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Effects of dietary carbohydrate and *myo*-inositol on metabolic changes in rats fed 1,1,1-trichloro-2,2-bis (p-chlorophenyl) ethane (DDT)

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Abstract

This study was conducted to examine the effects of dietary carbohydrate [starch or sucrose (500g/kg diet)] and *myo*-inositol (2g/kg diet) on metabolic changes in rats fed 1,1,1-trichloro-2,2-bis (p-chlorophenyl) ethane (DDT) (0.7g/kg diet). Dietary DDT enhanced serum and hepatic lipids and hepatic thiobarbituric acid reactive substances (TBA-RS), elevated hepatic activities of lipogenic enzymes such as malic enzyme (ME), glucose-6-phosphate dehydrogenase (G6PD) and fatty acid synthetase (FAS), increased hepatic cytochrome P-450 content and the activities of drug-metabolizing enzymes such as aminopyrine N-demethylase, glutathione S-transferase and 4-nitrophenol-UDP glucuronosyltransferase (4NP-UDPGT) and raised hepatic ascorbic acid and serum copper. Dietary sucrose promoted the increases in hepatic concentrations of total lipids, triglyceride and cholesterol, hepatic activity of ME, hepatic TBA-RS, cytochrome P-450 content and serum copper due to DDT feeding when compared to DDT administered in a starch based diet. Dietary *myo*-inositol significantly depressed the rises in hepatic concentrations of total lipids, triglyceride and cholesterol and the activities of ME and G6PD due to DDT feeding regardless of dietary carbohydrate quality. Dietary starch supplemented with *myo*-inositol potentiated the enhancements in hepatic activities of Phase II drug-metabolizing enzymes such as glutathione S-transferase and 4NP-UDPGT due to DDT feeding. These results suggest that dietary starch and *myo*-inositol can protect DDT fed rats against an accumulation of hepatic lipids, which might be mainly ascribed to the depression of hepatic lipogenesis. In addition, the present study implies that the supplementation of *myo*-inositol to high starch diet might improve the function of drug-metabolizing enzymes exposed to DDT.

Keywords: DDT; Carbohydrate; *myo*-inositol; Lipids; Hepatic lipogenic enzymes; Hepatic drug-metabolizing enzymes

1. Introduction

Feeding of xenobiotics such as DDT or PCB to rats causes many metabolic changes that include: i) accumulation of hepatic lipids [1–4], ii) elevation in serum cholesterol and copper [5–6], iii) increase in lipid peroxidation [7–8], iv) enhancement of ascorbic acid in tissue and urine [9], and v) induction of drug-metabolizing enzymes [10]. *Myo*-inositol is broadly distributed in mammalian tissues and cells, higher plants, fungi, and some bacteria where it provides important biological functions [11]. *Myo*-inositol is an essential growth factor for many cells in tissue culture [11]. One of the most exciting developments in the field of *myo*-inositol functions in the cells has arisen very recently with the recognition of a dynamic role for membrane phosphoinositides in providing for the release of the second messengers 1,2-diacylglycerol and inositol triphosphate in stimulated cells [11]. A number of studies have shown that dietary *myo*-inositol prevents the development of a fatty liver in rats [11–13]. But many nutrition scientists argue that *myo*-inositol, a vitamin-like substance, is not an essential nutrient because the body can synthesize it from glucose [11–14]. Recently, we have demonstrated that dietary *myo*-inositol can protect sucrose-fed rats against accumulation of hepatic lipids, which may be ascribed to the depression in the hepatic lipogenesis [15–17]. In addition, dietary *myo*-inositol also prevented the development of a fatty liver induced by DDT intake [18–19]. However, the mechanism by which the fatty liver was prevented by *myo*-inositol in rats fed DDT remains unknown. On the other hand, lipogenic effect of dietary sucrose is higher than that of starch, which is implicated in various disease states such as arteriosclerosis [20–23]. So we hypothesized that dietary sucrose exacerbates a fatty liver in rats fed DDT. In the present study, we investigated that the influence of dietary

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Table 1
Effects of dietary carbohydrate and *myo*-inositol on growth and tissues weights in rats fed 0.07% DDT[1]

Diets	Gains in body wt. (g/14 days)	Liver wt. (% of body wt.)	Kidneys wt. (% of body wt.)	Spleen wt. (% of body wt.)
Normal				
Starch (5)[2]	106[a,b]	4.81[a]	0.911[a,b]	0.477
+*myo*-inositol (5)	111[b]	4.81[a]	0.851[a]	0.506
Sucrose (5)	108[b]	5.42[a]	0.881[a]	0.507
+*myo*-inositol (5)	106[a,b]	4.97[a]	0.904[a,b]	0.460
DDT				
Starch (5)	99[a]	7.71[b,c]	0.925[a,b]	0.496
+*myo*-inositol (6)	98[a]	7.09[b]	0.958[b]	0.449
Sucrose (5)	99[a]	8.62[c]	0.950[b]	0.477
+*myo*-inositol (6)	99[a]	8.04[b,c]	0.980[b]	0.453
Pooled SEM	3	0.3	0.027	0.029
Source of Variation		Analysis of variance, p value		
Carbohydrate	NS	0.0077	NS	NS
myo-inositol	NS	0.0260	NS	NS
DDT	0.0000	0.0000	0.0037	NS
Carbohydrate × myo-inositol	NS	NS	NS	NS
Carbohydrate × DDT	NS	NS	NS	NS
myo-inositol × DDT	NS	NS	NS	NS
Carbohydrate × *myo*-inositol × DDT	NS	NS	NS	NS

[1] Values are means and Pooled SEM. Within a column, values followed by different letters are significantly different ($P<0.05$).
NS=not significant. [2] Number in parentheses is the number of rats in each group.

carbohydrate and *myo*-inositol on hepatic lipids levels and lipogenic enzymes activities or a rate-limiting enzyme of fatty acid oxidation in rats fed DDT.

Xenobiotics are metabolized in the liver in two distinct, enzymically-mediated, phases. Phase I reactions include oxidation and reduction and are catalyzed by a group of cytochrome P-450. The increases in the activities of phase I enzymes, mainly the cytochrome P-450, are involved in the activation of carcinogens [24–25]. Phase II reactions involve conjugation of the phase products with a range of substances including glucuronic acid. Conjugation results in the formation of more water soluble derivatives so phase reactions are generally regarded as defensive detoxification mechanisms [26]. Treatment of rats with xenobiotics enhances hepatic ascorbic acid and cholesterol biosynthesis [27–29]. Alterations in tissues levels of ascorbic acid and serum level of cholesterol were correlated with the induction of drug-metabolizing enzymes in rats fed xenobiotics [30]. In addition, serum cholesterol generally relates to serum copper when the animals given xenobiotics [6]. Therefore, we also investigated the effects of dietary carbohydrate and *myo*-inositol on hepatic activities of drug-metabolizing enzymes, hepatic ascorbic acid and serum levels of cholesterol and copper in rats fed DDT.

2. Methods and materials

2.1. Animals and diets

After feeding commercial stock diet (MF Oriental Yeast Co., Tokyo) for 3 days, forty-two male Wistar rats (Hiroshima Laboratory Animal Center, Hiroshima, Japan) weighing 59 ~ 76g were arranged into 8 weight-matched groups averaging 68g each. Half of the groups were fed diets containing 0.07% DDT (Tokyo-kasei Ind. Ltd., Tokyo) and uncontaining DDT. These groups were assigned the diets of starch-based diet and sucrose-based diet with or without 0.2% *myo*-inositol (Tsuno Rice Fine Chemicals Ltd., Wakayama). Room temperature was kept at 24 °C with a 12-h light: dark cycle (lights on, 0800-2000h). Composition of the basal diet was (in g/kg diet): casein, 200; carbohydrate, 652 [high starch (potato starch, 502; sucrose, 150) or high sucrose (potato starch, 150; sucrose, 502)]; cellulose powder, 50; corn oil, 50; AIN-93 mineral mixture, 35; AIN-93 vitamin mixture, 10; DL-methionine, 3. The dietary addition of DDT and *myo*-inositol were made at the expense of potato starch or sucrose. All the diets and deionized water were supplied *ad libitum.* After feeding them for 14 ~ 15 days, the diets were removed from cages at 8:00 a.m.; the animals were lightly anesthetized with ether and killed between 1:00 p.m. and 3:00 p.m. Blood was collected by heart puncture and serum samples were isolated by centrifugation at 4°C. The liver was homogenized in 4 volumes of 0.14M KCl. The homogenates were centrifuged at 10,000 × g for 10 min. From one part of the postmitochondrial supernatant, the activity of aminopyrine N-demethylase was measured. Addition of solid $CaCl_2$ (8.0mM final concentration) to another part of postmitochondrial supernatant allowed complete sedimentation of the microsomes at 25,000 × g in 15 min [31]. The sediment was homogenized in 0.14M KCl and mixed with glycerol (20% final concentration). The microsomes were used for the determination of the amount

Table 2
Effects of dietary carbohydrate and *myo*-inositol on the levels of liver and serum lipids in rats fed 0.07% DDT[1]

Diets	Liver				Serum		
	Total lipids (mg/g tissue)	Triglyceride (μ mol/g tissue)	Cholesterol (μ mol/g tissue)	Phospholipid (μ mol/g tissue)	Triglyceride (mmol/L)	Cholesterol (mmol/L)	Phospholipid (mmol/L)
Normal							
Starch (5)[2]	94^{a}	$37.7^{a,b}$	6.97^{a}	$38.2^{a,b}$	$1.08^{a,b}$	$2.63^{a,b,c}$	2.93^{a}
+*myo*-inositol (5)	76^{a}	22.3^{a}	6.59^{a}	$41.6^{b,c}$	1.22^{a}	2.45^{a}	$3.10^{a,b}$
Sucrose (5)	$100^{a,b}$	$53.6^{b,c}$	8.09^{a}	34.9^{a}	$1.23^{a,b}$	$2.69^{a,b,c}$	$3.39^{b,c}$
+*myo*-inositol (5)	90^{a}	$38.0^{a,b}$	7.91^{a}	$41.5^{b,c}$	$1.28^{a,b}$	$2.56^{a,b}$	$3.47^{b,c,d}$
DDT							
Starch (5)	149^{c}	69.6^{c}	13.49^{b}	47.9^{c}	1.84^{b}	$3.05^{a,b,c}$	$3.69^{c,d}$
+*myo*-inositol (6)	$99^{a,b}$	$39.1^{a,b}$	$10.36^{a,b}$	46.0^{c}	$1.65^{a,b}$	$3.07^{a,b,c}$	$3.77^{c,d}$
Sucrose (5)	234^{d}	126.0^{d}	20.39^{c}	47.7^{c}	$1.35^{a,b}$	3.28^{c}	$3.74^{c,d}$
+*myo*-inositol (6)	$141^{b,c}$	75.4^{c}	13.62^{b}	45.5^{c}	$1.67^{a,b}$	$3.15^{b,c}$	3.89^{d}
Pooled SEM	11	8.1	1.11	1.96	0.22	0.15	0.13
Source of Variation	Analysis of variance, p value						
Carbohydrate	0.0006	0.0000	0.0012	NS	NS	NS	0.0190
myo-inositol	0.0001	0.0000	0.0027	NS	NS	NS	NS
DDT	0.0000	0.0000	0.0000	0.0000	0.0068	0.0001	0.0000
Carbohydrate × *myo*-inositol	NS	NS	NS	NS	NS	NS	NS
Carbohydrate × DDT	0.0114	0.0099	0.0398	NS	NS	NS	NS
myo-inositol × DDT	0.0290	NS	$0.05<p<0.1$	0.0173	NS	NS	NS
Carbohydrate × *myo*-inositol × DDT	NS	NS	NS	NS	NS	NS	NS

[1] Values are means and Pooled SEM. Within a column, values followed by different letters are significantly different ($P<0.05$).
NS=not significant. [2] Number in parentheses is the number of rats in each group.

of cytochrome P-450. The rest of postmitochondrial supernatant was centrifuged at 105,000 × g for 60 min, and the microsomal pellets were suspended in 0.14M KCl. The resulting supernatant was stored at −80°C until needed for assays of lipogenic enzymes and glutathione S-transferase. The microsomal suspension was also stored at −80°C until needed for assays of 4-nitrophenol-UDP glucuronosyltransferase (4NP-UDPGT) and carnitine palmitoyltransferase I (CPTI) which is a rate-limiting enzyme of fatty acid oxidation.

2.2. Biochemical assays

Liver lipids were extracted by the method of Folch et al. [32], and liver total lipids was gravimetrically determined. Liver triglyceride and cholesterol were measured by the method of Danno et al. [33], using enzymatic kits (Triglyceride G-Test Wako, Total cholesterol C-Test Wako, respectively; Wako Pure Chemical, Osaka, Japan). Total lipid phosphorus was measured by the method of Bartlett [34], and the values were multiplied by 25 to obtain the phospholipid content. Serum levels of cholesterol, phospholipid and triglyceride were determined using enzymatic kits (Total cholesterol C-Test Wako, Phospholipids B-Test Wako, Triglyceride G-Test Wako, respectively; Wako Pure Chemical, Osaka, Japan). Hepatic thiobarbituric acid reactive substances (TBA-RS) were measured according to the method of Masugi et al. [35]. A portion of liver was homogenized with ice-cold 5% metaphosphoric acid and then centrifuged at 1500 x g for 10 min. Ascorbic acid in the supernatant was measured by the 2,4-dinitrophenylhydrazine method [36]. Serum copper was determined with a commercial available kit (Cu-Neotest, Sinotest Ltd., Tokyo).

2.3. Enzymatic assays

Hepatic activities of glucose-6-phosphate dehydrogenase (G6PD) and malic enzyme (ME) were measured spectrophotometrically at 340nm by the methods of Freedland [37]. Fatty acid synthetase (FAS) activity in liver was determined by the method of Martyn et al. [38]. The activities were expressed as mU/mg protein or U/100g body weight, where 1mU is the amount catalyzing the formation of 1nmol product/min. The activities of these enzymes were determined at 25°C. Activity of CPT I was measured using L-carnitine, palmitoyl CoA and 5,5′-dithio-bis (2-nitrobenzoic acid) according to the method of Bieber and Fiol [39].

Hepatic cytochrome P-450 content was determined by the dithionite difference method of Omura and Sato [40]. Hepatic activity of aminopyrine N-demethylase was determined by measuring the liberated formaldehyde produced during the incubation [41]. Formaldehyde was measured according to the method of Nash [42]. The activity of glutathione S-transferase was determined spectrophotometrically at 340 nm by measuring the formation of the conjugate of reduced glutathione and 1-chloro-2,4-dinitrobenzene at 25°C according to the method of Habig et al.

Table 3
Effects of dietary carbohydrate and *myo*-inositol on the activities of serum glutamic pyruvic transaminase (GPT), glutamic oxaloacetic transaminase (GOT) and liver thiobarbituric acid substances in rats fed 0.07% DDT[1]

	Liver		Serum	
Diets	TBA-RS (^{A}532/g tissue)	(^{A}532/100g body wt.)	GPT activity (IU/l)	GOT activity (IU/l)
Normal				
Starch (5)[2]	1.79^{a,b}	8.60^{a}	5.04	29.4
+*myo*-inositol (5)	1.76^{a,b}	8.43^{a}	5.60	37.8
Sucrose (5)	1.61^{a}	8.68^{a}	4.08	32.8
+*myo*-inositol (5)	1.59^{a}	7.90^{a}	3.12	33.0
DDT				
Starch (5)	3.39^{b,c}	25.8^{b}	5.67	32.4
+*myo*-inositol (6)	4.26^{c,d}	31.1^{b,c}	4.32	33.4
Sucrose (5)	4.84^{c,d}	40.7^{c}	5.40	32.5
+*myo*-inositol (6)	5.63^{d}	46.7^{c}	4.88	32.6
Pooled SEM	0.43	3.64	1.10	2.54
Source of Variation	Analysis of variance, p value			
Carbohydrate	NS	0.0387	NS	NS
myo-inositol	NS	NS	NS	NS
DDT	0.0000	0.0000	NS	NS
Carbohydrate × *myo*-inositol	NS	NS	NS	NS
Carbohydrate × DDT	0.05<p<0.1	0.0424	NS	NS
myo-inositol × DDT	NS	NS	NS	NS
Carbohydrate × *myo*-inositol × DDT	NS	NS	NS	NS

[1] Values are means and Pooled SEM. Within a column, values followed by different letters are significantly different ($P<0.05$).
NS=not significant. [2] Number in parentheses is the number of rats in each group. ^{A}532; Absorbance at 532 nm.

[43]. 4NP-UDPGT activity was estimated by the method of Horio et al. [44]. The microsomal suspension was treated with Triton X-100 (final concentration, 0.25% (w/v)) on ice for 15min immediately before the assay of 4NP-UDPGT activity. The 4NP-UDPGT assay mixture contained 50mM Tris/HCl (pH 7.4), 3.3mM magnesium chloride, 3mM saccharic acid-1, 4-lactone, 1.4mM 4-nitrophenol, 6mM UDP glucuronic acid, and enzyme sample. The mixture (0.3ml) was incubated for 12min at 37°C, and the reaction was stopped by the addition of trichloroacetic acid to 125mM. After the precipitate had been removed by centrifugation, the supernatant was diluted with 1N NaOH, and 4NP was measured spectrophotometrically at 400nm. The enzymes activities are shown as mU/mg protein or U/100g body weight. Protein was measured by the method of Lowry et al. [45].

The activities of serum glutamic pyruvic transaminase (GPT) and glutamic oxaloacetic transaminase (GOT) were measured using commercial kits (GPT-UV Test Wako, GOT-UV Test Wako, respectively; Wako Pure Chemical, Osaka, Japan).

2.4. Statistics

Data were statistically analyzed by three-way analysis of variance (ANOVA). The sources of variation were DDT, carbohydrate and *myo*-inositol. Duncan's multiple range test [46] was used only if the ANOVA demonstrates a significant F score.

3. Results

As shown in Table 1, growth was less in rats fed DDT than in controls. Dietary carbohydrate and *myo*-inositol had no effect on the growth depression in the present study. Dietary DDT significantly raised liver weight. In addition, dietary sucrose also increased liver weight. Dietary *myo*-inositol depressed the increase in liver weight due to DDT feeding. Dietary DDT also enhanced kidneys weight regardless of dietary carbohydrate and *myo*-inositol. Spleen weight was not influenced by the present dietary manipulation.

Table 2 describes the effects of dietary carbohydrate and *myo*-inositol on serum and hepatic lipids in rats fed DDT. Dietary DDT significantly elevated hepatic concentrations of total lipids, triglyceride, cholesterol and phospholipid. Dietary sucrose promoted the increases in hepatic concentrations of total lipids, triglyceride and cholesterol in rats fed DDT. Dietary *myo*-inositol significantly depressed the rises in hepatic concentrations of total lipids, triglyceride and cholesterol induced by DDT regardless of dietary carbohydrate quality. Serum levels of triglyceride, cholesterol and phospholipid were also elevated in rats fed DDT. In the rats fed DDT, dietary carbohydrate and *myo*-inositol had no significant influence on serum levels of triglyceride, cholesterol and phospholipid.

Table 3 shows hepatic TBA-RS content and the activities of serum GPT and GOT in rats fed DDT. Dietary DDT

Table 4
Effects of dietary carbohydrate and *myo*-inositol on liver activities of lipogenic enzymes in rats fed 0.07% DDT[1]

	Liver					
	ME		G6PD		FAS	
Diets	(mU/mg protein)	(U/100g body wt.)	(mU/mg protein)	(U/100g body wt.)	(mU/mg protein)	(U/100g body wt.)
Normal						
Starch (5)[2]	40.5^{a,b}	17.4^{a}	66.3^{a,b}	28.4^{a}	1.58^{a}	0.66^{a}
+*myo*-inositol (5)	37.8^{a}	16.4^{a}	69.5^{a,b}	30.3^{a,b}	1.52^{a}	0.68^{a}
Sucrose (5)	59.3^{a,b,c}	28.6^{a,b}	104.1^{c,d}	51.8^{a,b}	1.69^{a}	0.81^{a}
+*myo*-inositol (5)	48.2^{a,b}	21.6^{a}	79.0^{b,c}	35.5^{a,b}	2.43^{a,b}	1.11^{a,b}
DDT						
Starch (5)	80.7^{d}	51.4^{c,d}	80.1^{b,c}	49.9^{a,b}	2.13^{a,b}	1.52^{a,b}
+*myo*-inositol (6)	61.9^{b,c,d}	40.0^{b,c}	47.5^{a}	29.8^{a,b}	1.49^{a}	1.01^{a,b}
Sucrose (5)	125.6^{e}	89.5^{e}	111.5^{d}	79.4^{c}	3.39^{b}	2.49^{c}
+*myo*-inositol (6)	79.5^{c,d}	58.8^{d}	62.9^{a,b}	46.4^{a,b}	2.67^{a,b}	2.02^{b,c}
Pooled SEM	5.8	4.06	8.7	5.57	0.51	0.32
Source of Variation	Analysis of variance, p value					
Carbohydrate	0.0000	0.0000	0.0016	0.0005	0.0240	0.0108
myo-inositol	0.0001	0.0001	0.0006	0.0002	NS	NS
DDT	0.0000	0.0000	NS	0.0005	0.05<p<0.1	0.0004
Carbohydrate × *myo*-inositol	0.05<p<0.1	0.05<p<0.1	NS	NS	NS	NS
Carbohydrate × DDT	0.05<p<0.1	0.0101	NS	NS	NS	NS
myo-inositol × DDT	0.05<p<0.1	0.05<p<0.1	0.0401	0.0368	NS	NS
Carbohydrate × *myo*-inositol × DDT	NS	NS	NS	NS	NS	NS

[1] Values are means and Pooled SEM. Within a column, values followed by different letters are significantly different ($P<0.05$).
NS=not significant. [2] Number in parentheses is the number of rats in each group.

significantly enhanced hepatic TBA-RS content. A promoting effect of the enhancement was observed in rats fed the high sucrose diets when the data was expressed per g tissue. When expressed per 100g body weight, this promoting effect was significant. The enhancement in hepatic TBA-RS due to DDT was not significantly influenced by dietary *myo*-inositol. Dietary DDT, Carbohydrate and *myo*-inositol had no effect on serum GPT and GOT.

The effects of dietary carbohydrate and *myo*-inositol on hepatic activities of lipogenic enzymes in rats fed DDT were presented in Table 4. Since the liver weight was varied with dietary treatment, the data were expressed in both per mg protein and per total liver per 100g body weight. Dietary sucrose increased hepatic activities of ME, G6PD and FAS regardless of DDT. When the data was expressed per mg protein, dietary addition of DDT raised hepatic activity of ME. Similar trends were observed in hepatic activities of G6PD and FAS when feeding with DDT. All of lipogenic enzymes were significantly increased by DDT when expressed per 100g body weight. Hepatic activities of these enzymes were higher in rats fed high sucrose diet containing DDT than any other groups. The supplementation of *myo*-inositol to high sucrose diet significantly suppressed the elevations of hepatic activities of ME and G6PD induced by DDT feeding. A similar suppressing effect of dietary starch supplemented with *myo*-inositol on hepatic activities of these enzymes was observed in rats fed on DDT. The increase in FAS activity due to DDT feeding was not significantly influenced by dietary *myo*-inositol. Hepatic activity of CPT I was not affected by the present dietary manipulation (data not shown).

Table 5 represents the data on the influence of dietary carbohydrate and *myo*-inositol on hepatic cytochrome P-450 content and the activities of drug-metabolizing enzymes. The amount of cytochrome P-450 and all of drug-metabolizing enzymes in the liver were significantly higher in DDT groups than in control groups. Dietary sucrose significantly amplified the increased amount of cytochrome P-450 caused by DDT when the data was expressed per 100g body weight. The raise in hepatic activity of aminopyrine N-demethylase due to DDT feeding was unaffected by dietary carbohydrate and *myo*-inositol. When expressed per 100g body weight, dietary *myo*-inositol potentiated the enhancements in hepatic activities of glutathione S-transferase and 4NP-UDPGT caused by DDT feeding. The potentiating effect of *myo*-inositol on these phase II enzymes activities was prominent in rats fed the high starch diet.

There was a trend that dietary sucrose decreased serum level of copper in rats without receiving DDT (Table 6). On the other hand, DDT enhanced serum copper, which was potentiated with the high sucrose diets. Dietary *myo*-inositol had no effect on serum copper in either starch or sucrose diet with or without DDT addition. The hepatic concentration of ascorbic acid was significantly increased in rats fed DDT (Table 6). Dietary *myo*-inositol promoted the increase in hepatic concentration of ascorbic acid when the data was expressed per g tissue, while it was not affected by *myo*-inositol when expressed per 100g body weight. Dietary

Table 5
Effects of dietary carbohydrate and *myo*-inositol on liver drug-metabolizing enzymes in rats fed 0.07%DDT[1]

Diets	Liver							
	Cytochrome P-450		Aminopyrine N-demethylase		Glutathione S-Transferase		4NP-UDPGT	
	(nmol/mg protein)	(nmol/100g body wt.)	(mU/mg protein)	(U/100g body wt.)	(mU/mg protein)	(U/100g body wt.)	(mU/mg protein)	(U/100g body wt.)
Normal								
Starch (5)[2]	0.170[a]	20.3[a]	1.96[a]	0.599[a]	546[a]	232[a]	6.58[a]	0.79[a]
+*myo*-inositol (5)	0.216[a]	25.7[a]	1.83[a]	0.555[a]	557[a]	236[a]	8.87[a,b]	1.08[a]
Sucrose (5)	0.238[a]	29.9[a]	1.80[a]	0.628[a]	477[a]	228[a]	7.36[a]	0.95[a]
+*myo*-inositol (5)	0.194[a]	22.5[a]	1.78[a]	0.565[a]	499[a]	226[a]	15.04[a,b,c]	1.73[a]
DDT								
Starch (5)	0.472[b]	101.2[b]	3.87[b]	1.865[b,c]	1068[b]	719[b]	18.99[b,c]	3.93[b]
+*myo*-inositol (6)	0.448[b]	88.0[b]	3.91[b]	1.784[b]	1453[c]	1015[c]	34.41[d]	6.39[c]
Sucrose (5)	0.651[c]	140.3[c]	4.00[b]	2.151[c]	1100[b]	799[b]	19.49[b,c]	4.11[b]
+*myo*-inositol (6)	0.498[b]	108.1[b]	3.88[b]	2.040[b,c]	1139[b]	829[b]	24.09[c,d]	5.21[b,c]
Pooled SEM	0.034	7.0	0.19	0.100	87	41	3.25	0.49
Source of Variation	Analysis of variance, p value							
Carbohydrate	0.0083	0.0083	NS	NS	NS	NS	NS	NS
myo-inositol	0.0283	0.0138	NS	NS	NS	NS	0.0134	0.0214
DDT	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Carbohydrate × *myo*-inositol	0.0351	NS	NS	NS	NS	NS	NS	NS
Carbohydrate × DDT	0.05<p<0.1	0.0360	NS	NS	NS	NS	NS	NS
myo-inositol × DDT	NS	NS	NS	NS	0.05<p<0.1	0.0032	NS	0.0458
Carbohydrate × *myo*-inositol × DDT	NS	NS	NS	NS	0.05<p<0.1	0.0155	NS	NS

[1] Values are means and Pooled SEM. Within a column, values followed by different letters are significantly different (P<0.05). NS=not significant.
[2] Number in parentheses is the number of rats in each group.

carbohydrate had no effect on hepatic concentration of ascorbic acid.

4. Discussion

As demonstrated by Oda et al. [1], dietary DDT remarkably enhanced hepatic concentrations of total lipids, triglyceride, cholesterol and phospholipid (Table 2). As expected, the present study demonstrated that dietary sucrose exacerbated a fatty liver in rats fed DDT as compared with dietary starch. In our previous study, it was observed that dietary supplementation of *myo*-inositol to high sucrose diet containing DDT prevented the development a fatty liver [18–19]. The present study showed that dietary *myo*-inositol also depressed the rises in hepatic concentrations of total lipids, triglyceride and cholesterol in rats fed high starch diet containing DDT (Table 2). These results indicate that dietary *myo*-inositol can protect DDT-fed rats against an accumulation of hepatic lipids regardless of carbohydrate quality.

Dietary addition of DDT raised hepatic activities of ME, G6PD and FAS (Table 4). These findings agree in large part with those of Hitomi et al., who reported that administration of xenobiotics to rats increased the activities of ME and G6PD [47]. Further, the present study showed that hepatic activities of lipogenic enzymes were higher in rats fed the high sucrose diet containing DDT than in rats fed the high starch diet containing DDT. Dietary *myo*-inositol also decreased the elevations in hepatic activities of G6PD and ME induced by DDT feeding regardless of dietary carbohydrate (Table 4). The present study demonstrated that dietary *myo*-inositol had no effect on hepatic activity of carnitine palmitoyltransferase I in rats fed DDT (data not shown), which plays a pivotal role in the regulation of fatty acid oxidation. In addition, dietary *myo*-inositol did not affect the increases in serum lipids induced by DDT feeding. These results suggest that dietary *myo*-inositol prevents DDT-mediated hepatic lipids accumulation by mechanism not involving an enhancement in hepatic fatty acid oxidation. The preventive effect of dietary *myo*-inositol on an accumulation of hepatic lipids due to DDT feeding may be mainly ascribed to the depression of hepatic lipogenesis.

Dietary DDT remarkably elevated hepatic TBA-RS that is an indicator of lipid peroxidation (Table 3). Similar results have been reported by Kobayashi et al. [7]. Interestingly, the high sucrose diet promoted the raise in hepatic TBA-RS due to DDT exposure compared with the high

Table 6
Effects of dietary carbohydrate and *myo*-inositol on the levels of serum Cu and liver vitamin C in rats fed 0.07% DDT[1]

Diets	Serum	Liver	
	Cu (μmol/L)	Vitamin C (μmol/g tissue)	(μmol/100g body wt)
Normal			
Starch (5)[2]	17.0[a,b]	1.53[a]	7.39[a]
+*myo*-inositol (5)	16.8[a,b]	1.48[a]	7.15[a]
Sucrose (5)	14.3[a]	1.45[a]	7.84[a]
+*myo*-inositol (5)	13.7[a]	1.34[a]	6.76[a]
DDT			
Starch (5)	20.6[b,c]	2.51[b]	19.37[b]
+*myo*-inositol (6)	20.0[b,c]	2.79[c]	19.77[b]
Sucrose (5)	21.1[c]	2.45[b]	20.85[b]
+*myo*-inositol (6)	22.0[c]	2.61[b,c]	21.08[b]
Pooled SEM	1.18	0.08	0.80
Source of Variation		Analysis of variance, p value	
Carbohydrate	NS	$0.05<p<0.1$	NS
myo-inositol	NS	NS	NS
DDT	0.0000	0.0000	0.0000
Carbohydrate × *myo*-inositol	NS	NS	NS
Carbohydrate × DDT	0.0322	NS	NS
myo-inositol × DDT	NS	0.0072	NS
Carbohydrate × *myo*-inositol × DDT	NS	NS	NS

[1] Values are means and Pooled SEM. Within a column, values followed by different letters are significantly different ($P<0.05$).
NS=not significant. [2] Number in parentheses is the number of rats in each group.

starch diet (Table 3). Lipid peroxidation has been suggested to play a role in carcinogenesis [48]. Some reports indicated that dietary sucrose acted as promoters in the development of *preneoplastic* lesions in rats treated with carcinogens [49–50]. These findings, together with those from the present study, speculated that the enhancements in the number of *preneoplastic* lesions caused by dietary sucrose might be partly mediated through an increased lipid peroxidation. Obviously, until more direct experimental evidence is obtained, this idea remains speculative.

In accordance with previous reports [10], hepatic cytochrome P-450 content and hepatic activities of drug-metabolizing enzymes were induced by DDT feeding (Table 5). In the rats fed DDT, the supplementation of *myo*-inositol generally potentiated the enhancements in hepatic activities of phase II drug-metabolizing enzymes without increasing phase I drug-metabolizing enzyme. The promoting effect of *myo*-inositol was prominent with high starch diet (Table 5). On the other hand, dietary sucrose amplified the increased amount of cytochrome P-450 in the rats fed DDT (Table 5). The increases in the activities of phase I enzymes and cytochrome P-450 are involved in the activation of carcinogens [24–25]. Phase II drug-metabolizing enzymes inactivate chemical carcinogens into less toxic or inactive metabolites [26], so phase II are generally regarded as defensive detoxification mechanisms. These results imply that dietary supplementation of *myo*-inositol to high starch diet might improve the function of drug-metabolizing enzymes exposed to DDT.

Fields et al. demonstrated that dietary sucrose decreased serum copper compared with dietary starch [51]. In accordance with their result, the present study showed a trend of decrease in serum copper of rats fed the high sucrose diet uncontaining DDT (Table 6). Ohchi et al. have shown that feeding of xenobiotics including DDT caused an increase in serum copper [6]. Therefore, we also investigated the effect of dietary carbohydrate and *myo*-inositol on serum levels of copper in rats fed DDT. In accordance with previous report, dietary DDT increased serum copper (Table 6). Interestingly, the enhancement in serum copper due to DDT feeding was promoted by dietary sucrose, while dietary *myo*-inositol had no effect on serum copper (Table 6). These results in the present study suggest that dietary sucrose might promote the effect of DDT on serum copper in rats, although the metabolic relationship between serum copper and hepatic lipids in rats fed DDT is unclear.

In conclusion, this study indicates that dietary sucrose exacerbates the effects of DDT on an accumulation of hepatic lipids, the enhancements in TBA-RS content, hepatic activities of lipogenic enzymes, cytochrome P-450 content and serum level of copper in rats when compared with dietary starch. In addition, the present study also indicates that dietary supplementation of *myo*-inositol can have a preventive effect on fatty liver and potentiate the increases in hepatic activities of phase II drug-metabolizing enzymes caused by DDT, especially in the high starch diet, without increasing phase I enzyme. Thus, we speculated that dietary starch and *myo*-inositol may be of potential value to rats fed DDT. Further studies are required to determine the serum and liver *myo*-inositol levels when *myo*-inositol was sup-

plied through the diet and in absence of dietary *myo*-inositol to investigate the metabolic interrelationship between tissues levels of *myo*-inositol and hepatic and serum lipids status or hepatic activities of drug-metabolizing enzymes.

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