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Spermatogenesis in Rats After Administration of the Anthracycline Antibiotic Pharmorubicin

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It is established that Wistar rat testes demonstrate stable morphological changes attended by a diminishment of the stem cell population, depletion of the other layers of the spermatogenic epithelium, a decrease of the number of tubules with meiosis stage XII, and intensified desquamation of epithelial cells during 30 days after administration of pharmorubicin in the maximal permissible dose. The depletion of the stem cell population may result in long-term damage to the reproductive function and manifest itself long after antibiotic treatment.

Key Words: *testis; spermatogenesis; pharmorubicin*

Antitumor antibiotics from the anthracycline family are widely used in the drug therapy of malignant neoplasms [4,5]. Their marked antiblastomic effect is known to be accompanied by a toxic effect on cell systems of the organism with a rapid rate of renewal such as the bone marrow, and gastrointestinal epithelium, as well as the reproductive tissues [1,3,5,6]. Yet information on the effect of anthracycline antibiotics on the reproductive organs is scant and controversial.

The aim of the present investigation was to study the effect of the anthracycline antibiotic pharmorubicin (PR) on rat spermatogenesis.

MATERIALS AND METHODS

Experiments were carried out on 60 male Wistar rats weighing 110-130 g, 10 of which were controls. PR was injected i.v. at the maximal permis-

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TABLE 1. Morphological Indexes of Rat Spermatogenesis after a Single Administration of PR in the Maximal Permissible Dose ($M \pm m$)

Index	Control	Days after PR administration				
		2	5	10	15	30
Index of spermatogenesis, arb. units	3.84±0.07	3.92±0.06	3.70±0.08	3.76±0.19	3.70±0.06	3.05±0.21*
Mean number of spermatogonia	10.28±0.51	6.54±0.41*	4.21±0.32*	3.76±0.58*	2.53±0.11*	0.27±0.10*
Tubules with desquamated epithelium, %	0.75±0.30	0.40±0.20	1.10±0.23	1.80±0.20*	1.40±0.24*	0.60±0.04
Tubules with stage XII of meiosis, %	2.62±0.50	1.20±0.30	1.30±0.32	1.00±0.34	0.44±0.24*	0.44±0.20*

Note: An asterisk indicates $p \leq 0.05$ as compared to the control.

sible dose of 7.5 mg/kg, calculated by the method of graphic probit-analysis, over a 30-day observation period [2]. Control animals were treated with an equivalent volume of solvent.

For a study of morphological changes occurring in the testes the rats were sacrificed by dislocation of the neck on the 2nd, 5th, 10th, 15th, or 30th day after PR administration. During autopsy, the testes were removed and fixed in Carnoy fluid. Paraffin sections (5 μ) were stained with hematoxylin-eosin. The number of layers of spermatogenic epithelium was counted in each individual tubule examined on the testis section and the index of spermatogenesis was calculated according to the formula: $J=\Sigma a/A$, where J is the index of spematogenesis; a is the number of layers of spermatogenic epithelium found in each tubule; A is the number of tubules counted. In addition, the mean number of normal spermatogonia, the number of tubules with meiotic stage XII, and the number of tubules with desquamated spermatogenic epithelium per 100 tubules were counted. The significance of differences in statistical processing of the experimental material was estimated using the Wilcoxon-Mann-Whitney nonparametric test.

RESULTS

On the 2nd day after PR administration karyorrhexis and karyopyknosis of spermatogonia and a significant decrease of their number to 63% of the control (Table 1) are found in the testes against the background of slight interstitial edema. Heads of mature spermatozoa are often hyperchromic. The signs of edema are preserved and the number of stem cells remains low on the 5th day of the experiment. Primary spermatocytes are swollen and their chromatin is marginally condensed. A portion of mature spermatozoa have hyperchromic heads. On the 10th day after antibiotic administration the morphological signs of damage of the spermatogenic epithelium become still more evident. A further depletion of the stem cell population occurs (Table 1). The number of primary and secondary spermatocytes as well as of spermatids drops markedly. Giant primary spermatocytes with clearly defined margination of chromatin and disrupted meiosis are common. The number of tubules with meiotic stage XII is insignificantly below the control. Some spermatozoa are hyperchromic and there is an increased number of tubules with desquamated spermatogenic epithelium and necrotized cells in their lumen. The number of stem cells remains lowered on the 15th day. Spermatogonia with deformed and wrinkled nuclei are encountered. Degenerative changes are preserved in spermatocytes. During this period of observation the number of tubules with meiotic stage XII is significantly lowered and the number of tubules with desquamated spermatogenic epithelium is increased (Table 1). On the 30th day after PR administration the number of spermatogonia dips to the lowest level during the whole period of observation. In some tubules stem cells are absent altogether, resulting in a significant decrease of the index of spermatogenesis (Table 1). Other layers of spermatogenic epithelium are also maximally depleted during this period. The number of tubules with meiotic stage XII is significantly lower as compared to the control, whereas the number of tubules with desquamated epithelium does not differ from that in the control. The boundaries of spermatogenic epithelial cells, especially of spermatids, are not clearly visible.

Thus, this study has established that stable morphological changes develop in rat testes during 30 days after a single antibiotic administration in the maximal permissible dose. The ability to bind with DNA, inhibiting its synthesis, lies at the root of the damaging effect of PR as well as of other anthracycline antibiotics. DNA replication is known

to occur during spermatogenesis in spermatogonia and primary spermatocytes at the proleptotene stage and this is probably the reason that these cells are the main target of the antibiotic's toxic effect. However, a direct toxic effect of the drug on nonproliferating cells of the spermatogenic epithelium, particularly on mature spermatozoa, is not ruled out. This is indicated by the hyperchromia of the nuclei of these cells observed during 10 days after PR administration. At the same time, such long-term damage to the morphological picture of the spermatogenic epithelium is primarily due to the progressive devastation of the stem cell population. The depletion of the proliferative pool of normal spermatogonia may result in long-term damage to the reproductive function and may manifest itself long after PR treatment. That is why only the surviving portion of stem cells should determine the ability of the spermatogenic epithelium to be regenerated.

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