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Delayed Damaging Effects of Anthracycline Antibiotics on the Reproductive System of Rats

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Destructive changes and disturbances of spermatogenesis are found to occur in the testes of Wistar rats 1 and 3 months after a single injection of the anthracycline antibiotic pharmorubicin in the maximum permissible dose. The morphological picture normalizes 6 months postinjection. Disturbances in reproductive function are observed as early as 3 months after treatment. The number of dominant lethal mutations rises one month postinjection.

Key Words: *anthracycline antibiotic pharmorubicin; delayed effects; testis*

Anthracycline antibiotics are widely used in the chemotherapy of malignant neoplasms [3,4]. However, their strong toxic effect on healthy organs and tissues with a high proliferative activity calls for scrupulous experimental studies of these preparations [6]. The reproductive organs, bone marrow and gastrointestinal epithelium are among the organism's systems which renew themselves rapidly.

Anthracycline antibiotics induce disturbances of spermatogenesis in rats shortly after injection [2,5]. In veiw of the fact that these drugs can sometimes considerably prolong the life of cancer patients, their delayed toxic effects on the reproductive system assume great importance.

The present study was aimed at investigating the state of the reproductive system of male Wistar rats in the long term after treatment with anthracycline antibiotics. For our experimental model we used pharmorubicin (PR).

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MATERIALS AND METHODS

The experiments were performed on 90 male and 120 female Wistar rats weighing 150-200 g, 45 males and 60 females of which were included in the control groups. The males received a single intravenous injection of PR (Farmitalia Carlo Erba) in the maximum permissible dose (MPD) of 7.5 mg/kg, calculated by the methods of graphic probit-analysis over a 30-day observation period [1]. The control animals were injected with an equivalent volume of vehicle. For the study of morphological alterations in the reproductive organs males were sacrificed by cervical dislocation 1, 3, and 6 months after injection of PR (5 rats in the control and experimental groups per point). The testes were removed and fixed in Carnoy fluid. Paraffin sections (5 μ) were prepared, stained with hematoxylin and eosin, and used for morphologi-

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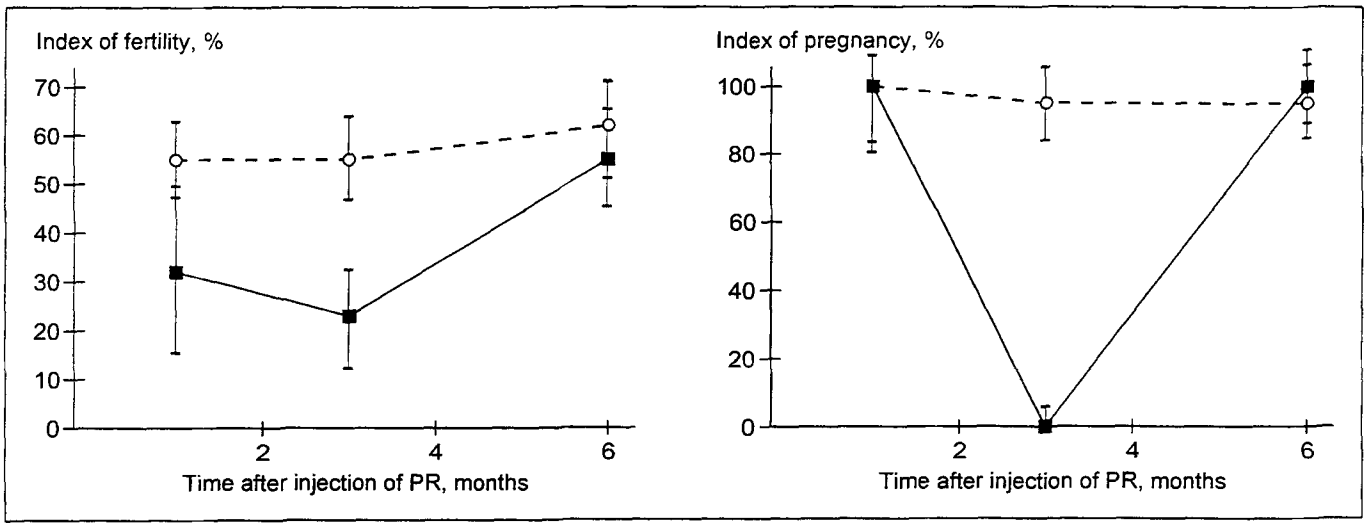

Fig. 1. Fertility of male rats in the long term after a single administration of PR in the MPD. Dashed line: control.

cal analysis (index of spermatogenesis, number of normal spermatogonia, number of tubules with meiotic stage XII, and tubules with desquamated epithelium). In addition, we counted degenerative forms of spermatozoa and the total number of sperm per epididymis (TNS). Mature spermatozoa were obtained from the epididymis by cutting it longitudinally in physiological saline. The suspension of sperm was placed on a slide and smears were prepared and stained with hematoxylin and eosin. TNS in the epididymis was determined using a homogenate in a certain volume of physiological saline and a leukocyte mixer and Goryaev counting chamber [7].

For evaluation of the functional state of the reproductive system 1, 3, and 6 months following PR administration, 30 experimental rats and 30 controls were caged with 120 intact females (1:2 ratio) for 10 days (duration of two estrous cycles). Mating was judged from vaginal smears. The indexes of fertility and pregnancy were calculated [7]. Pregnant females were sacrificed on day 20 of gestation and the number of corpora lutea in the ovaries, of implantation sites in the uterus, and of live and dead fetuses were counted. Then the indexes of pre- and postimplantation mortality were calculated [7]. Statistical processing of the results was carried out using the Student and Wilcoxon-Mann-Whitney tests and the Fisher z-transformation.

RESULTS

One month after injection of PR a mild edema, vascular hyperemia, and lymphoid infiltration of interstitial tissue are observed in the testes. The number of normal spermatogonia is sharply reduced in comparison with that in the control group (Table 1), and in some seminiferous tubules they are absent altogether, lowering the index of spermatogenesis. Other epithelial layers in the convoluted seminiferous tubules are also maximally depleted. The number of tubules with meiotic stage XII is reliably decreased, whereas the number of tubules with desquamated epithelium does not differ from that in the control (Table 1). Three months after PR administration the vascular reaction is not strongly expressed, and clusters of leukocytes are seen among the interstitial endocrinocytes. The number of normal spermatogonia in higher than that observed one month post-

TABLE 1. Morphological Parameters of Spermatogenesis in Rats at Different Times after Injection of PR in the MPD ($M \pm m$)

Parameter	1 months		3 months		6 months	
	control	PR	control	PR	control	PR
Number of spermatogonia	10.28±0.56	0.27±0.19*	18.14±2.14	5.28±0.90*	15.71±0.97	8.88±0.63*
Index of spermatogenesis, arb. units	3.84±0.07	3.03±0.21*	3.76±0.02	2.60±0.25*	3.62±0.06	3.53±0.08
Tubules with meiotic stage XII, %	2.62±0.49	0.40±0.24*	1.00±0.63	1.40±0.24	1.17±0.31	3.00±1.25
Tubules with desquamated epithelium, %	0.75±0.52	0.60±0.40	0.83±0.40	1.60±0.68	0.83±0.81	1.50±0.50

Note. Here and Table 2: *$p<0.05$ in comparison with the control.

TABLE 2. Parameters of Reproductive Function of Female Rats Mated with Males Given PR in the MPD 1 and 6 months before Mating (*M*±*m*)

Group	No. of corpora lutea per female	No. of implantation sites per female	No. of live fetuses per female	Preimplantation mortality, %	Postimplantation mortality, %
PR injected to males 1 month before mating	10.40±0.68	5.60±1.47*	5.20±1.35*	44.60±14.39*	5.00±4.2
Control	13.00±0.68	11.75±0.88	11.00±0.90	9.75±3.83	6.62±1.59
PR injected to males 6 months before mating	11.92±0.24	11.07±0.38	10.09±0.49	7.13±3.00	4.19±1.34
Control	10.20±0.37	10.00±0.45	9.40±0.40	2.00±2.00	5.82±2.40

injection, but still below the control value. Among the spermatogonia are many dividing cells. Some tubules have the normal structure, possibly attesting to a process of reparative regeneration. At the same time, some tubules are depleted to the extent that only sustentocytes are preserved on the basal membrane. The index of spermatogenesis is lowered. The number of tubules with meiotic stage XII and with desquamated epithelium does not differ from that in controls (Table 1). Six months after PR injection the morphological picture normalizes. Hardly any of the studied parameters of spermatogenesis differ from the control values. However, the number of normal spermatogonia is still reduced and accounts for 50% of the control (Table 1). TNS per epididymis and the number of degenerative forms of spermatozoa one and 6 months postinjection are found to be similar in both groups. A sharp drop of TNS (to 5.7% of the control value) and an increased number of degenerative forms (42.55±6.25% *vs.* 22.75±1.84%) are noted 3 months postinjection.

The indexes of fertility and pregnancy one month after the injection of PR do not differ reliably from these in controls (Fig. 1). However, 3 months postinjection the index of fertility drops sharply, not one fertilized female being pregnant. Fertility and ability to conceive are restored 6 months after PR administration (Fig. 1).

In females mated with males treated with PR one month beforehand, the number of corpora lutea (per female) is the same (Table 2), whereas the number of implantation sites is only half as high as that in controls, resulting in increased preimplantation mortality. This parameter increases 5-fold in the experimental group. The number of live fetuses per female decreases due to a greatly reduced number of implantation sites. However, postimplantation mortality does not rise (Table 2). Since zygotes with multiple chromosome aberrations usually die before implantation, the increased preimplantation mortality suggests numerous abnormalities in the genetic apparatus of the sex cells [7]. As mentioned above, not a single rat became pregnant obtained 3 months after PR administration. When PR was injected to males 6 months before mating, embryonal mortality did not differ from the control values (Table 2).

Thus, marked destructive alterations and disturbances of spermatogenesis are seen in the testes one month after injection of PR. Three months later the morphological picture is still abnormal, but some signs of reparative regeneration appear. The structure of the testes returns to normal 6 months after PR treatment. Fertility and the ability to conceive are disrupted 3 months postinjection. This is evidently related to oligospermia and an increased number of degenerative forms of mature spermatozoa and is a result of the depletion of the spermatogonium population 1 month postinjection. This male sterility is transient and the reproductive function is restored after 6 months. The increased preimplantation mortality in females mated with males treated 1 month earlier with PR suggests an increased level of dominant lethal mutations at the stage of primary spermatocytes. The preparation does not increase embryonal mortality at the stage of spermatogonia. On the other hand, the possibility of defective offspring being born cannot be ruled out.

Our findings suggest that the anthracycline antibiotic pharmorubicin exerts delayed toxic effects on the morphofunctional state of the reproductive system of male rats and on the genetic apparatus of the sex cells.

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