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[COMMUNICATION]

Critical Period of Induction by Tamoxifen of Genital Organ Abnormalities in Male Mice

SATOKO IRISAWA, TAISEN IGUCHI[1] and NOBORU TAKASUGI[1]

Laboratory of Biology, Tokyo Kasei Gakuin University, Machida 194-02, and
[1] Department of Biology, Yokohama City University,
Kanazawa-ku, Yokohama 236, Japan

ABSTRACT—C57BL/Tw male mice given 5 daily injections of 100 μg tamoxifen (Tx) starting on the day of birth (0 day) were examined at 5, 10, 15, 20 and 30 days of age. Body and organ weights and diameter of seminiferous tubules in Tx mice were significantly smaller than those of the age-matched controls. Spermatogenesis was found in 30-day-old control mice, but was completely suppressed in Tx mice at the same age. In addition, 60-day-old male mice given neonatal injections of 100 μg clomiphene (Clm) or nafoxidine (Naf), and those given injections of 100 μg Tx beginning at different early postnatal ages were also examined. Tx caused more damage to testis than did Clm and Naf. Mean spermatogenic index and diameters of seminiferous tubules in mice given Tx beginning at 0 day were significantly smaller than those in mice given Tx starting at 5 days. These findings suggest that the critical period of producing the deleterious effects of Tx on the genital organs is present within 3 days after birth.

INTRODUCTION

Perinatal treatment with natural and synthetic estrogens including diethylstilbestrol (DES) induces permanent suppression of spermatogenesis in the testes of rats and mice [1–12]. Tamoxifen (Tx), one of triphenylethylene derivatives, inhibits estrogen action by competing with the hormone in binding the estrogen receptor. Tx, therefore, has been widely used for the therapy of estrogen-receptor positive human breast cancer [13, 14]. On the other hand, Tx has been reported as producing estrogenic effects on uteri and vaginae of various mammals [15–23]. In male mice, neonatal exposure to Tx resulted in permanent suppression of spermatogenesis and atrophy of genital organs [24] as reported in mice treated neonatally with estrogenic hormones [3–6]. Recently, various abnormalities of pelvis [25, 26] and os penis [27] were found in male mice treated neonatally with Tx. Other antiestrogens, clomiphene (Clm) and nafoxidine (Naf) also caused various abnormalities in genital organs of mammals [28]. However, none of the previous studies examined the responsiveness of mouse genital organs to administration of Tx starting at different early postnatal ages. The present study was aimed at examining the Tx-induced sequential changes in genital organs and their responsiveness to Tx in neonatal and early-postnatal male mice.

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MATERIALS AND METHODS

C57BL/Tw male mice were kept under 12 hr light/12 hr dark condition at 23–25°C temperature. Mice were given 5 daily subcutaneous injections of 100 μg tamoxifen (Tx, mw=563.6) (Sigma, St. Louis. MO), suspended in 0.02 ml of saline and of the vehicle alone, starting within 24 hr after birth and killed by ether anesthesia at ages of 5, 10, 20, 30 and 60 days. Mice were also given daily injections of 100 μg Tx and of the vehicle alone for 5 days starting on the day of birth (0 day), 3, 5, 7 and 10 days. In addition, two groups of mice were neonatally given 5 daily injections of 100 μg clomiphene citrate (Clm, mw=598.1) (Sigma) and

100 μg nafoxidine hydrochloride (Naf, mw= 462.0) (Sigma) beginning on the day of birth, respectively. These animals were killed at 60 days.

Pairs of testes, seminal vesicles with coagulating glands and epididymides were weighed separately. These organs were fixed in Bouin's solution, embedded in paraffin and serially sectioned at 8 μm. The sections were stained with Delafield's hematoxylin and eosin. In 5 transverse sections randomly selected from each testis, 20 seminiferous tubules were examined to count the number of seminiferous tubules containing mature spermatozoa. Percent ratio of the tubules showing active spermatogenesis was calculated for each mouse on the basis of 200 tubules and was used as the index of spermatogenic activity [5]. Diameters of seminiferous tubules were measured with a micrometer. Data were analysed by Duncan's multiple range test and Fisher's exact probability test.

RESULTS AND DISCUSSION

Weights of body, testes and epididymides in 60-day-old mice treated neonatally with a daily dose of 100 μg of Tx, Clm or Naf were significantly smaller than those in the controls except for the testis weight in Naf mice (Table 1). In mice given Clm injections neonatally, however, the body weights were significantly greater than in mice given Tx injections starting at 0 day. Weight of testes in mice given Tx starting at 5, 7 and 10 days, respectively, were significantly greater compared with that in mice given Tx starting at 0 day. Testes and epididymides in Naf mice, were also significantly heavier than those in mice given Tx from 0 day.

As shown in Figure 1, body weights in neonatally Tx-treated mice at ages of 10, 30 and 60 days were significantly smaller than those in the age-matched controls. Weights of epididymides (at 30 and 60 days), seminal vesicles with coagulating glands (at 60 days) and testes (at 30 and 60 days) were significantly smaller than those of the corresponding controls. The testes in 5-day-old Tx and control mice contained seminiferous tubules with spermatogonia and spermatocytes and interstitial cells.

Spermatozoa and spermatids were found in the tubules of control mice at 30 and 60 days. In the controls, the mean spermatogenic index was 30.6

TABLE 1. Body and organ weights in 60-day-old C57BL/Tw male mice treated with antiestrogens

Treatment and period (days of age)	No. of mice	Body weight (g)	Organ weights (mg/20 g body weight)	
			testes	epididymides
Saline				
*0–4	10	18.4±0.7[a]	135.7±8.4	52.1±3.3
100 μg tamoxifen				
0–4	10	12.4±0.9[b]	43.8±5.6[b]	20.4±3.0[b]
3–7	15	12.3±0.9[b]	63.2±5.0[b]	18.5±1.9[b]
5–9	10	14.3±0.9[b]	68.1±7.1[be]	22.0±1.3[b]
7–11	10	15.0±0.9[c]	105.4±12.0[cd]	34.9±3.3[bd]
10–14	13	15.1±1.1[c]	104.1±9.9[cd]	33.7±1.8[bd]
100 μg clomiphene				
0–4	6	15.5±1.0[ce]	66.6±4.3[b]	23.2±1.3[b]
100 μg nafoxidine				
0–4	5	12.0±1.3[b]	123.4±15.6[d]	39.0±4.6[bd]

* The day of birth is indicated as 0; a, Mean±S.E.
[b] $P<0.01$ vs controls (Duncan's multiple range test)
[c] $P<0.05$ vs controls (Duncan's multiple range test)
[d] $P<0.01$ vs 100 μg tamoxifen 0–4 (Duncan's multiple range test)
[e] $P<0.05$ vs 100 μg tamoxifen 0–4 (Duncan's multiple range test)

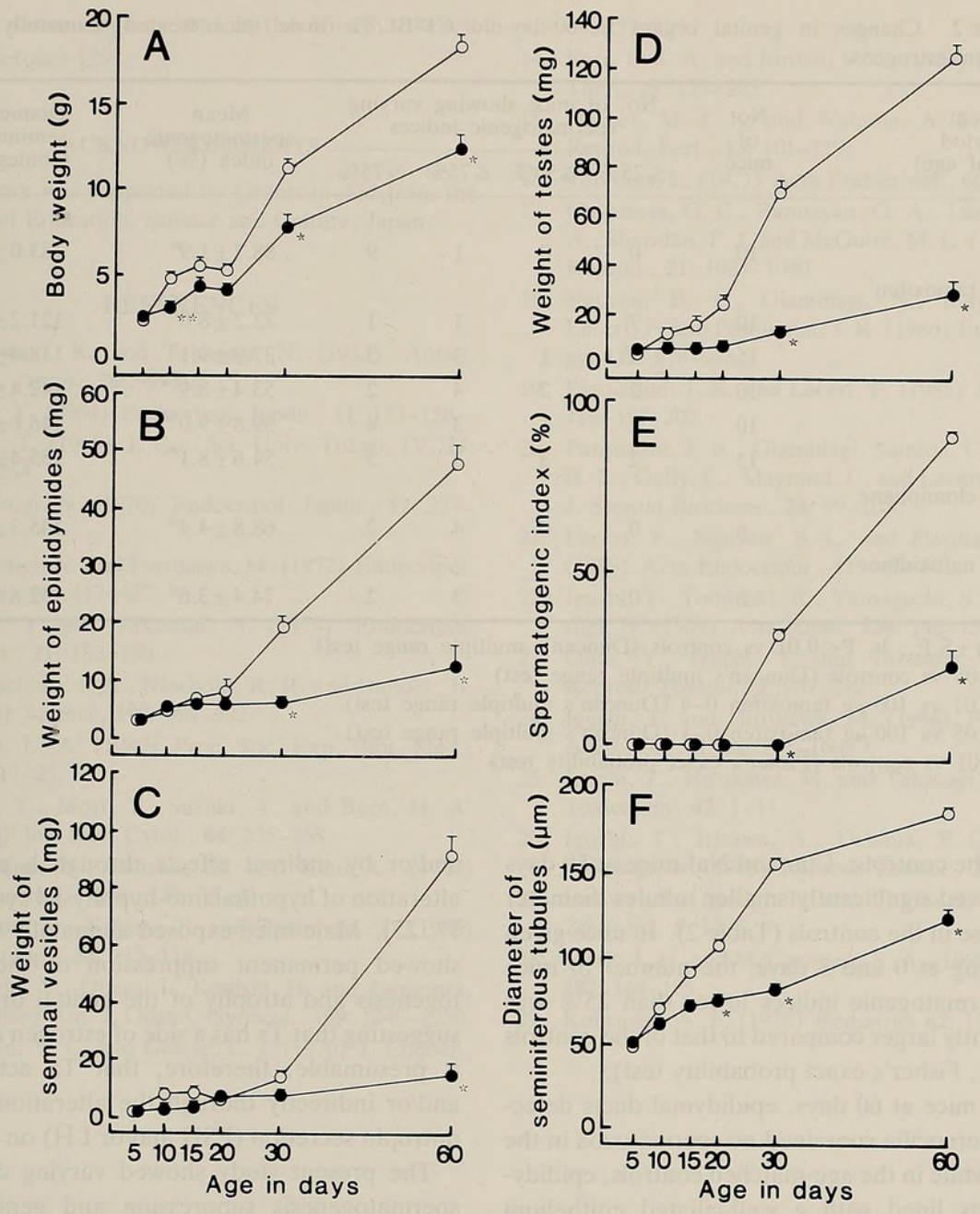

FIG. 1. Sequential changes in body weight (A), weights of epididymides (B), seminal vesicles with coagulating glands (C), testes (D), spermatogenic index (E) and diameters of seminiferous tubules (F) in mice given daily injections of 100 μg Tx (●) or the vehicle alone(○) starting on the day of birth. *$p<0.01$, **$p<0.05$ *vs* respective controls.

$\pm 0.9\%$ at 30 days, and $88.7 \pm 1.9\%$ at 60 days. In contrast, the testis of Tx mice began to show spermatogenesis later than 30 days of age. Seminiferous tubules in Tx mice at ages of 20 and 30 days were smaller in diameter, lacking spermatids and spermatozoa. In 60-day-old Tx mice, spermatogenic index ($22.2 \pm 8.0\%$) was significantly lower than the value in the age-matched controls. In Clm and Naf mice at 60 days, spermatogenic indices were not significantly lower than that in the controls (Table 2). Diameters of seminiferous tubules increased with age, from 5 to 60 days, in both control and Tx mice, though the diameters in Tx mice were significantly smaller than those in the corresponding controls after 20 days.

In all groups of 60-day-old mice given 5 daily Tx injections, spermatogenic indices and diameters of seminiferous tubules were significantly smaller

TABLE 2. Changes in genital organs in 60-day-old C57BL/Tw male mice treated neonatally with antiestrogens

Treatment and period (days of age)	No. of mice	No. of mice showing varying spermatogenic indices				Mean spermatogenic index (%)	Diameter of seminiferous tubules (μm)
		≦25%	≦50%	≦75%	>75%		
Saline							
0–4	10	0	0	1	9	88.7±1.9[a]	183.0±3.0
100 μg tamoxifen							
0–4	10	7[f]	1	1	1	22.2±8.0[b]	121.2±4.4[b]
3–7	15	8[f]	1	3	3	37.9±9.1[b]	118.4±5.2[b]
5–9	10	2	2	4	2	53.4±8.9[be]	142.4±6.2[be]
7–11	10	2	1	3	4	59.8±9.0[cd]	146.6±7.5[bd]
10–14	13	2	3	3	5	54.6±8.1[be]	155.4±6.5[bd]
100 μg clomiphene							
0–4	6	0	0	4	2	68.8±4.4[d]	145.3±8.7[bd]
100 μg nafoxidine							
0–4	5	0	0	3	2	74.4±3.6[d]	152.8±6.3[bd]

[a] Mean±S.E.; b, $P<0.01$ vs controls (Duncan's multiple range test)
[c] $P<0.05$ vs controls (Duncan's multiple range test)
[d] $P<0.01$ vs 100 μg tamoxifen 0–4 (Duncan's multiple range test)
[e] $P<0.05$ vs 100 μg tamoxifen 0–4 (Duncan's multiple range test)
[f] $P<0.01$ vs controls (Fisher's exact probability test)

than in the controls. Clm and Naf mice at 60 days also showed significantly smaller tubules diameter than those in the controls (Table 2). In mice given Tx starting at 0 and 3 days, the number of mice with spermatogenic indices lower than 25% was significantly larger compared to that of the controls ($p<0.01$, Fisher's exact probability test).

In Tx mice at 60 days, epididymal ducts defective in stereocilia contained no spermatozoa in the lumen, while in the age-matched controls, epididymal ducts lined with a well-ciliated epithelium contained numerous spermatozoa. Seminal vesicles of these control mice contained a large amount of eosinophilic secretion in the lumen, whereas no such secretion was found in Tx mice at the same age. These findings suggest that the testis of 60-day-old Tx mice failed to secrete an amount of androgen sufficient for maintaining the sex accessory organs or that the testis was decreased in responsiveness to gonadotropin in Tx mice.

Previous studies have demonstrated that in neonatally estrogen-treated male rats and mice, the long-lasting suppression of spermatogenesis is caused by direct effects of estrogen on the testis and/or by indirect effects through a permanent alteration of hypothalamo-hypohysial system [6, 9, 17, 25]. Male mice exposed neonatally to Tx also showed permanent suppression of the spermatogenesis and atrophy of the genital organs [24], suggesting that Tx has a side of estrogen agonist. It is presumable, therefore, that Tx acts directly and/or indirectly thorugh the alteration of gonadotropin secretion (FSH and/or LH) on the testis.

The present study showed varying degrees of spermatogenesis suppression and genital organ abnormalities in male mice given Tx starting at 0, 3, 5, 7 and 10 days, the highest degree being found in two groups of mice given Tx beginning at 0 and 3 days. It is suggested, therefore, that in mice, the critical period of Tx induction of male genital organ dysfunction and abnormalities is present within 3 days after birth. Tx has been used for the therapy of estrogen-reactive human breast cancer; however, on the basis of the present study, the possibility cannot be excluded that male fetuses of pregnant women treated with Tx for the breast cancer exhibits postnatally a long-lasting testicular dysfunction, since neonatal mice are approximate-

ly at the same stage as that of the 16- to 20-week human fetuses [29].

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