THE EFFECT OF FLUTAMIDE ON PUBERTY IN MALE RATS: AN EVALUATION OF THE PROTOCOL FOR THE ASSESSMENT OF PUBERTY DEVELOPMENT AND THYROID FUNCTION

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Introduction

For assessing the potential of pesticides and other chemicals to disrupt endocrine function in humans and wildlife, the Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC) recommended a screening and testing program. In developing the recommended Tier 1 Screening (T1S) battery, many existing and potential assays were evaluated for their relative strengths and weaknesses. Rodent 20-day thyroid/pubertal assay is one of the alternative assays, which is expected to detect some biological activities as estrogen agonism, androgen agonism and antagonism, and thyroid related effects. Adolescence is a time of dramatic neuroendocrine changes that are required for sexual maturation. Hormonal mimicking or inhibiting chemicals may cause significant impairment during this critical period and a disproportionate alteration in normal sexual maturation. This concern was recognized by EDSTAC, which acknowledged the need for the development and standardization of a protocol for the assessment of the impact of endocrine-disrupting chemicals in the pubertal male and recommended inclusion of an assay of this type as an alternative test in the EDSTAC T1S[1]. Flutamide is a pure antiandrogen, which exerts a variety of effects after being metabolized to hydroxyflutamide by displacing androgens from specific receptors in target tissues[2], forming biologically inactive complexes with nuclear androgen receptors. In the present report, we document the results of the rodent 20-day thyroid/pubertal assay of flutamide performed in our laboratory and discuss the practical application to the sensitivity of new parameters for detecting endocrine-related effects of test chemicals.

Method and Materials

Pregnant Sprague-Dawley crl:CD rats, acquired from Laboratory of Animal Resources, NITR, KFDA, were housed individually in clear plastic cages with wood chips as bedding and given pellet rodent diet and tap water *ad libitum* under controlled environmental conditions. Prior to treatment, male weanlings were assigned to each group by randomization method based upon body weight, so no significant difference in mean body weight was observed among the groups. Immature male rats (33 day of age, 10 rats/dose) were treated by gavage with flutamide (0, 1, 5, 25 mg/kg/day) for 20 days and the corn oil was used as the vehicle, and the dose volume was 5.0 *ml/kg* body weight. The animals were inspected daily 9:00 and 10:00 for prepuce separation (PPS). PPS is considered complete when the prepuce can be completely retracted to expose the glans penis[3]. One day after the last dosing, all animals were euthanized by blood withdrawal from the abdominal aorta under light ether anesthesia. All rats were kept quietly at least one hour

prior to blood sampling, to avoid the effects of transfer stress on hormone levels. Testes, epididymes, ventral prostate, seminal vesicles plus coagulating glands and fluid (SVCGF), Cowper's gland, glans penis and levator ani plus bulbocarvernosus muscles (LABC) were weighed and organ weights, also, calculated relative to the body weight. The thyroid glands were placed in 10% formalin fixative and examined microscopically. The blood was allowed to clot at room temperature after which the serum was collected and stored in aliquots in capped vials at -80°C until analyzed for serum hormone concentrations. Serum testosterone, estradiol and thyroxin levels were measured by RIA kits. The mean and standard deviation of the mean were calculated for body weights, organ weights, and PPS for the control and experimental groups of rats. The means were compared using Dunnett's test after one-way ANOVA using Sigma Plot program. Significant differences between values are indicated by $P<0.05$.

Results and Discussion

Body weight was not affected by flutamide treatment (Fig. 1). Significant delay was observed in the timing of PPS at flutamide doses greater than 5 mg/kg (Fig. 2). PPS occurred at a heavier body weight in flutamide-treated rats, but the weight was appropriate for the age. But, the flutamide-treated animals were indistinguishable from the vehicle-injected controls, when average body weight and weight gain per day were compared. The absolute and relative weights of ventral prostate, SVCGF, LABC, Cowper's gland and glans penis were significantly decreased in rats treated with flutamide in a dose dependent manner(Table 1). Flutamide, as well as its main metabolite, hydroxyflutamide, binds to the androgen receptor and effectively blocks recognition of androgens, resulting in decreased intracellular androgenic stimulus and ultimately decreased weights for the androgen-dependent tissues[2]. In addition, the epididymes weights were significantly decreased at 5 and 25 mg/kg/day, while no changes were observed in testes at all doses examined. The adrenal gland weight was increased at 25 mg/kg/day. There were no effects of flutamide treatment on the serious systemic toxicity. No microscopic changes were observed in the thyroid gland after flutamide administration. Serum testosterone and estradiol levels were significantly increased at the flutamide treated group (Table 2). As a result of the decreased intracellular androgenic stimulus, gonadotropin releases from the anterior pituitary is increased. In response to the increase in gonadotropins and a blockade of Leydig cell androgen receptors, serum testosterone increases[4]. Secondary to the increase in testosterone, serum levels of estradiol (the aromatization of product of testosterone) also increase. It is possible that the antiandrogenic effects of flutamide blocked the negative feedback mechanism of testosterone on the hypothalamus or pituitary gland, resulting in an increase in gonadotrophin release. But, no significant difference with respect to thyroxin levels was observed. The present rodent 20-day thyroid/pubertal assay of flutamide, androgen receptor antagonist, demonstrated the usefulness of the test system in rodents as a screening tool for the detection of endocrine-related effects and the sensitivity of several parameters for this purpose. Taking account of the present study, it would appear that further improvements are required regarding more sensitive and accurate parameters to detect endocrine-related effects.

Acknowledgments

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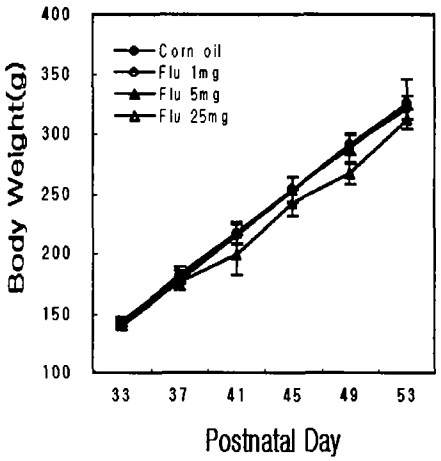

Fig.1 The effects of flutamide on body weight. No changes Were observed by the treatment.

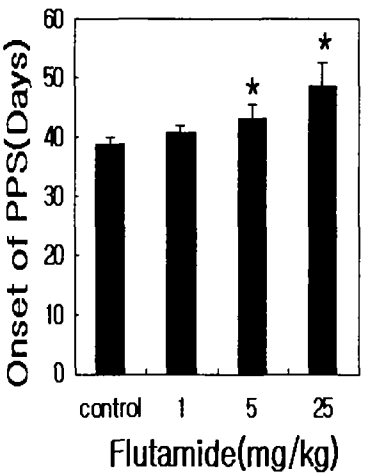

Fig.2 Flutamide-induced delay of PPS.

Table 1. Absolute(A) and Relative(R) Organ Weights in Control and Flutamide Treated Male Rats

Dose (mg/kg)		Combined Adrenal (g)	Combined Epididymis(g)	Ventral Prostate(g)	Seminal Vesicle(g)	LABC(g)	Cowper's Gland(g)	Glans Penis(g)
0	A	0.046±0.007[a]	0.32±0.03	0.28±0.04	0.54±0.04	0.76±0.07	0.056±0.01	0.085±0.006
	R	0.015±0.001	0.107±0.008	0.091±0.015	0.177±0.022	0.251±0.023	0.018±0.002	0.028±0.003
1	A	0.049±0.004	0.29±0.02	0.27±0.03	0.36±0.06*	0.68±0.07*	0.048±0.014	0.090±0.006
	R	0.016±0.001	0.095±0.001*	0.086±0.009	0.117±0.022*	0.219±0.023*	0.016±0.004	0.029±0.002
5	A	0.052±0.005	0.24±0.02*	0.17±0.02*	0.24±0.03*	0.50±0.04*	0.032±0.007*	0.075±0.006
	R	0.018±0.001	0.087±0.009*	0.064±0.006*	0.085±0.010*	0.178±0.015*	0.011±0.002*	0.026±0.002
25	A	0.056±0.009*	0.22±0.02*	0.14±0.05*	0.13±0.03*	0.44±0.07*	0.021±0.007*	0.051±0.010*
	R	0.018±0.002	0.074±0.008*	0.049±0.015*	0.045±0.011*	0.148±0.023*	0.007±0.002*	0.017±0.004*

a: Values are means derived from ten animals, ±SD.
*: Significantly different from the control value(*P<0.05).

Table 2. Serum Hormone Levels in Male Rats Treated from PND 33 to PND 53

Dose(mg/kg)		Testosterone (ng/ml)	Estradiol (pg/ml)	Thyroxine (ng/ml)
Vehicle Control		2.1±0.31[a]	23.6±5.46	56.6±5.51
Flutamide	1	1.6±0.53	24.8±5.60	44.4±12.82
	5	3.2±1.05*	23.7±1.88	47.3±14.16
	25	3.1±0.25*	31.0±7.84*	51.3±10.21

a: Values are means derived from ten animals, ±SD.

*: Significantly different from the control value(*P<0.05).