Antihypertensive Effects of Onion on NO Synthase Inhibitor-induced Hypertensive Rats and Spontaneously Hypertensive Rats

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This study was designed to show the effects of onion on blood pressure in N\textsuperscript{G}-nitro-L-arginine methyl ester (L-NAME) induced-hypertensive rats and stroke prone spontaneously hypertensive rats (SHRSP) using dried onion at 5% in their diets. For the experiment with L-NAME induced-hypertensive rats, male 6-weeks-old Sprague-Dawley rats were given tap water containing L-NAME to deliver 50 mg/kg BW/day. In this experiment, we found distinct antihypertensive effects of onion on the L-NAME induced-hypertensive rats and the SHRSP. Dietary onion decreased the thiobarbituric acid reactive substances (TBARS) in plasma in these hypertensive rats. Also, onion increased the nitrate/nitrite (products of nitric oxide (NO)) excreted in urine and the NO synthase (NOS) activity in the kidneys in SHRSP. These results suggested that the increased NO caused by the greater NOS activity, and additionally by the increased saving of NO by the antioxidative activity of onion, was one of the cause of the antihypertensive effect of onion in SHRSP. In the L-NAME induced hypertensive rats, onion did not significantly block the inhibition of NOS activity by L-NAME, and decreased nitrate/nitrite excretion in urine was not restored. The mechanism of the antihypertensive effect of onion probably involves increased saving of NO by antioxidative activity of onion in L-NAME induced-hypertensive rats.

Key words: hypertension; onion; NO synthase inhibitor; SHRSP; antioxidant

Onion (Allium cepa) and garlic (Allium sativum) are said to have a beneficial medicinal effect on various aspects including blood platelet aggregation and cholesterol and glucose levels in serum.1-6) For the effects of blood pressure, the antihypertensive effects of garlic on the hypertensive patient,7,8) spontaneously hypertensive rats (SHR),9) and nitric oxide synthase (NOS) inhibitor-induced hypertensive rats10) have been studied. The effects of onion on blood pressure have been studied less extensively, the only study we found being a report of Louria et al. who administered crude onion extract to hypertensive patients.11) Kiviranta et al. failed to show the antihypertensive effects of an ethanolic extract of onion on SHR.12)

Recent studies show the antihypertensive effects of antioxidative components in food including α-tocopherol13,14) and ascorbic acid.14-17) Onion is a good source of quercetin, which is one of the most abundant flavonol-type flavonoids in fruits and vegetables.18) Quercetin is present in onion in glycoside forms, and most of it is in a highly absorbable glucoside form.19) Frémont et al. showed that dietary flavonoids (quercetin + catechin, 2:1) reduced lipid peroxidation in rats fed polyunsaturated fat diet.20) Onion also contains many kinds of sulfur compounds. These are alkyl cysteine sulfoxides such as S-propenylcysteine sulfoxide, S-propylcysteine sulfoxide, and S-methylcysteine sulfoxide.21,22) Sulfoxides have been shown to be strongly antioxidative.21-25)

Nitric oxide (NO) is produced from arginine by NOS, and is responsible for acetylcholine-mediated vascular relaxation. The blockade of NO synthesis by the injection of NOS inhibitors, such as N\textsuperscript{G}-nitro-L-arginine methyl ester (L-NAME), causes hypertension.26,27) Reduced NOS activity or decreased NO concentration might lead to an elevated blood pressure.

In this study the effects of onion on the blood pressure in the L-NAME induced-hypertensive rats and the stroke-prone spontaneously hypertensive rats (SHRSP) were studied. The effects of onion on the NO metabolism were also studied.

Materials and Methods

Animals and experimental diets. For the experiment with L-NAME induced-hypertensive rats, male 6-weeks-old Sprague-Dawley rats weighing approximately 200 g were obtained from Clea Japan, Inc. (Osaka, Japan). For 4 or 5 days the animals were
given free access to water and a basal diet. They were randomly divided into three test groups of six  

animals each to average the initial body weight and systolic blood pressure. One group received a control 

diet and, for drinking, tap water (Control group). The other two groups were fed a control diet (LN 

group) or an onion diet (LN-ON group), and were given tap water containing l-NAME to deliver 50 mg 
/kg BW/day. The concentration of l-NAME in tap water was adjusted on a daily basis to ensure proper 

dosing. For the experiment with SHRSP, male and female SHRSP were obtained from the colonies estab-

lished in Kinki University (Nara, Japan) and were bred in our laboratory. Male 15 week old SHRSP 

were randomly divided into 2 groups (n=6) to average the initial body weight and systolic blood 

pressure, and were fed a control diet (Control group) or an onion diet (ON group) with tap water to drink. 

For experiments with l-NAME induced hypertensive rats and with SHRSP, the animals were housed 

individually in cages with wire mesh bottoms in a room kept at 22±1°C and with a dark period from 

20:00 to 8:00 h. Food and water were given ad libitum for the 4-week duration of the experiment. The 

composition of a 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 starch to make 100. The compositions of the control 

diet and the onion diet were the same as the basal diet except that 5

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was randomly divided into 3 groups (n=9) to 

average the initial body weight and systolic blood 

pressure. One group received a control diet and, for drinking, tap water (Control group) or an onion diet (ON group) with tap water to drink. 

For experiments with l-NAME induced hypertensive rats and with SHRSP, the animals were housed 

individually in cages with wire mesh bottoms in a room kept at 22±1°C and with a dark period from 

20:00 to 8:00 h. Food and water were given ad libitum for the 4-week duration of the experiment. The 

composition of a 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. The compositions of the control 
diet and the onion diet were the same as the basal diet except that 5% of the corn α-starch was replaced with cellulose and onion powder, respectively. For the preparation of onion powder, onion harvested on 

June 2000 in Izumisano was used. The onions were crushed, heated to 80–90°C by a microwave heater 
to cause denaturation of enzyme activity, and freeze-dried. The composition of the mineral mixture is 
AIN-93G-MX and that of vitamin mixture is AIN-93-VX.29

After the 4-week growing period, the rats were starved overnight, anesthetized with ether, and their 

blood was collected in a heparinized syringe from the abdominal aorta. The plasma was separated by 
centrifugation at 10,000×g at 4°C for 15 min, and stored at −80°C while awaiting biochemical analy-
sis. Immediately after blood sampling, the abdomi-
nal aorta and kidneys were harvested, cleaned, blotted, and stored at −80°C until they were processed. 

All experiments were done under the Guideline for 

Animal Experiment in Osaka City University and the 

Notification No. 6 of the Japanese government.

Blood pressure. The systolic blood pressure was measured at the end of each week using the tail-cuff 

method30 with an instrument (BP98, Softron Inc. Japan). The tails of rats were heated in an oven and 

the average of three readings was taken as the final value.

TBARS measurement. Lipid peroxides in blood plasma were measured as the thiobarbituric acid 

reactive substances (TBARS) by the spectrometry method30 and were expressed as the amount of MDA 
in plasma.

NOS activity. NOS activity was measured by the 

method of Breder and Snyder.31 A kidney and the ab-

dominal aorta were homogenized with five volumes of 

50 mM HEPES (pH 7.4) containing 1 mM dithiothreitol, 1 mM EDTA, 5 μg/ml phenylmethyl-
sulphonyl fluoride, 5 μg/ml of pepstatin, and 5 μg/ml of aprotinin. The homogenate mixture was centrifuged 

for 5 min at 11,000×g, and the supernatant was used for the 

NOS assays. Assay mixture consisted of 

12.5 mM HEPES (pH 7.3), 1.2 mM MgCl2, 0.96 mM 

CaCl2, 3 μm tetrahydrobiopterin, 1 μm FAD, 1 μm 

FMN, 0.024 mM l-arginine, 0.01 μmol l-[U-14C]-arginine (1.5 kBq), 0.12 mM NADPH, and 0.02 ml of 

homogenate supernatant in a final volume of 0.1 ml. Activity was expressed as the amount of citrulline 
produced per protein. Protein was measured by the 

method of Lowry et al.32

Nitric oxide in urine. Urine was collected at the last 3 
days of the growing period by placing the rats in 

metabolic cages. The total nitrate and nitrite concen-

tration in urine was measured by reacting with Greiss 

reagent following the procedure described by Wu 
et al.33 Results were expressed as total nitrate and 
nitrite per creatinine in urine.

Statistical analysis. The data was expressed as 

mean±SEM for six rats. Data were analyzed by the 
analysis of variance (one way ANOVA) and multiple 

range comparisons by Fisher's protected least sign-
ificant difference (PLSD) procedure or an unpaired 

Student's t-test using StatView, Abacus Concepts, 
Inc., Berkeley, CA. A P value of <0.05 was consi-
dered significantly different.

Results

Effects of onion on l-NAME induced-hypertensive 

rats

As Table 1 shows, average body weight gain and 

food intake for 4 weeks in the Control, LN, and LN-

ON groups were not different from each other. 

The NOS activity in kidney and aorta was sign-
ificantly lower in rats of LN and LN-ON groups 
than that in rats of the Control group, although the activity in the aorta in the LN-ON group was not 

significantly different from that in the Control group (Table 1).

The average systolic blood pressure of three groups at the start of experiment was about 135 mmHg 
(Fig. 1). The blood pressure was increased gradually in rats of the LN group, and rose to about 180 mmHg
Fig. 1. Effects of Dietary Onion on Systolic Blood Pressure in \(L\)-NAME Induced Hypertensive Rats.

\(\circ\), Control group; \(\square\), LN group; \(\bullet\), LN-ON group. Values with different superscript letters at a same time are significantly different from each other \((p < 0.05)\).

Fig. 2. Effects of Dietary Onion on the TBARS in Plasma in \(L\)-NAME Induced Hypertensive Rats.

Values with different superscript letters are significantly different from each other \((p < 0.05)\).

Table 1. Effects of Dietary Onion on Growth, Food Intake, and NOS Activity in Rats Administered \(L\)-NAME

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>LN</th>
<th>LN-ON</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight gain (g/day)</td>
<td>6.63±0.47</td>
<td>6.30±0.39</td>
<td>6.42±0.23</td>
</tr>
<tr>
<td>Food intake (g/day)</td>
<td>20.5±0.6</td>
<td>19.3±0.6</td>
<td>18.9±0.7</td>
</tr>
<tr>
<td>NOS activity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kidney (pmol/min/mg protein)</td>
<td>0.312±0.061(^a)</td>
<td>0.176±0.019(^b)</td>
<td>0.200±0.019(^b)</td>
</tr>
<tr>
<td>Aorta (pmol/min/mg protein)</td>
<td>0.122±0.014(^a)</td>
<td>0.082±0.007(^b)</td>
<td>0.094±0.011(^b)</td>
</tr>
</tbody>
</table>

Mean±SEM \((n = 6)\). Values within the same row with different superscripts are significantly different from each other \((p < 0.05)\).

Antihypertensive Effects of Onion

at the end of the 4-week growing period. The blood pressure in rats of the LN-ON group increased more slowly and grew to about 160 mmHg at the end of 4 weeks. The significant antihypertensive effect of onion was observed from 1 to 4 weeks.

The TBARS in plasma was significantly higher in rats of the LN group than the Control group (Fig. 2). The value was decreased to the level of the Control group by feeding the onion diet to the LN-ON group.

The NO excreted in urine was significantly lower in rats of the LN group compared to the Control group (Fig. 3). In the LN-ON group, NO in urine was not significantly different from that in the Control group.

Effects of onion on SHRSP

As Table 2 shows, the growth and food intake in SHRSP of the ON group for the 4-week’s duration of the experiment were not different from those of Control group.

Average systolic blood pressure of the two groups at the start of experiment was about 200 mmHg. The blood pressure increased gradually in the Control group, and rose to about 235 mmHg at the end of the growing period (Fig. 4). The blood pressure of the ON group increased much more slowly, attaining about 215 mmHg after 4 weeks, and the significant antihypertensive effect of onion was observed from 1 to 4 weeks.

The TBARS in plasma was significantly lower in the ON group than that of the Control group (Fig. 5). The NOS activity in kidney and aorta was higher in the ON group than that of the Control group, although the difference was not significant for the aorta (Fig. 6).

The NO excreted in urine was significantly higher in the ON group than the Control group (Fig. 7).

Discussion

The effects of onion on blood pressure have not been well established in previous literature. Kiviranta
Table 2. Effects of Dietary Onion on Growth and Food Intake in SHRSP

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>ON</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight gain (g/day)</td>
<td>1.08±0.17</td>
<td>0.91±0.07</td>
</tr>
<tr>
<td>Final body weight (g)</td>
<td>280±5</td>
<td>277±10</td>
</tr>
<tr>
<td>Food intake (g/day)</td>
<td>15.0±0.2</td>
<td>14.4±0.2</td>
</tr>
</tbody>
</table>

Mean±SEM (n=6).

Fig. 3. Effects of Dietary Onion on the NO Excretion in Urine in L-NAME Induced Hypertensive Rats.

The NO excreted in urine was expressed as total nitrate and nitrite per creatinine in urine. Values with different superscript letters are significantly different from each other (p<0.05).

Fig. 4. Effects of Dietary Onion on Systolic Blood Pressure in SHRSP.

○, Control group; ●, ON group. *p<0.05 vs. Control group at the same time.

Fig. 5. Effects of Dietary Onion on the TBARS in Plasma in SHRSP.

* p<0.05 vs. Control group.

et al. failed to show any antihypertensive effects of an ethanolic extract of onion on SHR by the oral administration for up to 7 weeks. In this experiment, we showed distinct antihypertensive effects of onion on L-NAME induced-hypertensive rats and SHRSP by using 5% dried onion in the diets. The reason of the difference of this result with the result of Kiviranta et al. is not clear. The possible difference in the chemical composition and dose levels between an ethanolic extract of onion and whole onion may be the reason for the different results for antihypertensive effect.

Several recent studies have provided compelling evidence for the generation of increased levels of reactive oxygen species (ROS) in the vascular tissues of SHR. The role of oxidative stress on the genesis and maintenance of hypertension has been supported by amelioration of hypertension in SHR by the administration of many kinds of antioxidants. In this experiment, onion was antioxidative for both L-NAME induced-hypertensive rats and SHRSP, suggesting that the antioxidative components in onion are responsible for the antihypertensive activity.

There are many components in onion known as potent antioxidants, and one of them is quercetin. The antioxidative activity of quercetin has been shown in rats fed a polyunsaturated fat diet. Manach et al. detected an antioxidative activity of quercetin recovered from human plasma.
also contains antioxidative sulfur compounds. Both onion and garlic contain volatile sulfur compounds, and have long been used as flavoring agents. S-allylcysteine sulfoxide (alliin), a major sulfur component in garlic, is known to inhibit lipid peroxidation.23–25) The sulfur compounds in onion are alkyl-compounds such as S-propenylcysteine sulfoxide (major component), S-propylcysteine sulfoxide, and S-methylcysteine sulfoxide.21,22) Allyl-compounds in onion were detected in a trace quantity by Calvey et al.38) Sulfur rich onion oil was found to be effective antioxidative in rats administered nicotine.39) For the effect of in vivo oxidation on hypertension, superoxide and other ROS might contribute to the generation and maintenance of hypertension in SHR by several mechanisms, including inactivation of endothelium-derived NO. ROS have been shown to react quickly with NO, and its consequent inactivation of NO is responsible for the blockade of acetylcholine-mediated vascular relaxation.40–42) ROS may also contribute to the depletion of the NOS cofactor tetrahydrobiopterin. Recently, it has been reported that the absence of tetrahydrobiopterin leads to an uncoupling of the L-arginine-NO pathway and then causes superoxide formation instead of NO formation.43) Supplementation with tetrahydrobiopterin suppressed the development of hypertension in SHR.44) These results suggest that antioxidants might protect the depletion of tetrahydrobiopterin and then increase NOS activity.

The possible effects of onion components other than quercetin and sulfur compounds on the antihypertensive activity of onion are considerable. Ingested nitrate/nitrite from onion in the diet may be effective physiologically, and may play a part in the increase in urinary nitrate/nitrite. Also, arginine from onion in the diet may increase NO. Much research has shown that intravenous or oral administration of arginine increases NO synthesis in animal and human experiments.45,46) Additional studies must be done to understand the roles of these components on antihypertensive activity of onion.

References


38) Calvey, E. M., Matusik, J. E., White, L. D.,
Antihypertensive Effects of Onion


