BIOMEDICAL AND ENVIRONMENTAL SCIENCES **19**, 273-276 (2006)

Effects of Terephthalic Acid on Rat Lipid Metabolism[1]

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Objective To study the effect of terephthalic acid (TPA) on lipid metabolism in Sprague-Dawley (SD) rats. **Methods** Five groups of SD rats that ingested 0%, 0.04%, 0.2%, 1%, and 5% TPA, respectively, were included in a 90-day subchronic feeding study. Effects of TPA on levels of serum protein, total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL), total antioxidative capability (T-AOC), superoxide dismutase (SOD) and malondialdehyde (MDA) were observed. Urine samples were collected and analyzed for concentration of ion. **Results** TPA decreased the level of serum T-AOC in a dose dependent manner. The contents of serum and bladder MDA significantly decreased in 1% and 5% TPA ingestion groups. Serum CuZn superoxide dismutase (CuZnSOD) lowered in groups of 0.2%, 1%, and 5% TPA. TPA subchronic feeding had no significant influences on serum TC, LDL or HDL, but increased serum TG, TP and ALB after administration of 0.04% and/or 0.2% TPA. Concentrations of urinary Ca^{2+}, Mg^{2+}, Na^{+}, and K^{+} were elevated in 1% and 5% TPA groups. **Conclusion** Antioxidative potential decreased after TPA exposure. MDA increase in serum and bladder tissues was one of the most important reactions in rats which could protect themselves against TPA impairment. The decrease of serum CuZnSOD was related to the excretion of Zn^{2+}.

Key words: Terephthalic acid; SD rat; Serum; Bladder; SOD; MDA; T-AOC; Lipoprotein

INTRODUCTION

Since McCord *et al.*[1] reported that superoxide dismutase (SOD) is a marker of oxidative injury, it has been found that mechanism of oxidative stress is not only related with many diseases such as cancer, Alzheimer's disease, familial amyotrophic lateral sclerosis (FALS), and several neurological disorders[2-4], but also involves toxic reactions of agricultural chemicals, petrochemicals and other compounds[5-8]. Terephthalic acid (TPA), which belongs to non-genotoxic compounds, is extensively used in the world. Animal studies[9-10] showed that 3%-5% TPA exposure induced bladder stone within nearly two weeks, followed by bladder hyperplasia and ultimately carcinomas after 90-day subchronic and 2-year chronic feeding studies respectively. Epidemiologic investigations[11-15] demonstrated that changes in serum angiotensin-converting enzyme, pulmonary function and ventilation, and urinary ions among occupational workers are significantly related with TPA exposure. To investigate the effect of TPA on the oxidative defense system and elucidate its mechanism, we detected urinary ions, serum lipoprotein and T-AOC, and the content of SOD and malondialdehyde (MDA) in serum and bladder samples from rats which had been exposed to TPA for 90 days.

MATERIALS AND METHODS

Chemicals

Terephthalic acid was obtained from Yi Zheng Chemical Fiber Co. (Jiangsu, China). Total antioxidative capability (T-AOC), superoxide dismutase (SOD) and malondialdehyde (MDA) diagnostic agents were purchased from Nanjing Jiancheng Bioengineering Institute (Jiangsu, China). Serum total protein (TP), albumin (ALB), globulin (GP), total cholesterol (TC), triglycerin (TG), and high-density lipoprotein (HDL) diagnostic agents

[1]This work was supported by the National Natural Science Foundation of China (No. 30170798), the Preliminary Study of an Important Project in the National Basic Research, MOST (No. 2001CCA04900) and the Greatest Project in the National Basic Research (No. 2002CB512908).

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0895-3988/2006
CN 11-2816/Q

were obtained from Nanjing Kehua Biochemical Co. Ltd. All other chemicals used were commercial products of the highest purity.

Animals and Treatments

Male and female Sprague-Dawley (SD) rats were purchased from Shanghai Animal Center (Shanghai, China), and quarantined for 7 days before the experiments. Room temperature and relative humidity were controlled at 22℃±3℃ and 60%± 10%, respectively. Fluorescent lighting was provided in a 12 h light/dark cycle. The animals were randomly divided into five groups including 0%, 0.04%, 0.2%, 1%, and 5% TPA, and weighed every week. The treatment was continued for 90 days.

Sample Collection and Storage

All rats were sacrificed on the 90th day. Blood was collected from the retro-orbital venous plexus. After having clotted for 2 h at room temperature, serum were removed and stored at -20℃. Bladder tissues were weighed, and stored at -70℃. Fresh urine (100-1000 μL) was collected between 7:00 and 9:00 a.m. on the day before the animals were sacrificed and stored at -20℃.

Urinary Ions

Urine Ca^{2+}, Mg^{2+}, Zn^{2+}, Na^{+}, and K^{+} were analyzed with an AA-6501F atomic absorption flame emission spectrophotometer (Japan) and calibrated with creatinine that was analyzed by a Lisa-500 automatic analyzer (French).

Serum Protein and Lipoprotein

Serum protein, total cholesterol (TC), triglycerin (TG), and high-density lipoprotein (HDL) were measured with a Lisa-500 automatic analyzer (French).

T-AOC, SOD, and MDA Measurement

Bladder was cut into small pieces, homogenized (1 g/mL) in pre-chilled Tris-HCl buffer (Tris-HCl 0.01 mol/L, EDTA-2Na 0.0001 mol/L, Glucose 0.01 mol/L, and NaCl 0.1 mol/L, pH 7.4) in a tissue homogenizer, then centrifuged at 3000×g for 15 min at 4℃, and the collected supernatant was assayed immediately. The content of tissue protein was determined by Coomassie brilliant blue G_{250}. Levels of T-AOC, SOD, and MDA in bladder and serum were measured according to the product instructions, and their absorbencies were measured by a Beckman DU650 spectrophotometer (USA).

Statistical Analysis

Student's *t*-test was employed to calculate the significance of difference between control and experimental values. $P<0.05$ was considered statistically significant.

RESULTS

Body Weight Gain and Urinary Ions

The mean body weight gains of SD rats in all experimental groups had no significant change (Table 1). Levels of urinary Ca^{2+}, Mg^{2+}, Zn^{2+}, Na^{+}, and K^{+} increased in TPA treatment rats in a dose dependent manner (Table 2).

Serum Protein and Lipoprotein

Serum TP, ALB, and TG increased in TPA treatment groups respectively. Levels of HDL, LDL, and TC had no marked changes in all experimental groups (Table 3).

Levels of T-AOC, SOD, and MDA

TPA decreased serum T-AOC and CuZnSOD in a dose dependent manner (Fig. 1). The mean content of MDA also decreased in bladder and serum after TPA exposure (Figs. 1 and 2).

TABLE 1

Changes in Body Weight (g) and Weight Gain (g) in SD Rats After 90-day Subchronic Feeding of TPA ($\bar{x} \pm s$)

Group	Male				Female			
	n	Initial Weight	Final Weight	Weight Gain	*n*	Initial Weight	Final Weight	Weight Gain
Control	12	138±25	369±42	231±30	12	118±11	254±17	135±18
TPA 0.04%	12	131±13	361±49	230±41	12	114±12	249±30	135±28
0.2%	11	132±17	370±37	238±33	12	113±13	266±25	153±29
1%	12	128±14	356±37	228±33	12	119±11	246±21	127±24
5%	12	135±15	343±51	208±45	12	119±9	249±19	130±15

TABLE 2

Changes of Urinary ion in SD Rats After 90-day Subchronic Feeding of TPA ($\bar{x} \pm s$)

	n	Ca^{2+} (g/mol Cr)	Mg^{2+} (g/mol Cr)	Zn^{2+} (g/mol Cr)	K^{+} (g/mmol Cr)	Na^{+} (g/mmol Cr)
Control	23	4.50±0.75	20.92±2.64	0.03±0.00	0.19±0.02	0.38±0.05
TPA 0.04%	24	5.07±0.80	37.41±4.49	0.12±0.01	0.34±0.02	0.49±0.06
0.2%	24	5.23±0.91	44.46±5.75*	0.11±0.01	0.25±0.03	0.29±0.04
1%	24	17.08±2.27**	46.66±5.51**	0.18±0.02*	0.49±0.06**	0.40±0.16
5%	17	84.34±22.02**	103.25±24.64**	0.48±0.13**	0.32±0.08**	0.80±0.20*

Note. Compared with control, $^{*}P<0.05$, $^{**}P<0.01$.

TABLE 3

Changes in Serum Protein of SD Rats After 90-day Subchronic Feeding of TPA ($\bar{x} \pm s$)

	n	Cr (μmol/L)	TP (g/L)	ALB (g/L)	GP (g/L)	A/G	TC (mmol/L)	TG (mmol/L)	HDL (mmol/L)
Control	12	38.93±5.90	64.62±4.88	31.08±2.32	33.54±3.07	0.93±0.07	1.20±0.36	0.42±0.10	0.82±0.26
TPA 0.04%	12	35.93±5.46	69.12±4.95*	34.47±2.30**	34.65±3.84	1.00±0.13	1.34±0.33	0.63±0.26	0.96±0.22
0.2%	12	39.68±4.88	67.63±4.57	34.71±1.34**	32.93±3.67	1.06±0.11**	1.20±0.25	0.88±0.43**	0.89±0.18
1%	12	34.80±5.75	61.18±5.77	30.13±2.51	31.05±3.67	0.98±0.08	1.23±0.41	0.59±0.32	0.95±0.30
5%	12	42.97±10.82	62.54±8.74	30.46±2.92	32.09±6.26	0.97±0.13	1.25±0.41	0.43±0.21	0.89±0.24

Note. Compared with control, $^{*}P<0.05$, $^{**}P<0.01$.

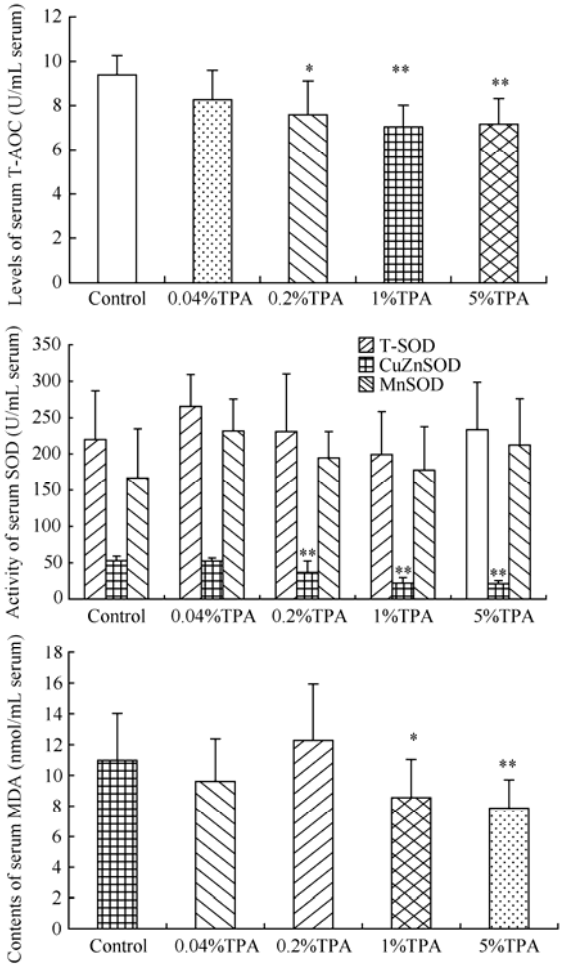

FIG. 1. Effects of TPA on levels of serum T-AOC, SOD, and MDA. ($\bar{x} \pm s$) compared with control, $^{*}P<0.05$, $^{**}P<0.01$.

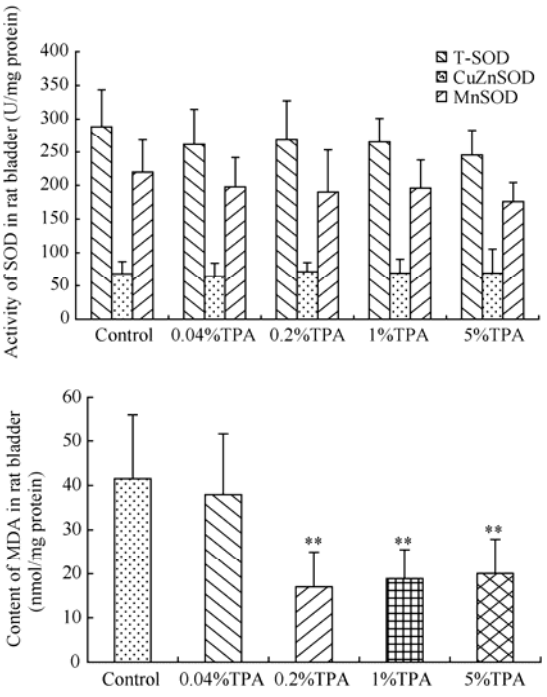

FIG. 2. Effects of TPA on levels of SOD and MDA in rat bladder. (n=8, $\bar{x} \pm s$) compared with control, $^{**}P<0.01$.

DISCUSSION

TPA, a kind of organic industrial chemical, is extensively used worldwide. The manifestations of toxicity in baby *ctenopharyngodon idellus*[16] and the induction of bladder stone, epithelial cell proliferation and ultimately carcinomas by TPA as a

non-genotoxic chemical has aroused deep concern over the world[9-10].

Oxidative stress is a self-defense system protecting body against the damage of free radicals, and its dysfunction is always observed in diseases such as tumor and during chemical exposure[2-7]. The present study illustrated that TPA affected neither the body weight nor weight gain in rats after 90-day subchronic feeding, but it decreased serum T-AOC in a dose dependent manner, representing the total antioxidative capability of the body. In addition, TPA significantly decreased contents of MDA in both serum and bladder tissues, especially after 5% TPA ingestion, suggesting that oxidative defense system initiates to protect the body from TPA induced toxic effects[17-18]. Our data also showed that the concentration of serum CuZnSOD decreased after TPA treatment. CuZnSOD was distributed in cellular membranes, while the circulating enzymes derived from both hemolysis and peripheral tissues were transported by HDL and/or LDL[19]. Hall *et al.*[20] reported that TPA 20 mg/kg/d decreased serum cholesterol and triglyceride levels in rats. Hence, decreased serum CuZnSOD might be the result of the effects of TPA on lipid metabolism. To elucidate this mechanism, we detected serum protein and lipoprotein in rats after 90-day feeding of TPA. The results have shown that except for TP, ALB, and TG increase in 0.04% and/or 0.2% TPA groups, levels of HDL and LDL have no difference compared with the control. However, TPA treatment significantly increases the concentration of urinary Ca^{2+}, Mg^{2+}, Zn^{2+}, Na^{+}, and K^{+}. Thus, decreased serum CuZnSOD is related to the increased excretion of Zn^{2+}.

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(*Received September* 28, 2004 *Accepted October* 12, 2005)