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Different effects of 26-week dietary intake of rapeseed oil and soybean oil on plasma lipid levels, glucose-6-phosphate dehydrogenase activity and cyclooxygenase-2 expression in spontaneously hypertensive rats ☆

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ABSTRACT

We intended to determine whether or not dietary canola oil (CO) elevates plasma lipids and oxidative stress, since both of these are, possibly, related to the CO-induced life shortening through exacerbation of hypertension-associated vascular lesions found in stroke-prone spontaneously hypertensive rats (SHRSP). Spontaneously hypertensive rats (SHR) were used in this study to avoid a potential bias in the results due to the irregular death by stroke seen in SHRSP. SHR were fed for 26 weeks on a chow containing either, 10 wt/wt% of CO or soybean oil (SO), i.e., the control. Elevated plasma lipids and glucose-6-phosphate dehydrogenase (G6PD) activation in the liver and erythrocyte were found in SHR fed CO compared to that fed SO, while anti-oxidative enzymes other than G6PD were not activated. The CO diet brought about significant vascular lesions in the kidney, in which abundant cyclooxygenase-2 (COX-2) positive foci were immunochemically located in the juxtaglomerular apparatus. These results suggest that dietary CO induces a hyperlipidemic condition, in which G6PD may serve as an NADPH provider, and aggravates genetic diseases in SHR (also, probably, in SHRSP). The increased COX-2 expression indicates a role of renin-angiotensin-aldosterone system activation in the increased vascular lesions, whereas the effects of oxidative stress remain unclear.

1. Introduction

Huang et al. (1996, 1997) reported that certain vegetable oils, including low-erucic acid rapeseed (canola) oil (CO), shortened life in stroke-prone spontaneously hypertensive rats (SHRSP) as compared with the control animals given soybean oil (SO) when each oil was added to a diet of 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 it had been reported by Minetoma et al. (1975) that laying hens given rapeseed meal revealed thyroid hypertrophy and the amounts of isothiocyanate and oxazolidinethione, catabolic products of glucosinolate in the meal were propotional to the thyrotoxicity. In SHRSP, however, the sulfur-containing compounds did not shorten survival time (Huang et al., 1996). The study by Minetoma et al. (1975) also indicated that some other factor(s) than the sulfur-containing compounds in the rapeseed meal cause hemorrhage and vascular abnormalities in the liver of the hen. In 1998, Miyazaki et al. found that neither the fatty acid fraction obtained by lipase-treatment of low-erucic rapeseed oil nor unsaponifiable fraction of the oil shortened the life of SHRSP. Thus the life shortening was though to be attributed to contents other than fatty acids or unsaponifiable substances. Although in our recent study we tried to fractionate the causative(s) in CO by super critical gas extraction technique and obtained a less toxic fraction than CO, we could not find fractions containing the causative(s) (Ohara et al., 2006).

Abbreviations: CO, canola oil; G6PD, glucose-6-phosphate dehydrogenase; NADPH, nicotinamide adenine dinucleotide phosphate; PBS, phosphate buffered saline; SO, soybean oil; SHR, spontaneously hypertensive rats; SHRSP, stroke-prone spontaneously hypertensive rats; WKY rats, Wistar Kyoto rats.

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While phytosterols are included in the unsaponifiable fraction of the oil and considered not to possess life shortening effects (Miyazaki et al., 1998), the amount of phytosterols in CO was reported as one of the causes for the life shortening, since replacement of cholesterol with, or accumulation of, CO-derived phytosterols in cell membrane possibly makes the membrane fragile and leads to tissue rupture (Ratnayake et al., 2000a,b; Naito et al., 2003; Ogawa et al., 2003). However, conflicting results have been reported (Tatematsu et al., 2004; Ohara et al., 2006), and very recently a potential stroke-stimulating substance, dihydro-vitamin K1, in CO has also been suggested as a candidate causative (Okuyama, 2007). Considering that decisive substances have not yet found despite of the efforts mentioned above, it is worthwhile to examine further the physiological and pathological changes due to CO ingestion in the rat in order to find a clue to identify the causatives.

In a previous study intended to survey the CO-induced pathophysiologic changes in spontaneously hypertensive rats (SHR) in comparison with their normotensive genetic counter part, Wistar Kyoto (WKY) rats, it was demonstrated that a 26-week ingestion of CO as the sole dietary fat increased plasma lipids in rats of both strains compared with those in the control groups given SO (Naito et al., 2000a). Similar changes were also observed in WKY rats fed on CO diet for an even shorter period, i.e., 13 weeks (Naito et al., 2000b). In both studies, CO ingestion elevated blood pressure in the animals compared with the control animals. Moreover, in the 13-week feeding study, CO ingestion significantly increased glucose-6-phosphate dehydrogenase (G6PD) activity in the liver of WKY rats (Naito et al., 2000b). If the increase in G6PD activity is a general consequence of CO ingestion in rats, the activation of G6PD may play a role in the observed aggravation of hypertension-related conditions, especially in SHR or SHRSP, because G6PD, the first enzyme in the pentose phosphate pathway, provides nicotinamide adenine dinucleotide phosphate (NADPH) for lipogenic enzymes (Salati and Amir-Ahmady, 2001) and because the hyperlipidemic state accelerates inflammatory events, including hypertension (Park et al., 2006; Lloyd et al., 2007; Savoia and Schiffrin, 2007).

In the 26-week feeding study mentioned above, histological examinations revealed significant lesions, especially in the heart and the kidney, in SHR given CO (Naito et al., 2000a). Similar tissue injuries were also found in WKY rats given CO, but those injuries were far less severe than that in SHR. In addition to the facilitation of lipogenesis, an increase in oxidative stress by G6PD may play a role in the more severe lesions in SHR given CO, since decreased NADPH production by G6PD deficiency may reduce NADPH oxidase-derived superoxide anion and lower vascular atherosclerotic lesion growth in mice (Matsui et al., 2005, 2006). This suggests that the increased NADPH production by G6PD possibly results in an enhanced production of superoxide anion. Therefore, the increased NADPH production by G6PD may lead to atherosclerotic lesions, since superoxide anion inactivates nitric oxide and reduces biologically available nitric oxide for endothelium-dependent vasodilation (Gryglewski et al., 1986). Such an increase in oxidative stress by G6PD is paradoxical, because G6PD has also been thought to be an anti-oxidative enzyme (Salvemini et al., 1999; Leopold and Loscalzo, 2000; Salati and Amir-Ahmady, 2001) that provides NADPH to maintain glutathione in a reduced form and is upregulated by oxidative stress (Ursini et al., 1997). However, the CO-induced exacerbation of hypertension and vascular lesions with an increased G6PD arouses our interest in finding whether or not the fact that CO ingestion-induced greater tissue injury in SHR than in WKY rats was due to some effect of CO to enhance oxidative stress, with that effect being synergistically augmented in SHR (and SHRSP), since the genetic hypertension-related vascular lesions have been reported to be closely related to increased oxidative stress via uncoupled nitric oxide synthase in SHR (Racasan et al., 2005; Paliege et al., 2006; Li et al., 2006) and SHRSP (Hamilton et al., 2004).

In the above mentioned 13-week study in WKY rats, on the other hand, the activities of the anti-oxidative enzymes, superoxide dismutase and catalase were examined in addition to G6PD, for compensatory increases in oxidative stress, since such compensatory changes had been considered to be a possible consequence in animals given CO. In that study, however, WKY rats given CO showed even lower activities of superoxide dismutase and catalase in the liver than animals given SO (Naito et al., 2000b). Therefore, it is of interest to determine whether or not a longer CO ingestion in SHR changes lipogenesis or oxidative stress, or both, and whether or not changes in the activities of anti-oxidative enzymes are related to the more severe vascular lesions seen in SHR (and ultimately, also in SHRSP) given CO compared with that given SO.

In the present study we intended to confirm whether or not a 26-week CO ingestion-induced elevation of plasma lipids is reproduced and to examine whether or not concomitant changes in anti-oxidative enzyme activities including G6PD occur also in SHR. Besides, in this study the kidney, the organ in which the vascular lesions due to CO ingestion were most evident in the previous studies in SHR (Naito et al., 2000a) and SHRSP (Ohara et al., 2006), was histologically examined by means of immunochemical staining for cyclooxygenase-2 (COX-2). It has been reported that COX-2 expression in the kidney is regulated by nitric oxide (Cheng et al., 2006; Yang et al., 2006), and NADPH provided by G6PD is utilized for nitric oxide generation (Leopold et al., 2003). On one hand, COX-2 stimulates renin release (Paliege et al., 2004; Harris et al., 2004), which plays a role in hypertension and peripheral vascular lesions via the renin-angiotensin-aldosterone system.

2. Materials and methods

2.1. Animal husbandry

Twenty males, SHR (Charles River Japan, Tsukuba), 5 weeks of age, were used. Ten animals were assigned to the CO group; and 10, to the SO (control) group. Animals in the CO group were fed with a fat-free AIN-93 diet (Oriental Yeast, Tokyo, Japan) supplemented with 10 wt/wt% CO (Japan Oilseed Processors Association, Tokyo); and the animals in the SO group were fed with the same diet containing, instead, 10 wt/wt% SO (Japan Oilseed Processors Association, Tokyo). In this study the single concentration of 10 wt/wt% (24.8 energy percentage) was adopted because the purpose of the study was not to find any dose-dependent change due to CO ingestion. In this study we intended to confirm whether 10 wt/wt% CO-induced hyperlipidemia and lesions in the kidney that were found in both, previous 13-week and 26-week feeding studies in SHR and WKY rats (Naito et al., 2000a,b) and survival time studies in SHRSP (Huang et al., 1997; Naito et al., 2003; Ohara et al., 2006) in which 10 wt/wt% supplementation of the oil was adopted. The mean daily intake of oil calculated from daily food consumption that was measured once a week throughout the experimental period, without considering the amount spilt, was 6.5 ± 0.5–7.9 ± 0.6 g/kg day for CO and 6.6 ± 0.5–7.8 ± 0.7 g/kg day for SO.

Fatty acid compositions of the oils are shown in Table 1. These animals were given distilled and deionized water containing 1% NaCl for drinking. Such NaCl loading is known to enhance the development of hypertension in the rat (Sapirstein

Table 1
Fatty acid compositions (%) of soybean oil (SO) and canola oil (CO)

Fatty acid		SO	CO
14:0	Myristic acid	0.1	0
16:0	Palmitic acid	10.5	3.8
16:1	Palmitooleic acid	0	0.2
18:0	Stearic acid	3.6	1.9
18:1	Oleic acid	23.2	59.0
18:2	Linoleic acid	54.5	21.2
18:3	Linolenic acid	7.2	11.2
20:0	Arachidic acid	0.3	0.5
20:1	Eicosaenoic acid	0.2	1.5
22:0	Behenic acid	0.4	0.3
22:1	Erucic acid	0	0.4