The metabolic effects of di (2-ethyl hexyl) phthalate medium dose on lipid profiles in serum and liver tissue

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Abstract

Background: Di (2-ethyl hexyl) phthalate is the most widely used plasticizer in blood storage bag for transfusion. This substance can modify lipid metabolism. This study was aimed to elucidate the metabolic effects of di (2-ethyl hexyl) phthalate medium dose on lipid profiles in serum and liver tissue.

Methods: Sprague Dawley rats were fed 1.0% di (2-ethyl hexyl) phthalate diet (DEHP group, n=5) or a non-supplemented diet (control group, n=5) for 10 days. The rats were allowed to freely access each food. Serum lipid concentrations were measured using enzyme assay kits. Lipids of liver tissues were extracted and the lipid contents were determined. A appeal of liver was prepared to determine the activities of malic enzyme and carnitine palmitoyl transferase-1 (CPT-1).

Results: Serum lipid concentrations (mg/dL) of DEHP group decreased compared to control (P<0.05). The serum triglyceride (TG) concentrations of control and DEHP groups were respectively (100.5±16.5) and (31.2±1.7); phospholipid (PL), (143.3±7.8) and (88.9±3.2); total cholesterol, (88.7±4.6) and (51.9±2.3); and liver TG content of control and DEHP group increased compared to control (P<0.05), in which their increases were approximately by 4.35- and 2.33-folds, respectively.

Conclusion: The di (2-ethyl hexyl) phthalate medium dose attenuates lipids secretion from the liver cells into the bloodstream. The increase of liver PL level accompanied with the promotions of malic enzyme and the CPT-1 activities are the key factors of the dietary di (2-ethyl hexyl) phthalate effects in rats to attenuate the lipid secretions from the livers.

Key words: Di (2-ethyl hexyl) phthalate, hyperphospholipids, lipolysis, liver lipids, serum lipids

Di (2-ethyl hexyl) phthalate is the most widely used plasticizer in polyvinyl chloride plastic. It was reported that di (2-ethyl hexyl) phthalate used in blood storage bags leaches out in significant amounts into the blood stored and the blood products resulted from exposure of patients to this compound during transfusion.1-3 It was also reported that after 21 days storage of blood used to transfuse in human recipients, the blood storage bags averagely leaches out 10 mg di (2-ethyl hexyl) phthalate/100 mL blood. Furthermore, a number of reports are available on the toxicity of di (2-ethyl hexyl) phthalate, particularly studied in the liver.4-7 Almost all of those studies carried

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out the treated doses reached 200mg/100g body weight (BW). Gayathri et al.6 administered the rats with a dose of 0.75 mg/100g BW which is equivalence with transfusion of ten unit of blood in a human recipient. These authors did not find the serious toxic effects as evidenced by lack of any histopathological changes in the liver or significant alterations in many biochemical parameters. Overall, the doses of di (2-ethyl hexyl) phthalate that had been treated have ranges 750μg-200mg per 100g BW. Therefore, the dose of 75 mg di (2-ethyl hexyl) phthalate/100g BW that was used in the present study might be considered medium dose.

Currently, di (2-ethyl hexyl) phthalate, a phthalate plasticizer, belongs to a peroxisome proliferators class of rodent nongenotoxic hepatocarcinogens.7,8 This phthalate modulates the peroxisome proliferators-activated receptor (PPAR).9 PPARα is known as lipid-activated transcription factors expressed in the liver that belongs to the nuclear hormone superfamily. Numerous authors7,9 reported that di (2-ethyl hexyl) phthalate is the essential transcription factors regulating key cellular functions that include lipid metabolism.

Some biochemical parameters in lipid metabolism such as serum lipid profiles are constantly altered during normal states or disorders of metabolisms. Very-low density lipoprotein is a lipoprotein handling the lipid transportation from the synthesized lipid in the liver into extra hepatic tissues. Hence, serum lipid profiles generally indicate how lipid metabolism occurred in the liver. Commonly, disorders of lipid metabolism in the liver such as fat infiltration induce hepatic steatosis.10-12 The impacts of the hepatic steatosis is similar to those seen in patients with alcoholic liver disease and range from mild hepatic steatosis to steatohepatitis, liver fibrosis, and cirrhosis,13 and, rarely, to hepatocellular carcinoma.14

Considering di (2-ethyl hexyl) phthalate is widely used in consumer products in common society such as food packaging materials and children’s toys15 and used for tubing and containers for blood transfusions and blood products, etc., di (2-ethyl hexyl) phthalate constantly and directly or indirectly interacts with human and animal health cells. Therefore, its effect on lipid profiles in serum and liver tissue is of interested to evaluate. The present study was conducted to elucidate the metabolic effects of di (2-ethyl hexyl) phthalate medium dose on lipid profiles in serum and liver tissue using sprague-dawley (SD) rats as animal model.

METHODS

Animals and experimental design

All aspects of the experiment were conducted according to guidelines provided by the ethical committee of experimental animal care at Saga University (Saga, Japan). Male SD-rats aged 5 weeks were housed individually in an air-conditional room (24oC) with a 12-h light/dark cycle. After one week acclimatization, rats were assigned to two groups (five rats each). Control diet (as control group) was prepared according to recommendations of the American Institute of Nutrition (AIN) and contained (in weight %) 20 of casein, 10 of safflower oil, 1 of vitamin mixture (AIN-93), 3.5 of mineral mixture (AIN-93), 10 of sucrose, 0.25 of choline bitartrate, 0.3 of L-Cystein, 0.0014 of t-BHQ, 5 of cellulose, 13.2 of α-cornstarch, and β-cornstarch to make 100. The di (2-ethyl hexyl) phthalate diet (as DEHP group) was prepared by replacement of 1.0% β-cornstarch with di (2-ethyl hexyl) phthalate to the control diet. Considering evidences in food intake and final BW of the dietary di (2-ethyl hexyl) phthalate in male SD-rats, the 1% of food intake is equally to a range of 73-77 mg di (2-ethyl hexyl) phthalate/100g BW dose. The animals received the diets for 10 days. At the end of the feeding period, rats were killed by decapitation after a 9-h starvation. Livers were excised immediately, and serum was separated from blood.

Analyses of serum and liver lipids

Liver lipids were extracted according to the method of Folch et al.,17 and concentrations of TG, cholesterol, and phospholipids (PL) were measured by the methods used elsewhere.10-12,18,19 Serum TG, PL, cholesterol, and glucose were measured using enzyme assay kits from Wako Pure Chemicals according to the manufacture’s instructions.

Preparation of liver sub cellular fractions

The mitochondrial and cytosol of liver sub cellular fractions were prepared as previously reported by Nagao et al.19 Protein content was determined by the method used in our previous studies.10-12,18,19

Assays of hepatic enzyme activity

The malic enzyme (ME, EC1.1.1.40), the carnitine palmitoyl transferase-1 (CPT-1; EC2.3.1.23), glucose 6-phosphate dehydrogenase (G6PDH; EC1.1.1.49), fatty acid synthase (FAS; EC2.3.1.85), phosphatidate phosphohydrolase (PAP, EC3.1.3.4) activities were
determined by the methods used in our previous studies.\textsuperscript{10-12,18,19} The glutathione peroxidase (GSH-Px; EC1.11.1.9) was determined by the methods used elsewhere.\textsuperscript{20,21}

**Statistical analyses**

All values are expressed as mean ± standard error of the mean (SEM). Data were analyzed by one-way analysis of variance, and all differences were inspected by Duncan’s new multiple-range test using SPSS statistical software (SPSS inc., Chicago, IL, USA).

**RESULTS**

**Dietary di (2-ethyl hexyl) phthalate promoted liver weight**

The daily food intake is shown in Table 1. The food intake of DEHP group decreased in comparison to control. The low level of food intake in the group was equivalent with the reduction of body weight. However, the weights of liver were significantly higher than that of the control group (P<0.05).

<table>
<thead>
<tr>
<th>Group</th>
<th>Control</th>
<th>DEHP*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial body weight (g)</td>
<td>132.8 ± 3.4</td>
<td>134.6 ± 2.5</td>
</tr>
<tr>
<td>Final body weight (g)</td>
<td>206.1 ± 3.5$^a$</td>
<td>188.4 ± 6.3$^b$</td>
</tr>
<tr>
<td>Food intake (g/day)</td>
<td>18.7 ± 0.8$^a$</td>
<td>14.1 ± 0.8$^b$</td>
</tr>
<tr>
<td>Liver weight (g/100 g body weight)</td>
<td>4.0 ± 0.1$^a$</td>
<td>6.7 ± 0.2$^b$</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM of five rats. Clearly define a & b indicates significant difference at P < 0.05. *DEHP, di(2-ethyl hexyl) phthalate

**Effects of di (2-ethyl hexyl) phthalate on glucose blood level, serum and liver lipid levels**

As shown in Figure 1, the lipid levels in serum of DEHP group decreased significantly (P<0.05), in which serum TG, PL, total cholesterol, and HDL-cholesterol levels decreased approximately by 70%, 38%, 41%, and 46%, respectively. Although failed to reach significant level, the glucose blood level decreased by approximately 9%.

![Figure 1. Serum lipids and glucose levels](image)

Values are expressed as mean± SEM of five rats. Clearly define a & b regarding difference of significance at P < 0.05.
Figure 2 shows the differences in liver lipid contents. Dietary di (2-ethyl hexyl) phthalate promoted significantly the liver PL content than that of the control group (P<0.05). The level was promoted approximately by 15%. Both liver TG and liver cholesterol contents, however, decreased significantly in the group (P<0.05). The decreases were approximately by 73% and 44%, respectively.

**Effects of di (2-ethyl hexyl) phthalate on the activities of malic enzyme and carnitine palmitoyl transferase-1**

Figure 3 shows the activities of enzymes determined in present study. Compared to control, both malic enzyme and CPT-1 activities of DEHP group increased significantly (P<0.05), in which the increases were approximately by 4.35- and 2.33-folds, respectively. The activities of G6PDH and PAP slightly increased that were approximately by 12% and 5%, respectively. However, FAS activity decreased slightly. Furthermore, the activity of antioxidant enzyme represented by GSH-Px decreased significantly (P<0.05).
DISCUSSION

Di (2-ethyl hexyl) phthalate modulates the PPAR\(^\alpha\) and is an essential transcription factors regulating lipid metabolism.\(^7\)\(^,\)\(^9\)\(^,\)\(^16\) The present study found that orally intake of 1.0\% di (2-ethyl hexyl) phthalate diet reduced markedly serum lipid level. The intake also attenuated liver TG and cholesterol contents, however promoted liver phospholipids level. The malic enzyme and CPT-1 activities increased markedly. The promotions of these enzyme activities and the liver phospholipids level are the key factors of the metabolic effects of di (2-ethyl hexyl) phthalate in attenuations of serum lipids and the liver TG and cholesterol contents.

The di (2-ethyl hexyl) phthalate dose applied in present treatment was 1.0\% of food intake; and by considering those food intakes and the body weights of the animal treated (Table 1), the dose of the present treatment was of 75 mg di (2-ethyl hexyl) phthalate per 100 g BW. This dose was in the range of currently treated which is 750 μg-200 mg per 100 g BW.\(^6\) As shown in Table 1, the liver weight of DEHP group increased significantly compared to control (P<0.05). The decrease in final body weight however might be suitable with low food intakes of the rats in the group. Overall, the applied 75 mg di (2-ethyl hexyl) phthalate/100 g BW dose to the rats developed hepatomegaly.

Hepatomegaly is known as an abnormal enlargement of the liver. The enlargements of the liver size by this treatment (Table 1) indicate a disorder of metabolism including lipid metabolism in the liver tissue. Therefore, serum and liver lipids levels were important to determine. The decreases of serum lipid levels (Fig. 1) suggested that dietary di (2-ethyl hexyl) phthalate attenuated lipids secretion from the liver tissue into the bloodstream. The decrease of lipid levels secreted from the liver therefore might cause their content in the liver decreased. The decrease of liver TG and cholesterol content accompanied with a promotion of liver PL level (as shown in Fig. 2) is consistent with the reports of Yanagita et al.\(^16\) that intake of 1\% di (2-ethyl hexyl) phthalate in rats induces hyperphospholipids of the liver tissue but attenuates TG and cholesterol levels. Therefore, di (2-ethyl hexyl) phthalate intake in the present study attenuated the serum lipid level and also the liver TG and cholesterol levels, but promoted liver PL content.

The increased PL content of liver tissue might indicate a promotion of PPAR\(\alpha\), the PPAR expressed in the liver. Burns and Heuvel\(^22\) reported that phosphorylation modulates PPAR\(\alpha\) and affects these receptors activities to control a variety of target genes involved in lipid homeostasis. Latruffe et al.\(^23\) and Howarth et al.\(^24\) found that di (2-ethyl hexyl) phthalate administered to rats lead to increase the volume and density of peroxisomes in the liver cells. Both those later authors also reported that during peroxisome proliferations, di (2-ethyl hexyl) phthalate stimulate the transcription of genes encoding enzymes of lipid metabolism, particularly fatty acid beta-oxidation in a PPAR-dependent manner. Our previous study found that the increased fatty acid degradation is associated with the promotion of liver PL level.\(^11\) The promotions of liver PL level might indicate that those receptors are activated and therefore fatty acid degradation increased. The increased fatty acid degradation (Figure 3A) accompanied with unchanged activities of FAS and PAP between the groups might indicate a decrease of fatty acyl-CoA level, a substrate provided for TG biosynthesis; therefore liver TG content decreased (Figure 2). Furthermore, the increased fatty acid degradation in the liver tissue might indicate an enhancement of Krebs cycle to deliver adenosine triphosphate. The enhancement of Krebs cycle rate can be elucidated from some parameters of anaplerotic reactions. Anaplerotic reaction is a filling up mechanism of Krebs cycle catalyzed by several enzymes. One biocatalyst involved in this mechanism is malic enzyme. Malic enzyme converses pyruvate to malate, a key molecule in nucleic acid nucleotide formations. This mechanism was reasonable because serum glucose level decreased (Figure 1) although it failed to reach significant level (P<0.05). Hence, the decreased serum glucose level might indicate a promotion of glucose uptake by the liver cell as results of gradient concentration occurred between bloodstream and liver cytosolic cellular fractions. Furthermore, the decreased activities of antioxidant enzyme represented by GSH-Px might indicate that toxicity of medium dose of di (2-ethyl hexyl) phthalate used in present treatment might still appear in liver. Overall, considering all alterations of those enzyme activities, the flow chart
In conclusion, the ingestion of di (2-ethyl hexyl) phthalate in rats decreased levels of serum and liver lipids, except liver phospholipids. The promotion of liver phospholipids content induced activities of malic enzyme and CPT-1. Both these enzymes play essential roles in metabolic responses regulated by the di (2-ethyl hexyl) phthalate in lipid metabolism. The application of di (2-ethyl hexyl) phthalate in accurately dose might be one of the beneficial strategies in development of hypolipidemic drugs to secure the vessel blood from the attached lipids.

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REFERENCES


