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Note

Antioxidant, Lipid-Lowering and Antihypertensive Effects of Red Welsh Onion (*Allium fistulosum*) in Spontaneously Hypertensive Rats

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The physiological effects of red Welsh onion were examined and compared with those of white Welsh onion. Male 6-week-old spontaneously hypertensive rat (SHR) was fed a control diet or diets high in fat and sucrose (HFS) with or without 5 % Welsh onion (red or white variety) for 4 weeks. A significant effect to suppress the increase in the blood pressure and the increase in the lipids in plasma and liver was observed in rats fed the red, but not white, Welsh onion compared to the rats fed the HFS diets. Red and white Welsh onion was effective to suppress the increase in the lipid peroxides in plasma, and in liver in case of white Welsh onion. The activity of antioxidant enzymes was downregulated by feeding the red and white Welsh onion. These results suggest that, although the antioxidant activity of red Welsh onion is weaker than the white one, antioxidant activity together with hypolipidemic effect of red Welsh onion might work favorably to suppress the increase in blood pressure. Detection of flavonoids, especially quercetin, and anthocyanins suggests that these compounds might be physiologically active components in red Welsh onion.

Keywords: red Welsh onion, spontaneously hypertensive rats, antioxidant effect, antihypertensive effect, hypolipidemic effect

Welsh onion (*Allium fistulosum* L.), a vegetable of the *Allium* family, is an important flavoring foodstuff in Asian countries. However, only a few reports have studied its medicinal effects. Chen *et al.* reported vasodilatory effects (1999) and blood pressure lowering and antithrombotic effects (2000) of Welsh onion juice, but did not identify the varieties of Welsh onion used or propose the exact mechanisms of these effects. In our previous experiment, green, but not white, Welsh onion had antihypertensive effects on rats fed diets high in fat and sucrose (HFS) (Yamamoto *et al.*, 2005).

In addition to the green and white varieties of Welsh onion, a red variety is increasingly harvested in northern part (Gunma, Ibaragi, etc.) of Japan. The *in vitro* antioxidant activity of red Welsh onion was reported to be much higher than that of white onion (Aoyama and Yamamoto, 2006). These results suggest that the red Welsh onion is a source of antioxidants that could play important roles in physiological protection against reactive oxygen species (ROS) generated in the body. In the present experiment, the effects of red Welsh onion on the development of hypertension, increases in the lipids in plasma and liver, and lipid peroxidation in a spontaneously hypertensive rat (SHR) fed the HFS diets were examined.

Two varieties of Welsh onion, red- and white-sheath varieties, harvested in Ibaragi, Japan, were used. Since the green part of these vegetables is not eaten in Japan, the "edible" red or white part was used for the experiments (Fig. 1). These vegetables were crushed, heated to 80 – 90 °C for about 5 min by microwave oven to cause denaturation of enzyme activity, freeze-dried, and pulverized to pass through a 30 mesh sieve. Twenty-eight 6-week-old male spontaneously hypertensive rats (SHR) were obtained from SLC Japan, Inc (Shizuoka, Japan). They were housed individually in cages with wire mesh bottoms in a room kept at 22 ± 1 ºC and with a 12:12-h light:dark cycle (light period from 8:00 to 20:00 h).

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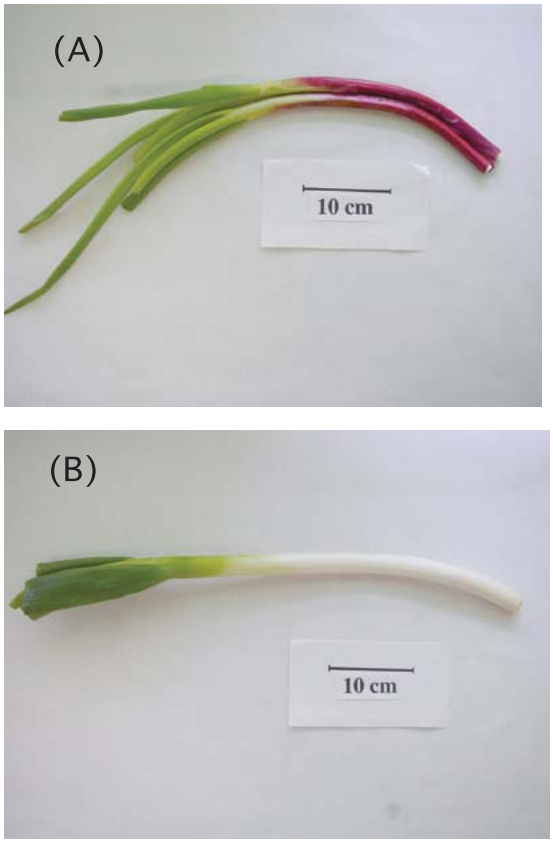

Fig. 1. Appearance of red (A) and white (B) Welsh onions.

After acclimatization for 5 days, the animals were randomly divided into four test groups of seven animals each to average the systolic blood pressure and the initial body weight. One group received a control diet (control group), and the other three groups were fed a diet high in fat and sucrose (HFS group), and with 5.0 % red (RWO group) or white Welsh onion (WWO group). The composition of the control diet was as follows (wt %): corn oil, 5.0; mineral mixture, 3.5; vitamin mixture, 1.0; choline bitartrate, 0.25; casein, 20.0; cellulose, 5.0; and corn α-starch to make 100. The composition of the HFS diet was as follows (wt %): corn oil, 5.0; lard, 15.0; cholesterol, 0.5; mineral mixture, 3.5; vitamin mixture, 1.0; choline bitartrate, 0.25; casein, 20.0; sucrose, 40.0; cellulose, 5.0; and corn α-starch to make 100. The composition of the diets containing Welsh onion was the same as the HFS diet except that 5.0 % of the powdered red or white Welsh onion was replaced with cellulose to keep isocaloric. The composition of the mineral mixture is AIN-93G-MX and that of the vitamin mixture is AIN-93-VX (Reeves, 1997). Food and water were given *ad libitum* for the 4-week duration of the experiment. All experiments were conducted in accordance with the Ethics Guideline for Animal Experiments of Osaka City University. The systolic blood pressure was measured at the start and at the end of 2- and 4-week feeding periods using the tail-cuff method with an instrument (BP98, Softron Inc., Japan). After the 4-week growing period, the rats were starved overnight for 14 h, anesthetized with ethylether, and their blood was collected in a heparinized syringe from the abdominal aorta. The plasma was separated by centrifugation at 1,000 × *g* at 4 ºC for 15 min, and was stored at -80 ºC until being used for biochemical analysis. Epididymal fat tissue and liver were harvested, rinsed gently with chilled saline, blotted, weighted, and then stored at -80 ºC until the use. Concentrations of total cholesterol, HDL-cholesterol, triacylglycerol (TG) in the plasma were determined using a commercial diagnostic kit from Wako Pure Chemicals Co., Ltd. (Osaka, Japan). A portion of the liver was homogenized in 4 volumes (v/w) of ice-cold 10 mmol/L Tris-HCl buffer (pH 7.4) containing 150 mmol/L KCl. Total lipids in the liver homogenate were extracted by the method of Folch *et al.* (1957) and were measured by the gravimetric method. The total cholesterol and TG contents in this lipid extract were measured using the same diagnostic kits as those used for the plasma analysis. Lipid peroxides in blood plasma and liver were measured as the thiobarbituric acid reactive substances (TBARS) by the spectrometry method (Ohkawa *et al.*, 1979). The activity of superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) in the cytosol of liver was measured by the nitrite method (Oyanagui, 1984) and the absorbance at 340 nm (Yamamoto and Takahashi, 1993), respectively. The results of these enzyme activities were expressed as units per milligram of protein. Protein was measured by the method of Lowry *et al.* (1951). The flavonoids from red and white Welsh onion were hydrolyzed and then analyzed according to the method of Ewald *et al.* (1999). Flavonoids used as the standard were quercetin, kaempferol, myricetin, luteolin and apigenin. Total anthocyanins were measured according to Benvenuti *et al.* (2004). Derivatives of ascorbic acid and dehydroascorbic acid were detected at 300 nm after post column reaction with $NaBH_4$ (Yasui and Hayashi, 1991). The result was expressed as the sum of ascorbic acid and dehydroascorbic acid. Each data value for the animal experiments is expressed as the mean ± SEM of seven rats. Data were subjected to a one-way analysis of variance (ANOVA) and multiple-range comparison by Fisher's protected least significant difference (PLSD) procedure. Data of antioxidant contents were expressed as mean ± SD for three experiments, and were subjected to the *t*-test.

The growth, food intake and liver and fat-tissue weights in the HFS, RWO, and WWO groups were not significantly different from each other (Table 1). The total cholesterol in plasma in the RWO group was lower, and the percentage of HDL cholesterol in total cholesterol in the RWO and WWO group was higher than the HFS group. Total cholesterol and

Table 1. Effects of dietary addition of red or white Welsh onion on the growth, dietary intake, liver and fat weight, and lipids in plasma and liver in SHR fed with a high-fat high-sucrose diet for 4 weeks.

Groups	CON	HFS	RWO	WWO
Body weight gain (g/day)	3.2±0.3*	3.5±0.2	3.4±0.3	3.4±0.3
Food intake (g/day)	15.8±0.3^{a}	14.1±1.1^{b}	13.6±0.7^{b}	13.3±0.6^{b}
Food efficiency (%)	20.1±2.1^{b}	24.6±2.7^{a}	24.7±1.8^{a}	25.7±1.9^{a}
Liver (g/100 g BW)	2.82±0.09^{b}	3.61±0.21^{a}	3.52±0.27^{a}	3.63±0.47^{a}
Epididymal fat (g/100 g BW)	3.78±0.15^{b}	4.67±0.32^{a}	4.19±0.08^{ab}	4.52±0.12^{ab}
Lipids in plasma				
Total cholesterol (mmol/L)	1.85±0.11^{a}*	1.86±0.15^{a}	1.57±0.23^{b}	1.76±0.07^{ab}
HDL cholesterol (%)**	40.4±2.0^{ab}	35.4±1.9^{b}	47.0±3.7^{a}	46.5±0.7^{a}
Triacylglycerol (mmol/L)	0.72±0.04	0.76±0.05	0.73±0.08	0.68±0.02
Lipids in liver				
Total lipids (mg/g)	57.4±3.5^{b}	126±12^{a}	106±7^{a}	108±6^{a}
Total cholesterol (μmol/g)	9.37±0.30^{c}	76.5±6.7^{a}	58.1±3.5^{b}	66.4±4.7^{ab}
Triacylglycerol (μmol/g)	35.8±3.6^{c}	125±14^{a}	94.1±6.0^{b}	102±7.5^{ab}

*Mean±SEM, n=7. Values within a row with different superscript letters are significantly different from each other (p<0.05).

**(HDL cholesterol/total cholesterol) × 100.

TG in the liver of rats of the RWO group, but not the WWO group, were significantly lower than those in the rats of the HFS group. These results suggested that the red Welsh onion was more effective than the white Welsh onion in lowering lipids in the plasma and liver. The blood pressure in rats of the RWO group, but not the WWO group, was lower than that of the HFS group at 4 weeks (Fig. 2). In our previous experiment, we suggested that green but not white Welsh onion had a significant antihypertensive effect on normotensive rats fed HFS diets (Yamamoto *et al.*, 2005). From these and the present results, we came to a conclusion that red and green, but not white, varieties of Welsh onion are effective in suppressing increases in blood pressure. The TBARS in the plasma and liver of rats of the HFS group was higher than that of the control group. Red and white Welsh onions were effective in suppressing the accumulation of lipid peroxides in plasma, and also in liver, in the case of white Welsh onion (Fig. 3). The activities of SOD and GSH-Px in the liver were higher in the SHR fed the HFS diet than those fed the control diets. The activity of SOD in the rats of the RWO and WWO groups, and the activity of GSH-Px in the rats of the WWO group, was lower than that for the HFS group.

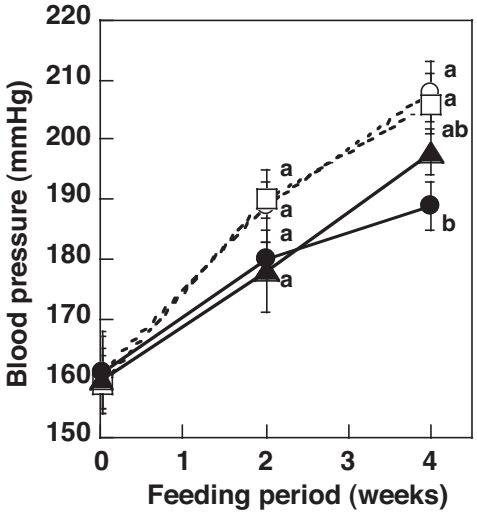

Fig. 2. Effects of red Welsh onion on the systolic blood pressure of SHRs fed a high-fat high-sucrose diet. ○, Control group; □, HFS group; ●, RWO group; ▲, WWO group. Each point represents the mean ± SEM, n=7. Values with different superscript letters are significantly different from each other (p<0.05).

Allium vegetables are a source of various antioxidant components that could potentially play important roles in physiological protection against ROS generated in the body. One of these components is a group of flavonoids that includes quercetin and kaempferol, which were detected in red and white Welsh onion, respectively (Table 2). Other flavonoids, including myricetin, apigenin and luteolin, were not detected in both varieties of Welsh onion. Anthocyanins were detected in the red, but not white, Welsh onion. From these results and the results of food intake in Table 1, intake of quercetin and anthocyanins in rats fed the diets with red Welsh onion were calculated to be 3.6 and 0.35 mg/day, respectively. Additionally, ascorbic acid is an antioxidant component found in Welsh onions, and the ascorbic acid content in red Welsh onion was lower than that of white Welsh onion.

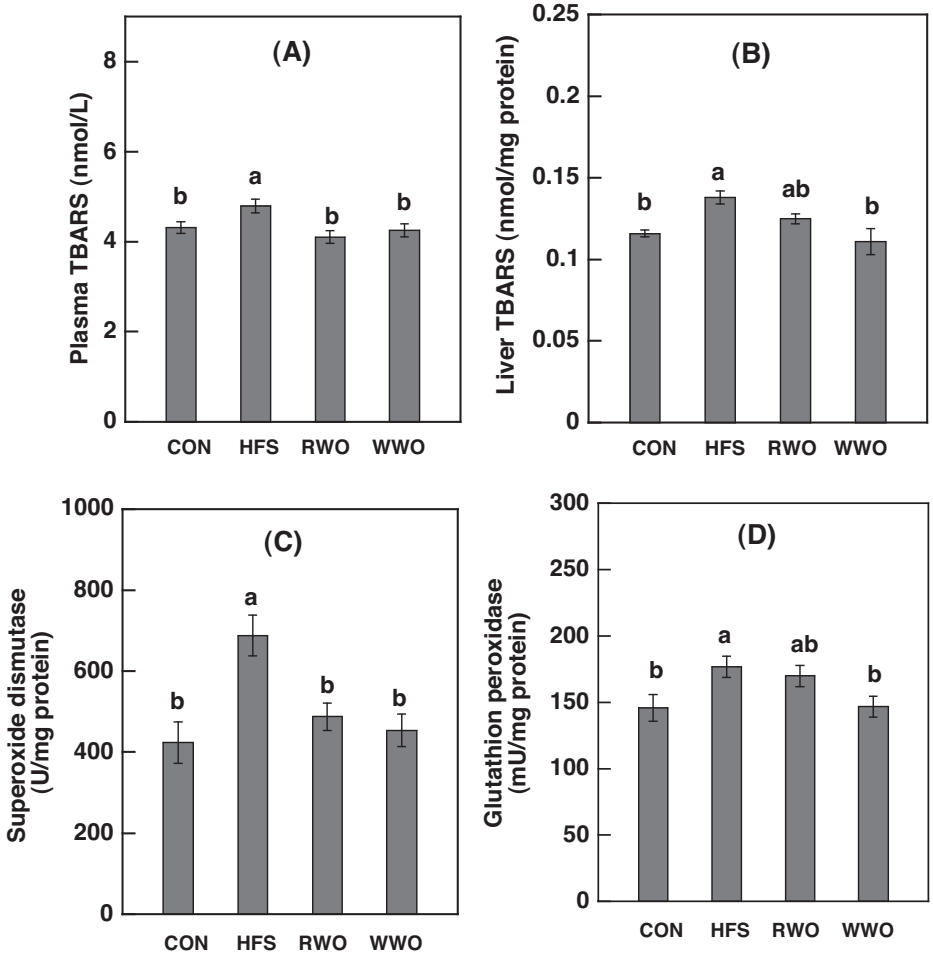

Fig. 3. Effects of red Welsh onion on the TBARS in plasma (A) and liver (B) and superoxide dismutase (C) and glutathione peroxidase (D) activities in the liver of SHRs fed a high-fat high-sucrose diet. Each bar indicates the mean ± SEM, n=7. Values with different superscript letters are significantly different from each other ($p<0.05$).

Table 2. Flavonoid, ascorbic acid, and anthocyanin in red and white Welsh onions.

	Red Welsh onion	White Welsh onion
	(mg /100 g fresh weight)	
Flavonoids		
Quercetin	53.1±2.1*	nd[1]
Kaempferol	nd	0.63±0.06
Anthocyanins	5.06±0.11	nd
Ascorbic acid	7.73±0.44	11.5±0.2

*Mean±SEM (n=3). [1]not detected.

The role of oxidative stress on the genesis and maintenance of hypertension has been supported by many studies on SHR. The ROS in the vascular tissues were high levels in the SHR (Zalba *et al.*, 2001; Hamilton *et al.*, 2001; Suzuki *et al.*, 1995), and the hypertension in SHR was ameliorated by the administration of many kinds of antioxidants (Newaz *et al.*, 1999; Chen *et al.*, 2001; Vasdey *et al.*, 2001). The weaker *in vivo* antioxidant activity of red Welsh onion compared to white Welsh onion was inconsistent with the results of a previous *in vitro* experiment suggesting stronger antioxidant activity in red Welsh onion (Aoyama and Yamamoto, 2006). Although the activity of red Welsh onion on lower lipid peroxides in plasma and liver was weaker than that of the white Welsh onion, antioxidant components in the red Welsh onion might be effective to protect vascular tissues against ROS, such as superoxides generated in the SHR. Furthermore, the combined antioxidant and hypolipidemic activities of red Welsh onion might be effective in decreasing the generation or increasing the scavenging of ROS in the vascular tissues, and suppressing increases in blood pressure. Detection of flavonoids, especially quercetin, and anthocyanins in red Welsh onion suggests that these compounds might play important roles in protecting against ROS generated in the body.

In the present experiment, we suggested that a red variety of Welsh onion is one of the hypertensive, lipid-lowering and oxidative stress-suppressing vegetables. Further studies

should be done to elucidate the antioxidant and antihypertensive components in red Welsh onion and to understand the mechanisms of physiological activities of these components.

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