Elucidation of the Toxic Mechanism of the Plasticizers, Phthalic Acid Esters, Putative Endocrine Disrupters: Effects of Dietary Di(2-ethylhexyl)phthalate on the Metabolism of Tryptophan to Niacin in Rats

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We have previously reported that the administration of a large amount of di(n-butyl)phthalate (DBP) increased the conversion ratio of tryptophan to niacin in rats. In the present experiment, the effect of di(2-ethylhexyl)phthalate (DEHP) on the conversion ratio and how altering the conversion ratio of tryptophan to niacin depended on the concentration of DEHP were investigated to elucidate the toxic mechanism of phthalic acid esters (PhE). Rats were fed with a diet containing 0%, 0.01%, 0.05%, 0.1%, 0.5%, 1.0%, or 3.0% DEHP for 21 days. To assess the conversion ratio of tryptophan to niacin, urine samples were collected at the last day of the experiment and measured for metabolites on the tryptophan-niacin pathway. The conversion ratio increased with increasing dietary concentration of DEHP above 0.05%; the conversion ratio was about 2% in the control group, whereas it was 28% in the 3.0% DEHP group. It is suggested that the inhibition of α-amino-β-carboxymuconate-ε-semialdehyde decarboxylase (ACMSD) by DEHP or its metabolites caused this increase in the conversion ratio. We conclude that PhE such as DEHP and DBP disturbed the tryptophan-niacin metabolism.

Key words: phthalic acid ester; di(2-ethylhexyl)phthalate; conversion ratio of tryptophan to niacin; endocrine disrupter; niacin toxicity

Phthalic acid esters (PhE) are used in the industrial production of lubricants, glues, insect repellents, dielectric fluids, and plastics.1) Among the phthalate esters, di(2-ethylhexyl) phthalate (DEHP) is one of the most frequently used additives in the manufacture of elastic polyvinyl chloride.2) Many studies have demonstrated that treating rats with DEHP induced testicular atrophy with liver enlargement,3) although the precise nature and mechanism for the action of DEHP on these organs has remained unclear.

To clarify the mechanism for the toxicity of PhE, Shibata et al.4) have found that the growth of weaning rats fed with a niacin-free and tryptophan (Trp)-limited diet was promoted by the administration of di-n-butylphthalate (DBP). Furthermore, they revealed that the conversion of Trp to niacin was significantly promoted by feeding with the DBP diet.5) However, it is not known whether this is common phenomenon associated with PhE, since the effects of PhE on living bodies are not the same; for example, DEHP induced liver enlargement and proliferation of hepatic peroxisomes, while di-n-hexylphthalate caused no peroxisome proliferation but caused fat accumulation in the liver.6) We therefore investigated whether or not DEHP, one of the most frequently used PhE, would also increase the conversion ratio of Trp to niacin, and how this conversion ratio depended on the amount of dietary DEHP administrated.

Materials and Methods

Chemicals. NAD+ was purchased from Sigma Chemical Company (St. Louis, MO, U.S.A.). Vitamin-free milk casein, sucrose, t-methionine, Nam, Trp, and AnA were purchased from Wako Pure Chemical Industries (Osaka, Japan). Kynurenic sulfates, MNA chloride, XA, KA, and 3-HA were obtained from Tokyo Kasei Kogyo (Tokyo, Japan). 2-Py and 4-Py were respectively synthesized by the methods of Pullman and Colowick7) and of Shibata et al.8) The mineral and vitamin mixtures were obtained from Oriental Yeast Kogyo (Tokyo, Japan). All other chemicals were used of the highest purity available from commercial sources.

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Abbreviations: DEHP, di(2-ethylhexyl)phthalate; PhE, phthalic acid esters; DBP, di-n-butylphthalate; Trp, L-tryptophan; NiA, nicotinic acid; Nam, nicotinamide; MNA, N′-methyl nicotinamide; 2-Py, N′-methyl-2-pyridone-5-carboxamide; 4-Py, N′-methyl-4-pyridone-5-carboxamide; AnA, anthranilic acid; KA, kynurenic acid; XA, xanthurenic acid; 3-HA, 3-hydroxyanthranilic acid; QA, quinolinic acid; ACMSD, α-amino-β-carboxymuconate-ε-semialdehyde decarboxylase

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Animals and diets. The animal room was maintained at a temperature of around 22°C and at about 60% humidity with a 12-hr light/12-hr dark cycle. Body weight and food intake were measured daily at around 10:00 a.m., and food and water were renewed daily.

The care and treatment of the experimental animals conformed to The University of Shiga Prefecture guidelines for the ethical treatment of laboratory animals. Male rats of the Wistar strain (6 weeks old) were obtained from Clea Japan (Tokyo, Japan) and immediately placed in individual metabolic cages (CT-10; Clea Japan). They were then divided into seven groups, and fed ad libitum for 21 days (Table 1). Urine samples on the last day (10:00 a.m. to 10:00 a.m. 24-hr urine) were collected in amber bottles with 1 ml of 1 M HCl and stored at -25°C until needed for use. The rats were killed by decapitation after the last urine samples had been collected, a 10-μl sample of blood was taken from the carotid artery, and the liver was removed.

Analysis. The contents of Nam, 2-Py, and 4-Py in the urine were simultaneously measured by the HPLC method of Shibata et al., while the content of MNA in the urine was measured by the HPLC method of Shibata. The contents of KA,XA, 3-HA and Ana in the urine were measured by conventional HPLC methods.

The concentrations of NAD (NAD⁺ + NADH) and NADP (NADP⁺ + NADPH) in the blood and liver were respectively measured by the colorimetric method of Shibata and Murata, and by the method of Shibata and Tanaka.

Statistical analysis. Each data value is expressed as the mean ± SEM. Differences between groups were determined by ANOVA. If significance was indicated, the Student-Newman-Keuls post hoc test was used to determine where the significance occurred, P<0.05 being considered statistically significant.

Results

Effect of various concentrations of DEHP on the body weight gain, food intake, and liver weight A 0.01-0.5% amount of DEHP in the diet did not affect the body weight or food intake (Figs. 1(A) and 1(B)). The body weight gain and food intake of the rats fed on 1.0% and 3.0% DEHP were significantly lower than in the other groups (Figs. 1(A) and 1(B)). The liver weight increased with increasing DEHP concentration (Fig. 1(C)).

Effect of various concentrations of DEHP on the NAD and NADP contents in the liver and blood

The liver content of NAD (NAD⁺ + NADH) was significantly higher in the 0.1-3.0% DEHP groups, but not in the 0.01% and 0.05% groups, than in the control group (Fig. 2(A)). 0.5-3.0%, but not 0.01-0.1%, DEHP in the diet increased the liver content of NADP (NADP⁺ + NADPH) (Fig. 2(B)).

The concentrations of blood NAD and NADP were not affected by feeding DEHP (Figs. 2(C) and 2(D)).

Effect of various concentrations of DEHP on the urinary excretion of upper metabolites on the Trp-niacin pathway

The urinary outputs of anthranilic acid (AnA), kynurenic acid (XA), xanthurenic acid (XA), and 3-hydroxyanthranilic acid (3-HA) are expressed in terms of nmol/g of diet, since the food intake was not the same among the groups. The AnA output to urine was not affected in the 0.01-1.0% DEHP groups, XA was not affected in the 0.01-0.5% groups, and KA and 3-HA were not affected by DEHP (Fig. 3).

Effect of various concentrations of DEHP on the urinary excretion of lower metabolites on the Trp-
niacin pathway

The urinary outputs of quinolinic acid (QA), nicotinamide (Nam), N'-methylnicotinamide (MNA), N'-methyl-2-pyridone-5-carboxamide (2-Py), and N'-methyl-4-pyridone-5-carboxamide (4-Py) are also expressed in terms of nmol/g of diet. Under the present experimental conditions, QA, Nam, MNA, 2-Py, and 4-Py originated from dietary Trp, since the diets were niacin-free. The urinary output of QA formed from 3-HA increased with increasing dietary concentration of DEHP (Fig. 4(A)), although the urinary 3-HA output was not affected (Fig. 3(D)). Nam is a direct catabolic metabolite of NAD and NADP, Nam being metabolized to MNA which is then metabolized to 2-Py or 4-Py. MNA, 2-Py, and 4-Py are not niacin-active compounds. The outputs of these compounds were also increased by feeding DEHP (Figs. 4(B), 4(C), 4(D) and 4(E)).

Effect of various concentrations of DEHP on the conversion ratio of Trp to niacin

The conversion ratio of Trp to niacin increased with increasing dietary DEHP concentration (Fig. 5). A severely adverse effect was observed in the group...
Fig. 3. Comparison of the Urinary Excretion of AnA (A), KA (B), XA (C), and 3-HA (D) by Rats Fed on Diets Containing Various Amounts of DEHP.
Each value is the mean ± SEM (n = 5 per group). A different superscript letter means significant difference at p < 0.05.

Fig. 4. Comparison of the Urinary Excretion of QA (A), Nam (B), MNA (C), 2-Py (D), and 4-Py (E) by Rats Fed on Diets Containing Various Amounts of DEHP.
Each value is the mean ± SEM (n = 5 per group). A different superscripts letter means significant difference at p < 0.05.
fed with the 3.0% DEHP diet as determined by the data for the body weight and food intake (Fig. 1). Under these conditions, the conversion ratio reached 28% (Fig. 5), this value being about 14-fold higher than the value of 2% in the control group.

Discussion

We have previously reported that DBP, a plasticizer and phthalic acid ester, increased the conversion ratio of Trp to niacin,\(^3\) and that the growth of weaning rats fed with a niacin-deficient diet was significantly promoted by adding DBP.\(^4\) In the present study, DEHP also increased the conversion ratio with increasing concentration of more than 0.05%. The addition of 1% DBP to the niacin-free, 20% casein diet increased the conversion ratio five-fold,\(^5\) while 1.0% DEHP increased it seven-fold, and 3.0% DEHP increased it fifteen-fold. These findings demonstrate that feeding a phthalic acid ester such as DBP and DEHP disturbed the Trp-niacin metabolism. Whether other phE however, would affect the Trp-niacin metabolism remains to be elucidated.

Trp-niacin metabolism follows two pathways, the Trp-\(\alpha\)-amino-\(\beta\)-carboxymuconate-\(\epsilon\)-semialdehyde (ACMS) pathway and ACMS-niacin pathway, since the Trp-niacin pathway branches at ACMS into the ACMS-acetyl CoA pathway and ACMS-niacin pathway. ACMS metabolized to \(\alpha\)-aminomuconate-\(\epsilon\)-semialdehyde is led to the ACMS-acetyl CoA pathway by aminocarboxymuconate-semialdehyde decarboxylase (ACMSD), while ACMS is spontaneously cyclized to QA.\(^5\) It is considered that QA formation and QA metabolism are the important regulatory events on the Trp-niacin pathway, and that ACMSD and quinolinate phosphoribosyltransferase (QPRT) are the rate-limiting enzymes on the Trp-niacin pathway.\(^6\) In the present study, the urinary excretion of the metabolites, AnA, KA, XA and 3-HA, on the Trp-ACMS pathway was not affected by feeding DEHP, whereas DEHP feeding increased the metabolites, QA, Nam, MNA, 2-Py and 4-Py, on the ACMS-niacin pathway. These results suggest that DEHP affected ACMSD to form QA. DEHP or its metabolites may inhibit ACMSD activity or gene expression of ACMSD. Although phthalic acid, a hydrolysis product of DEHP, inhibited QPRT activity,\(^20\) DEHP seem unlikely to have decreased the formation of lower metabolites from QA. In fact, feeding DBP did not affect the liver QPRT activity.\(^5\)

Some amount of niacin is needed to maintain a healthy body, but an excess of niacin causes an adverse effect.\(^26\) Supplementation of the diet with 1% Nam in rats caused growth inhibition.\(^25\) A similar phenomenon has been reported that growth retardation was observed with about 50 mg (400 \(\mu\)mol) of Nam/100 g of body weight/day in rats.\(^26\) 3.0% DEHP in the diet increased the conversion ratio of Trp to niacin to 28%. This means that about 16 \(\mu\)mol/100 g of body weight/day of niacin was biosynthesized to form Trp, whereas the rats fed on the control diet biosynthesized 1.6 \(\mu\)mol of niacin/100 g of body weight/day. In the case of administering excess niacin, most of this niacin is soon excreted into the urine, and the utilization rate is low.\(^27\) Therefore, whether or not the amount of niacin causes an adverse effect might depend on its origin, i.e., endogenously synthesized or exogenously administered. Although little is known about the relationship between the amount of biosynthesized niacin and its adverse effect, there is the possibility that the increased conversion ratio by DEHP causes an adverse effect. DEHP induced testicular atrophy with liver enlargement.\(^5\) However, it is unclear whether excess biosynthesized niacin affected the testis or liver. More work is needed to clarify the relationship between the effect of DEHP and niacin metabolism.

In summary, the conversion ratio of Trp to niacin increased with increasing on the dietary concentration of DEHP. This phenomenon was observed at a DEHP concentration of more than 0.05%. Thus, DEHP activated the ACMS-niacin pathway, and not the Trp-ACMS pathway, suggesting the inhibition of ACMSD by DEHP or its metabolites. We conclude that such phE compounds as DEHP and DBP disturbed the Trp-niacin metabolism.

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References


