The Rat Spermatogenesis after Injection of Paclitaxel (Antitumor Agent)

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Morphological and functional characteristics of spermatogenesis in rats were studied during the early and delayed periods after single injection of paclitaxel (antitumor agent) in the maximum tolerated dose. The drug induced structural changes in the gonads associated with oligospermia and increased number of pathological forms of spermatozoa with reduced functional activity.

Key Words: *paclitaxel; spermatogenesis; rats*

Paclitaxel is the first drug of the taxane group introduced into clinical practice and widely used in the treatment of the most incident tumors, such as lung cancer, breast and prostatic cancer, epithelial tumors of the head and neck, *etc.* [1,3]. Treatment with this drug produces a pronounced clinical effect in patients resistant to other antitumor drugs and prolongs the total and relapse-free survival [7]. The antiproliferative effect of paclitaxel is based on its capacity to stimulate the assembly of abnormal microtubules from tubulin dimers [11,12]. Encouraging results of treatment by protocols including this drug attracted special attention to the problem of sterility, a side effect of cytostatics on actively proliferating tissues of gonads [10]. Long-term treatment with this drug in low doses (0.1-1.0 mg/kg) leads to reduction of male rat fertility [14].

Since high-dose paclitaxel therapy is used in clinical practice, we studied the state of spermatogenesis in rats after single injection of paclitaxel in the maximum tolerable dose (MTD).

MATERIALS AND METHODS

Experiments were carried out on 50 male Wistar rats (25 controls and 25 experimental animals; 250-300 g) from Laboratory of Biological Simulation, Institute of Pharmacology. The animals were kept in accordance with the regulations approved by the European Convention for Vertebrate Protection (Strasbourg, 1986). Antitumor drug paclitaxel (mitotax, Dr. Reddy's) was intravenously injected in a dose of 7.6 mg/kg (MTD calculated by graphical probit analysis). Controls were injected with an equivalent volume of the solvent.

Spermatogenesis was studied during periods corresponding to the effects on different stages of sex cell maturation: days 1 and 5 for spermatozoa, day 16 for spermatides, day 36 for spermatocytes, and day 90 for spermatogonia [6]. For evaluation of morphological disorders developing in the testes, animals of both groups were sacrificed (5 per term of the experiment) by cervical dislocation. The testes and caudal parts of the epididymides were collected. The testes were fixed in Carnoy fluid. Paraffin sections (5 μ) were stained with hematoxylin and eosin. Spermatogenic epithelium layers were counted in each examined tubule on section and the spermatogenesis index was calculated. Spermatogonias, tubules with meiosis stage 12 and with desquamated spermatogenic epithelium were counted (per 100 tubules) [8]. For evaluation of spermatozoon morphology, their total count per epididymis was determined and the percent of pathological forms was calculated. The total count of spermatozoa (TCS) was evaluated in homogenized cell suspension of one epididymis in dosed portion of saline using a leukocytic melanger and a Goryaev chamber. The other epididymis was dis-

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sected, a smear was prepared, fixed in methylene, and stained with azur II and eosin. Functional state of mature male sex cells was evaluated by maximum duration of their movement and by the percent of mobile forms in the suspension of sex cells from the epididymis (on a slide in a humid chamber) [8].

The results were statistically processed using nonparametric Mann—Whitney test.

RESULTS

During the first 24 h after injection of paclitaxel, we observed pyknosis of spermatogonia nuclei with blurred interface between the spermatogenic epithelial cells against the background of interstitial edema and vascular hyperemia. Later these changes disappeared, but spermatogenic epithelium remained thinned until the end of the experiment.

Quantitative evaluation of structural abnormalities showed significant reduction in the number of tubules with meiosis stage 12 on day 5 of the experiment. The increase of this parameter on days 36 and 90 indicated reparative regeneration processes (Fig. 1, *a*). The number of tubules with desquamated epithelium virtually did not change early (within 5 days) after injection of paclitaxel, but later their number increased (Fig. 1, *b*). This can be explained by the formation (under the effect of the drug) of a generation of pathologically modified cells subjected to elimination; this is confirmed by cytogenetic findings [9]. Estimation of the spermatogonia cell populations showed its reduction during all periods of observation (Fig. 1, *c*). A reduction in the count of these cells on day 1 of experiment is presumably a result of their partial death. The absence of positive shifts in this parameter during the later periods can attest to the toxic effect of the

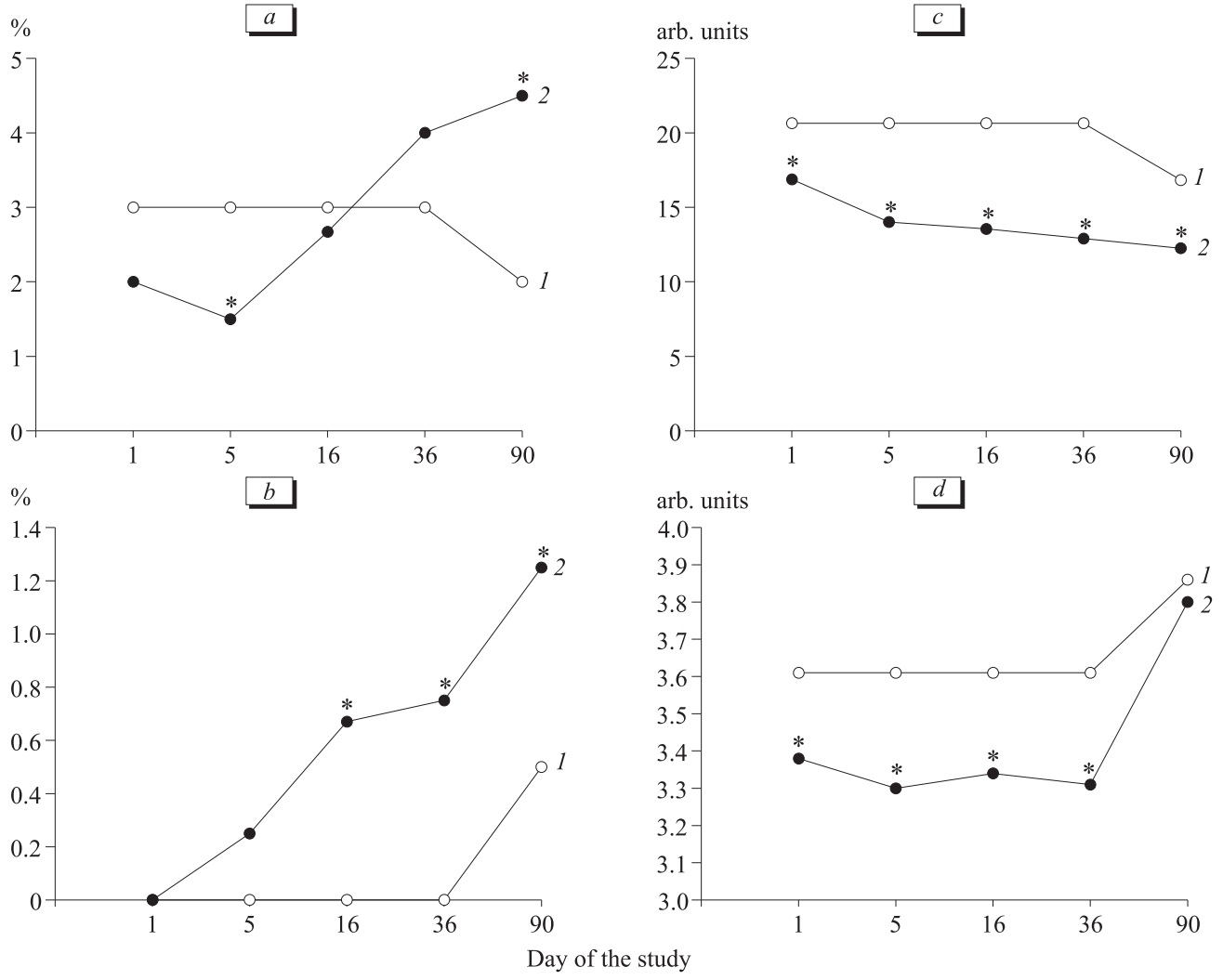

Fig. 1. Morphology of the rat spermatogenesis during early and delayed periods after a single injection of paclitaxel MTD. *a*) number of twisted seminal tubules with meiosis stage 12; *b*) number of tubules with desquamated epithelium; *c*) number of spermatogonias; *d*) spermatogenesis index. Here and in Fig. 2: *1*) saline (control); *2*) paclitaxel (experiment). *$p<0.05$ compared to the control.

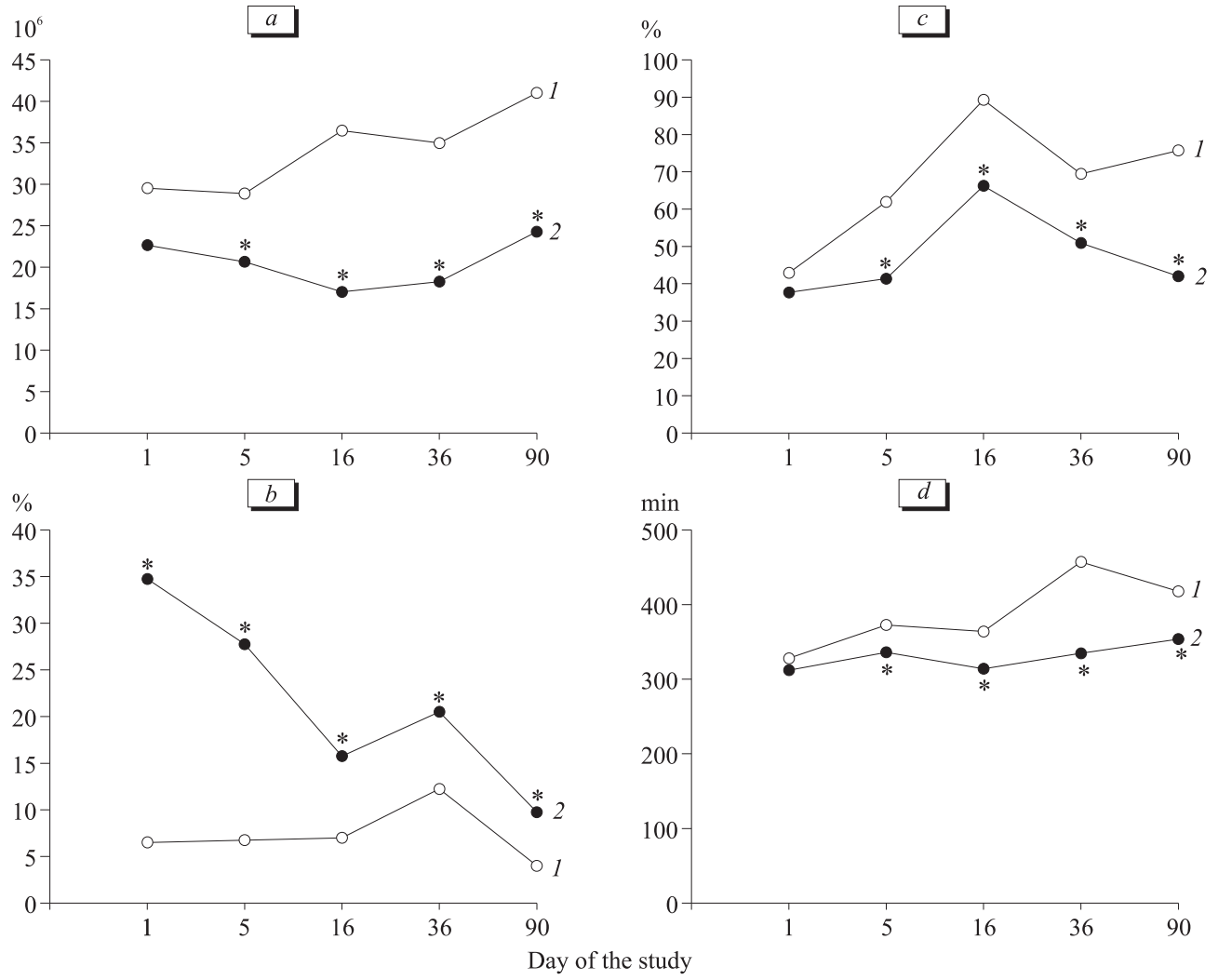

Fig. 2. Morphological and functional parameters of the rat male sex cells after a single injection of paclitaxel MTD. *a*) total spermatozoon count; *b*) number of abnormal spermatozoa; *c*) number of mobile spermatozoa; *d*) maximum duration of spermatozoon movement.

drug on all types of spermatogonias, including A_0 (stem cells). Experiments on mice showed that stem spermatogonias are the targets for taxoid toxicity [9]. Damage to spermatogenic epithelium stem cells is not characteristic of all types of cytostatic treatment [4]. However, injury to these spermatogonias leads to sterility in the delayed periods after antitumor therapy, which can deteriorate quality of life in patients of reproductive age in complete lasting remission after chemotherapy.

Thinning of spermatogenic tissue was observed during almost all stages of the experiment (judging from spermatogenesis index), but by the end of the experiment this parameter increased to the level observed in the control (Fig. 1, *d*), presumably as a result of activation of epitheliocyte meiosis during delayed periods after the drug injection. Loosening of the spermatogenic tissue during this period was due to a decrease in gonocyte count, rather than number of layers.

Evaluation of TCS per epididymis showed that productivity of spermatogenesis was low during the early and delayed periods after paclitaxel injection. The most pronounced oligospermia was detected after influence on spermatides and spermatocytes (days 16 and 36; Fig. 2, *a*). TCS decreased on these days to 48-54% of the control. Paclitaxel increased the number of pathological spermatozoa during all periods of the study. The parameter increased most significantly (4-5-fold) as a result of direct effect on mature sex cells (days 1-5; Fig. 2, *b*).

Morphological changes in the spermatogenic tissue in rats treated with paclitaxel were paralleled by inhibition of functional activity of mature sex cells throughout the entire experimental period. The percent of mobile forms and maximum duration of movement are the most significant criteria of ejaculate fertility. Low content of mobile forms of mature sex cells in the ejaculate (<50%) can be the cause of infertility [5].

Analysis of the results showed that the drug decreased the number of mobile spermatozoa during all periods of the study, the minimum values were detected on days 5 and 90 (up to 41-42%; Fig. 2, *c*). Paclitaxel treatment reduced the maximum duration of spermatozoon movements (by 10-27%) throughout the entire experiment (Fig. 2, *d*).

These data indicate that paclitaxel damages actively dividing cells (spermatogonias and spermatocytes) and postmeiotic epitheliocytes (spermatides and spermatozoa). Disorders in the division spindle due to the toxic effect of the drug on microtubules underlies its antiproliferative effect. Presumably, this effect of paclitaxel explains damage to mitotically and meiotically dividing epitheliocytes. Damage to non-dividing cells can result from inhibition of spermatogenesis, which is paralleled by the formation of microtubules. The toxic effects on spermatogenic cells in general can be due to apoptosis inducing capacity of the drug [13]. It is also possible that paclitaxel violates the integrity of cell membranes via LPO activation. This latter fact is a common result of cytostatic treatments [2].

Hence, single injection of paclitaxel in MTD to male rats induced structural and functional disorders in gonocytes of all progenesis stages. Judging from their severity, this can lead to reduction of the ejaculate fertility during the early and delayed periods after drug injection.

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