Effects of Anthracyclines on Reproductive Function in Rats

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A single injection of antitumor anthracycline antibiotic in a maximum tolerated dose decreased mating capacity in male rats (doxorubicin, farmorubicin) and decreased the efficiency of mating (farmorubicin). In female rats mating capacity did not decrease, but some of them became sterile. The majority of toxic effects were reversible. The incidence of embryonal death before implantation was increased in intact females mated with males treated with farmorubicin. In females treated with anthracycline antibiotics high incidence of pre- and postimplantation embryonal deaths was noted.

Key Words: *anthracycline antibiotics; reproductive function*

Antitumor anthracycline antibiotics are effective broad-spectrum cytostatics [6]. Doxorubicin and farmorubicin, close by their chemical composition, are most often used. Doxorubicin is effective in 17 tumors [4] and its analog farmorubicin is as effective, but is characterized by lower hemato- and cardiotoxicity [3,5]. Encouraging results of the treatment of many oncological diseases with anthracycline antibiotics attracted much interest to the problem of sterility as a consequence of toxic effect of these drugs on the gonads [7, 10,12]. The data of clinical studies are contradictory. N. A. Rastrygin [7] noted that these drugs exert pronounced toxic effects on male and female gonads, while R. L. Schilsky [12] showed that doxorubicin only rarely induced reproductive dysfunction in males. Reproductive function after antibiotic treatment has been studied experimentally [11,13], but not extensively.

We investigated early and delayed effects of doxorubicin and farmorubucin on the function of male and female reproductive system.

MATERIALS AND METHODS

Experiments were carried out on 360 Wistar rats (250-300 g) from Rassvet Breeding Center, Tomsk; half of these were controls. The animals were handled in accordance with the European Convention (Strasbourg, 1986). Before and during the experiment the animals were kept under standard vivarium conditions in plastic cages, up to 15 animals per cage with wood chips, and fed standard fodder PK 120-3. Doxorubicin (adriablastine) and farmorubicin (both from Farmitalia Carlo Erba) were intravenously injected to males and females in single maximum tolerable doses (4.0 and 7.5 mg/kg, respectively). The doses were estimated by the graphic probit analysis after 30-day observations [1].

The reproductive function in males and females was evaluated by their fertility and the rate of embryonic death. Experimental and control animals were caged with intact rats 1-10, 30-40, 90-100, and 180-190 days after cytostatic treatment (2 females per male) for 10 days (duration of two estral cycles). Experimental and control groups consisted of at least 20 females and 10 males. Mating was confirmed by vaginal smears. The mating capacity (fertility index, FI) and efficiency of mating (pregnancy index, PI) [8]. On day 20 of pregnancy the females were sacrificed by cervical dislocation and the number of corpora lutea in the ovaries, implantation sites and live and dead fetuses in the uterus were counted, after which pre- and postimplantation mortality indexes were estimated [8].

The results were statistically processed using Wilcoxon, Mann—Whitney tests, and Fisher angular transformation.

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RESULTS

During the first 10 days after doxorubicin injection FI in male rats decreased by 2.5 times in comparison with the control (Table 1). In other days of experiment this index did not differ from the control. In males treated with farmorubicin the mating capacity also decreased 2-fold but later than after doxorubicin (30 days postinjection). Experimental males were inert and apathetic during these periods; the decreased FI was presumably a result of impaired sexual instinct which is controlled by systems regulating the reproductive function and hormonal status. Presumably the drugs exert toxic effects on the central nervous system; it is known that antiblastomogenic agents induce depression [9]. In females treated with anthracycline antibiotics PI did not differ from the control during the entire experiment (Table 1). However it was previously shown that anthracycline antibiotics, in particular, doxorubicin in high doses reduce fertility in female mice [13].

The efficiency of mating did not decrease in males treated with doxorubicin during the entire experiment. By contrast, PI of intact females mated with farmorubicin-treated males was decreased during the first 10 days of the experiment. Moreover, all females mated 90 days postinjection were not fertilized. Sterilization of males in this experimental group was transitory, and their fertility was restored by the end of the experiment. Suppression of the reproductive function in males after farmorubicin treatment is a result of previous oligospermia due to pronounced toxic effect of the drug on spermatozoa and spermatogonias (with consideration for the duration of spermatogenesis stages in rats) [2].

Injections of doxorubicin and farmorubicin to female rats decreased PI during the first 10 days of the experiment (Table 1) and the efficiency of mating at these terms decreased 3.5 times after doxorubicin and 2-fold after farmorubicin. Interestingly, that females mated 1 day after cytostatic treatment were not pregnant; this was apparently due to toxic effect of the drug on mature follicles in which meiotic division was activated. At other terms PI in experimental females was similar to that of controls. The only exception were females injected with doxorubicin 180 days before mating. Only 67.7% of them got pregnant after mating with intact males (Table 1).

Pre- and postimplantation mortality in intact females mated with experimental males did not differ

TABLE 1. Effect of Doxorubicin (D) and Farmorubicin (F) on Reproductive Function in Rats (% of Control)

Parameter		Terms of mating after drug injection, days			
		1-10	30-40	90-100	180-190
FI					
males	D	37.5*	72.8	93.7	101.6
	F	76.4	51.6*	75.0	90.3
females	D	87.5	108.2	97.9	87.7
	F	96.6	104.4	93.5	88.6
PI					
males	D	100.0	93.8	91.7	92.3
	F	80.0*	100.0	0.0	95.0
females	D	26.6*	95.7	95.7	67.7*
	F	57.5*	87.5	112.3	83.0
Preimplantation mortality					
males	D	181.2	98.4	139.6	67.0
	F	95.6	459.8*	—	36.6
females	D	124.3	247.3*	191.0	71.3
	F	59.6	425.6*	193.5	512.8*
Postimplantation mortality					
males	D	128.5	100.0	225.5	133.7
	F	41.6	75.7	—	82.3
females	D	1194.3*	1160.8*	1012.1*	233.3*
	F	1167.7*	49.0	190.0	473.2*

Note. *$p<0.05$ *vs.* the control.

from the control at all terms of observation. Preimplantation mortality was increased only in females mated with males injected with farmorubicin 30 days before mating (Table 1). The duration of spermatogenesis stages in rats suggests that the drug affects spermatocytes.

The preimplantation mortality increased in females injected with anthracycline antibiotics 30 days (doxorubicin, farmorubicin) and 180 days (farmorubicin) before mating. It is noteworthy that postimplantation mortality was high in experimental females, in contrast to intact females mated with antibiotic-treated males. The highest rate of embryonal death after implantation was observed during the first 10 days of experiment in doxorubicin-treated females, this parameter being increased during all periods of observation. Recently an increased level of dominant lethal mutations in female sex cells under the effect of anthracycline antibiotics was demonstrated [14]. Therefore, the possibility of genotypical effect of the drugs on oocytes at different stages of maturation cannot be ruled out. The damaging effect of antibiotics on maternal organism can also contribute to this toxic effect.

Hence, antitumor anthracycline antibiotics decreased mating capacity in male rats (doxorubicin, farmorubicin) and efficiency of mating (farmorubicin). In female rats mating capacity did not decrease, but some of them became sterile. The majority of toxic effects were reversible. Preimplantation embryonal mortality increased in intact females mated with farmorubicin-treated males. High rates of pre- and postimplantation embryonal deaths were observed in females injected with anthracycline antibiotics. Differences in the damaging effects of anthracycline antibiotics on the reproductive function consisted mainly in the time and degree of manifestation: farmorubicin was more toxic for the male reproductive system and doxorubicin for females.

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