Antihypertensive Effect of *Pleurotus nebrodensis* in Spontaneously Hypertensive Rats

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Abstract: In this study, we examined the effects of *Pleurotus nebrodensis* on systolic blood pressure of spontaneously hypertensive rats. Single-dose and continuous-dose tests with sample diets made from the fruit body of the mushroom, *P. nebrodensis* were carried out on SHR and control rats. Sample diets included 6% dry powder of fruit body (6% dry powder), hot water extract, polysaccharide fraction, protein fraction, dialyzable fraction and non-dialyzable fraction. Polysaccharide and protein fractions were obtained by hot water extraction. The protein fraction was separated to the dialyzable fraction and non-dialyzable fraction by dialysis.

In the single-dose test, protein fraction, hot water extract and polysaccharide fraction decreased systolic blood pressure. Blood pressure was lowered after administration for 2 h, and it returned to the pre-administration blood pressure after 48 h. In the continuous-dose test, spontaneously hypertensive rats were fed each of the diets for 16 weeks. The 6% dry powder group showed significantly inhibited elevation of blood pressure compared with the control group and there was no influence on total cholesterol and triglyceride levels. The non-dialyzable fraction showed suppression of increase in blood pressure from the start of the continuous oral administration. Effects on the rennin angiotensin system and renal function were also indicated. The antihypertensive action effect of *P. nebrodensis* can be expected to not only prevent but also to improve hypertension.

Key words: *Pleurotus nebrodensis*, mushroom, spontaneously hypertensive rat, blood pressure

1 INTRODUCTION

The pharmacological effects of mushrooms vary greatly, ranging from antihypertensive actions, cholesterol lowering actions to anti-tumor activities, and various mushrooms have been used in Chinese medicine and folk medicine for a very long time.

Antihypertensive actions with such mushroom species as *Pleurotus nebrodensis*, *Ganoderma lucidum*, *Lentinula edodes*, and *Mycoleptodonoides aitchisonii* have been reported in the literature. Mushrooms have been suggested to lower blood pressure by improving lipid metabolism and kidney function and hindering angiotensin-converting enzymes.

In the past, we investigated the antihypertensive actions of *L. edodes*, *Grifola frondosa* and *G. lucidum* using spontaneously hypertensive rats (SHR). We reported that the strength of antihypertensive action varied with mushroom species and we ascertained the effects of different combinations of mushrooms with antihypertensive actions. *P. nebrodensis* is native to Southern Europe, Central Asia and China, and the hot water extract and dry powder of the fruit body of cultured *P. nebrodensis* have been shown to prevent hypertension. The color, shape and size of mushroom fruit bodies are affected by culture environments. In addition, the culture environment has an effect on the pharmacological constituents, but few studies have been conducted on the effects of culture environment on pharmacological effects. In a previous study, we investigated the effects of culture additives on the pharmacological properties of *P. nebrodensis* by measuring antihyperten-
sive indicators and documenting the efficacy of nucleic acid-related compounds in cultured cells. In the present study, the hot water extract of *P. nebrodensis* was treated with enzymes to obtain different subsamples, and their effects on systolic pressure were investigated.

2 EXPERIMENTAL

2.1 Mushrooms

*P. nebrodensis* (FERM P-19370) from the collection at Takasaki University of Health and Welfare was used.

2.2 Sample preparation

Fruit bodies of *P. nebrodensis* cultured in the conventional manner were used. Using a mushroom fruit body drying rack, fruit bodies were air-dried for 24 h by maintaining the temperature between 40 and 60°C and then pulverized using a Waring blender to make a dry powder. To 9 g of the dry powder, 600 mL of distilled water was added, and the resulting solution was held at 80°C in a waterbath for extraction. After cooling, the supernatant was filtered, and the filtrate was placed in a flask and brought up to a volume of 600 mL with distilled water (hereafter, hot water extract). The hot water extract was treated with either peptidase to obtain the polysaccharide fraction or glucosidase to obtain the protein fraction. The protein fraction was subjected to dialysis using a dialysis tube (molecular weight cutoff: 3000 Da) to obtain dialyzable and non-dialyzable fractions.

The dry powder was mixed with a commercially available diet (MF powder, Oriental Yeast Co., Ltd, Tokyo, Japan) at a concentration of 6% (hereinafter, 6% dry powder). The other samples were administered to rats at doses equivalent dosage of 9 g/60 kg/600 mL/day in humans.

2.3 Animals

Five-week-old male spontaneously hypertensive rats (SHR/NCrI-Crlj) and Wistar Kyoto rats (WKY/NCrI-Crlj) were purchased (Charles River Laboratories Japan, Inc., Yokohama, Japan). The rats were kept in metabolic cages with the temperature and humidity of the laboratory set at 22 ± 1°C and 60 ± 10%, respectively and the laboratory was dark from 1900 to 7:00. For one week after receipt, the rats were acclimatized and had free access to MF powder and distilled water.

In the present study, single-dose and 16-week continuous-dose tests were conducted on SHR to investigate the effects of mushroom on blood pressure. In both tests, MF powder enriched with 8% sodium chloride was given starting at the age of 6 weeks up to the start of the tests to increase blood pressure of SHR. Just prior to each test, rats were held in groups to achieve an as even as possible average systolic pressure.

2.4 Single-dose administration

The test used 8-week-old SHR. The following four groups (n=3 each) were established with SHR: hot water extract, polysaccharide fraction, protein fraction and distilled water (SHR control). The rats were never fasted, including before administration, and they had free access to MF powder and distilled water. Each agent was administered orally using a stainless steel oral tube for rats. Blood pressure was measured a total of six times: before administration, and 2, 6, 12, 24 and 48 h after administration. Blood pressure (systolic and diastolic) and heart rate were measured using a noninvasive automatic sphygmomanometer (BP-98A, Softron Co., Ltd, Tokyo, Japan) after placing each animal in a 38°C warmer for several minutes. Three continuous measurements were made and averaged.

2.5 Continuous-dose administration

The test was initiated at the age of 12 weeks when systolic pressure reached 180 mmHg. The following six groups (n=6 SHR each) were established: hot water extract, polysaccharide fraction, dialyzable fraction, non-dialyzable fraction, 6% dry powder and distilled water. In addition, the following two groups (n=8 each) with WKY were established: distilled water (WKY control) and hot water extract (WKY hot water extract). In the 6% dry powder and distilled water groups, distilled water was orally administered once daily for 16 consecutive weeks, and in the other groups, doses were administered orally using an oral tube once daily for 16 consecutive weeks. For the 6% dry powder group, the rats had free access to distilled water and MF powder was mixed with the dry powder of the fruit body, and for the other groups, the rats had free access to MF powder and distilled water. Blood pressure, heart rate and body weight were measured once weekly. A urine sample was collected two days before the end of the test. On the last day of the test, all rats were fasted for one day and anesthetized by injecting 45 mg/kg of Nembutal intraperitoneally. A blood sample was collected from the left cardiac ventricle using a 20G needle and subjected to biochemical test (Automatic Chemical Analyzer: Auto Lab, Radioimmunoassay) and an endocrinological test to measure renin and angiotensin II levels (radioimmunoassay: RIA).

2.6 Statistical analysis

Numerical data were expressed as mean ± SE, and a t-test was used to compare the SHR control group to the other groups with the level of significance set at p<0.05 (two-tailed).
3 RESULTS AND DISCUSSION
3.1 Single-dose administration

In the single-dose test, each P. nebrodensis sample was administered orally, and antihypertensive actions were ascertained by measuring blood pressure at different time points. The changes in systolic blood pressure are shown in Fig. 1. For the control group, average blood pressure before administration was 168.3 ± 1.6 mmHg (100%), and was 96, 98 and 100% at 2, 12 and 48 h after administration, respectively. For the protein fraction, hot water extract and polysaccharide fraction groups, the average blood pressure 2 h after administration was 84, 85 and 88%, respectively. For the protein fraction group, the average blood pressure 12 and 48 h after administration was 95 and 98%, respectively. Blood pressure increased gradually from 6 to 48 h after administration, and at 48 h after administration, blood pressure returned to close to the pre-administration value. This tendency was also seen for the hot water extract and polysaccharide fraction groups.

In the present study, blood pressure was measured by the tail-cuff method following warming and immobilization. Consequently, the blood pressure measurement may reflect a reaction to stress9. Further consideration is required to avoid warming or restraining rats. Der-Zen et al. reported the use the tail-cuff method, which is commonly used to measure blood pressure in rats (as in this study) in a noninvasive automatic way after warming each animal in a heater for several min9-11.

Single-dose studies using SHR have been conducted for M. aitchisonii, G. lucidum and Lyophyllum decastes9,12,13). Comparison of preadministration blood pressure with that after oral administration of L. decastes showed a lowering of blood pressure by 20% in 4.5 h, while intraperitoneal administration lowered blood pressure by 18% in 1.5 h10). Compared to intraperitoneal administration, oral administration acted more slowly but had longer lasting effects, and as a result, oral administration is more suitable for the prevention of hypertension in routine settings. In studies where G. lucidum13 or M. aitchisonii9 were administered orally, significant hypotension was achieved 3 or 4 h after administration. It appears that the duration of antihypertensive action differs among mushroom species.

The present test showed that the duration of the antihypertensive action for P. nebrodensis is comparable to that for M. aitchisonii, and when compared to other mushrooms, P. nebrodensis lowers blood pressure more quickly.

Persistent antihypertensive action has been reported with some antihypertensive agents, such as azelnidipine and telmisartan14,15). Due to its high affinity to vascular tissue, azelnidipine has a long retention in vascular tissue where it continuously expresses its antihypertensive action, even after the drug disappears from blood14). Similarly, telmisartan, which was developed as an angiotensin II type 1 receptor antagonist, shows a longer half-life than its analogs because of its slow dissociation from type 1 receptor, and as a result, persistent antihypertensive effects are achieved15). The duration of action of antihypertensive agents varies depending on uptake by the body or action points; thus, the antihypertensive action of mushroom fractions may differ based their constituents and action mechanisms. In recent years, the prolonged action of antihypertensive agents has been believed to be beneficial in preventing cardiovascular events due to the sudden increase in blood pressure experienced upon waking in humans. Thus, P. nebrodensis appears to be a useful agent in lowering blood pressure.

3.2 Continuous-dose test

In the continuous-dose test, SHRs were fed each of the P. nebrodensis diets for 16 consecutive weeks to ascertain their effects on blood pressure. Because the dietary composition of the 6% dry powder group was different from that of the other diet groups, the effects on blood pressure were investigated by making a comparison to the SHR control group (distilled water).

Figures 2 and 3 show the blood pressure results at weeks 9 and 16 of administration, respectively. For the SHR control group (distilled water), the average blood pressure was 185.4 ± 7.4 mmHg before the start of the test and it gradually increased to 215.9 ± 11.9 mmHg at week 16. At week 9, the blood pressure for the non-dialyzable fraction group was significantly lower compared to the SHR control group. At week 16, blood pressure for the 6% dry powder group was 169.8 ± 4.4 mmHg, showing clear antihyperten-

Fig. 1 Effects of the Mushroom Fruit Body Extracts on Systolic Blood Pressure of Spontaneously Hypertensive Rats Examined for Single-dose Administration.

Blood pressure was measured 0, 2, 6, 12, 24 and 48 h after administration. The result indicated 0 h as 100%

Values are means ± SEM (n=3).
sive effects. For the non-dialyzable and dialyzable fraction groups, antihypertensive effects were observed continuously from week 1, but no marked differences were seen at week 16 compared to the SHR control group.

In one study, SHR with an average body weight of 116 g fed a diet mixed with 5% *L. edodes* or *G. frondosa* dry powder and water with 0.5% sodium chloride showed significant reductions in blood pressure after a few weeks\(^3\). Although *G. frondosa* has been shown to significantly lower blood pressure in SHR with high blood pressure, *L. edodes* has not been shown to lower blood pressure\(^4\). In one study where *P. nebrodensis* dry powder was administered for a long time before blood pressure began to increase, significant antihypertensive effects were confirmed. Therefore, *P. nebrodensis* appears to be effective in both preventing and improving hypertension. The results of a study on *A. blazei* showed that the antihypertensive effects of mushrooms were extremely dose-dependent\(^16\). We have also confirmed dose dependency for *L. edodes*, *L. decastes* and *P. nebrodensis*. Therefore, it will be necessary to investigate the dose dependency of the various extracts of *P. nebrodensis* prepared in the present study.

The results of biochemical tests using blood and urine samples and the level of urinary sodium are shown in Table 1. The triglyceride and total cholesterol levels for the SHR control group were lower than those for the WKY group. The triglyceride level for the polysaccharide group was high but was lower compared to the WKY group. In the body, urea nitrogen is the final product of protein metabolism, and its level is known to increase due to excretion disorders, such as decreased glomerular filtration or increased permeability, or increased production of uric acid in the body\(^17\). In the present study, the level of plasma urea nitrogen was higher for the non-dialyzable fraction and dialyzable fraction groups compared to the SHR control group. In one study, protein was administered to rats in which 5/6 of the kidneys were removed, and the results showed that protein restriction improved kidney function and lowered the plasma level of urea nitrogen. In general, urea nitrogen is used as an indicator of kidney function, and an increase in urea nitrogen may affect protein metabolism in the kidney or the body. However, no significant differences were seen in urea nitrogen between SHR and WKY administered distilled water, suggesting that the possibility for hypertension to directly reduce kidney function is low. Administered protein appears to have impact on body metabolism. However, the level of urinary sodium for the non-dialyzable fraction and 6% dry powder groups was lower compared to the SHR control group.

In the renin-angiotensin system, renin acts on angiotensinogen to release angiotensin I, which reacts with angiotensin converting enzyme to form angiotensin II. Angiotensin II facilitates the secretion of aldosterone,
Table 1  Effects of *Pleurotus nebrodensis* on Biochemical Changes in the Blood and Urine of Spontaneously Hypertensive Rats.

<table>
<thead>
<tr>
<th></th>
<th>Triglyceride (mg/dl)</th>
<th>Total cholesterol (mg/dl)</th>
<th>Blood urea nitrogen (mg/dl)</th>
<th>Sodium (urine) (mEq/l)</th>
<th>Sodium (blood) (mEq/l)</th>
<th>Renin (ng/ml/h)</th>
<th>Angiotensin I (pg/ml)</th>
<th>Angiotensin II (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHR control</td>
<td>22.3 ± 4.7</td>
<td>43.0 ± 2.1</td>
<td>25.8 ± 2.5</td>
<td>61.7 ± 1.5</td>
<td>145.0 ± 0.6</td>
<td>27.2 ± 3.8</td>
<td>16667 ± 1764</td>
<td>1270 ± 200</td>
</tr>
<tr>
<td>Hot water extract</td>
<td>18.6 ± 3.3</td>
<td>43.0 ± 2.9</td>
<td>26.3 ± 1.0</td>
<td>66.0 ± 12.2</td>
<td>145.4 ± 0.5</td>
<td>28.1 ± 3.2</td>
<td>19600 ± 2676</td>
<td>1258 ± 114</td>
</tr>
<tr>
<td>Polysaccharide fraction</td>
<td>26.6 ± 3.3</td>
<td>46.2 ± 2.2</td>
<td>26.6 ± 0.8</td>
<td>57.0 ± 4.6</td>
<td>145.6 ± 0.2</td>
<td>31.7 ± 4.3</td>
<td>17200 ± 1908</td>
<td>692 ± 91</td>
</tr>
<tr>
<td>Dialyzable fraction</td>
<td>19.0 ± 5.3</td>
<td>39.3 ± 4.9</td>
<td>31.1 ± 1.0</td>
<td>68.7 ± 6.9</td>
<td>145.3 ± 0.7</td>
<td>30.1 ± 12.2</td>
<td>18267 ± 9303</td>
<td>1750 ± 250</td>
</tr>
<tr>
<td>Non-dialyzable fraction</td>
<td>20.5 ± 3.5</td>
<td>40.5 ± 3.5</td>
<td>29.5 ± 2.6</td>
<td>46.5 ± 10.5</td>
<td>144.5 ± 1.5</td>
<td>24.8 ± 12.0</td>
<td>15500 ± 2500</td>
<td>825 ± 175</td>
</tr>
<tr>
<td>6% dry powder</td>
<td>18.0 ± 1.7</td>
<td>45.3 ± 3.2</td>
<td>26.6 ± 1.7</td>
<td>46.7 ± 7.4</td>
<td>145.7 ± 1.2</td>
<td>32.3 ± 5.5</td>
<td>16000 ± 2082</td>
<td>1103 ± 170</td>
</tr>
<tr>
<td>WKY control</td>
<td>31.3 ± 3.4</td>
<td>104.3 ± 2.1</td>
<td>24.7 ± 0.6</td>
<td>70.5 ± 8.2</td>
<td>146.5 ± 0.5</td>
<td>20.3 ± 0.3</td>
<td>8850 ± 868</td>
<td>380 ± 27</td>
</tr>
</tbody>
</table>

Values are mean ± SEM(SHR : n = 6, WKY : n = 8). *Significantly different from SHR-Control at p < 0.05.
which in turn suppresses the secretion of Na+ in the kidney and causes peripheral vascular constriction\textsuperscript{36}. No significant differences existed in plasma renin levels between WKY and SHR, including the control and experiment groups. However, compared to WKY, the levels of angiotensin I and II were significantly higher for the SHR control group, suggesting their involvement in hypertension. While no significant differences were present in angiotensin I between the SHR control group and the various \textit{P. nebrodensis} component groups, the level of angiotensin II for the polysaccharide and non-dialyzable fraction groups was lower, but not significantly so. Studies have shown the antihypertensive effects of \textit{M. aitchisonii}\textsuperscript{38} and \textit{P. cornucopiae}\textsuperscript{39} via the inhibition of angiotensin conversion. As a major antihypertensive agent, isoleucyl tyrosine (dipeptide) has been isolated from \textit{M. aitchisonii}. While many studies have documented the antihypertensive actions of food products, there have been not many studies on mushrooms.

According to the studies that we have conducted to date, long-term administration of \textit{P. nebrodensis} in both dry powder and hot-water extract fractions to SHR before blood pressure began to increase resulted in dose-dependent lowering of blood pressure along with improved neutral lipids, total cholesterol, urea nitrogen and renin-angiotensin system\textsuperscript{37}. Because hypertension in SHR was established in the present study, the antihypertensive action of the hot-water extract was less likely to be expressed, and it will be necessary to confirm dose dependency. The results of the present study suggest that the 6% dry powder diet lowers blood pressure via a mechanism other than blood lipid metabolism, renal function or renin-angiotensin system. Furthermore, the preventative effects of \textit{P. nebrodensis} on hypertension may also contribute to the prevention of arteriosclerosis and accompanying ischemic heart disease and stroke by improving triglyceride and total cholesterol levels.

The present study suggests that because \textit{P. nebrodensis} can suppress hypertension even when blood pressure is already high, it should contribute to the promotion and maintenance of human health.

4 CONCLUSIONS

The antihypertensive actions of \textit{P. nebrodensis} were investigated using SHR. In single-dose administration, antihypertensive effects were seen with the hot water extract of \textit{P. nebrodensis} fruit body and polysaccharide and protein fractions. In continuous-dose administration, significant antihypertensive effects were confirmed with 6% dry powder. The results suggest that \textit{P. nebrodensis} is effective in not only preventing, but also improving hypertension.

References


