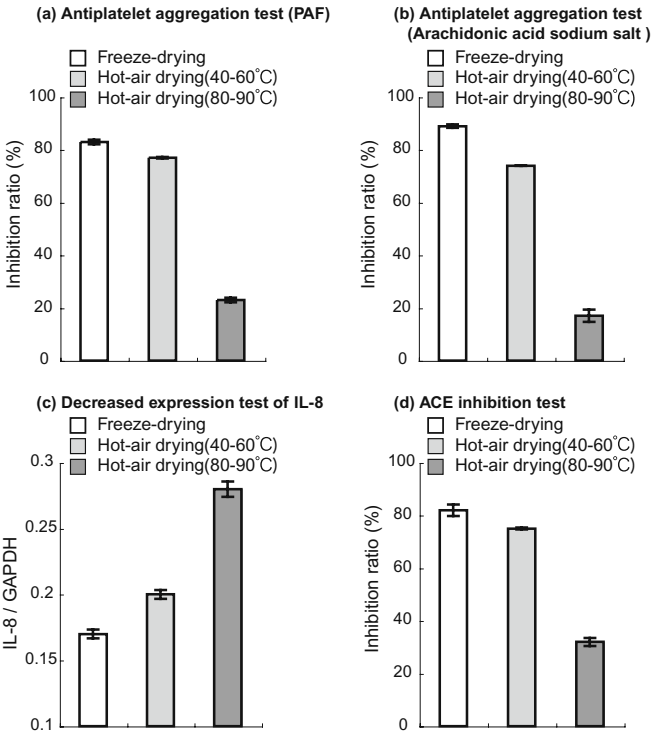

Fig. 4a–d. Functional tests of 30% methanol extracts of *Ganoderma lucidum* dried by various methods. Dried fruiting bodies of one strain collected from ume tree were tested for differences in functional activities among drying methods. **a, b** Inhibition test for platelet aggregation: inhibitory effects on platelet aggregation induced with PAF and arachidonic acid. **c** Inhibition of IL-8 gene expression. **d** ACE inhibition assay. *Bars* indicate SE ($n = 3$)

Differences in functional activities due to extraction method

For comparison of functional activities obtained with different extraction methods, *G. lucidum* was grown on a single ume tree (Fig. 5). Inhibitory activities of PAF and arachidonic acid-induced platelet aggregation and ACE inhibitory activity for 30% methanol extracts and 30% ethanol extracts were higher than those of hot-water extracts. The 30% methanol extracts led to the lowest expression of IL-8. A convincing reason for the result is that only water-soluble components were extracted by the hot-water extraction, but water-soluble and water-insoluble components were extracted by 30% methanol and 30% ethanol. In addition, heat-sensitive components might have been inactivated during incubation at 80°C in the hot-water extraction method.

Single-dose administration test of extracts with SHR

For investigation of the effectiveness of extracts on essential hypertension, fruiting body extracts of *G. lucidum* grown

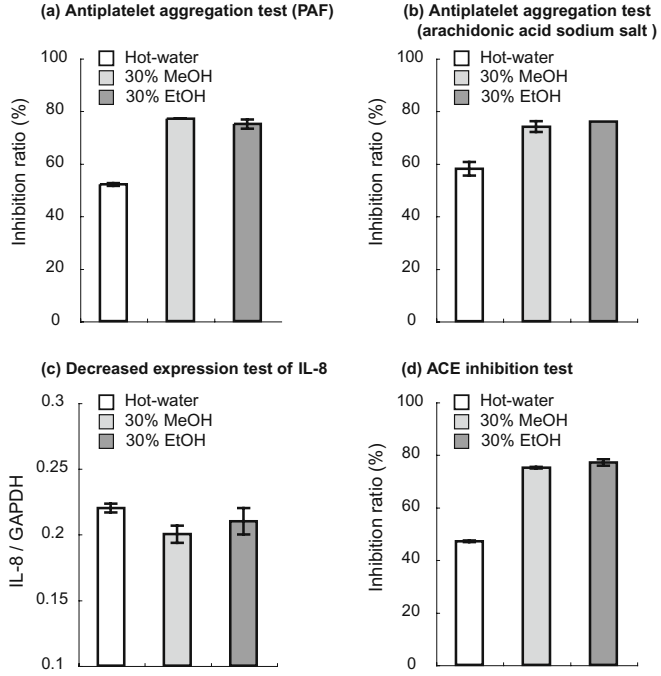

Fig. 5a–d. Functional tests of extracts of *Ganoderma lucidum* obtained by various methods. **a, b** Inhibition test for platelet aggregation: inhibitory effects on platelet aggregation induced with PAF and arachidonic acid. **c** Inhibition of IL-8 gene expression. **d** ACE inhibition assay. *Bars* indicate SE ($n = 3$)

on the ume tree were orally administered by force and changes in blood pressure were investigated (Fig. 3). In all test groups, blood pressure began to decrease as early as 30 min after administration of the extract and the peak decrease was within 6–12 h after administration. The blood pressure decrease in the group administered the hot-water extract was less than in the group administered the ethanol or methanol extract. These results suggest that both water-soluble and water-insoluble components are able to be extracted by methanol and ethanol extraction but not by hot-water extraction, and that some effective components were inactivated by heat treatment as mentioned in the section describing functional evaluation of various extraction methods.

Differences in functional activities obtained among sawdust media

Functional activity tests were made with 30% methanol extracts of mycelia of *G. lucidum* cultured on sawdust medium using ume stemwood or other kinds of wood (Fig. 2). PAF-induced platelet aggregation was most markedly inhibited by extracts obtained from mycelia cultured on sawdust medium using ume stems followed by those cultured using pruned ume branches or oak. Arachidonic acid-induced platelet aggregation was also most markedly inhibited by extracts obtained from mycelia cultured on sawdust medium using ume stems, followed by those cultured using ume branches and beech. Extracts of mycelia grown on medium with ume stemwood was most effective in inhibition of platelet aggregation. Furthermore, the ACE inhibitory activity of mycelia was highest in the order of those cultured on ume stems, ume branches, and cherry. The inhibition of IL-8 gene expression was most marked with extracts of mycelia from cherry wood followed by ume branches, oak, and ume stems, suggesting that use of ume trees as the culture medium would have considerable influence on the functional activities of extracts. Therefore the availability of ume pruned branches that are typically treated as orchard wastes means they can be utilized in culture media for production of *G. lucidum* having highly functional activities. Differences in pharmacological properties of extracts obtained from different culture media must be due to the wood component influencing the functional activities of mycelia. In this study, the C/N ratio was not adjusted in preparing the culture medium so that differences in the C/N ratio could have produced some differences in the natural products produced by the mycelia. However, in a previous report about effects of culture medium on *Agaricus blazei*, the C/N ratio of the culture medium might have produced changes in nitrogen content of the fruiting body, but there was no correlation between such changes and pharmacological properties.[19] Accordingly, we could not conclude that the differences in the pharmacological properties of mycelia were attributable to the C/N ratio of the culture medium. Also, some differences in growth of mycelia might have been produced by physicochemical differences in the trees used as culture medium. We have to examine this possibility. For *G. lucidum*, it has been reported that addition of ethanol to culture medium enhances growth of mycelia as well as production of polysaccharide,[26] and that the production of exo-polysaccharide was influenced by the pH of the culture medium.[27] Therefore, it seems necessary to make an exploratory study that allows isolation of the main active components with pharmacological activity and elucidation of the components in the culture medium that are related to the differences in pharmacological activities of the mycelia observed in the present study. Although this study was made with mycelia of *G. lucidum*, we have to develop a culture method to stably produce fruiting bodies and investigate the effects of the culture medium on their functional activities.

Conclusions

Fruiting bodies of *Ganoderma lucidum* grown on ume trees exhibited higher functional activities than those grown on other kinds of trees or cultured artificially. This is scientific evidence for traditional ideas and indicates that some pharmacological components accumulate more abundantly in *G. lucidum* grown on ume than on other trees, or that only those mushrooms grown on ume contain functional compounds. Higher functional activities were obtained by freeze drying or air drying *G. lucidum* at low temperature, and 30% ethanol extracts and 30% methanol extracts had higher activities than hot-water extracts. Mycelia of *G. lucidum*

grown on ume exhibited higher activities than that grown on other broadleaf trees such as beech, oak, cherry, and cedar. This indicates that *G. lucidum* not only has strain-specific functional activities, but also that the culture medium (ume) has some influence on these activities. Therefore, we considered that the pruned branches of ume that are currently treated as industrial waste are usable as a culture medium for production of highly functional *G. lucidum*. Because the fruiting bodies used in the present study were naturally produced, the fruiting bodies of *G. lucidum* cultured on ume trees may not always have higher functional activities. Therefore, we plan to prepare medium using pruned branches of ume for culture of *G. lucidum* under the same conditions using an identical strain to examine the reproducibility of the functional activities observed in the present study.

References

1. Yamanaka K (1995) Mushroom production and mushroom science (in Japanese). Mokuzai Gakkaishi 14:795–804
2. Lin ZB, Zhang HN (2004) Anti-tumor and immunoregulatory activities of *Ganoderma lucidum* and its possible mechanisms. Acta Pharmacol Sin 25:1387–1395
3. Cao QZ, Lin ZB (2004) Antitumor and anti-angiogenic activity of *Ganoderma lucidum* polysaccharides peptide. Acta Pharmacol Sin 25:833–838
4. Gao JJ, Min BS, Ahn EM, Nakamura N, Lee HK, Hattori M (2002) New triterpene aldehydes, lucialdehydes A-C, from *Ganoderma lucidum* and their cytotoxicity against murine and human tumor cells. Chem Pharm Bull (Tokyo) 50:837–840
5. el-Mekkawy S, Meselhy MR, Nakamura N, Tezuka Y, Hattori M, Kakiuchi N, Shimotohno K, Kawahata T, Otake T (1998) Anti-HIV-1 and anti-HIV-1-protease substances from *Ganoderma lucidum*. Phytochemistry 49:1651–1657
6. Kabir Y, Kimura S, Tamura T (1988) Dietary effect of *Ganoderma lucidum* mushroom on blood pressure and lipid levels in spontaneously hypertensive rats (SHR). J Nutr Sci Vitaminol (Tokyo) 34:433–438
7. Kanmatsuse K, Kajiwara N, Hayashi K, Shimogaichi S, Fukinbara I, Ishikawa H, Tamura T (1985) Studies on *Ganoderma lucidum*. I. Efficacy against hypertension and side effects (in Japanese). Yakugaku Zasshi 105:942–947
8. Hong KJ, Dunn DM, Shen CL, Pence BC (2004) Effects of *Ganoderma lucidum* on apoptotic and anti-inflammatory function in HT-29 human colonic carcinoma cells. Phytother Res 18:768–770
9. Akihisa T, Nakamura Y, Tagata M, Tokuda H, Yasukawa K, Uchiyama E, Suzuki T, Kimura Y (2007). Anti-inflammatory and anti-tumor-promoting effects of triterpene acids and sterols from the fungus *Ganoderma lucidum*. Chem Biodivers 4:224–231
10. Yang XJ, Liu J, Ye LB, Yang F, Ye L, Gao JR, Wu ZH (2006) In vitro and in vivo protective effects of proteoglycan isolated from mycelia of *Ganoderma lucidum* on carbon tetrachloride-induced liver injury. World J Gastroenterol 12:1379–1385
11. Zhang GL, Wang YH, Ni W, Teng HL, Lin ZB (2002) Hepatoprotective role of *Ganoderma lucidum* polysaccharide against BCG-induced immune liver injury in mice. World J Gastroenterol 8:728–733
12. Liu J, Kurashiki K, Shimizu K, Kondo R (2006) 5alpha-reductase inhibitory effect of triterpenoids isolated from *Ganoderma lucidum*. Biol Pharm Bull 29:392–395
13. Shimizu K, Liu J, Miyamoto I, Kondo R (2006) Utility and function of mushrooms: the preventative effect of benign prostatic hyperplasia and osteoporosis by *Ganoderma lucidum* (in Japanese). Food Food Ingred 211:124–133
14. Matsumoto K, Ito H (1997) The eating habit which raises immunocompetence (in Japanese). Bunrishoin, Tokyo, pp 101–117
15. Takai Y, Kikuzaki H, Akiyama K, Kohno B, Tujita T, Nakazawa M, Ueda M Nakatani N, Miyatake K (2004) Effects of culture substrates on antioxidant activities of extracts from *Ganoderma lucidum* (Rei-shi). Mushroom Sci Biotechnol 12:113–118
16. Liu J, Fujita R, Sato M, Shimizu K, Konishi F, Noda K, Kumamoto S, Ueda C, Tajiri H, Kaneko S, Suimi Y, Kondo R (2005) The effect of strain, growth stage, and cultivating condition of *Ganoderma lucidum* on 5α-reductase inhibition. J Wood Sci 51: 189–192
17. Kitamoto Y (2006) Current progress in breeding of edible and pharmaceutical mushrooms (in Japanese). Mokuzai Gakkaishi 52:1–7
18. Shen Q, Royse DJ (2001) Effects of nutrient supplements on biological efficiency, quality and crop cycle time of maitake (*Grifola frondosa*). Appl Microbiol Biotechnol 57:74–78
19. Yoshimoto H, Eguchi F, Higaki M, Ohga S (2005) Influence of culture medium components on the pharmacological effects of *Agaricus blazei* (in Japanese). Mokuzai Gakkaishi 51:389–393
20. Cushman DW, Cheung HS (1971) Spectrophotometric assay and properties of the angiotensin-converting enzyme of rabbit lung. Biochem Pharmacol 20:1637–1648
21. Shimizu A, Yano T, Saito Y, Inada Y (1985) Isolation of an inhibitor of platelet aggregation from a fungus, *Ganoderma lucidum*. Chem Pharm Bull (Tokyo) 33:3012–3015
22. Morigiwa A, Kitabatake K, Fujimoto Y, Ikekawa N (1986) Angiotensin converting enzyme-inhibitory triterpenes from *Ganoderma lucidum*. Chem Pharm Bull (Tokyo) 34:3025–3028
23. Choi HS, Cho HY, Yang HC, Ra KS, Suh HJ (2001) Angiotensin I-converting enzyme inhibitor from *Grifola frondosa*. Food Res Int 34:177–182
24. Lee DH, Kim JH, Park JS, Choi YJ, Lee JS (2004) Isolation and characterization of a novel angiotensin I-converting enzyme inhibitory peptide derived from the edible mushroom *Tricholoma giganteum.* Peptides 25:621–627
25. Eguchi F (2005) Biological activities and functions of mushrooms (in Japanese). CMC, Tokyo, pp 16–25
26. Yang HL, Wu TX, Zhang KC (2004) Enhancement of mycelial growth and polysaccharide production in *Ganoderma lucidum* (the Chinese medicinal fungus, "Lingzhi") by the addition of ethanol. Biotechnol Lett 26:841–844
27. Kim HM, Park MK, Yun JW (2006) Culture pH affects exopolysaccharide production in submerged mycelial culture of *Ganoderma lucidum*. Appl Biochem Biotechnol 134:249–262