REPRODUCTIVE TOXICITY OF METHYLTESTOSTERONOE AND FLUTAMIDE, REFERENCE MATERIALS TO SCREEN ENDOCRINE DISRUPTING CHEMICALS MIMICKING ANDROGENS AND ANTIANDROGENS, IN ADULT MALE RATS

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Introduction

The hypothalamo-pituitary unit and the reproductive system are delicately regulated by interactions of various endocrine, paracrine and autocrine factors. Hypophysial hormones play a vital role in regulating gonadal function, while gonadal steroid hormones are in the control of their secretion by a feedback mechanism. To operate normal functions of reproductive system, the maintenance of appropriate hormone balance is essential[1]. This balance is easily impaired by various exogenous chemicals disrupting endocrine system[2].

Recently, it is acknowledged that reproductive disorders observed in wild animals could be related to a variety of man-made chemicals. Additionally, in human, the cause of decreases in sperm counts and fertility during last decades, are ascribed to these chemicals[2]. Their toxic effects are induced principally by mimicking endogenous hormones, especially sex steroid hormones. Thus, several governments and agencies make an effort to find screening methods for detection of endocrine disrupting chemicals, so-called endocrine disruptors(EDS)[3]. In this study, we executed the experiment to screen reproductive toxicity of methyltestosterone and flutamide, reference materials suggested by OECD to detect endocrine disrupting chemicals displaying androgen and antiandrogen-like activities, respectively, in adult male rats.

Methods and Materials

Nine-week-old male Sprague-Dawley rats were obtained from Han-lim Laboratory. After a quarantine and adaptation period of approximately 1 week, the rats were allotted to four groups by treatment materials: vehicle (CON), methyltestosterone (MET), flutamide (FLU) and two material combination (MET+FLU). A control group was administrated with vehicle (corn oil). Three treatment groups were sub-divided by different dosages: low (1 mg/kg B.W.), middle (10 mg/kg B.W.) and high (100 mg/kg B.W.) dose. At 10 weeks of age, all rats were administrated with each material by oral gavage. Following daily treatments during 1 week, some animals were sacrificed on the next day (Sac

I) of final treatment, and the others were sacrificed after 5 weeks (Sac II) following final treatment. On the sacrifice day, testis and accessory sex organs were weighed, and disposed to histologic procedures through fixation, embedding in paraffin, sectioning and staining. The number of sperm heads in cauda epididymis and testis was assessed and motions of sperms obtained from vasa deference were analyzed using SAIS 10.1 (Medical supply, Ltd, Seoul, Korea). Serum androgens levels were also determined.

Results and discussion

Sacrifice I

As shown in figure 1, paired testicular and epididymal weights in the high dose groups of FLU and MET+FLU were significantly reduced, compared to controls. Prostate weights obtained from the high dose group of MET were significantly increased, while those from all dose groups of FLU and the middle and high dose groups of MET+FLU were significantly decreased, compared to controls. Reductions in seminal vesicle weights were seen at all dose groups of FLU, and the middle and high dose of MET+FLU groups, compared to controls.

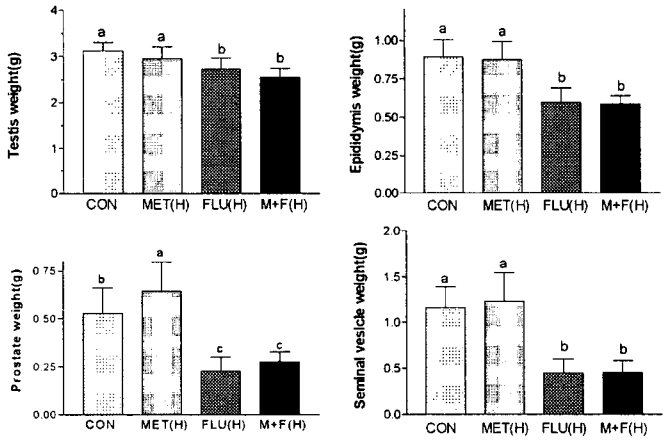

Fig. 1. Weight comparison of the testis and accessory sex organs in adult male rats following treatments with high dose of methyl-testosterone (MET), flutamide (FLU), and two material combination (MET+FLU) for 7 consecutive days. The means with no superscripts(lowercase letters) in common are significantly different($p<0.05$).

Testicular microscopic changes in the high dose groups of all materials were presented in figure 2. Slightly degenerative changes in spermatocytes were observed at early stages of spermatogenesis in MET group, while at later stages, VI-VIII of spermatogenesis in FLU group. In of MET+FLU group, markedly degenerative changes in spermatocytes at VI-VIII stages of spermatogenesis were noticed.

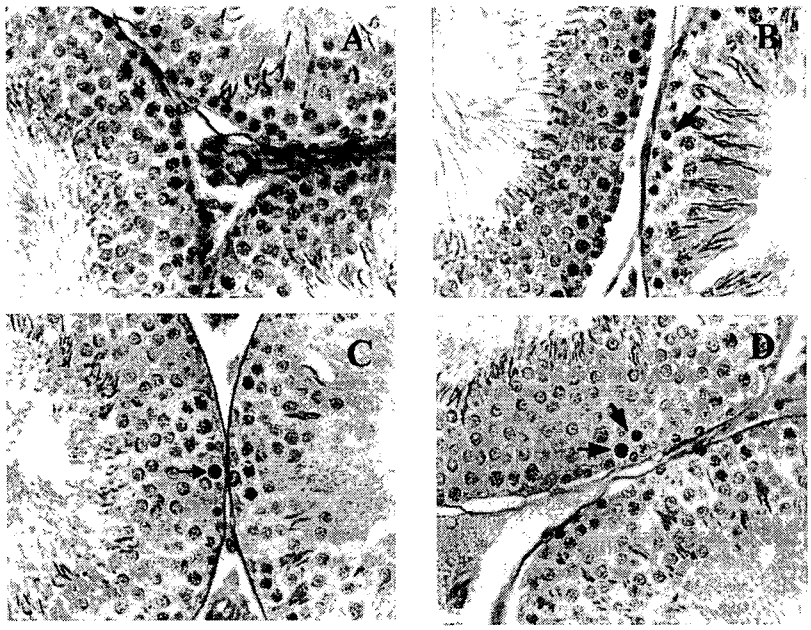

Fig. 2. Testicular histology of adult male rats treated with high dose of methyltestosterone (MET), flutamide (FLU), and two material combination (MET+FLU) for 7 consecutive days. A: CON group, B: MET group, C: FLU group, D: MET+FLU group. All magnifications are ×400.

In prostate gland (Fig. 3), epithelial cells characterized by hypertrophied cells forming many folds were observed in the high dose group of MET. In the high dose group of FLU, well developed stroma occupying glandular portions and low height epithelial cells were examined. In the high dose group of MET+FLU, epithelial cell layer was characterized by low height.

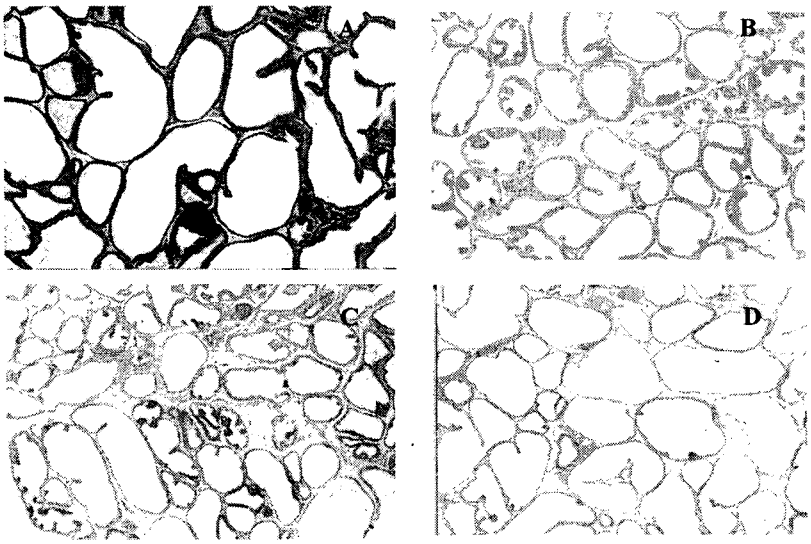

Fig. 3. Histology of prostate gland in adult male rats following treatments with high dose of methyltestosterone (MET), flutamide (FLU), and two material combination (MET+FLU) for 7

consecutive days. A: CON group, B: MET group, C: FLU group, D: MET+FLU group. All magnifications are ×40.

In seminal vesicle (Fig. 4), highly hypertrophied epithelial cells in the high dose group of MET were examined. In the high dose groups of FLU and MET+FLU, epithelial cells were atrophied and muscle layers characterized by thickening due to narrowing of inner space were observed. Weight changes in prostate and seminal vesicle following material treatments were further supported by these histological findings.

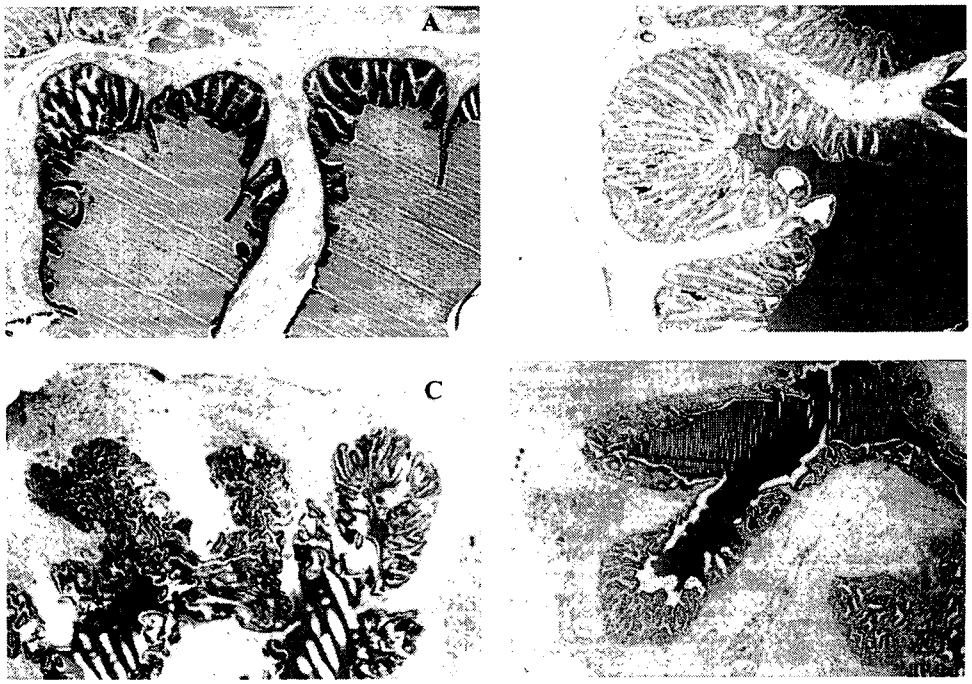

Fig. 4. Histology of the seminal vesicle in adult male rats following treatments with high dose of methyltestosterone (MET), flutamide (FLU), and two material combination (MET+FLU) for 7 consecutive days. A: CON group, B: MET group, C: FLU group, D: MET+FLU group. All magnifications are ×40.

In cauda epididymal sperm counts(table 1), significant increase was observed in the middle dose group of MET. On the contrary, in the high dose groups of FLU and MET+FLU, these were significantly decreased, compared to controls.

Table 1. The counts of sperms recovered from cauda epididymis in adult male rats following treatments with methyltestosterone (MET), flutamide (FLU), and two material combination (MET+FLU) for 7 consecutive days.

Materials / Dose	CON	MET	FLU	MET+FLU
LOW	83.0±17.5	96.3±28.9	91.5±30.8	74.1±20.5
MID		109.6±25.9 *	85.0±16.0	73.4±26.0
HIGH		60.1±18.5	44.5±16.6 *	37.3±12.9 *

Values($x10^6$/organ) are Mean±S.D.

In serum androgens concentrations (Fig. 5), significant increase in the high dose group of FLU was determined. On the other hand, in the high dose groups of MET and MET+FLU, serum androgens levels were markedly decreased, compared to controls.

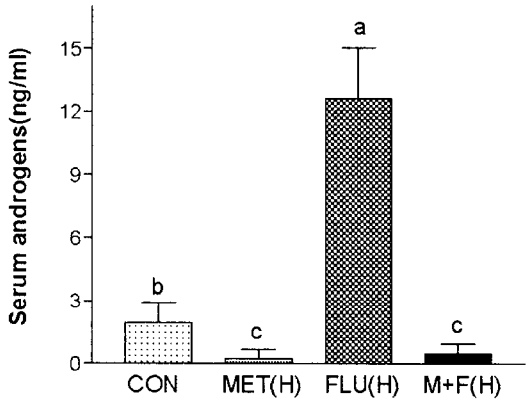

Fig. 5. The changes in serum androgen levels in adult male rats following treatments with high dose of methyltestosterone (MET), flutamide (FLU), and two material combination (MET+FLU) for 7 consecutive days. The means with no superscripts(lowercase letters) in common are significantly different ($p<0.05$).

Sacrifice II

In the sacrifice II, significant differences were observed in sperm motion parameters(Fig. 6). Sperm motility was significantly decreased in the high dose group of MET+FLU, compared to controls. In other parameters of curvilinear velocity, straight-line velocity, average path velocity, beat cross frequency and lateral head displacement, significant decrements were also observed in the high dose group of FLU, and, furthermore, in the high dose group of MET+FLU, compared to controls.

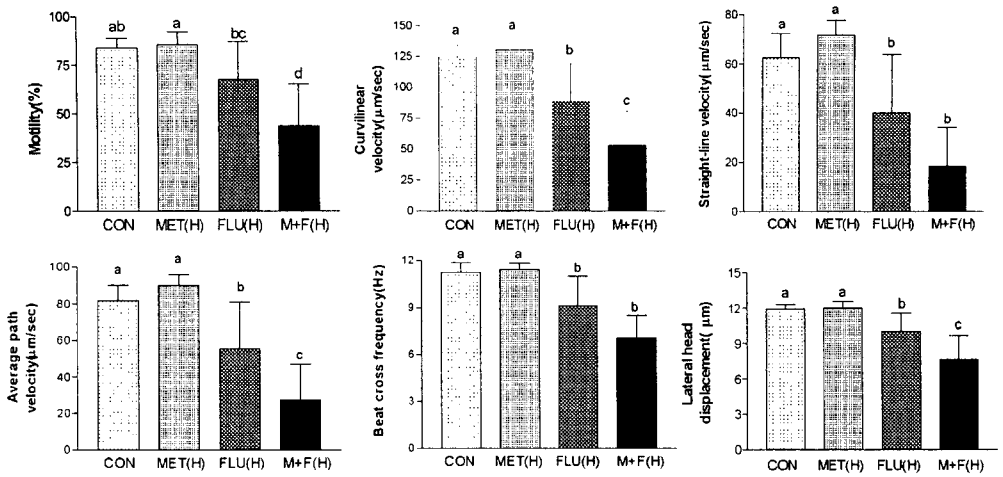

Fig. 6. Motion parameters of sperm retrieved from vasa deference at five weeks following treatments with high dose of methyltestosterone(MET), flutamide(FLU), and two material combination (MET+FLU). The means with no superscripts(lowercase letters) in common are significantly different($p<0.05$).

Daily methyltestosterone treatment to adult male rats during 1 week increased the weights of prostate gland and seminal vesicle, and hypertrophied epithelial cells in these organs. On the contrary, treatment of flutamide and two material combination decreased the weights of sex organs, induced degeneration of testicular spermatocytes. Flutamide treatments increased serum androgens concentrations, while treatments of methyltesotsterone and two material combination decreased them.

On sacrifice executed after 5 weeks following the final treatment, the time when spermatocytes affected by treatment materials are present in vas deference, motion parameters of sperms retrieved from vasa deference showed significant decrement in flutamide group and two material combination group.

The overall results indicate that the treatments of methyltestosterone and flutamide during 7 consecutive days in the adult male rat could be associated with a partial failure in reproductive system.

References

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3. Gray LE, Kelce WR, Wiese T, Tyl R, Gaido K, Cook J, Klinefelter G, Desaulniers D, Wilson E, Zacharewski T, Waller C, Foster P, Laskey J, Reel J, Giesy J, Lasw S, Mclachlan J, Breslin W, Cooper R, Giulio RD, Johnson R, Purdy R, Mihaich E, Safe S, Sonnenschein C, welshons W, Miller R, Mcmaster S, Colborn T. (1997) Reprod. Toxicol. 11,719.