

NOTE

Possible Reproductive Toxicity of Styrene in Peripubertal Male Mice

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Abstract. Environmental estrogens (endocrine disruptive chemicals) have been shown to affect reproduction in wild life and it has been reported that maternal exposure to those chemicals has adverse effects on the male reproductive tract. However, little is known about the potential effects of prepubertal or pubertal exposure to environmental estrogens on the male reproductive tract. Here we examine plasma hormone levels of mice following 4-week oral administration of styrene. Plasma free testosterone levels were dramatically decreased following 4 weeks of styrene treatment compared with control group. No differences in plasma corticosterone and luteinizing hormone levels were seen between styrene and control groups. Thus, exposure to styrene around pubertal period may directly disrupt the male reproductive tract. These facts suggest that more detailed studies regarding assessment of the risk to the developing human testes from exposure to styrene and other environmental estrogens in prepubertal and pubertal period are warranted.

Key words: Corticosterone, Free testosterone, Luteinizing hormone, Styrene monomer, Testis
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STYRENE is an extremely important commodity chemical used extensively in the manufacture of numerous polymers and copolymers, including polystyrene, acrylonitrile-butadiene-styrene, styrene-acrylonitrile, styrene-butadiene latex, and styrene-butadiene rubber. Styrene is a component of cigarette smoke and automobile exhaust, and it may occur naturally at low levels in various types of foods [1]. The biomedical effects of styrene have been studied both experimentally and epidemiologically. There are extensive publications on the mutagenicity and carcinogenicity [2–6]. Recently, migration of styrene from thermoset polyester cookware into foods has been studied during normal cooking applications, with testing for 2 h at 175°C into olive oil resulting in significantly higher migration of styrene

than seen for other foods [7]. Although recent reports demonstrated that exposure to styrene monomer significantly affected behavior and development in rats [8, 9], little is known about the potential direct effects of prepubertal or pubertal exposure to styrene to the male reproductive tract. In the present study, we evaluated the effects of styrene on the male reproductive tract of young mice following 4-week oral administration with low and high doses by measuring plasma levels of free testosterone (FT), corticosterone (B), luteinizing hormone (LH).

Material and Methods

Animals

All studies were carried out in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by Kochi Medical School. Male C57BL/6 mice (5 weeks old) were purchased from JAPAN SLC Inc. (Shizuoka, Japan).

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All animals were housed individually in a light (12 h on/off) and temperature-controlled room, with food and water available *ad libitum*.

Styrene treatment

Styrene monomer was purchased from Nakarai Chemical Co. (Tokyo, Japan). Styrene monomer was finally dissolved in 0.005% ethanol. Water, 0.005% ethanol (control), styrene monomer (5 µg/ml) or styrene monomer (50 µg/ml) were administered p.o. (as drinking water) for 4 weeks. In order to keep the concentration of styrene monomer stable, we changed the drinking water twice per week although the exact stability of styrene monomer is not known. The beds of mice were kept clean to avoid the exposure of styrene monomer through the skin. The mice were sacrificed by decapitation and both testes and spleen were weighed. One testis per mouse was taken for the histological studies with hematoxylin-eosin staining. Trunk blood was collected in siliconized 1.5 ml eppendorf tubes containing disodium EDTA. Whole blood was centrifuged in a TOMY microfuge for 10 min at 3000 rpm to separate red blood cells from plasma.

Plasma free testosterone, corticosterone and luteinizing hormone levels

Plasma free testosterone, corticosterone and luteinizing hormone levels were measured using commercially available RIA and EIA assay kits (DPC Corp., Los Angeles, CA; ICN Biomedicals Inc., Costa Mesa, CA and Amersham International plc, Buckinghamshire, England, respectively). The inter- and intra-assay coefficients of variation were 3.7%

and 4.0% at 19 pg/ml for free testosterone, 6.5% at 150 ng/ml and 7.1% at 166 ng/ml for corticosterone and were 10.9% at 17.2 ng/ml and 10.7% at 16 ng/ml for LH assay, respectively.

Data analysis

Data were analyzed by ANOVA followed by Fisher's protected least squares difference test.

Results

The average water intake, body weight, testis and spleen weight in control and styrene treated groups are shown in Table 1. The parameters such as average water intake, body weight, testis weight, testis weight/body weight, spleen weight were not different among control, styrene monomer 5 µg and 50 µg/ml group in 4-week treatment. Spleen weight/body weight in styrene monomer 5 µg and 50 µg/ml group in 4-week treatment was significantly higher than in control group. No differences in all parameters were seen between water and control groups following 4 weeks of the treatment (data not shown).

Styrene monomer (5 µg/ml) did not affect plasma free testosterone levels following 4 weeks of treatment. In contrast, plasma free testosterone levels were dramatically decreased following 4 weeks of styrene monomer (50 µg/ml) treatment compared with the control group (Fig. 1). No differences in plasma free testosterone were seen between water and control groups following 4 weeks of treatment (data not shown). In addition, no differences in histological studies with hematoxylin-eosin staining were seen

Table 1. Effect of 4-week oral administration of styrene monomer on average water intake, body weight, testis weight and spleen weight in male C57/BL6 mouse

	Ave. water intake (ml/day)	Body weight (g)	Testis weight (mg)	Testis weight/ Body weight (%)	Spleen weight (mg)	Spleen weight/ Body weight (%)
Control	5.77 ± 0.19	23.7 ± 0.3	222.7 ± 4.1	0.939 ± 0.007	66.4 ± 2.1	0.280 ± 0.006
Styrene monomer 5 µg/ml	5.58 ± 0.11	23.0 ± 0.4	211.9 ± 6.4	0.921 ± 0.023	69.6 ± 2.1	0.302 ± 0.007*
Styrene monomer 50 µg/ml	5.77 ± 0.12	23.7 ± 0.4	224.9 ± 7.0	0.950 ± 0.023	70.6 ± 1.5	0.298 ± 0.003*

*p < 0.05 vs Control

Data represent the mean ± SEM (n = 7). Note: Body weight was measured at 9 weeks of age for the 4-week styrene monomer treatment.

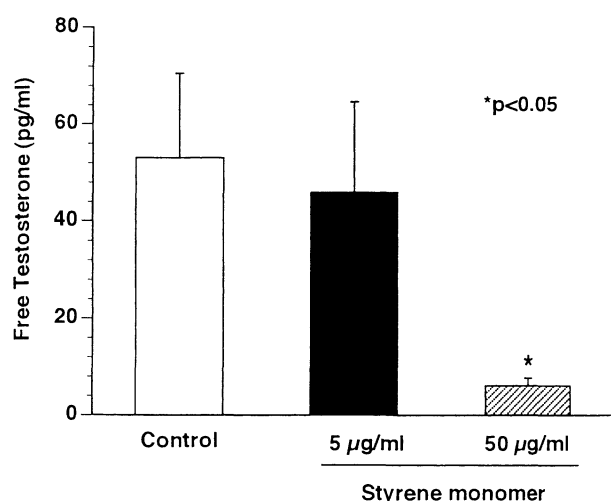


Fig. 1. Effects of styrene treatment for 4 weeks on plasma free testosterone levels in male C57BL/6 mice. Styrene treatment was performed as described in Methods. Data represent the mean \pm SEM ($n=7$). * represents significant alterations at $p<0.05$ vs control group.

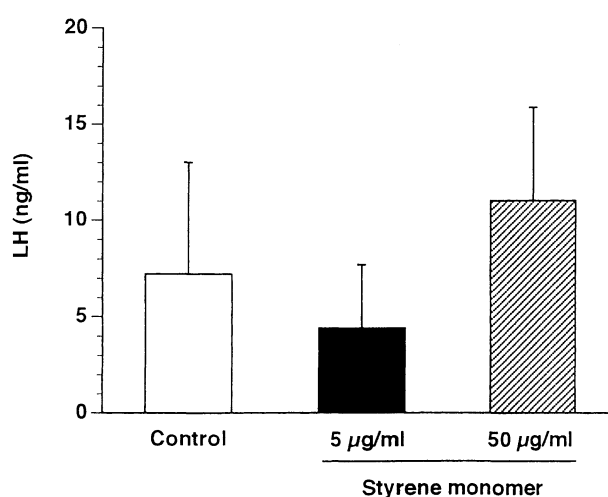


Fig. 3. Effects of styrene treatment for 4 weeks on plasma luteinizing hormone levels in male C57BL/6 mice. Styrene treatment was performed as described in Methods. Data represent the mean \pm SEM ($n=7$).

among four groups following 4 weeks of the treatment.

Next, we examined the effect of styrene on plasma corticosterone levels. Styrene monomer (5 μ g and 50 μ g/ml) tended to decrease plasma corticosterone levels following 4 weeks of treatment, but not significantly so (Fig. 2). No differences in plasma cor-

ticosterone were seen between water and control groups following 4 weeks of treatment (data not shown).

To determine whether styrene directly affects the male reproductive tract or modulates the pituitary function, plasma luteinizing hormone (LH) was measured following the 4 weeks of treatment regimen. Plasma LH levels were not significantly different among styrene monomer (5 μ g and 50 μ g/ml) and control groups following 4 weeks of treatment (Fig. 3). No differences in plasma LH levels were seen between water and control groups following 4 weeks of treatment (data not shown).

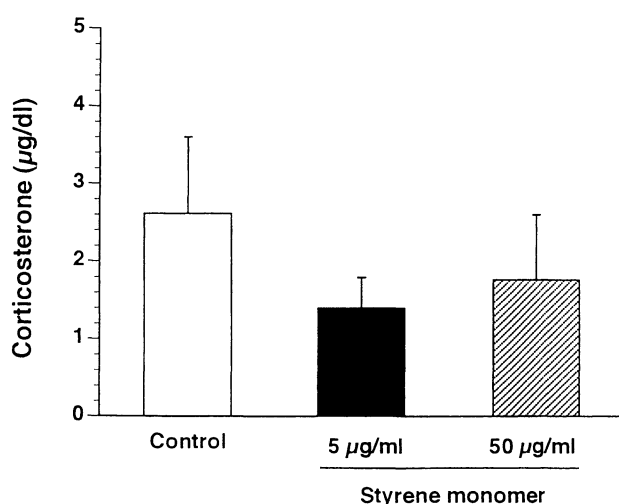


Fig. 2. Effects of styrene treatment for 4 weeks on plasma corticosterone levels in male C57BL/6 mice. Styrene treatment was performed as described in Methods. Data represent the mean \pm SEM ($n=7$).

Discussion

A previous report demonstrated significant losses in body weight and length in the offspring of female rats after inhalation exposures of 0.35, 1.18 and 12 ppm styrene during gestation [10]. In addition, Sikov *et al.* exposed female rats before and during gestation to 100 ppm styrene and found decreased fetal weight and increase in minor malformation [11], suggesting that the fetal animals are very sensitive to maternal exposure to this compound. In another study, styrene monomer treated rats showed a mild but somewhat inconsistent reduction in activity and

gripstrength during the course of exposure [9]. In human study, women who were the most highly exposed to styrene at the workplace had offspring with adjusted birth weights of 4% less than the offspring of unexposed women [12]. Although Beliles *et al.* studied three generations of rats fed styrene monomer in the drinking water and found no effects on litter size, pup survival, sex ratio, or pup body weight [13], plasma free testosterone levels were dramatically decreased following 4-week treatment of styrene in the present study. The differences between the two groups could be attributed to the use of different animals and/or different dose of this compound. As puberty is reached at around 6 or 7 weeks of age in the mouse, it is suggested that not only maternal exposure but also prepubertal and/or pubertal exposure to the compound specifically disrupt male reproductive functions in mice.

The mechanism underlying the decreases of free testosterone, and the unchanged testis weight and increased spleen weight/body weight in response to styrene is unknown. The potential for toxicity to fetuses is enhanced because styrene monomer is a lipophilic low molecular weight compound that crosses the maternal-placenta barrier and is easily absorbed [12]. In fact, styrene has been found in fetal and umbilical cord blood at levels proportional to those in maternal blood [14]. Taken together the fact that plasma corticosterone levels in styrene treated group were not different from the control group in the present study, styrene might affect the enzymes other than P450scc and 3 β -hydroxysteroid

dehydrogenase. More detailed molecular and histological studies would be needed to clarify this mechanism.

In a recent study dopamine depletion has been reported as a neurochemical basis of the neurotoxicity of styrene and styrene epoxide has also been reported to deplete glutathione and cause lipid peroxidation possibly leading to neuronal membrane damage [15], suggesting this compound may affect reproductive tract function by affecting pituitary function. The possibility also exists that styrene may affect gonadotropin releasing hormone (GnRH) in the hypothalamus and thus play a role in modulating LH secretion. The present data showed that plasma LH levels were unchanged following 4-week styrene treatment. Although plasma LH levels were not elevated via feedback mechanism, it is tempting to speculate that styrene affects the male reproductive system at least partly via a direct mechanism in the testis during these periods as pituitary function is not fully developed in prepubertal period. Nor should the effects of longer term exposure to styrene on pituitary and/or hypothalamic modulation be excluded.

The comparison of the results from animal investigation to the human adverse effect is difficult. However, styrene is a widely used monomer in food-packing polymer [16] including styrene foam and residual styrene in foods has been determined by several methods [17, 18]. More emergent and detailed studies are helpful to assess the possible risk to the development of the human testis from exposure to styrene and other environmental estrogens.

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