EFFECTS OF DIETHYLSTILBESTROL ON PUBERTY IN MALE RATS: AN EVALUATION OT THE PROTOCOL FOR THE ASSESSMENT OF PUBERTAL 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]. The estrogen receptors are distributed widespread in the testis and reproductive tract from fetal life through adulthood, and the exposure of the developing male to exogenous estrogenic compounds either in utero or neonatally can result in a range of abnormalities of reproductive development and function. Therefore estrogens are considered to play a critical role in normal male reproductive development[2, 3, 4]. Diethylstilbestrol (DES) is a nonsteroidal synthetic estrogen capable of producing all the pharmacologic and therapeutic responses attributed to natural estrogens. It is indicated in the treatment of breast cancer with metastatic disease and prostatic carcinoma as palliative therapy. In the present report, we document the results of the rodent 20-day thyroid/pubertal assay of DES 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 SD 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 DES (10, 20, and 40 ug/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 LH, 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

Statistically significant decreases in mean body weights were observed at the highest dose (40 ug/kg) in rats treated with DES (Fig. 1). A significant delay was observed in the timing of PPS at doses greater than 20 ug/kg (Fig. 2a), however, no changes were observed in the mean body weight on PPS day because of the decrease of body weight of the treated animals in spite of the delayed PPS onset (Fig. 2b). The weight of ventral prostate and SVCGF were significantly reduced in the 20 and 40 ug/kg/day (Table 1). The LABC and glans penis were reduced in weight at all dose levels and the adjacent Cowper's gland was significantly smaller at 20 and 40 ug/kg/day (Table 1). Relative weights of ventral prostate, SVCGF, LABC, Cowper's gland and glans penis were statistically significantly decreased in rats treated with DES (Table 1). The weight of testes was significantly decreased at 20 and 40 ug/kg/day (Table 2). The adrenal gland weight was increased at all doses examined (Table 2). The weight of liver was increased at 10 and 20 ug/kg/day, but the relative weight increased in all dose group due to the decrease of body weight in the highest group. As for other point such as food intake and weights of thyroid glands, hypophysis, kidney, there were no effects of DES treatment on the serious systemic toxicity. No microscopic changes were observed in the thyroid gland after DES administration (data not shown). For hormone measurement, serum testosterone and LH levels were significantly decreased at all DES-treated group but serum estradiol levels were slightly decreased (not significant). No significant difference with respect to thyroxin levels was observed (Table 3). The present rodent 20-day thyroid/pubertal assay of DES, an estrogen receptor agonist, 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.

Acknowledgments

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References

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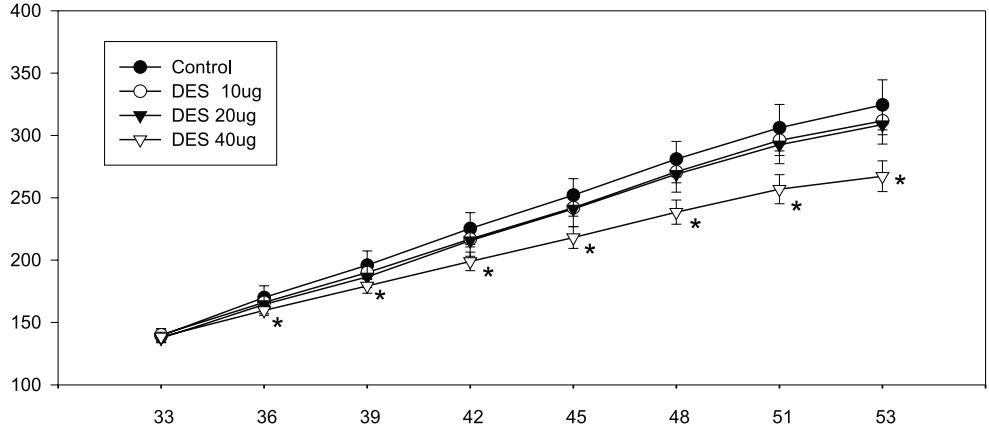

Figure 1. Body weight changes of male rats treated with DES for 20 days from postnatal day 33. Values are given as mean±SD. * Significantly different from the control group (P<0.05).

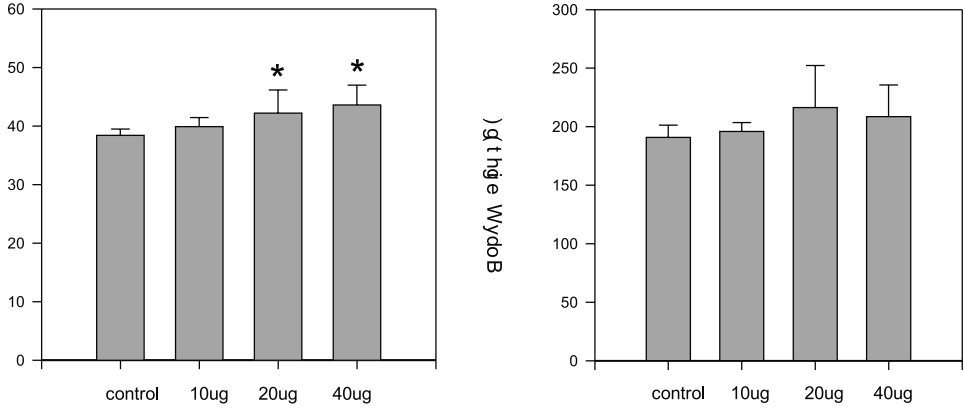

Figure 2. Effects of DES on the onset of PPS (A) and the body weight on day of PPS (B). Values are given as mean±SD. * Significantly different from the control group (P<0.05).

Table 1. Absolute (A) and Relative (R) Weights of Reproductive Organs and Accessory Sex Glands in Control and DES Treated Male Rats

Group		Combined Testes	Combined Epididymides	Ventral Prostate	SVCGF	LABC Gland	Cowper's Penis	Glans
Corn oil	A	2.73±0.16[a]	0.33±0.03	0.28±0.04	0.54±0.09	0.76±0.07	0.056±0.009	0.085±0.006
	R	0.89±0.07	0.107±0.008	0.09±0.01	0.18±0.02	0.25±0.02	0.018±0.003	0.028±0.003
DES	A	2.64±0.12	0.32±0.03	0.29±0.02	0.52±0.04	0.63±0.03*	0.051±0.007	0.073±0.003*
10ug/kg	R	0.88±0.03	0.105±0.007	0.10±0.01	0.17±0.01	0.21±0.01*	0.017±0.002	0.024±0.002*
DES	A	2.56±0.13*	0.30±0.03	0.19±0.02*	0.43±0.08*	0.62±0.04*	0.036±0.007*	0.074±0.005*
20ug/kg	R	0.85±0.03	0.102±0.007	0.06±0.01*	0.14±0.02*	0.20±0.01*	0.012±0.002*	0.024±0.003*
DES	A	2.47±0.13*	0.26±0.01*	0.14±0.03*	0.13±0.02*	0.39±0.03*	0.021±0.005*	0.061±0.004*
40ug/kg	R	0.96±0.05*	0.102±0.006	0.05±0.01*	0.05±0.01*	0.15±0.01*	0.009±0.001*	0.024±0.002*

a: Values are means derived from ten animals (g±SD).

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

Table 2. Absolute (A) and Relative (R) Organ Weights in Control and DES Treated Male Rats

Group		Liver	Heart	Combined Adrenals	Combined Kidneys	Thyroid Glands	Hypophysis
Corn oil	A	9.61±0.07[a]	1.01±0.07	0.046±0.007	2.44±0.10	0.013±0.003	0.011±0.001
	R	3.09±0.18	0.33±0.02	0.015±0.002	0.79±0.05	0.004±0.001	0.003±0.001
DES	A	12.12±0.65*	1.04±0.07	0.069±0.006*	2.42±0.31	0.017±0.003*	0.011±0.002
10µg/kg	R	4.01±0.16*	0.34±0.02	0.023±0.002*	0.80±0.10	0.006±0.001*	0.004±0.001
DES	A	12.94±0.58*	1.02±0.05	0.071±0.011*	2.31±0.12	0.010±0.003	0.010±0.003
20µg/kg	R	4.26±0.21*	0.34±0.01	0.024±0.003*	0.77±0.04	0.003±0.001	0.004±0.001
DES	A	9.84±0.73	0.87±0.03*	0.073±0.005*	2.20±0.21	0.013±0.001	0.011±0.001
40µg/kg	R	3.85±0.17*	0.34±0.02	0.029±0.002*	0.86±0.05	0.005±0.001	0.004±0.001*

a: Values are means derived from ten animals (g±SD).

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

Table 3. Serum Hormone Levels in Male SD Rats Treated with DES from PND 33 to PND 53

Group	Testosterone (ng/ml)	Estradiol (pg/ml)	Thyroxine (ng/ml)	LH (pg/ml)	
Corn oil	2.1±0.31[a]	28.7±8.89	56.6±5.51	23.3±8.52	
	10µg/kg	0.5±0.19*	22.4±3.11	75.6±17.43	7.9±1.03*
DES	20µg/kg	0.6±0.69*	22.9±11.57	69.1±13.05	10.7±5.18*
	40µg/kg	0.3±0.21*	23.5±4.92	54.1±13.48	5.1±0.54*

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

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