EFFECTS OF FLUTAMIDE ON THE ONSET OF PUBERTY IN SPRAGUE-DAWLEY FEMALE RATS

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Introduction

Due to the increasing public concern about Endocrine Disruptors (EDs) problems, many studies have been conducted to determine the probable adverse effects of EDs in both *in vivo* and *in vitro* [1,2]. Recently, the rodent 20-day pubertal onset assay was recommended as a testing method to detect EDs by Endocrine Disrupter Screening and Testing Advisory Committee (EDSTAC) [3]. Several studies have been demonstrated that the onset of puberty in the female is associated with the vaginal opening (VO) and first estrus [4,5]. Precocious VO can occur by estrogens or estrogen-like chemicals [6-8], whereas delayed by aromatase inhibitor or steroid synthesis inhibitors [9]. However, the effects of androgen receptor antagonist on the onset of puberty in female rats are clearly unknown. Therefore, this study is designed to investigate the onset of puberty in immature female rats exposed to flutamide during sexual maturation periods.

Materials and Methods

Study design

Sprague-Dawley Crl:CD female rats (Charles River Laboratories) were obtained from the Laboratory Animal Resources NITR/KFDA (Seoul, Korea) under SPF-conditions. Litters of 18-day old female rats were housed together by litter in clear polycarbonate cages for 3 days prior to the start of dosing. At 21-day of age, the animals were allocated to the various treatment groups by random sorting in accordance with body weight. The treatment groups were vehicle control and flutamide (1, 5, and 25 mg/kg/day). All test compounds were administered by oral gavage from 21-day of age for 20 days. The total amount of injection per rat was 3 ml/kg/day.

Clinical signs, body weight and organ weights

Throughout the study period, clinical signs and body weights were observed at least once a day after treatment. Twenty-four hours after the last treatment, each rat was anesthetized with CO_2 in the same sequence as the test substance was administered. The uterus and ovary were carefully dissected and weighed at once. The liver, heart, kidney, thyroid, thymus, pituitary glands, and adrenal glands were carefully dissected and weighted also.

Vaginal opening

Each animal was examined daily for VO. On the day that VO was first detected, the age and body weights were recorded.

Hormonal measurements

Commercially available radioimmunoassay (RIA) kits were used to measure serum concentrations of E2 and T4 (Amersham Corp., Arlington Height, IL, USA).

Statistical analysis

Statistical analysis was performed using one-way analysis of variance (ANOVA) and the Dunnett's test; p values of <0.05(*) were considered to be significant.

Results and Discussion

A physiological role of pure androgen receptor antagonist, flutamide, in the female pubertal onset assay has not been clearly determined. Thus, we examined the effects of flutamide on pubertal onset. During the study period, statistically significant changes in body weights were not observed at any doses of flutamide (data not present). Flutamide significantly decreased the absolute and relative ovary weight in 25 mg/kg-treated group compared to control (Table 1). Absolute liver weight was significantly increased in rats treated with flutamide 25 mg/kg (108% of control). Also, high dose of flutamide (25 mg/kg) significantly decreased the absolute pituitary glands weight (Table 2).

In our experimental condition, VO began at 30 days of age and continued until 35 days of age in control group (Fig. 1). Also, the mean age at VO was 32.4±1.6 days and the mean body weight at VO was 117.4±14.8 (Fig. 1, 2). However, high dose (25 mg/kg) of flutamide significantly accelerated the age at VO (26.1±0.3 days). As similar to VO days, the mean body weight at the age of VO was significantly lower (73.8±4.8) than that of control (117.4±14.8)(Fig. 1).

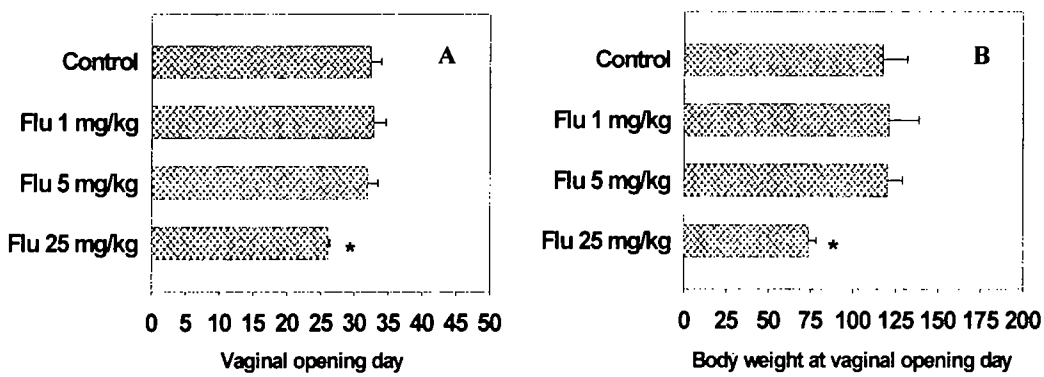

Fig. 1. Mean age at vaginal opening (A) and mean body weights at the time of vaginal opening (B) in Sprague-Dawley female rats treated with flutamide. Bars indicate mean age at which vaginal opening was first detected. Significantly different from control, *$p<0.05$.

Clark [10] reported that VO in rodents usually occurs around 33-42 days after birth. In the present study, the range of VO was 30-35 days in Sprague-Dawley female rats, which is consistent with those of previously reported data [11,12]. Several studies demonstrated that estrogenic chemicals such as methoxychlor, nonylphenol, and octylphenol have affected VO in rodents [3]. Serum hormone concentrations were evaluated individually in all female rats regardless of stage of estrous cycle. As shown in Fig. 2, serum estradiol (E2) and thyroid hormone (T4) concentrations were not changed after flutamide administration.

In conclusion, flutamide (25 mg/kg) significantly accelerated the age of VO days

(approximately 7 days earlier than the control) and decreased the ovarian weights. Flutamide effectively blocked the recognition of androgens by bind to the androgen receptor, and thus stimulation of gonadotropin release from the anterior pituitary resulted in the increase of serum testosterone concentration. Consequently, aromatase activity also increases in the vaginal epithelium of immature rats prior to the onset of puberty. Hence, the change in VO days is probably due to the local estrogen produced by aromatization. Thus, high dose of androgen receptor antagonist can alter the female pubertal onset, which could be important on the development of female reproductive function.

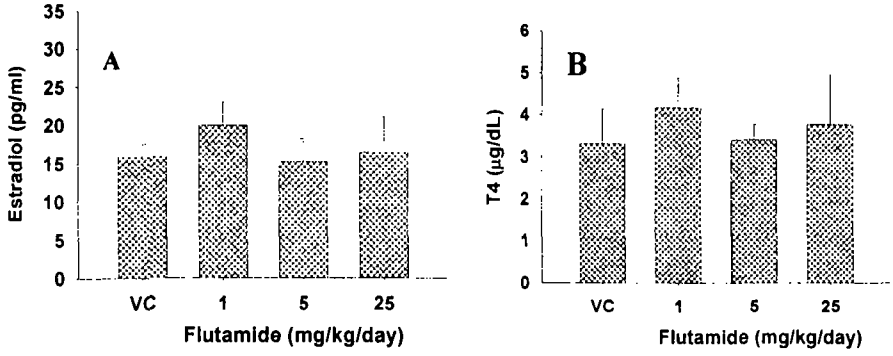

Fig 2. Serum concentrations of estradiol (A) and T4 (B) in Sprague-Dawley female rats treated with flutamide. Animals were dosed daily by oral gavage from PND 21 to PND 40.

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Table 1. Absolute and relative organ weights in Sprague-Dawley female immature rats treated with flutamide for 20 days

	Dosage	Initial B.W. (g)	Final B.W. (g)	Thyroid gland		Ovary		Uterus	
				Absolute (mg)	Relative (mg/100g b.w.)	Absolute (mg)	Relative (mg/100g b.w.)	Absolute (g)	Relative (g/100g b.w)
Control	0	52.5±3.6	156.7±5.72	8.91±1.68	5.66±0.99	54.77±9.11	34.86±6.21	0.28±0.07	0.18±0.05
Flutamide	1 mg/kg	51.1±2.2	157.4±11.9	8.44±1.83	5.39±1.16	52.26±5.01	33.41±4.39	0.27±0.05	0.17±0.04
	5 mg/kg	51.4±2.1	164.8±8.01	8.86±2.81	5.33±1.55	51.51±9.89	31.52±6.52	0.27±0.06	0.16±0.04
	25 mg/kg	51.9±3.2	159.7±6.17	8.28±2.43	5.16±1.41	41.73±7.65*	26.22±4.50*	0.23±0.04	0.14±0.02

Note. n = 10 animals per treatment group.
*Significantly different from control using Dunnett's test ($p < 0.05$)

Table 2. Absolute organ weights in Sprague-Dawley female immature rats treated with flutamide for 20 days

	Dosage	Initial B.W. (g)	Final B.W. (g)	Liver (g)	Heart (g)	Kidney (g)	Thymus (g)	Pituitary (mg)	Adrenals (mg)
Control	0	52.5±3.6	156.7±5.72	5.71±0.29	0.65±0.04	1.49±0.08	0.48±0.09	10.30±1.68	42.43±6.32
Flutamide	1 mg/kg	51.1±2.2	157.4±11.89	5.65±0.52	0.62±0.05	1.46±0.11	0.56±0.11	8.10±1.09*	35.86±4.96*
	5 mg/kg	51.4±2.1	164.8±8.01	6.12±0.58	0.65±0.05	1.44±0.09	0.52±0.07	8.61±2.49	38.42±4.76
	25 mg/kg	51.9±3.2	159.7±6.17	6.15±0.41*	0.61±0.05	1.41±0.05	0.53±0.07	6.71±1.64*	37.39±4.34

Note. n = 10 animals per treatment group.
*Significantly different from control using Dunnett's test ($p < 0.05$)