1 INTRODUCTION

Today we know that n-3 fatty acids are essential for normal growth and development and have many beneficial effects. Many epidemiological and animal studies have suggested that dietary \( \alpha \)-linolenic acid (ALA) prevents and improves hypertension\(^1\)\(^-\)\(^9\). In a recent epidemiological study, Djoussé\(^ et\)\(^ al.\)\(^9\) reported that dietary ALA is associated with a lower prevalence of hypertension and lower systolic blood pressure in Caucasian subjects.

It is known that blood pressure is regulated by the vasocostrictors and vasodilators. Rupp\(^ et\)\(^ al.\)\(^10\) reported that ALA supplementation increased prostaglandin I\(_2\) (PGI\(_2\)) metabolite, nitric oxide metabolites, and bradykinin, in the ALA group were significantly higher than those in the control group, but levels of vasoconstrictors, such as angiotensin II and thromboxane A\(_2\) metabolite, did not differ significantly. It is known that bradykinin induces prostaglandin I\(_2\) and nitric oxide. The present study shows that ALA reduced the systolic blood pressure of SHR, and its mechanism may be related to increases of prostaglandin I\(_2\) and nitric oxide through bradykinin stimulation.

Key words: \( \alpha \)-linolenic acid, flaxseed oil, hypertension, spontaneously hypertensive rats

2 EXPERIMENTAL

2.1 Animals and diets

All animals were treated in accordance with the guidelines for the care and use of laboratory animals (Notification of the Prime Minister’s Office in Japan). The experimental plan was approved by the Laboratory Animal Care Committee of the Research Laboratory, The Nisshin Oillio Group, Ltd.

* Correspondence to: Seiji Sekine, The Nisshin Oillio Group, Ltd., 1 Shinmei-cho, Yokosuka, Kanagawa 239-0832, JAPAN
E-mail: se-sekine@nisshin-oillio.com
Accepted April 17, 2007 (received for review April 13, 2007)
Journal of Oleo Science ISSN 1345-8957 print / ISSN 1347-3352 online
http://jos.jstage.jst.go.jp/en/
Seven-week-old male SHR of the Izumo colony (SHR/Izm, Japan SLC, Inc., Hamamatsu, Shizuoka, Japan) were individually housed in stainless steel wire cages and allowed free access to sterilized water. The temperature of the animal room was set at 23 ± 1°C, with a humidity of 50 ± 5% and illumination from 08:00 to 20:00 h. The animals were also allowed free access to a commercial stock diet (Picolab Rodent Diet 20, PMI Nutrition International, Richmond, IN, U.S.A.).

The rats, weighing 150-170 g, were randomized into two groups (n = 8). The rats were administrated orally 1 mL (density is 0.92 g/mL) of high oleic safflower oil (HOSO, The Nisshin Oillio Group, Ltd, Tokyo, Japan) or FSO (The Nisshin Oillio Group, Ltd, Tokyo, Japan) on day 1 and day 5. The fatty acid composition of the test lipids is shown in Table 1. HOSO and FSO were administered to the control and ALA groups, respectively. On day 1, the systolic blood pressure was measured 4 hours after oral administration. Four hours after oral administration on day 5, the blood was collected from the inferior vena cava. EDTA-2Na was used as an anticoagulant, and plasma was separated by centrifugation at 2,700 × g for 15 min at 4°C. All the animals were killed by cardiac puncture.

2.2 Measurement of systolic blood pressure
Systolic blood pressure was measured using a non-pre-heating, non-invasive blood pressure monitor for mice and rats (MK-2000, Muromachi Kikai Co., Ltd, Tokyo, Japan).

2.3 Analysis of plasma vasodilators and vasoconstrictors
Plasma 6-keto-prostaglandin F1α (6-keto-PGF1α) and thromboxane B2 levels were analyzed by means of enzyme immunoassay kits (Cayman Chemicals Co, Ann Arbor, MI, U.S.A.). 6-keto-PGF1α and thromboxane B2 are stable metabolites of PGI2 (also called prostacyclin) and thromboxane A2, respectively. Plasma BK level was analyzed by means of an enzyme immunoassay kit (Peninsula Laboratories Inc., San Carlos, CA, U.S.A.). Plasma angiotensin II level was measured using a radioimmunoassay kit (SRL Inc., Tokyo, Japan). Plasma NO metabolite (NO2 and NO3) level was analyzed by means of a colorimetric assay kit: NO2/NO3 Assay kit-C II (Dojindo Laboratories, Kumamoto, Japan). NO2 and NO3 are stable metabolites of NO after deproteinization of plasma by filtration using Centricon® (Millipore Co., Billerica, MA, U.S.A.).

2.4 Fatty acid composition of total plasma lipids and test lipids
Total liver lipids were extracted using the method described by Bligh and Dyer[13]. Total liver lipids and test lipids were saponified in 0.5 mol/L sodium hydroxide in methanol for 7 min at 100°C to liberate free fatty acids, and cooled on ice. After saponification, 2 mL of 14% boron trifluoride in methanol was added to a fatty acid sodium salts solution, and methylated for 5 min at 100°C. Fatty acid methyl esters were extracted using n-hexane. Fatty acid derivatives were analyzed by gas-liquid chromatography using a GC-2014 (Shimadzu Corporation, Kyoto, Japan) equipped with a capillary column (MEGAWAX™ 250, 30 m-long, 0.25 mm internal diameter, 0.25 μm thickness, Supelec, Inc., Bellefonte, PA, U.S.A.).

2.5 Statistical analysis
The results were analyzed for significant difference using Student’s t-test. Differences with a value of p < 0.05 were considered significant. A statistical software package (SPSS 13.0J, SPSS Japan Inc., Tokyo, Japan) was used for all of the statistical analyses.

3 RESULTS
Body weight did not differ significantly between the control and ALA groups (data not shown). Four hours after oral administration, systolic blood pressure in the ALA group was significantly lower than that in the control group (Fig. 1). In the plasma, 6-keto-PGF1α level in the ALA

---

Table 1 Major Fatty Acid Composition of the Test Lipids.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>ALA</th>
</tr>
</thead>
<tbody>
<tr>
<td>16:0</td>
<td>4.7</td>
<td>5.0</td>
</tr>
<tr>
<td>18:0</td>
<td>1.9</td>
<td>3.2</td>
</tr>
<tr>
<td>18:1(n-9)</td>
<td>77.1</td>
<td>18.1</td>
</tr>
<tr>
<td>18:2(n-6)</td>
<td>14.4</td>
<td>16.5</td>
</tr>
<tr>
<td>18:3(n-3)</td>
<td>0.2</td>
<td>49.3</td>
</tr>
</tbody>
</table>

*ALA, α-linolenic acid.
Effect of α-Linolenic Acid on Systolic Blood Pressure in Rats

The systolic blood pressure in the ALA group was significantly higher than that in the control group (*p < 0.05). Thromboxane B2 level did not differ significantly (*p > 0.05). BK level in the ALA group was significantly higher than that in the control group (*p < 0.05). Regarding angiotensin II level, no significant difference was seen between the control and ALA groups (*p > 0.05). NO metabolites (NO2 and NO3) level in the ALA group was significantly higher than that in the control group (*p < 0.05).

Table 2 shows the fatty acid composition of the total liver lipids in the SHR/Izm. The proportions of oleic acid and arachidonic acid in the ALA group were significantly lower than those in the control group. The proportions of ALA and eicosapentaenoic acid (EPA) in the ALA group were significantly higher than those in the control group.

**Fig. 2** Plasma 6-Keto-Prostaglandin F1α (A) and Thromboxane B2 (B) Concentrations in SHR/Izm. Values are mean ± SD, n = 8. *Mean values are significantly different from those of the control group (p < 0.05).

**Fig. 3** Plasma Bradykinin (A) and Angiotensin II (B) Concentrations in SHR/Izm. Values are mean ± SD, n = 8. *Mean values are significantly different from those of the control group (p < 0.05).

**Fig. 4** Plasma NO Metabolites (NO2 and NO3) Concentration in SHR/Izm. Values are mean ± SD, n = 8. *Mean values are significantly different from those of the control group (p < 0.05).
Table 2  Major Fatty Acid Composition in the Total Liver Lipids of SHR/Izm.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>ALA*</th>
</tr>
</thead>
<tbody>
<tr>
<td>16:0</td>
<td>18.0 ± 0.8</td>
<td>17.7 ± 1.7</td>
</tr>
<tr>
<td>18:0</td>
<td>18.2 ± 1.0</td>
<td>17.6 ± 1.3</td>
</tr>
<tr>
<td>18:1(n-9)</td>
<td>14.0 ± 1.5</td>
<td>8.6 ± 0.6*</td>
</tr>
<tr>
<td>18:2(n-6)</td>
<td>17.8 ± 0.9</td>
<td>18.6 ± 0.9</td>
</tr>
<tr>
<td>18:3(n-3)</td>
<td>0.1 ± 0.1</td>
<td>4.1 ± 2.3*</td>
</tr>
<tr>
<td>20:4(n-6)</td>
<td>18.3 ± 0.8</td>
<td>17.5 ± 0.9*</td>
</tr>
<tr>
<td>20:5(n-3)</td>
<td>0.3 ± 0.4</td>
<td>1.4 ± 0.6*</td>
</tr>
<tr>
<td>22:6(n-3)</td>
<td>7.5 ± 0.5</td>
<td>7.3 ± 0.3</td>
</tr>
</tbody>
</table>

*ALA, α-linolenic acid.
Value are mean ± SD, n = 8.
* Mean values are significantly different from those of the control group (p < 0.05).

4 DISCUSSION

Four hours after oral administration, systolic blood pressure in the FSO group was significantly lower than that in the HOSO group (Fig. 1). This result is similar to those reported in previous studies which used rats fed ALA-rich FSO. In the present study, we showed that the oral administration of ALA reduced systolic blood pressure.

Rupp et al. reported that vascular PGI2 formation was increased by dietary ALA intake in SHR. However, dietary lipid levels in their study differed from those in ours because Rupp et al. added different levels of FSO to the diets. Therefore, because PGI2 is synthesized from n-6 series fatty acid, the increase of PGI2 may be caused by the increase of n-6 series linoleic acid from supplementary FSO. This study was performed in such a way that the rats were administered the uniform lipid levels and the same n-6 fatty acid proportion. The present study showed that the plasma 6-keto-PGF1α level in the ALA group was significantly higher than that in the control group (Fig. 2A).

BK and NO were also elevated by ALA administration (Fig. 3A). BK has blood pressure-lowering effects that can be induced by secretion of secondary agents. Yamasaki et al. reported that BK stimulation increased cytosolic phospholipase A2 mRNA and prostaglandin H synthase (same as COX-1 and 2 mRNAs in human vascular endothelial cells. PGI2 is synthesized in response to the release of arachidonic acid from phospholipids followed by the transformation of arachidonic acid to PGH2 by PGH synthase and the subsequent transformation of PGH2 by PGI2 synthase. Therefore, PGI2 might be increased through the increase of BK with oral ALA administration. Gryglewski et al. reported that BK increases Ca2+ with the subsequent activation of constitutive COX-1 and NOS-3. The increase of NO may be also have been caused by the increase of BK when ALA was administered orally.

Thromboxane B2 (thromboxane A2 metabolite) and angiotensin II were not changed by the ALA administration (Figs. 2B and 3B). Therefore, vasoconstrictors may not play a role in the improvement of hypertension by ALA administration. Angiotensin II conversion and BK degradation were catalyzed by ACE (same as kininase II). Because angiotensin II did not change by following ALA administration, ACE activity might not be changed. The increase of BK may be affected by the increase of BK generation and/or a decrease of BK degradation through endopeptidase and others, but its mechanisms remain unclear.

On day 5, EPA proportion of liver was significantly higher in the ALA group. ALA can be rapidly converted to EPA. Fish oil containing EPA also exert an antihypertensive effect. Knapp and FitzGerald reported that a high dose of fish oil initially increased the formation of vasodilatory prostaglandins (PGI2 and I3). Therefore, in the present study, EPA converted from ALA may be partly associated with the lowering of systolic blood pressure and the increase of PGI2 with oral ALA administration.

5 CONCLUSION

The present study showed a lowering of blood pressure and increases of the levels of vasodilators, such as BK, PGI2, and NO, in SHR/Izm with following oral ALA administration. When ALA was administered orally, one of the antihypertensive mechanisms may be related to increases of PGI2 and NO through BK stimulation.

ACKNOWLEDGEMENTS

We would like to thank Mrs. Yumiko Ishikawa for her conscientious assistance with animal care.

References

Effect of α-Linolenic Acid on Systolic Blood Pressure in Rats


