Investigation of Male Rats Reproductive Function after Herbicide 2,4-DA Poisoning.

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Introduction

In conditions of increasing technical influence on the environment one of the most sensitive places in our organism is reproductive system [1,2]. Among numerous ecological toxicants there are chloroorganic compounds containing microscopic dioxins admixture, for example, highly effective and widely spread in agriculture herbicides on the base of 2,4-dichlorphenoxiacetic acid (2,4-D). Admission of compounds containing dioxins into an organism results in unfavourable consequences such as reproductive system diseases, fertility decreasing and heredity pathology [3,4,5]. However, the appearance and development mechanisms of male reproductive system disturbances due to admission of 2,4-D little and microscopic doses, i.e. in the real ecological situation, are not sufficiently clear up to nowadays. Therefore, the aim of our investigation was to study male rats reproductive function and general aspects of its regulation after chronicle intoxication by 2,4-DA (dimethylamine salt).

Materials and Methods

150 sexually mature male rats weighing 240-280 grams were used in the experiments. Chronicle intoxication was provided by means of 2,4-DA daily introduction into the stomach in 1/10 LD_{50} and LD_{50} total doses during 4 months according to rats spermatogenesis duration as well as time necessary for spermatozoons passing through epididimis. Indicated time allows to estimate the functional state of gametes which was under the toxicant influence during all period of their development. State of spermatozoons, seminiferous epithelium and Leydig's cells were studied after intoxication ending according to recommendation described in works [6].

Male rats being introducted herbicide were placed with intact female ones in order to study 2,4-DA influence on fertility. Male rats were taken away after a week and female rats were under constant supervision. The number of pregnant female rats as well as the number and

state of newborn rats in a brood was taken into consideration. Male rats were paired with female ones having normal anovulatory cycle after different time periods from toxicant annulment.

Radioimmunoassay of testosterone, estradiol, LH, FSH, prolactin, PGE_2 and I_2 was carried out in serum. Testosterone level was found also in testicles. Testosterone concentration in serum two hours later human chorionic gonadotropin (hCG) introduction in dose 100 ED for animal showed androgen testicles reserves. The test was estimated according to the ratio of hCG stimulated testosterone level in serum for basis one and was considered as positive, i.e. excepted enzyme block in hormone biosynthesis if this ratio was more than 2 [7].

Results and discussion

Lasting 2,4-DA introduction resulted in steady testicles endocrinal function depression manifested as testosterone level decreasing both in serum and in the testicles (table 1) which may be interpreted as androgens production depression.

Table 1

Some hormones levels in the rats serum and testicles after 2,4-DA herbicide chronicle intoxication.

Hormones	Control	1/10 LD_{50}	LD_{50}
Testosterone (testicles), pM/g	129.3±33.4	63.2±13.2*	55.8±15.1*
Testosterone (serum), nM/l	17.2±1.9	10.2±2.1*	9.5±1.8*
Estradiol, pM/l	190.1±23.8	233.4±21.4	274.8±24.1*
LH, ng/ml	0.68±0.09	0.47±0.05*	0.38±0.06*
FSH, ng/ml	7.65±0.69	6.08±1.10	4.7±0.49*
Prolactin, ng/ml	1.88±0.22	3.95±0.20*	4.17±0.27*
PGI_2, ng/ml	144.8±15.9	165.2±12.0	172.3±16.8
PGE_2, ng/l	279.1±15.0	214.6±26.3*	186.5±22.0*

*statistically significant changes ($p<0,05$)

Chorionic gonadotropin test showed that testicles remained sensitive to its stimulating effect. It was marked that testicles androgen reactivity in conditions of 2.4-DA introduction was even 1,3-1,5 times as large than the control animals one. So, hypotestosteronaemia after 2,4-DA intoxication is secondary and may be caused by endogenic LH deficiency (according to our data its level decreased to 58-69% compared with controls). At the same time FSH and prolactin levels changes were developing, i.e. FSH concentration decreased and prolactin one increased. Close correlation between LH, FSH and testosterone content during whole herbicide introduction period shows hypophysis leading role in the mechanisms of testes hormonal function inhibition. Honadotropines deficiency testifies LH-RH influence decreasing (luteinizing hormone-releasing hormone). Hypothalamus functional state is caused by prolactin concentration, high level of which depresses LH-RH secretion because

of dopamine content increasing in different brain structures. Besides, found estradiol level increasing may limit gonadotrophs reaction to LH-RH because estrogens reduces its impulses ratio [8].
Estrogenisation may be also followed by sexhormonbinding globulines increasing in serum and decreasing of free testosterone fraction which is readily available for tissues.

Correlation between testosterone and estradiol level as well as between PGI_2 one (r=-0,71 and 0,76 accordingly) was also found, i.e. testosterone inhibition effect on PGI_2 production was removed and estradiol stimulating effect prevailed. On the contrary, concentration of PGE_2, which stimulates LH releasing, decreased and it correlates with found in our experiment low level of this hormone. PG of classes E and I are the so called "protective" because they can limit cells injury in extremal state [9]. Our data shows relative decreasing of these classes PG level after 2,4-DA intoxication and it can result in cell protective possibility diminishing.

Androgen state violation resulted in morphofunctional changes of spermatogenesis indexes (table 2).

Table 2

Functional and morphological indexes of white rats spermatogenesis after 4 month 2,4-DA influence (M±m)

Index	Control	1/10 LD_{50}	LD_{50}
Spermatozoons mobility, min	261±11.9	215±14.5*	178±9.4*
Spermatozoons amount, 10^6	42±5.7	27.8±3.6*	21.1±3.6*
Spermatogenesis index	3.22±0.08	2.68±0.08*	2.07±0.13*
Normal spermatogonia amount	52.9±0.8	47.7±1.4*	37.4±0.8*
Tubules with desquamated epithelium, %	4.25±0.25	7.75±0.85*	10.5±0.96*
Testicles weight coefficients	0.73±0.06	0.78±0.07	0.70±0.05

Herbicide gonadotoxical effect was mainly due to changes of spermatozoons amount and percent of tubules with desquamated epithelium. Normal spermatogonia amount as well as testicles weight coefficients were the most resistant to the herbicide and decreased according to the dose only to 10-29% compared with controls. A relative maintenance of deep seminiferous epithelium layers in the presence of significant spermatozoons amount decreasing shows that spermatogenes cells damage mainly occurs during the stage of spermiogenesis. We can suppose that spermatogenesis index decreasing after the herbicide introduction is due to accelerated sex cells elimination and it is indirectly confirmed by found decreasing of seminiferous epithelium sloughing into the tubular lumen.

Microscopic testicle examination reveals Leydig's and Sertoli cells changes. Interstitial cells small forms amount, which are either immature or involutional with low functional activity, increased in the experiment animals. Follicular cells number also increased under the herbicide influence: Sertoli cells active production was not revealed in control rats but it was observed in 7-8 among 100 examined convoluted seminiferous tubules after poisoning

by 1/10 LD_{50} and after poisoning by LD_{50} in 15-18 ones. The interpretation of found results is not easy because of Sertoli cells multitude functions [10]. Sertoli cells number increasing can be an evidence of their active reaction on the toxical agent influence and has compensative meaning.

Changes of male rats reproductive function integral characteristic that is their fertility became the important result of spermatogenesis insufficiency. Fertility index decreased in 1,9 times after the herbicide introduction in 1/10 LD_{50} total dose, LD_{50} dose resulted in fertility absence but all control rats gave posterity. A month after herbicide annulment some restoration of experiment animals fertility observed and it was more expressive after poisoning by the 1/10 LD_{50} dose. But its full repair after larger dose influence did not occur even two months after herbicide annulment. Therefore, 2,4-DA lasting introduction causes transitorial sterility with long repair period in intact sexually mature male rats.

Testicles germinative function is maintained when gonadotropins and testosterone level is normal, because these hormones control sex cells development on the stages of spermatocytes and spermatides. High intratubular testosterone level necessary for gametogenesis is obtained by means of its transport to seminiferous epithelium cells in connection with androgenbinding proteins produced in Sertoli cells. Besides, spermatozoons fertility is also caused by PG of E class concentration. Complex system, in which testicles function is controlled by two hypophysis hormones and hypothalamus-hypophysis-gonads system function is controlled by wide endogenic modulators spectrum, makes very exact and fine mechanism of multiply control where testosterone and gonadotropins levels in serum and spermatozoons production have reverse influence on hypophysis and hypothalamus, regulating its own production speed. Evidently, found changes of androgen state and testicles morphofunctional state due character and grade of reproductive function damage under 2,4-D ecotoxicants influence and must be taken into consideration in the methods of their correction.

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