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Similar changes in clinical and pathological parameters in Wistar Kyoto rats after a 13-week dietary intake of canola oil or a fatty acid composition-based interesterified canola oil mimic

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ABSTRACT

Canola oil (CO) given as a dietary fat deteriorates hypertension-related condition and shortens the life of stroke-prone spontaneously hypertensive rats (SHRSP). Although substances other than fatty acids have been presumed as causatives, CO mimics consisting of oils other than CO also shorten the life. In this study we intended to examine whether or not fatty acid composition unique to CO participates in the adverse effect. CO or an interesterified CO mimic (ICOM) consisting of safflower oil, flaxseed oil and erucic acid was fed as a dietary fat for 13 weeks to Wistar Kyoto (WKY) rats, and clinical and pathological signs were compared. WKY rats were used to avoid the difficulty in evaluating the results in SHRSP due to irregular deterioration in conditions by stroke. Compared to a standard diet, both diets containing CO or ICOM similarly elevated blood pressure, increased plasma lipids, activated hepatic glucose-6-phosphate dehydrogenase, decreased platelets, shortened blood coagulation times and induced abnormalities in the kidney. Thus, CO-specific fatty acid composition appeared to affect the pathophysiology of the rat and produce consequent aggravation of pathological status, especially in SHRSP. However, the existence of causative factors other than fatty acids was suggested by increased neutrophil count exclusively induced by CO.

1. Introduction

Huang et al. (1996, 1997) reported that in stroke-prone spontaneously hypertensive rats (SHRSP), certain vegetable oils, including low erucic acid and low glucosinolate rapeseed (canola) oil (CO), shortened the survival time, compared with SHRSP given soybean oil (SO), when one of those oils was added at a rate of 10 wt/wt% to a standard rat chow. These authors also examined the effects of butyl, phenethyl and allyl isothiocyanate at concentrations comparable to those found in CO on the survival time of SHRSP, since Minetoma et al. (1975) reported that laying hens given rapeseed meal revealed thyroid hypertrophy and the amounts of isothiocyanate and oxazolidinethione, catabolic products of glucosinolate in the meal were proportional to the thyrotoxicity. However, the sulfur-containing compounds did not affect survival time. On the other hand, Miyazaki et al. (1998) found that the fatty acid fraction obtained by lipase-treatment of CO did not shorten the life of SHRSP, and presumed that the life-shortening was due to contents other than fatty acids. Thereafter, in our laboratory, CO has been extensively examined for adverse effects in the rat to find a clue for identifying the causatives (Naito et al., 2000a,b, 2003; Ohara et al., 2006, 2008a,b). However, so far the causatives have not yet been determined.

The volume of production of CO is the 3rd largest among the world oil production (http://www.soystat.com/2008/page:35.htm) and is the largest among the oils which have been reported to cause the life-shortening. The reason why we have exclusively studied on CO is that unbiased assessments must be performed to accumulate information about the effects of the widely used

Abbreviations: APTT, activated partial thromboplastin time; CO, canola oil; Cat, catalase; G6PD, glucose-6-phosphate dehydrogenase; GR, glutathione reductase; GPX, glutathione peroxidase; GST, glutathione-*S*-transferase; ICOM, interesterified canola oil mimic; LPO, lipid peroxide; NADPH, nicotinamide adenine dinucleotide phosphate; PT, prothrombin time; SO, soybean oil; SHR, spontaneously hypertensive rats; SHRSP, stroke-prone spontaneously hypertensive rats; SOD, superoxide dismutase; WKY rats, Wistar Kyoto rats.

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CO to reduce concern or find a beneficial intake for it. The results of clinical and pathological investigations demonstrated that the dietary CO deteriorates hypertension-related condition which would produce consequent aggravation of pathological status, especially in SHRSP (Naito et al., 2003; Ohara et al., 2006).

Although not only CO but also several oils including CO mimics consist of oils other than CO have been reported to shorten the life, no reports have yet been published on the effects on these oils on the clinical parameters and pathology of the rats, except for CO (Naito et al., 2000a,b, 2003; Ohara et al., 2006). The aim of this study was to confirm whether or not the fatty acid composition unique to CO has relevance to the changes in clinical and pathological signs found in rats given CO (Naito et al., 2000a,b, 2003; Ohara et al., 2006). For the purpose, Wistar Kyoto (WKY) rats were fed with a diet containing CO or an interesterified CO mimic (ICOM), at 10 wt/wt% as the sole dietary fat, for 13 weeks. Then clinical and pathological signs in the animals given CO or ICOM were compared. In the present study, WKY rats, the normotensive genetic counterpart of spontaneously hypertensive rats (SHR), and the line from which SHRSP stemmed, were used instead of SHRSP or SHR, since it would have been difficult to evaluate the results in SHRSP and SHR due to their spontaneously increasing blood pressure and irregular deterioration in conditions and their death due to stroke.

2. Materials and methods

2.1. Animal husbandry

Thirty male WKY rats, WKY/Izm, the direct descendant strain of Wistar Kyoto rat originated from Kyoto University (Disease Model Cooperative Research Association, Kyoto, Japan), 5 weeks old, were purchased from Japan SLC, Inc. (Hamamatsu, Japan), kept individually in metal hanging cages and acclimatized for one week. A standard pellet diet, CE-2 (CLEA Japan, Inc., Tokyo, Japan) was given to all the animals during the acclimatization. Male rats were used because all the previous studies on CO-induced life-shortening in SHRSP and on clinical and pathological changes by CO ingestion in SHRSP, SHR, WKY rats and Wistar rats were carried out using male animals. The animals were divided into 3 groups of 10 animals each and fed, respectively, with a fat-free dry powdered diet of AIN-93 ingredients (Oriental Yeast, Tokyo, Japan) supplemented with 10 wt/wt% CO (obtained from a low erucic acid and low glucosinolate variant of rapeseed, Japan Oilseed Processors Association, Tokyo, Japan), prepared without heating; the same diet, but supplemented with 10 wt/wt% ICOM (Japan Oilseed Processors Association); or a standard pellet diet, CE-2 containing 4.5% SO as the fat nutrient for 13 weeks. The adding rate of 10 wt/wt% for CO and ICOM was used following the rate used in the previous studies on CO-induced life-shortening in SHRSP and on clinical and pathological changes by CO ingestion in SHRSP, SHR, WKY rats and Wistar rats (Huang et al., 1996, 1997; Miyazaki et al., 1998; Naito et al., 2000a,b, 2003; Ohara et al., 2006, 2008a,b). The diets were replaced with new ones every fourth day. On the third day, acid values of CE-2, CO and ICOM dies were 0.22 ± 0.01, 0.20 ± 0.02 and 0.22 ± 0.01(means ± SEs, $n = 3$), and peroxide values were 4.36 ± 0.23, 5.30 ± 0.01 and 5.57 ± 0.12 ($n = 3$), respectively. The animals were allowed free access to the diet and drinking water (tap water). ICOM was prepared by mixing high-oleate safflower oil, high-linoleate safflower oil, flaxseed oil and erucic acid methyl ester, in the ratio of 8.5:74:17.5:0.5 (wt/wt%), respectively, and random esterification was done using sodium methylate as a catalyst followed by washing and dehydration. The random esterification was carried out in order to incorporate erucic acid into triglycerides in the mixture of oils. It has been reported that the metabolism and physiological activities of native triglycerides and randomized triglycerides may be different (Karupaiah and Sundram, 2007). However, no ICOM-specific changes were found in any parameters in the present study.

The ingredients and fatty acid compositions of the diets are shown in Table 1.

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

2.2. Gross observation, urinalysis and measurement of blood pressure

The general condition of the animals was examined everyday for 13 weeks. The animals were weighed on the day before starting the feeding and, thereafter, once a week. Food consumption was also measured once a week.

Systolic blood pressure was measured in 5 animals of each group with a tail-cuff sphygmomanometer (MK-1030, Muromachi, Tokyo, Japan) before starting the feeding and at the 4th, 8th and 12th weeks of the feeding; briefly, the rats were placed in a heating box and warmed to 38 °C for 10 min, and systolic blood pressure at the caudal artery was obtained as an average of three readings for each animal.

Table 1
Ingredients and fatty acid composition of diets.

	Standard diet	Diet containing 10 wt/wt%	
		Canola oil	Interesterified canola oil mimic
Ingredients			
Energy (kcal/100 g)	341	416	
Water (%)	8.9	9.0	
Total protein (%)	25.1	12.5	
Lipids (%)	4.5	10	
Fibre (%)	4.3	3.1	
Minerals (%)	7.1	2.4	
Ca (g/100 g)	1.2	0.50	
K (g/100 g)	1.07	0.36	
Na (g /100 g)	0.33	0.10	
Cl (g/100 g)	0.51	0.16	
Fatty acids (%)			
14:0 Myristic acid	0.4	0	0.1
16:0 Palmitic acid	15.4	4.1	5.4
16:1 Palmitooleic acid	1.3	0.2	0.1
18:0 Stearic acid	1.8	1.9	2.5
18:1 Oleic acid	22.7	60.2	61.3
18:2 Linoleic acid	49.4	20.5	19.9
18:3 Linolenic acid	3.4	9.8	8.7
20:0 Arachidic acid	0	0.6	0.4
20:1 Eicosaenoic acid	1.0	1.5	0.3
22:0 Behenic acid	0.2	0.3	0.3
22:1 Erucic acid	0.4	0.5	0.6
24:0 Lignoceric acid	0	0.2	0.2
24:1 Nervonic acid	0	0.2	0.2

In the 13th week, urine was collected for 24 h, for measuring urinary output and determination of urinary electrolyte (Na^+, K^+ and Cl^-) concentrations with an automatic electrolyte analyzer (EA05, A&T, Tokyo, Japan).

2.3. Hematology and blood chemistry

At the end of the 13-week feeding, the animals were fasted overnight and on the next day in the morning anesthetized with 50 mg/kg (i.v.) of sodium pentobarbital, and an abdominal incision was carried out. Blood was taken from the inferior caval vein, using ethylenediaminetetraacetate as an anticoagulant. Hematocrit, counts of red blood cell, platelet and white blood cell and white blood cell differentials were determined using a Coulter Counter® (S-PLUS IV, Coulter Electronics, Tokyo, Japan). Additionally, blood was also taken, using heparin, and centrifuged. Then, the plasma levels of the following items were measured by a COBAS-FARA® autoanalyser (Roche, Tokyo, Japan): concentrations of total protein, total cholesterol, free cholesterol, triglycerides, phospholipids, non esterified fatty acids, glucose, blood urea nitrogen, albumin, Na^+, K^+, Cl^- and Ca^{++}; the activities of asparate aminotransferase and alanine aminotransferase; and the albumin-globulin ratio (A/G, calculated using values of total protein and albumin). Prothrombin time (PT) and activated partial thromboplastin time (APTT) were measured with a coagulometer (CA-3000, TOA Electronics, Tokyo, Japan).

2.4. Measurement of enzyme activities in hepatic cytosol

In the present study, activities of several enzymes for maintaining the redox status were determined because, in the previous study, CO ingestion decreased activities of catalase (Cat) and superoxide dismutase (SOD), but increased glucose-6-phosphate dehydrogenase (G6PD), in WKY rats (Naito et al., 2000b) and SHR (Ohara et al., 2008a).

After drawing blood samples, the animals were exsanguinated, and the liver was removed. An isolated lobe of the liver was perfused with ice cold 1.15% KCl solution to remove blood, placed in 4 times its volume of the KCl solution, minced, and homogenized on ice with a Potter–Elvehjem homogenizer. The homogenate was centrifuged at 105,000 g for 60 min at 4 °C to obtain the cytosol fraction as the supernatant.

The activities of Cat (EC 1.11.1.6), glutathione peroxidase (GPX, EC 1.11.1.9) and glutathione-*S*-transferase (GST, EC 2.5.1.13), glutathione reductase (GR, EC 1.6.4.2), and G6PD (EC 1.1.1.49) were determined based on the methods of Aebi (1983), Flohe and Gunzler (1984), Goldberg and Spooner (1983) and Deutsch (1978), respectively. The activity of SOD (EC1.15.1.1) was measured by the method of (McCord and Fridovich, 1969). These were determined by automated assay methods by Wheeler et al. (1990) using a COBAS-FARA® autoanalyser. Lipid peroxide (LPO) was determined as malondialdehyde using thiobarbituric (Tien and Aust,