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Research Article

Effect of dilute Ethanol Intake on DEHP (di (2-ethylhexyl) phthalate)-induced Testicular Atrophy

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Abstract

Background: Di(2-ethylhexyl) phthalate (DEHP) is the most commonly used as a plasticizer for polyvinyl chloride (PVC), but recently, concern has arisen over the DEHP which may act as a reproductive toxicant to humans. On the other hand, ethanol is the most common supplement of beverages and foods, and so many persons ingest a large quantity of ethanol in daily life. However, interactions between ethanol and DEHP toxicity are not well known.

Method: To investigate the effect of dilute ethanol ingestion on the DEHP induced testicular atrophy, rats were received a 1% (w/w) DEHP diet and 2.5 or 5% (v/v) ethanol water for 7 days.

Result: The rats treated with DEHP-diet alone for 7 days were observed significant testicular weight loss. On the other hand, testicular weight loss was significantly suppressed in rats treated with DEHP diet and ethanol water. A significant negative correlation between relative testicular weight (as a percentage of body weight) and testicular MEHP concentration was found among rats treated with DEHP-free diet (Control) and DEHP diet alone. Most of the data plots for the DEHP diet plus ethanol water group were scattered above the regression line.

Conclusion: These results suggest that dilute ethanol may be effective in preventing DEHP testicular atrophy. However, the mechanism of prevention is unknown and further research is needed.

Keywords: di(2-ethylhexyl) phthalate (DEHP); ethanol; mono (2-ethylhexyl) phthalate (MEHP); testicular atrophy

Introduction

Di(2-ethylhexyl) phthalate (DEHP), one of the phthalate esters is the most commonly used as a plasticizer for polyvinyl chloride (PVC), but recently, concern has arisen over the DEHP which may act as a reproductive toxicant to humans [1-8].

Many studies have shown the toxic effects of DEHP on male reproductive organs in the rodents at growth stages [9-19]. Although the pathogenesis of testicular atrophy by DEHP is yet unclear, this injury has been suggested to be due to oxidative stress induced by mono (2-ethylhexyl) phthalate (MEHP), a metabolite of DEHP [20].

Since PVC containing DEHP is widely used in manufacturing consumer goods, food containers, toys, and medical instruments, DEHP contamination is widespread, and the general population is exposed to DEHP via food, water, and air, which arouses concern about human reproductive hazards [21]. On the other hand, ethanol is the most common supplement of beverages and foods, and so many persons ingest a large quantity of ethanol in daily life. However, interactions between ethanol and DEHP toxicity are not well known. The present study has investigated the effects of ethanol ingestion on the DEHP induced testicular injury in rats.

Materials and methods

Experimental animals

Sprague-Dawley (SD) male rats (aged 3weeks) were purchased from Charles River (Kanagawa, Japan) and kept in the Laboratory Animal Center of Kagawa University. They were maintained at 22–24 °C and 50–60% relative humidity with a 12 h light-dark cycle. The experiment protocol had the approval of Kagawa University Animal Committee and the experiment was undertaken obeying the Guide for Animal Experimentation of the Kagawa University.

Chemicals

DEHP and ethanol was purchased from Wako pure chemical industries Ltd. (Osaka, Japan). The chemical purities of DEHP and ethanol were found to be >97% and >99.7%, respectively. CE-2 diets (Clea, Tokyo, Japan) containing DEHP by 1 or 2 w/w% were prepared by Oriental Yeast Company (Chiba, Japan). MEHP was purchased from Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan). All other chemicals were the highest grade from commercial sources.

Experimental protocol

In the first experiment, four-week-old rats weighing 100-130g were divided into a control and treatment groups consisting of at least 6 animals. The control group received a DEHP-free diet plus tap water for 7 days. The treatment groups received 1% (w/w) DEHP diet plus tap water or 2.5% (v/v) ethanol water, or DEHP-free diet plus 2.5% (v/v) ethanol water for 7 days [22].

In the second experiment, 5% (v/v) ethanol water was tested in the same grouping as in the first experiment.

At the end of each experiment, rats were sacrificed by ether anesthesia. The testis was removed and weighed. The left testis was immediately fixed in Bouin's fluid. Blood samples collected from the heart were collected into heparinized tubes and plasma was separated from whole blood by centrifugation at 1500 g. Right testis and plasma were frozen at -40 ° C until MEHP measurement [23, 32].

Plasma and testicular MEHP Measurement

The concentrations of MEHP in plasma and testis were measured by high performance liquid chromatography. Analytical procedure and equipment are as reported [23, 32].

Statistics

Data were analyzed by using the SPSS for Windows computing program. Results were expressed as means ± standard deviations (SD). Statistical analyses were performed using one-way ANOVA procedure followed by Turkey HSD tests to detect difference between groups. Differences were regarded as significant at $P < 0.05$.

Results

The average DEHP intakes per rat were 1.0, 1.0 and 1.0 g/kg/day for the DEHP plus tap water group, DEHP plus 2.5% ethanol group and DEHP plus 5% ethanol group, respectively. The average daily water intakes per rat were 4.1 ml, 9.2 ml (ethanol: 1.27g/kg/day) and 9.5 ml (2.74g/kg/day) for the DEHP plus tap water group, DEHP plus 2.5% ethanol group and DEHP plus 5% ethanol group, respectively. In each experiment, testis weight in rats fed a 1% DEHP diet for 7 days was significantly reduced (Table 1, 2). On the other hand, in the rats treated with DEHP diet and ethanol water testicular weight loss was significantly suppressed (Table 1, 2). A significant negative correlation between relative testicular weight (as a percentage of body weight) and testicular MEHP concentration was found among rats treated with DEHP-free diet (Control) and DEHP diet alone. Most of the data plots for the DEHP diet plus ethanol water group were scattered above the regression line (Figure 1, 2). As shown in Figure 3, Plasma and testicular MEHP levels were closely correlated. These results suggest that dilute ethanol may have an inhibitory effect on DEHP testicular atrophy.

	Group			
	Control	2.5%Ethanol	1%DEHP	1%DEHP + 2.5%Etanol
n	12	12	12	12
Initial body weight (g)	117.9 ± 5.3	122.8 ± 3.6	119.3.0 ±3.8	117.9 ± 4.3
Final body weight (g)	177.2 ± 10.6	180.1 ± 3.5	165.1 ± 5.4	168.7± 10.4
Testes (g)	1.62 ± 0.08***	1.74± 0.17***	1.02 ± 0.27	1.23 ± 0.27*
Relative testicular weight (%)	0.92 ± 0.06***	0.97 ± 0.07***	0.61 ± 0.16	0.73 ± 0.14*
Plasma MEHP (μg / ml)	0.1<	0.1<	45.1 ±17.7	46.4± 15.8
Testicular MEHP (μg / g)	0.1<	0.1<	5.5 ± 2.4	8.0 ± 3.2

Table 1. *Body and testes weights and plasma and testicular MEHP concentrations in the rats exposed to 1 % (w/w) DEHP diet and 2.5 % (v/v) ethanol water for 7 days. * p < 0.05, *** p<0.001, as compared to DEHP alone group. Results were expressed as means ± standard deviations (SD).*

	Group			
	Control	5%Ethanol	1%DEHP	1%DEHP + 5%Etanol
n	12	6	12	12
Initial body weight (g)	108.6 ± 7.8	111.1 ± 12.9	109.0 ±10.2	111.2 ± 8.2
Final body weight (g)	172.8 ± 13.2	168.9 ± 13.2	158.9 ± 8.3	162.4± 10.8
Testes (g)	1.56 ± 0.16***	1.61± 0.15***	1.00 ± 0.16	1.21 ± 0.20*
Relative testicular weight (%)	0.90 ± 0.09***	0.95 ± 0.06***	0.63 ± 0.11	0.75 ± 0.13*
Plasma MEHP (μg / ml)	0.1<	0.1<	51.3 ±13.5	47.3 ± 12.4
Testicular MEHP (μg / g)	0.1<	0.1<	5.4 ± 1.6	5.6 ± 2.2

Table 2. *Body and testes weights and plasma and testicular MEHP concentrations in the rats exposed to 1 % (w/w) DEHP diet and 5 % (v/v) ethanol water for 7 days. * p < 0.05, *** p<0.001, as compared to DEHP alone group. Results were expressed as means ± standard deviations (SD).*

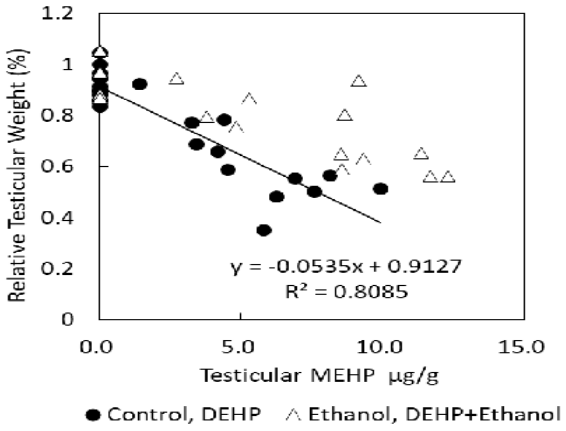

Figure 1. *Relationship between relative testicular weight and testicular MEHP concentration. Control and DEHP-alone groups: Closed circle, 5% ethanol and DEHP treatment groups: Open triangles.*

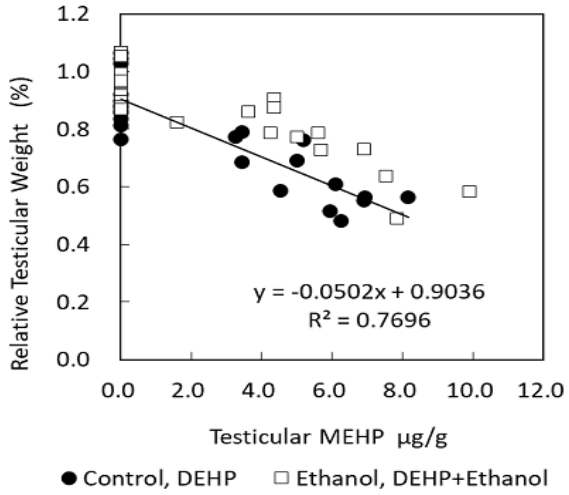

Figure 2. *Relationship between relative testicular weight and testicular MEHP concentrations. Control and DEHP-alone groups: Closed circle, 5% ethanol and DEHP treatment groups: Open squares.*

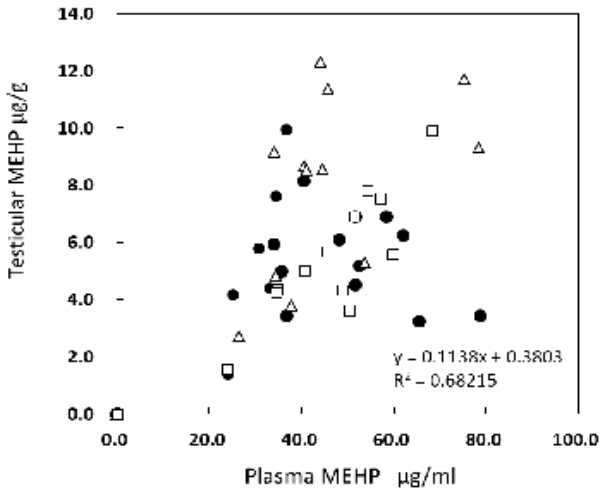

Figure 3. *Relationship between plasma MEHP concentration and testicular MEHP concentration. Control and DEHP-alone groups: Closed circle, 5% ethanol and DEHP treatment groups: Open triangles, 5% ethanol and DEHP treatment groups: Open squares.*

Discussion

DEHP administered orally is rapidly hydrolyzed by pancreatic lipase [24] and mucosal esterase [25] in the gastrointestinal tract to mono-(2-ethylhexyl) phthalate (MEHP), which is the most toxic metabolite of DEHP. MEHP has been shown to be a direct acting agent that disrupts the function of Sertoli and Leydig cells and also induces oxidative stress in the germ cells [26, 27, 20] leading to cellular apoptosis. On the other hand, ethanol is known to have a hydroxyl radical scavenging effect [28-31]. However, chronic high levels of alcohol exposure in male animals are associated with testicular atrophy and gonadal insufficiency [32, 33]. In this study, testicular weight loss was significantly suppressed in rats treated with a DEHP diet and dilute ethanol water. Previously, we reported that radical scavengers such as antioxidant vitamins and rare sugars could prevent DEHP-induced testicular atrophy [22, 23, 34-36]. The significant but not complete inhibitory effect on DEHP induced testicular atrophy may reflect the radical scavenging effect of short-term alcohol intake.

Conclusion

This study suggests that dilute ethanol may be effective in preventing DEHP testicular atrophy. However, the mechanism of prevention is unknown and further research is needed.

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Disclosure of conflict of interest

There is no conflict of interest in this work.

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