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ORIGINAL PAPER

Takeshi Shono · Sachiyo Suita

Dose-dependent effect of phthalate ester on testicular descent in pre-and post natal rats

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Abstract Mono-n-butyl phthalate (MBP) was administered to pregnant rats from the 15th to the 17th gestational day to investigate the dose-dependent effect of phthalate ester on testicular descent in both pre- and postnatal rats. Thirty pregnant rats (280–330 g) were separated into five groups and administered 1.0 g/kg/day MBP in group I, 0.5 g/kg/day in group II, 0.25 g/kg/day in group III, 0.125 g/kg/day in group IV from the day 15 to 17 of gestation. The rats in group V were only administered solvent and acted as controls. The position of the testis was evaluated in both pre- and postnatal rats. This was significantly higher in 20-day old fetuses in groups I and II than in groups III, IV or V. After birth, the incidence of undescended testis was also higher in groups I and II compared with those of groups III, IV or V. The undescended testes were located in the abdominal cavity in groups I and II, whereas they were located at the inguinal region in group III. The lowest dose that induced an adverse effect on testicular descent was 0.25 g/kg/day. In conclusion, maternal MBP inhibited testicular descent in prenatal rats and thus dose-dependently induced undescended testes postnatally. High-doses of MBP are thus considered to inhibit the transabdominal descent of the testis, probably due to its estrogenic activity, whereas low-doses of MBP may act as an anti-androgen and thereby inhibit inguinoscrotal testicular descent in postnatal rats.

Introduction

Various endocrine disrupting chemicals are considered to induce genitourinary abnormalities in animals and humans [4, 11, 13, 14, 15, 16, 17], however the exact mechanism by which they induce such abnormalities is unknown. Phthalate esters, which are one of major endocrine disrupting chemicals, have been commonly used as plasticizers for polyvinyl chloride and are widely distributed in the environment. In our previous studies, the prenatal administration of mono-n-butyl phthalate (MBP) was found to induce undescended testis (UDT) in postnatal rats [8]. However, no detailed studies of the dose-dependent effect of MBP on testicular descent have yet been reported. In this study the dose-dependent effect of MBP on testicular descent was investigated in pre-and postnatal rats.

T. Shono (✉) · S. Suita
Pediatric Surgery, Reproductive and Developmental Medicine,
Graduate School of Medical Sciences,
Kyushu University, 3-1-1 Maidashi Higashi-ku,
Fukuoka 812-8582, Japan
E-mail: shono@pedsurg.med.kyushu-u.ac.jp
Fax: +81-92-6425580

Materials and methods

Chemicals and animals

MBP (purity >98%) was purchased from the Tokyo Chemical Industry (Tokyo, Japan). It was used as a homogeneous mixture in sesame oil. Wister-King A (WKA) rats were obtained from a colony maintained at Seac Yoshitomi (Fukuoka, Japan). All rats were provided tap water and rat food ad libitum in an air-conditioned environment (22 ± 1°C) with a 12-hour light/dark cycle. Fertile females were placed with a male from 8:00 pm to 8:00 am during the dark phase. The animals were examined every morning for vaginal plugs and the day of the appearance of the plug was thus designated as day 0 of pregnancy.

Experimental protocols

Thirty pregnant rats (280–330 g) were separated into five groups. They were administered 1.0 g/kg/day of MBP by gavage in group I, 0.5 g/kg/day in group II, 0.25 g/kg/day in group III, 0.125 g/kg/day in group IV from day 15 to day 17 of gestation. In group V; the rats were only administered solvent and were used as controls. On day 20 of gestation, three rats in each group underwent a Caesarean section. The male fetuses were all killed by decapitation and then were fixed overnight in 10% formaldehyde. After preliminary fixation, the abdominal cavity was cleaned. The degree of transabdominal testicular ascent (DTA) was then determined by measuring the distance from the bladder to the lower pole of testis as previously described [8] using a micrometer eyepiece (×6.4). The measurements were standardized by defining the distance between

the bladder neck and the lower pole of the kidney as 100 units (Fig. 1). In the rest of the rats, the occurrence of UDT was examined from 60 to 70 days after birth.

We used Student's *t*-test and the χ^2 test to analyse the data, with *P*-values < 0.05 considered to be statistically significant.

Results

In 20-day-old fetuses, the testes were located high in the abdominal cavity in groups I and II (Fig. 2), and in the pelvis in groups III, IV and V (Fig. 3). The DTA was significantly larger in groups I and II than in groups III, IV or V (Table 1). A thin gubernaculum was noted in groups I and II. From 60 to 70 days after birth, UDTs were located in the abdominal cavity in groups I and II (Fig. 4), whereas they were located in the superficial inguinal position with the processus vaginals extended laterally while the tip of processus vaginalis adhered to the inguinal area in rats with UDT in group III (Fig. 5). No UDTs were observed in groups IV and V. The incidence UDT was also significantly higher in groups I and II compared with groups III, IV or V (Table 1). The lowest dose that induced an adverse effect on testicular descent was 0.25 g/kg/day.

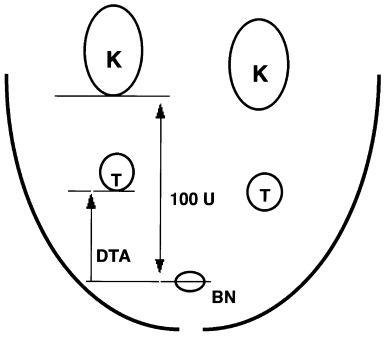

Fig. 1 The schema shows how the degree of transabdominal testicular ascent (*DTA*) was measured. The DTA was determined by measuring the distance from the bladder neck to the lower pole of the testis and was standardized by defining the distance between the bladder neck and lower pole of the kidney to be 100 units (*U*)

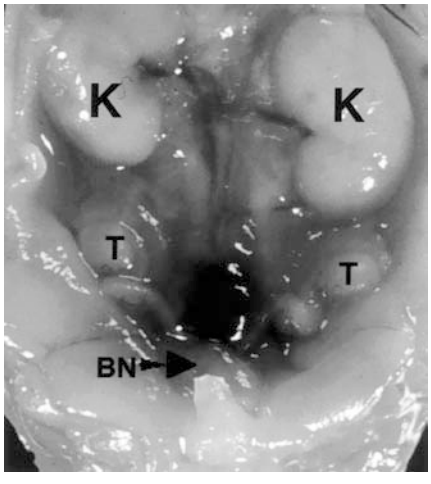

Fig. 2 A 20-day-old fetus in group I. Both testes show a high degree of ascent and remain in the position of the female ovaries below the kidney in the high-dose MBP-treated fetal rat. *T* testis, *K* kidney, *BN* bladder neck

Discussion

It has been hypothesized that testicular descent occurs in two steps which include the transabdominal migration of the testis and the inguinoscrotal descent of the testis [5]. The first step, the transabdominal migration of the testis, occurs within 10 to 15 weeks of gestation in humans [9] and from the 16th to 19th day of gestation in rats [21]. This phase has been shown to be inhibited by estrogen [6, 20]. It has been reported that the transabdominal migration of the testis does not express the real descent of the testis but instead demonstrates the relative ascent of the structures adjacent to the testis [19]. During this period, the testis remains stationary by being anchored to the inguinal region by the gubernaculum [19]. In the second step, the testis moves from the inguinal region to the scrotum under the control of androgen. The anti-androgen flumtamide is known to inhibit this phase [18, 20].

Phthalate esters are known to be one of the major endocrine disrupting chemicals, and they have been

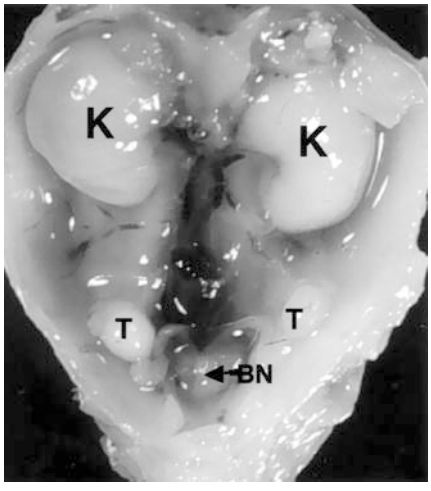

Fig. 3 A 20-day-old fetus in group III. Both testes are located in the lower abdominal cavity near the bladder neck in the low-dose MBP (0.25 g/kg/day)-treated rats, similarly to those of controls. *T* testis, *K* kidney, *BN* bladder neck

Table 1 The degree of transabdominal testicular ascent (DTA) with various concentrations of mono-n-butyl phthalate

	DTA (mean ± SE)	Undescended testis (%)
Group I	58.6 ± 2.1(n = 22)*'**	76.9 (n = 13)*'**
Group II	33.7 ± 2.8(n = 16)*'**	61.1 (n = 18)*'**
Group III	18.5 ± 1.9(n = 24)*	25.0 (n = 16)*
Group IV	9.5 ± 1.4(n = 20)	0.0 (n = 21)
Group V	8.5 ± 1.3(n = 28)	0.0 (n = 17)

*$P < 0.01$ (vs groups IV and V), **$P < 0.05$ (vs group III)

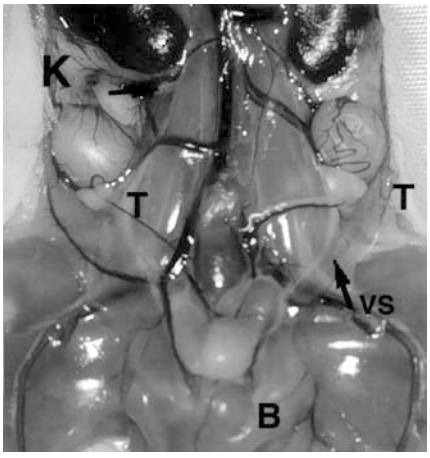

Fig. 4 A 60-day-old rat in group I. Both testes show a high degree of ascent just below the kidney, with atrophic vas deferens in high-dose MBP-treated rats. *T* testis, *K* kidney, *VS* vas deferens, *B* urinary bladder

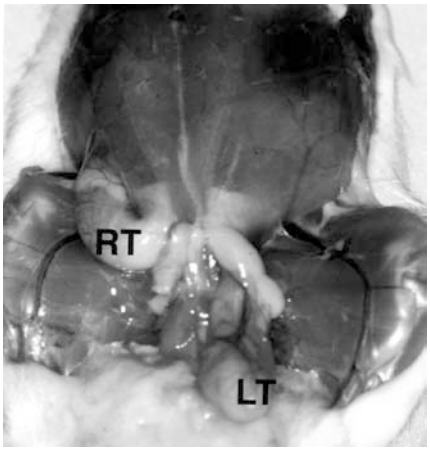

Fig. 5 A 60-day-old rat in group III. Left testis (*LT*) normally descended into the scrotum, however, the right testis (*RT*) is located at the superficial inguinal position and the tip of processus vaginalis adheres to the inguinal area in the low-dose MBP-treated rats

reported to cause both testicular atrophy and infertility in laboratory animals [1, 2, 4, 15]. Phthalate esters are also known to have both estrogenic and anti-androgenic activities in vivo [3] and in vitro [10]. In our present study, high doses of MBP (1.0 g/kg/day) induced a relative ascent of the testis in fetal rats and thereafter caused intra-abdominal UDT postnatally. These phenomena were similar to those found in prenatally estrogen-treated mice in which estrogen inhibited the transabdominal migration of the testis and caused intra-abdominal UDT [6, 12, 20]. On the other hand, a low dose of MBP (0.25 g/kg/day) inhibited inguinal scrotal descent and caused superficial inguinal UDT, and these effects of MBP were similar to those induced by the prenatal administration of anti-androgen using flutamide [17, 18]. Although MBP is known to have both estrogenic and anti-androgenic activities in vivo and in vitro, an estrogenic effect may only occur in high dose MBP-treated fetuses, which may thus inhibit transabdominal testicular descent as the estrogenic activities of MBP are known to be weak [3]. An alternative explanation is that testicular migration may depend on the estrogen-androgen balance in the organ, with a lower sensitivity of the earlier versus the later stages of migration to imbalances towards the estrogenic activities of MBP. The earlier stage of testicular migration could be inhibited only by high dosage MBP.

In conclusion, MBP showed a biphasic hormonal effect on testicular descent. High-dose MBP may act predominantly with an estrogenic effect in addition to an anti-androgenic effect, and prevented transabdominal testicular descent. On the other hand, low doses of MBP may act as an anti-androgen and inhibit inguinoscrotal testicular descent, thereby inducing superficial inguinal testis in rats.

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References

1. Agarwal DK, Maronpot RR, Lamb JC, Kluwe WM (1985) Adverse effects of butyl benzyl phthalate on the reproductive and hematopoietic systems of male rats. Toxicol 35: 189
2. Creasy DM, Foster JR, Foster PMD (1983) The morphological development of di-n-pentyl phthalate induced testicular atrophy in the rat. J Pathol 139: 309
3. Ema M, Miyawaki E, Kawashima k (1988) Further evaluation of developmental toxicity of di-n-butyl phthalate following administration during late pregnancy in rats. Toxicol Lett 98: 87
4. Gray LEJr, Ostby J, Furr J, Price M, Veeramachaneni DN, Parks L (2000) Perinatal exposure to the phthalates DEHP, BBP, and DINP, but not DEP, DMP, or DOTP, alters sexual differentiation of the male rat. Toxicol Sci 58: 350
5. Hutson JM (1985) A biphasic model for the hormonal control of testicular descent. Lancet 2: 419
6. Hutson JM (1987) Exogenous oestrogens prevent transabdominal testicular descent in mice with complete androgen resistance (testicular feminization) Pediatr Surg Int 2: 242
7. Hutson JM, Donahoe PK (1986) The hormonal control of testicular descent. Endocr Rev 7: 270
8. Imajima T, Shono T, Zakaria O, Suita S (1997) Prenatal phthalate causes cryptorchidism postnatally by inducing transabdominal ascent of the testis in fetal rats. J Pediatr Surg 32: 18
9. Jirasek JE (1971) Genital ducts and external genitalia: development and anomalies. Birth Defects 7: 131
10. Jobling S, Reynolds T, White R, Parker MG, Sumpter JP (1994) A variety of environmentally persistent chemicals, including some phthalate plasticizers, are weakly estrogenic. Environ Health Perspect 102: 380
11. Kelce WR, Stone CR, Laws SC, Gray LE, Kemppanien JA, Wilson EM (1995) Persistent DDT metabolite p,p'-DDE is a potent androgen receptor antagonist. Nature 375: 581
12. Luthra M, Hutson JM (1989) Late-gestation exogenous oestrogen inhibits testicular descent in fetal mice despite Müllerian duct regression. Pediatr Surg Int 4: 260
13. Moore RW, Rudy TA, Lin TM, Ko K, Peterson RE (2001) Abnormalities of sexual development in male rats with in utero and lactational exposure to the antiandrogenic plasticizer di (2-ethylhexyl) phthalate. Environ Health Perspect 109: 229

14. Mylchreest E, Wallace DG, Cattley RC, Foster PM (2000) Dose-dependent alterations in androgen-regulated male reproductive development in rats exposed to di (n-butyl) phthalate during late gestation. Toxicol Sci 55: 143
15. Parks LG, Ostby JS, lambright CR, Abbott BD, Klinefelter GR, Barlow NJ, Gray LEJr (2000) The plasticizer diethylhexyl phthalate induces malformations by decreasing fetal testosterone synthesis during sexual differentiation in the male rat. Toxicol Sci 58: 339
16. Piersma AH, Verhoef A, Biesebeek JD, Pieters MN, Slob W (2000) Developmental toxicity but of butyl benzyl phthalate in the rat using a multiple dose study design. Reprod Toxicol 14: 417
17. Sharpe RM (1995) Reproductive biology. Another DDT connection. Nature 375 : 538
18. Shono T, Ramm-Anderson S, Goh DW, Hutson JM (1994) The effect of flutamide on testicular descent in rats examined by scanning electron microscopy. J Pediatr Surg 29: 839
19. Shono T, Ramm-Anderson S, Hutson JM (1994) Transabdominal testicular descent is really ovarian ascent. J Urol 152: 781
20. Shono T, Hutson JM, Watts L, Goh DW, Momose Y, Middlesworth B, Zhou B, Ramm-Anderson S (1994) Scanning electron microscopy shows inhibited gubernacular development in relation to undescended testes in oestrogen-treated mice. Int J Androl 19: 263
21. Wensing CJG (1986) Testicular descent in the rat and a comparison of this process in the rat with that in the pig. Anat Rec 214: 154