Dietary intake of rapeseed oil as the sole fat nutrient in wistar rats — Lack of increase in plasma lipids and renal lesions —

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(Received August 7, 2008; Accepted August 21, 2008)

ABSTRACT — Dietary rapeseed (canola) oil (CO) given as the only fat nutrient shortens life in stroke-prone spontaneously hypertensive rats (SHRSP), compared with SHRSP given soybean oil (SO) instead of CO. CO ingestion increases plasma lipids and causes renal lesions in SHRSP and in spontaneously hypertensive rats (SHR), and increases plasma lipids also in Wistar Kyoto (WKY) rats, a normotensive counterpart of SHR. This study examined whether or not such unfavorable effects of CO are restricted to these closely related strains. For this purpose Wistar rats, the strain from which these strains were derived, were fed a diet containing 10% CO or SO as the sole fat nutrient for 10 weeks, and changes in clinical signs, urinalysis, blood biochemistry and pathology were compared. CO ingestion did not induce any abnormalities in Wistar rats, except significant increases in plasma concentrations of aldosterone and Na+, compared with the SO group. Thus, the unfavorable effects of CO ingestion appear to be restricted to SHRSP and its closely related strains. The role of increased aldosterone and Na+ in the unfavorable events caused by CO in SHRSP, SHR and WKY rats, and any factors which could induce such increases in aldosterone and Na+, remain to be elucidated.

Key words: CO, Wistar rat, Lack of adverse effects, Aldosterone

INTRODUCTION

Low erucic acid rapeseed (canola) oil (CO) has been reported to shorten survival times in stroke-prone spontaneously hypertensive rats (SHRSP), compared with SHRSP given soybean oil (SO) instead of CO. CO ingestion increases plasma lipids and causes renal lesions in SHRSP and in spontaneously hypertensive rats (SHR), and increases plasma lipids also in Wistar Kyoto (WKY) rats, a normotensive counterpart of SHR (Huang et al., 1996, 1997; Miyazaki et al., 1998). Previous studies suggested that CO ingestion shortens the life of SHRSP via an acceleration of hypertension-related conditions, including elevation of blood pressure and tissue lesions in the kidney and heart (Naito et al., 2000a, 2000b). However, such unfavorable effects have been found exclusively in SHRSP and its closely related strains, and there have been no reports on CO-induced effects using rats of other strains. Therefore, the present study examined whether or not such unfavorable effects of CO are restricted to SHRSP and its related strains. For this purpose Wistar rats, the strain from which WKY rats, SHR and SHRSP were derived, were fed a diet containing 10% CO as the sole fat nutrient for 10 weeks, and the resulting changes in clinical signs were compared with those in control rats fed SO instead of CO.

MATERIALS AND METHODS

Animals and breeding

Sixteen male Wistar rats, 4 weeks old, were purchased...
from Charles River Japan Inc. (Tsukuba, Japan), and acclimatized for 1 week. The animals were divided into 2 groups of 8 animals each and assigned to the CO group and SO group. The CO group was fed with a fat-free AIN-93 diet (Oriental Yeast, Tokyo, Japan) supplemented with 10 w/w% CO (Japan Oilseed Processors Association, Tokyo, Japan). The SO group was fed with the same diet, but containing 10 w/w% SO (Japan Oilseed Processors Association) instead of CO. The SO group was regarded as the control group, because the major component of fat in commercially available regular rat chow is SO. The fatty acid compositions of these diets are shown in Table 1. The animals were allowed free access to the diet and drinking water (tap water).

All the animals were used following the instructions by the Committee for Ethical Usage of Experimental Animals in Hatano Research Institute.

The 10-week feeding period was adopted based on the results of a previous study in which SHRSP were fed the same diets as in the present study, but for only 8 weeks. CO ingestion induced renal lesions and a tendency towards increased plasma lipids and decreased platelet counts (Ohara et al., 2006). In SHRSP, an 8-week period was thought to be a limit, as it would have been difficult to obtain and evaluate results due to their irregular deterioration in conditions and death due to stroke. On the other hand, elevated blood pressure, increased plasma lipids and decreased platelet count were also observed in WKY rats given a 10 w/w% CO diet for 13 weeks (Naito et al., 2000b). Therefore, in Wistar rats, any such changes induced by CO ingestion were expected to occur within a 10-week period.

In this study, the single concentration of 10 w/w% (24.8 energy percentage) was adopted because the purpose of the study was not to detect dose-dependent changes due to CO ingestion, but rather, merely to confirm whether or not the increase in plasma lipids and renal lesions found in SHRSP, SHR and WKY rats given 10 w/w% CO (Naito et al., 2000a, 2000b, 2003; Ohara et al., 2006) would also be found in Wistar rats.

**Gross observation and urinalysis**

Throughout the 10-week feeding period, the animals were examined for general condition every day, and weighed on the day before feeding the CO diet or SO diet and once a week thereafter. Twenty-four hr food consumption was measured every week. In the 10th week before scheduled autopsy, the animals were placed in metabolic cages for 24 hr to become acclimatized, and then urine was collected for 24 hr under fasting conditions. Urinary volume and urinary concentrations of electrolytes (Na+, K+ and Cl-) and creatinine were measured by an automatic electrolyte analyzer (EA05, A&T, Tokyo, Japan) and an autoanalyser (COBAS-FARA®, Roche, Tokyo, Japan), respectively.

**Hematology and blood chemistry**

At the end of the 10-week feeding, the animals were anesthetized with sodium pentobarbital (50 mg/kg, i.p.), and blood was taken from the inferior caval vein using ethylene diamine tetra-acetate as an anticoagulant. Hematocrit, red blood cell count, hemoglobin concentration, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, white blood cell count, platelet count and white blood cell differentials were determined with an automated hematology analyzer (Cell-Dyne® 3500SL Dainabot Japan Co., Ltd., Tokyo, Japan). Additionally, heparinized blood was taken, and the following items were measured with a COBAS-FARA® autoanalyser; concentrations of total cholesterol, free cholesterol, triglyceride, phospholipids, non-esterified fatty acids, glucose, blood urea nitrogen, creatinine, Na+, K+ and Cl−, and alkaline phosphatase activity. Plasma aldosterone concentration was determined by an aldosterone EIA kit (Cayman, Tokyo, Japan).

**Pathological examination**

Following the blood sampling, the animals were killed by exsanguination and autopsied. The brain, heart, lung,
liver, spleen, kidneys, adrenal glands, testes, epididymides and prostate were removed and examined macroscopically. These organs were weighed, immersed in 10% formalin buffer, and the fixed tissues were embedded in paraffin. Thereafter, the paraffin-embedded specimens were sectioned, stained with hematoxylin and eosin, and histologically examined under a microscope.

**Statistical methods**

Differences in the group mean values were evaluated by Student’s t-test for unpaired observations, and in the incidences of pathological findings were evaluated using Fisher’s exact test. The differences were considered to be significant when the P value was less than 0.05. A computer application, Prism®4.0b (GraphPad Software, Inc., San Diego, CA, USA) was used for the calculation.

**RESULTS**

**General condition, body weight gain, food consumption and urinalysis**

Throughout the 10-week experimental period, no abnormalities were noted, nor did any deaths occur. There were no differences in body weight gain or food consumption between the CO and SO groups (Fig. 1). The mean daily intake of oil calculated from daily food consumption that was measured once a week throughout the experimental period was 8.0 ± 3.3 – 9.1 ± 3.3 g/kg·day for CO, and 8.2 ± 2.5 – 9.4 ± 2.8 g/kg·day for SO (means ± S.D. in 8 animals for each). Urinary volume, urinary electrolytes and creatinine excretion were comparable in the 2 groups (Table 2).

**Hematology and blood biochemistry**

There were no significant differences in any of the hematology parameters (data not shown) and biochemistry between the CO and SO groups, except plasma aldosterone and Na+ concentrations, both of which were significantly higher in the CO group than in the SO group (Table 2).

**Gross and microscopic pathology**

There were no significant changes in absolute weights and relative weights to body weight of any organ examined. Moreover no abnormal findings were found in any organ in gross or microscopic pathology, except a slight myocardial degeneration in 1 of the 8 animals in the CO group. The incidence of this finding in the CO group compared with the SO group was not statistically significant.

**DISCUSSION**

It has been reported that survival time in SHRSP is shortened by dietary intake of CO as a 10 w/w% supplement to standard rat chow (Huang et al., 1996, 1997; Miyazaki et al., 1998), compared with control animals given a diet with the same amount of SO, instead of CO. In previous studies, tissue injuries in the kidney were specific to rats given the CO diet, and were found in SHRSP after ingestion of the CO diet for 8 weeks (Ohara et al., 2006). Also, in SHR and in its normotensive counterpart, WKY rats, both of which are genetically close to SHRSP, a 26-week ingestion of a diet containing 10% CO increased plasma lipids and decreased platelet counts, compared to control groups given an SO diet (Naito et al.,

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**Fig. 1.** Body weight gain and food consumption in male Wistar rats ingesting a diet containing 10 w/w% soybean oil or 10 w/w% canola oil. Symbols with bars represent means ± S.D. of 8 animals.
Moreover, with the 10% CO diet, an increased incidence of tissue lesions was found in the heart and kidney of both SHR and WKY rats although those injuries were less severe in WKY rats than in SHR (Naito et al., 2000a). Similar tissue injuries were also found in WKY rats fed the CO diet for an even shorter period, i.e., 13 weeks (Naito et al., 2000b). Although these unfavorable outcomes are a matter of concern, the CO-induced events have been reported exclusively in SHRSP (Huang et al., 1996, 1997; Miyazaki et al., 1998; Naito et al., 2003), SHR and WKY rats (Naito et al., 2000a, 2000b; Ohara et al., 2008). The increased plasma aldosterone and Na⁺ after CO ingestion noted in the present study indicates the possibility that CO activates the renin angiotensin aldosterone system (RAAS).

It was found in a recent study that the CO diet, comparing with the SO diet, brought about significant vascular lesions in the kidney in SHR, in which abundant cyclo-oxigenase-2 (COX-2) positive foci were immunohistochemically located in the juxtaglomerular apparatus (Ohara et al., 2008). COX-2 is constitutively expressed in the juxtaglomerular apparatus and plays a role in the homeostasis of renal physiology via production of prostanooids (Harris et al., 1998). However, excessive expression of COX-2 may stimulate RAAS (Harris et al., 2004; Paliege et al., 2004) and increase arterial pressure and promote peripheral vascular lesions in inflammatory diseases, including hypertension and diabetes (Zhao et al., 2006; Fiebeler et al., 2007). Thus, the renal tissue lesions

Table 2. Blood chemistry and urinalysis in male Wistar rats ingesting a diet containing 10 w/w% soybean oil or 10 w/w% canola oil

<table>
<thead>
<tr>
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<th>SO diet</th>
<th>CO diet</th>
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<tr>
<td><strong>Plasma</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>48.9 ± 6.6</td>
<td>48.1 ± 4.8</td>
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<tr>
<td>Free cholesterol (mg/dl)</td>
<td>9.08 ± 1.5</td>
<td>8.60 ± 1.1</td>
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<tr>
<td>Triglycerides (mg/dl)</td>
<td>28.0 ± 24</td>
<td>32.0 ± 16</td>
</tr>
<tr>
<td>Phospholipids (mg/dl)</td>
<td>81.1 ± 13</td>
<td>82.4 ± 9.7</td>
</tr>
<tr>
<td>Non-esterified fatty acid (mg/dl)</td>
<td>0.609 ± 0.13</td>
<td>0.616 ± 0.11</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>164 ± 14</td>
<td>156 ± 11</td>
</tr>
<tr>
<td>Alkaline phosphatase (U/l)</td>
<td>206 ± 19</td>
<td>221 ± 14</td>
</tr>
<tr>
<td>Blood urine nitrogen (mg/dl)</td>
<td>16.8 ± 1.5</td>
<td>17.4 ± 2.1</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.725 ± 0.10</td>
<td>0.688 ± 0.12</td>
</tr>
<tr>
<td>Aldosterone (pg/ml)</td>
<td>897 ± 24</td>
<td>1280 ± 250*</td>
</tr>
<tr>
<td>Plasma Na⁺ (mEq/l)</td>
<td>142 ± 1.0</td>
<td>143 ± 0.69*</td>
</tr>
<tr>
<td>Plasma K⁺ (mEq/l)</td>
<td>5.47 ± 0.87</td>
<td>5.12 ± 0.73</td>
</tr>
<tr>
<td>PlasmaCl⁻ (mEq/l)</td>
<td>105 ± 1.7</td>
<td>106 ± 1.4</td>
</tr>
<tr>
<td><strong>Urinary volume (ml/day)</strong></td>
<td>10.4 ± 3.4</td>
<td>10.3 ± 4.3</td>
</tr>
<tr>
<td>Na⁺ (mEq/kg·day)</td>
<td>0.987 ± 0.26</td>
<td>0.963 ± 0.26</td>
</tr>
<tr>
<td>K⁺ (mEq/kg·day)</td>
<td>3.43 ± 0.35</td>
<td>3.52 ± 0.53</td>
</tr>
<tr>
<td>Cl⁻ (mEq/kg·day)</td>
<td>1.15 ± 0.25</td>
<td>1.19 ± 0.21</td>
</tr>
<tr>
<td>Creatinine (mg/kg·day)</td>
<td>32.5 ± 3.0</td>
<td>32.5 ± 3.0</td>
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Values are means ± S.D. of 8 animals.
*p < 0.05 and **p < 0.01, significantly different from soybean oil group.
SO diet, CO diet; a fat-free AIN-93 diet supplemented with 10 w/w% soybean oil or canola oil.
accompanied by elevated blood pressure previously found in SHRSP (Naito et al., 2003), SHR and WKY rats (Naito et al., 2000a) fed the CO diet, might have occurred via CO-induced RAAS activation. In the present study in Wistar rats, CO ingestion did not cause any tissue injury in the kidney, but did elevate plasma levels of aldosterone and Na\(^+\) as compared with SO ingestion. The mechanisms underlying the CO ingestion-induced changes are unclear. Thus, if CO activates RAAS in rats of any strain, Wistar rats could be tolerant, but SHRSP and its related strains, SHR and WKY rats, could be susceptible to producing tissue injuries caused by the activated RAAS.

In summary, Wistar rats, the strain from which SHRSP, SHR and WKY rats are derived, fed a diet containing 10% CO as the sole fat nutrient for 10 weeks failed to present any of the unfavorable findings that had been found previously in SHRSP, SHR and WKY rats fed a similar CO diet for 8 weeks or longer. Concomitant increases in plasma levels of aldosterone and Na\(^+\) might be due to some factors in CO. It is possible that similar increases in plasma aldosterone and Na\(^+\) might also occur in SHR and its related strains and be involved in the unfavorable events caused by CO in these strains. Whether or not CO induces the undesirable effects appears to depend on the genetic background of restricted strains of rats. A longer lasting, multiple concentration CO-feeding study in rats of strains other than SHRSP and its related strains would be required to determine a safety margin for CO ingestion in the rat. Besides, CO has also been reported to have additional, unusual effects, for instance, decreased litter sizes and retarded growth in suckling pups of SHRSP dams given a 10% CO supplemented diet (Tatematsu et al., 2004), thrcenic changes in behavior in mice caused by a 10% CO supplemented diet (Kameyama et al., 1996), and hepatic hemorrhage caused by rapeseed meal in laying hens (Minetoma et al., 1975). Thus, to obtain a broad prospective on the safety of CO ingestion, surveying other effects than those considered in the present study would be necessary. Moreover, such a survey would need to consider the effects of other dietary vegetable oils and the results in species other than rodents.

REFERENCES

Scand., 181, 543-547.