A Phytochemical in the Edible Tamogi-take Mushroom (Pleurotus cornucopiae), D-Mannitol, Inhibits ACE Activity and Lowers the Blood Pressure of Spontaneously Hypertensive Rats

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D-Mannitol, one of the main phytochemicals of the edible Tamogi-take mushroom (Pleurotus cornucopiae), was found to inhibit an angiotensin I converting enzyme (ACE). The antihypertensive effect of D-mannitol and a hot water extract of Tamogi-take mushroom was demonstrated in spontaneously hypertensive rats (SHR) by oral administration.

Key words: angiotensin I converting enzyme (ACE) inhibitor; antihypertensive effect; D-mannitol; Tamogi-take; spontaneously hypertensive rat

There is increased medical and pharmaceutical interest in mushrooms as potential sources of novel immunomodulators,¹–³) antitumor agents,⁴–⁸) antibiotics,⁹) and antihypertensives.¹⁰–¹²) Tamogi-take (Pleurotus cornucopiae) is an edible mushroom that belongs to the Hira-take family and grows on standing and fallen elm trees on the Siberian peninsula of Russia and in the eastern and northern parts of Hokkaido, Japan. Angiotensin I-converting enzyme (ACE; kininase II; EC3.4.15.1) is potentially of great importance for controlling blood pressure in the rennin-angiotensin system.¹³–¹⁵) ACE converts the inactive decapptide angiotensin I to the potent vasopressor octapeptide angiotensin II.¹⁶) This article reports that D-mannitol, one of the phytochemicals from Tamogi-take mushroom, inhibits ACE activity and lowers the blood pressure of spontaneously hypertensive rats (SHR).

ACE inhibitory activity was estimated with 8 mU bovine lung ACE (Wako Pure Chemicals, Osaka, Japan) and 5 mM hippuril-histidyl-leucine as a substrate (Peptide Institute, Osaka, Japan) according to the method of Cushman and Cheung.¹⁷)

The ACE inhibitory fraction was separated from hot water extract of Tamogi-take mushroom (WETM) (Three B, Nanporo, Japan) and purified in the following manner: WETM was decolorized by charcoal activated powder. The decolorized extract was subjected to a YMC ODS-AQ 120-S50 column (4 × 2 cm, YMC, Kyoto, Japan) and eluted with H₂O, 10%, and 99.5% EtOH successively. ACE inhibitory activity was observed in the H₂O eluate. The H₂O eluate was concentrated under reduced pressure, and the residue (1 g) was chromatographed on silica gel (Wako Pure Chemicals, Osaka, Japan) using EtOAc:MeOH:H₂O (80:20:3) successively. The solution was separated into a silica gel column eluted with EtOAc–MeOH–H₂O (80:20:3), which resulted in an ACE inhibitory compound (21 mg) with white needle-shaped crystals. The spectral data of the compound were as follows: FD-MS m/z: 184 (M⁺).¹⁷

¹¹H-NMR (D₂O, 500 MHz): δ 3.84 (2H, dd, J = 3, 11.8 Hz, H-1, 6), 3.76 (2H, t, J = 8.6 Hz, H-2, 5), 3.73 (2H, m, H-3, 4), and 3.65 (2H, dd, J = 6.1, 11.9 Hz, H-1, 6).¹³¹C-NMR (D₂O, 500 MHz): δ 71.19 (C-2, 5), 69.63 (C-3, 4), and 63.58 (C-1, 6). The compound was identified as D-mannitol by direct comparison of the spectral data with the authentic compound.

Figure 1 shows dose-dependent inhibition of ACE by WETM and D-mannitol. The IC₅₀ value of WETM was

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Abbreviations: ACE, angiotensin I-converting enzyme; WETM, hot water extract of Tamogi-take mushroom; SHR, spontaneously hypertensive rats; SBP, systolic blood pressure
determined to be 6 mg/ml. D-Mannitol derived from WETM and the authentic compound showed similar ACE inhibitory activity, 3 mg/ml of IC$_{50}$ (Fig. 1). In the experiment described below the authentic compound was used.

Figure 2 shows dose-dependent inhibition of ACE by various sugars. D-Mannitol, D-sorbitol, and D-dulcitol, which are classified as sugar alcohols (IC$_{50}$: 16.4 mM), were the most effective inhibitors of ACE. Disaccharides, including D-maltose and α-lactose, were weaker inhibitors of ACE. Monosaccharides containing D-mannose, D-glucose, and D-galactose ranked in the middle (IC$_{50}$: 60 mM).

The antihypertensive effect of the compound was studied by single oral administration in male SHR. This operation was conducted at the Safety Research Institute for Chemical Compounds Co., Ltd., Hokkaido, Japan. Male rats (12 weeks old) of the SHR/Hos from Hoshino Laboratory Animals were housed under housing conditions of 22°C, and 50% humidity with a 12 h light/dark cycle. Food in the form of dry pellets and water was available ad libitum for 15 d before the start of the experiment. The animals were then separated into three groups of five rats each. The rats were dosed with 10 ml/kg of 4% D-mannitol solution (wt/wt) and 10 ml/kg of 25% WETM (wt/wt). These were orally administered to SHR by gastric intubation together with distilled water as a control. Systolic blood pressure (SBP) was measured before administration and then afterward at 2 h intervals for 8 h by the tail-cuff method with a programmed electrosphygmomanometer (MK-2000, Muromachi Kikai, Tokyo, Japan). The protocol of the study accorded with the standards relating to the care and management of experimental animals (Notification No. 6, March 27, 1980 of the Japanese Prime Minister’s Office). Figure 3 shows the antihypertensive effect of D-mannitol and WETM. The initial average blood pressure at about 178 mmHg decreased to 157 mmHg 4 h after administration of D-mannitol. Similarly, when WETM was administrated, SBP significantly decreased 180 mmHg to 165 mmHg (Fig. 3). The antihypertensive effect of WETM to SHR might be due to D-mannitol.
because the amount of d-mannitol given to SHR was adjusted to replicate that contained in WETM. But there were other fractions chromatographed on silica gel that indicated ACE inhibitory activity (data not shown), which might have yielded hypotensive compounds. Further research on these fractions is in progress. Since sugar alcohol might prevent an increase in blood pressure by other mechanisms, such as osmotic diuretic effect, further studies are necessary to clarify the actual mechanism in vivo.

Many mushrooms have been reported to contain sugar alcohols as their main phytochemical components. This is the first report to indicate that sugar alcohol from mushrooms provides an ACE inhibitory effect and hypotension of blood pressure in SHR. In conclusion, it is suggested that the Tamogi-take mushroom has the physiological effect of controlling blood pressure by inhibiting ACE activity.

References