Effects of Boiling on the Antihypertensive and Antioxidant Activities of Onion

Eiko KAWAMOTO1, Yoko SAKAI1, Yuko OKAMURA2 and Yukiko YAMAMOTO1,*

1Graduate School of Human Life Science, Osaka City University, Sugimoto 3–3–138, Sumiyoshi-ku, Osaka 558–8585, Japan
2Faculty of Human Life Science, Osaka City University, Sugimoto 3–3–138, Sumiyoshi-ku, Osaka 558–8585, Japan

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Summary This study was designed to show the effect of boiling on the antihypertensive and antioxidant activities of onion in N\textsuperscript{\textcircled{\textgreek{n}}}-nitro-L-arginine methyl ester (L-NAME) induced-hypertensive rats and spontaneously hypertensive rats (SHR). Male 6-wk-old Sprague-Dawley rats were made hypertensive by being given distilled water containing L-NAME at a dose of 50 mg/kg BW/d. These rats were fed diets containing raw or boiled onion at a concentration of 5%. Raw onion significantly reduced the increase in systolic blood pressure in both L-NAME induced-hypertensive rats and SHR, and inhibited the increase in thiobarbituric acid reactive substances (TBARS) and conjugated dienes in the plasma and tissues of SHR. The antihypertensive effect of boiled onion was not found, and the antioxidant activity of it was much weaker than that of raw onion. The excretion of nitric oxide metabolites (NOx) in urine was enhanced by raw onion in both L-NAME induced-hypertensive rats and SHR, and was enhanced by boiled onion only in SHR. In conclusion, our results suggested that the antihypertensive activity of raw onion disappeared during boiling, and the disappearance of the antioxidant activity of onion, with a consequent reduction in the saving of nitric oxide (NO). In conclusion, our results suggested that the antihypertensive activity of onion disappeared during boiling, and the disappear of the antihypertensive activity of raw onion after boiling might come, in part, from a decrease of the antioxidant activity of onion, with a consequent reduction in the saving of nitric oxide (NO).

Key Words onion, NO synthase inhibitor, spontaneously hypertensive rats, antioxidant, boiling

Onion (Allium cepa) has been a source of medicinal compounds for a long time. Though current interest in health foods has spurred many studies on the beneficial medicinal effects of onion on, for example, blood platelet aggregation and levels of cholesterol and glucose in serum (1–3), little has been reported on the effect of onion on blood pressure. Recently, we reported a distinct antihypertensive effect of onion in rats whose hypertension had been induced by dosing nitric oxide synthase (NOS) inhibitor N\textsuperscript{\textcircled{\textgreek{n}}}-nitro-L-arginine methyl ester (L-NAME), and also on stroke prone spontaneously hypertensive rats (SHRSP) (4). In that study, we suggested that the mechanism of the antihypertensive effect of onion probably included the increased saving of nitric oxide (NO), which is responsible for acetylcholine-mediated vascular relaxation, by the antioxidant activity of onion.

The evaluation of the impact of common domestic food processing on the health benefits of vegetables is of great practical importance, but only a few studies have been reported on the effects of heat processing on the physiological activity of vegetables. With regard to the heat sensitivity of the effects of Allium vegetables, Chen et al. have reported that the juice of raw, but not heated, Welsh onion had beneficial effects on hypertensive and antithrombotic actions in normal rats (5). However, we have never seen any reports on the effects of heat treatment on the hypertensive and antioxidant effects of onion.

Onion is a good source of quercetin, one of the most abundant flavonol-type flavonoids in fruits and vegetables, and the antioxidant activity of quercetin has been demonstrated in rats and humans (6–8). There are many reports showing that flavonoids such as quercetin and its glucosides are stable during the heat treatments involved in food processing (9–11). In the present experiment, the effects of boiling on the antihypertensive and antioxidant activities of onion were studied for L-NAME induced-hypertensive rats and spontaneously hypertensive rats (SHR).

MATERIALS AND METHODS

Animals and experimental diets. For experiments with N\textsuperscript{\textcircled{\textgreek{n}}}-nitro-L-arginine methyl ester (L-NAME) induced-hypertensive rats, male 6-wk-old Sprague-Dawley rats weighing approximately 150 g were obtained from Japan SLC (Shizuoka, Japan). The animals were allowed free access to water and a basal diet for 4 to 5 d. They were randomly divided into four test groups of six animals each to average the initial body weight and systolic blood pressure. A control group...
received tap drinking water and a control diet. The other three groups were given tap water containing L-NAME at a concentration to deliver 50 mg/kg BW/d, and were fed a control diet (LN group) or a diet containing raw or boiled onion (LN-RO or LN-HO group). The concentration of L-NAME in tap water was adjusted on a daily basis to ensure proper dosing. For the experiment with spontaneously hypertensive rats (SHR), male 6-wk-old SHR were obtained from Japan SLC. After feeding a basal diet for 1 wk, they were randomly divided into three groups of six animals each to average the initial body weight and systolic blood pressure. They were fed a control diet (control group) or a diet containing raw or boiled onion (R-ON or H-ON group) with tap water to drink. The animals were housed individually in cages with wire mesh bottoms in a room kept at 22±1°C, with a dark period from 20:00 to 8:00 h. Food and water were given ad libitum for the 3-wk duration of the experiment. The composition of the basal diet was as follows (wt%): corn oil, 5.0; mineral mixture, 3.5; vitamin mixture, 1.0; choline bitartrate, 0.1; casein, 20.0; and corn α-starch to make 100.0. The composition of the control diet, and raw or boiled onion diet was the same as that of the basal diet except that corn α-starch, at a concentration of 5.0% in diets, was replaced with cellulose or raw or boiled onion powder. The composition of the mineral mixture was AIN-93G-MX and that of the vitamin mixture was AIN-93-VX (12). Brown-skinned onions, harvested in June 2002 in Osaka Prefecture, were used. To prepare raw onion powder, the onion bulb was minced, heated to 80–90°C for 5 min by a microwave heater (to cause denaturation of enzyme activity), freeze-dried, and crushed to a fine powder. To prepare boiled onion powder, onion bulbs were cut into 8 pieces and boiled with distilled water for 60 min. After cooling, both onion bulb and boiling water were freeze-dried and crushed to a fine powder. Sampling procedures. After a 3-wk growing period, the rats were starved overnight and were anesthetized with diethylether. Their blood was then collected in a heparinized syringe. The plasma was separated by centrifugation at 1,200×g at 4°C for 15 min, and stored at −80°C. In the SHR experiments, the liver, heart and lungs were quickly removed after blood sampling. The organs were rinsed with ice-cold saline water, blotted dry with tissue paper, weighed and stored at −80°C while awaiting biochemical analysis. All experiments were performed under the Guideline for Animal Experiments in Osaka City University and the Notification No. 6 of the Japanese government.

Blood pressure. The systolic blood pressure was measured at the start and at the end of 2 and 3 wk of the experiment using the tail-cuff method (13) with an instrument (BP98, Softron Inc. Japan). The rat-tails were preheated in an oven and the average of three readings was taken as the final value.

Lipid peroxides. Lipid peroxides in blood plasma, liver, heart, and lung in L-NAME induced-hypertensive rats and SHR were measured as the thio孙urburic acid reactive substances (TBARS) by the spectrometry method (14) and were expressed as the amount of malondialdehyde (MDA) in plasma and tissues. In addition, lipid peroxides in liver, heart, and lung in SHR were measured as the amounts of conjugated dienes. For the assay of conjugated dienes, total lipids in tissue homogenates were extracted by the methods of Folch et al. (15). The lipid extracts were evaporated and the residue was dissolved in cyclohexane, and the absorbance at 233 nm was determined (16). The amount of conjugated dienes was calculated using a molar extinction coefficient of 2.52×104 M−1.

Nitric oxide metabolites in urine. Urine was collected during the last 3 d of the growing period by placing the rats in stainless steel metabolic cages. The total nitrate and nitrite concentration was measured by reacting with Greiss reagent following the procedure described by Wu et al. (17). Results were expressed as total nitrate and nitrite per creatinine in urine.

Lipid analysis. Lipid analysis was performed in the SHR experiment. Concentrations of total cholesterol and triacylglycerol in plasma were measured using a commercial diagnostic kit (Cholesterol C-test Wako and Triglyceride G-test Wako, respectively) from Wako Pure Chemicals Co. (Osaka, Japan). A portion of the liver was homogenized in 4 volumes (v/w) of ice-cold 10 mM Tris-HCl buffer (pH 7.4) containing 150 mM KCl. Total lipids in the liver homogenate were extracted by the method of Folch et al. (15). The total cholesterol and triacylglycerol contents in this extract were measured using the same diagnostic kits used for plasma analysis.

Statistical analysis. The data were expressed as mean±SEM for six rats, and were analyzed by the analysis of variance (one way ANOVA) and multiple range comparisons by Fisher’s protected least significant difference (PLSD) procedure or unpaired Student’s t-test using StatView, Abacus Concepts, Inc., Berkeley, CA. A p value of <0.05 was considered significantly different.

RESULTS

Effects of raw and boiled onion on L-NAME induced-hypertensive rats

Systolic blood pressure increased gradually in rats of the LN group, rising to about 200 mmHg at the end of the 3-wk growing period (Fig. 1). The blood pressure in rats of the LN-RO group increased more slowly growing to about 180 mmHg after 3 wk, and a significant hypotensive effect of raw onion was observed after 2 and 3 wk. The blood pressure in LN-HO group at 2 and 3 wk was not significantly different from that of the LN group.

As Table 1 shows, average body weight gain and food intake for 3 wk in all groups were not different from each other. The TBARS in plasma were significantly higher in rats of the LN group compared to the control group, and were lowered to the level of the control group by feeding the raw onion diet. The TBARS in plasma of LN-HO group were not significantly different from those of the LN group.

The NO metabolites excreted in urine were significantly lower in rats of the LN group compared to those of the control group (Fig. 2). The NO metabolites in
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urine in rats fed raw, but not boiled, onion were significantly higher than those of the LN group.

Effects of raw and boiled onion on SHR

Systolic blood pressure increased gradually in SHR of the control group, and rose to about 210 mmHg at the end of the 3-wk growing period (Fig. 3). The pressure for SHR of the R-ON group increased much more slowly, reaching about 200 mmHg after 3 wk, and a significant hypotensive effect of raw onion was observed at 2 and 3 wk. The hypotensive effect of boiled onion was not observed at the end of 2 and 3 wk.

As Table 2 shows, the body weight gain and food intake for the 3 week's duration of the experiment in SHR of the R-ON and H-ON groups were not different from those of the control group. In addition, the total cholesterol and triacylglycerol in plasma were not different in SHR of the R-ON and H-ON groups compared to those of the control group. Relative weight of heart in the H-ON group was lower, and that of lung in the R-ON group was higher, than those of the control group.

The TBARS in the plasma of SHR in the R-ON group, but not the H-ON group, were significantly lower than those of the control group (Table 3). The TBARS in the liver and heart were the same for all three groups. The TBARS in the lung and the conjugated dienes in the liver, heart, and lung in the R-ON group were significantly lower than those of the control group. The conjugated dienes in the liver and heart in SHR in the H-ON group were significantly lower than those of the control group, but were higher than those of the R-ON group. The NO metabolites excreted in the urine of SHR in the R-ON and H-ON groups were significantly higher than those of the control group (Fig. 4). The NO metabolites excreted in the urine of SHR fed boiled onion were lower, but not significantly, than those of the rats fed raw onion.

Table 1. Effects of raw or boiled onion added in diets on growth, food intake and plasma TBARS in rats administered L-NAME.

|                          | Control          | LN              | LN-RO           | LN-HO           |
|--------------------------|------------------|-----------------|-----------------|-----------------|
| Body weight gain (g/d)   | 6.84±0.50        | 6.25±0.17       | 6.38±0.26       | 6.66±0.56       |
| Food intake (g/d)        | 20.0±0.3         | 18.5±0.4        | 18.3±0.7        | 18.5±0.7        |
| Plasma TBARS (μmol/L)    | 1.64±0.09        | 2.47±0.21       | 1.93±0.14       | 2.07±0.14       |

Mean±SEM (n=6).
Values within a same row with different superscripts are significantly different from each other (p<0.05).
Table 2. Effects of raw or boiled onion on the growth, food intake, organ weights, and plasma lipids in SHR.

|                          | Control       | R-ON          | H-ON          |
|--------------------------|---------------|---------------|---------------|
| Body weight gain (g/d)   | 2.81±0.17*    | 2.91±0.19     | 2.72±0.23     |
| Food Intake (g/d)        | 17.5±0.3      | 17.2±0.4      | 16.6±0.4      |
| Organ weight (g/100 g body weight) |            |               |               |
| Liver                    | 3.08±0.05     | 3.21±0.03     | 3.15±0.05     |
| Heart                    | 0.48±0.01a    | 0.45±0.01b    | 0.43±0.01b    |
| Lung                     | 0.48±0.02b    | 0.59±0.06a    | 0.51±0.03ab   |
| Plasma lipids            |               |               |               |
| Total cholesterol (mmol/L) | 1.43±0.06    | 1.59±0.14     | 1.50±0.06     |
| Triacylglycerol (mmol/L)  | 0.60±0.05     | 0.65±0.03     | 0.57±0.05     |

*Mean±SEM, n=6. Values within a row with different superscripts letters are significantly different from each other (p<0.05).

Table 3. Effects of raw or boiled onion on the TBARS and conjugated dienes in plasma and organs in SHR.

|                          | Control       | R-ON          | H-ON          |
|--------------------------|---------------|---------------|---------------|
| Plasma                   |               |               |               |
| TBARS (μmol/L)           | 2.56±0.10a    | 1.84±0.03b    | 2.19±0.25ab   |
| Liver                    |               |               |               |
| TBARS (μmol/100 g)       | 46.9±1.3      | 47.1±2.2      | 47.0±1.3      |
| Conjugated dienes (μmol/100 g) | 230±10a   | 103±1c         | 132±6b        |
| Heart                    |               |               |               |
| TBARS (μmol/100 g)       | 28.9±1.8      | 27.5±0.9      | 26.6±2.0      |
| Conjugated dienes (μmol/100 g) | 77.3±6.5a   | 33.0±4.2c     | 52.7±5.2b     |
| Lung                     |               |               |               |
| TBARS (μmol/100 g)       | 38.4±1.6a     | 26.4±2.1b     | 32.5±3.3b     |
| Conjugated dienes (μmol/100 g) | 39.0±4.8b    | 20.9±4.3b     | 29.9±3.1ab    |

*Mean±SEM, n=6. Values within a row with different superscript letters are significantly different from each other (p<0.05).

Fig. 4. Effects of raw or boiled onion on the excretion of NO metabolites in the urine of SHR. The NO metabolites excreted in the urine were expressed as total nitrate and nitrite per creatinine in urine. Bars are means±SEM, n=6. Data with different superscript letters are significantly different from each other (p<0.05).

DISCUSSION

Although onion is one of the most widely used Allium vegetables in the world, few reports were found on the antihypertensive effects of onion. Recently, we showed a distinct antihypertensive effect of onion for L-NAME induced-hypertensive rats and the SHRSP (4). Furthermore, we showed in this experiment that the antihypertensive effect of onion in the L-NAME induced-hypertensive rats and SHR disappears during boiling.

The role of oxidative stress on the genesis and maintenance of hypertension has been supported by many studies on SHR. The reactive oxygen species (ROS) in the vascular tissues in SHR were at high levels (18-20), and the hypertension in SHR was ameliorated by the administration of many kinds of antioxidants (21-23). The results of this experiment also showed an increase in the TBARS and conjugated dienes accumulated in the plasma and tissues in SHR. For the L-NAME induced-hypertension in normal rats, earlier reports referring to the enhanced oxidation in these rats were not found. In this experiment, we showed an enhanced oxidation in the L-NAME induced-hypertensive rats. As the superoxide and other ROS react quickly with NO (24-26), a decreased NO concentration in these rats might lead to store ROS, and then enhance oxidation.

The development of increased blood pressure in SHR was attributed to reduced NOS activity and decreased NO availability due to the enhanced oxidation (21, 27-29). On the contrary, enhanced NOS activity and increased NO excretion in SHR have been reported by different investigators (30-32). Vaziri et al. explained this apparent contradiction by the possibility that enhanced NOS activity and increased NO availability
during the early phase of evolution of hypertension in SHR may be followed by reduced NOS activity and decreased NO availability with advanced hypertension (30). Decreased excretion of NO metabolites in SHR in this experiment might be an indication that these rats were at the advanced phase of hypertension. Further studies are required to examine this possibility.

Antioxidants might be effective for saving of NO, which plays an important role in the regulation of blood pressure. Treatment with many kinds of antioxidants increased NO availability in hypertensive rats (21, 27–29). In this and our previous report (4), onion was effective for increasing the urinary excretion of NO metabolites in SHR and L-NAME induced-hypertensive rats. These results suggest a possibility that the antihypertensive effect of onion might come from an increase in NO saving due to the antioxidative activity of onion.

With regard to the heat stability of the antioxidative activity of onion, Cazzani et al. assessed the effect of boiling for 30 min on the in vitro antioxidative activity of onion juice (33, 34). The antioxidant activity of onion juice against the coupled oxidation of β-carotene plus linoleic acid (33) or against lipid peroxidation of rat liver microsome (34) was decreased to 63% or 94% of the initial activity before boiling for 30 min, respectively. In this experiment, we assessed the effects of boiling for 60 min on the in vivo antioxidative activity of onion. Raw onion added to diets significantly decreased the TBARS and conjugated dienes accumulated in the plasma and tissues in hypertensive rats, and the antioxidative activity of raw onion was much lower after boiling. Furthermore, the effects of raw onion on increasing the excretion of NO metabolites in urine were lower after boiling in both the SHR and L-NAME induced-hypertensive rats. This suggests our tentative conclusion, namely, that the antihypertensive effect of raw onion might disappear after boiling, in part, from a decrease of the antioxidative activity of onion, with a consequent reduction in the saving of NO.

There are many components in onion known as potent antioxidants, and one of them is quercetin (6–8). Many reports have shown the stability of quercetin in onion during heat treatment (9–11). Onion also contains antioxidative sulfur compounds (35–37), but we have never seen any reports on the heat stability of sulfur compounds in onion. The possible effects of onion components other than quercetin and sulfur compounds on the antihypertensive activity of onion may not be negligible. Ingested nitrate/nitrite or arginine from onion in the diet may take part in increasing urinary nitrate/nitrite. Further studies should be undertaken to clarify if any components in onion are responsible for antioxidant and antihypertensive activities, and might be responsible for instability by boiling.

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