Spermatogenesis in Rats after Injection of the Antitumor Drug Vepeside

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Stable morphological changes and disorders of spermatogenesis develop in the testicles of Wistar rats during 30 days after a single injection of vepeside in the maximum tolerance dose. By the end of experiment the morphology of the testicles gradually normalizes, but some quantitative parameters characterizing spermatogenesis remain shifted. The detected disorders are caused by the damaging effect of the drug on the cells of all layers of spermatogenic epithelium.

Key Words: *rat; spermatogenesis; vepeside*

Vepeside, a synthetic derivative of podophillotoxins, is widely used in practical oncology. It is effective in lymphogranulomatosis, acute nonlymphocytic leukemia, and nonseminomatous tumors of the testicle [7]. Drug therapy of these diseases leads to complete remission in 60-80% of cases and in many cases to cure [2,7]. This antitumor drug remains an object of clinical studies [6]. Suppression of hemopoiesis is the factor limiting its use [6]. Therapy with vepeside leads to prolongation of the life span of cancer patients and even to cure, and therefore it is important to investigate its side effects on the reproductive organs. There are reports about toxic effect of vepeside on male gonads [5]. Manifest detrimental effect of the drug on the testicles necessitates its profound experimental investigation.

We investigated the effect of vepeside on the rat spermatogenesis.

MATERIALS AND METHODS

Experiments were carried out on 53 Wistar rats weighing 250-300 g. Vepeside (Bristol-Myers) was injected intravenously in a single maximum tolerance dose estimated by graphic probit-analysis, and the animals were followed up for 30 days [1]. Control animals were injected with the solvent in an equivalent dose. The rats were sacrificed by cervical dislocation on days 2, 5, 7, 14, 21, and 28 after drug injection. The testes were removed, weighed, and weight coefficients were calculated. Spermatozoa were counted in the epididymis (homogenate in normal saline) using leukocytic melanger and Goryaev's chamber [3]. For counting abnormal mature spermatozoa, the epididymis was dissected longitudinally in normal saline. The suspension of spermatozoa was applied on a slide, and smears were made and stained with hematoxylin and eosin. For morphological analysis, the testes were fixed in Carnoy's fluid, and 5 μ paraffin slices were cut and stained with hematoxylin and eosin. The layers of spermatogenic epithelium were counted and the spermatogenesis index calculated. Mean number of normal spermatogonia, number of canaliculi with meiosis stage 12, canaliculi with desquamated spermatogenic epithelium per 100 canaliculi, and the number of interstitial endocrinocytes were counted [3,4]. The significance of differences was evaluated using the Wilcoxon—Mann—Whitney's test.

RESULTS

Weight coefficients of the testicles decreased almost by half by day 7 of experiment (Table 1). Later this value increased, but did not reach the control level

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by the end of the follow-up. Total number of mature sex cells in experimental rats was significantly lower than in the control throughout the entire experiment and accounted for 12% by day 14. The percentage of abnormal mature spermatozoa increased almost twofold *vs.* the control on day 5 after vepeside administration and remained increased virtually during the entire observation period.

On day 2 after vepeside, there was a slight interstitial edema in the testicles, swelling of spermatocytes and spermatides and of interstitial endocrinocytes: the cytoplasm of these cells was opaque, the interface between the cells blurred, the nuclei of spermatocytes enlarged, with marginal condensation of chromatin in the cells. Contrary to this, the nuclei of spermatogonia were pyknotic, often with karyorrhexis. On day 5, vascular changes progressed, basal membrane of twisted seminal canaliculi was detached or destroyed because of pronounced intracanalicular edema (Fig. 1). Some canaliculi were necrotic, in others the spermatogenic epithelium was thinned, with dead cells sometimes seen in the lumina. Only spermatogonia were often seen on the basal membrane of seminal canaliculi (Fig. 2). Canaliculi with spermatides and spermatozoa disappeared. On day 7 after vepeside, morphological signs of seminal injury were still observed, and later the interstitial edema was less expressed. The interface between the cells became more and more clear. On the other hand, giant spermatogonia were present in some twisted seminal canaliculi (Fig. 3). By the end of experiment, the structure of the majority of canaliculi was normal, although the epithelium remained thinned in some of them.

Quantitative analysis of structural elements of the testicles showed a significantly lower spermatogenesis index in experimental animals than in the controls throughout the whole experiment (Table 1). The count of normal spermatogonia significantly decreased by day 5 of experiment, their number in the testicles was just 33% of the control. On day 7, the number of normal spermatogonia remained decreased. Later the number of cellular population of normal spermatogonia reached the control level (Table 1).

The number of canaliculi with desquamated epithelium increased on days 2-5 of experiment. Twisted canaliculi with meiosis stage 12 virtually disappeared on days 5-7 after vepeside administration. On day 14, cells with the second division metaphase reappeared, but their number remained low till the end of experiment. The count of interstitial endocrinocytes in the testicles of experimental rats was virtually normal during the entire experiment (Table 1).

Thus, a single injection of vepeside in the maximum tolerance dose induced stable morphological changes and impaired spermatogenesis. By day 28 of experiment, the morphology of testicles gradually normalized, but some quantitative parameters characterizing spermatogenesis remained low. The observed disorders were due to damaging effect of the drug on the cells of all layers of spermatogenic epithelium. Vepeside blocks cell cycle at stages S, G_2,

TABLE 1. Morphological Changes in the Testes after a Single Injection of Vepeside (40 mg/kg, $X \pm m$)

Parameter	Day after vepeside						
	control	2	5	7	14	21	28
Weight coefficients of testicles, %	0.51±0.02	0.58±0.02	0.58±0.01	0.28±0.06*	0.46±0.06*	0.41±0.03*	0.33±0.02*
Total count of spermatozoa, 10^6	57.45±6.08	37.90±3.13*	21.30±3.13*	35.23±3.23*	5.05±2.64*	18.14±2.40*	32.55±1.64*
Abnormal mature spermatozoa, %	5.89±0.70	6.50±0.29	10.25±2.63*	10.00±2.42*	11.50±1.68*	5.00±0.49	11.40±2.16*
Spermatogenesis index, arb. units	3.77±0.44	3.60±0.03*	2.93±0.04*	2.16±0.26*	3.11±0.20*	2.73±0.13*	2.17±0.39*
Count of normal spermatogonia	17.69±2.14	13.11±1.72	5.92±0.62*	5.11±0.43*	14.12±2.37	13.32±1.58	17.57±3.04
Canaliculi with desquamated epithelium, %	0.75±0.48	2.00±0.58	2.75±1.03	1.00±0.00	0.50±0.29	0.50±0.28	0.25±0.25
Canaliculi with meiosis stage 12, %	2.50±0.42	1.25±0.25	0.00±0.00*	0.00±0.00*	0.25±0.25*	0.25±0.25*	0.25±0.25*
Count of interstitial endocrinocytes	14.80±1.79	12.09±1.48	13.17±0.76	11.32±1.40	9.45±0.26	13.00±1.25	11.80±1.76

Note. *$p \leq 0.05$ *vs.* the control.

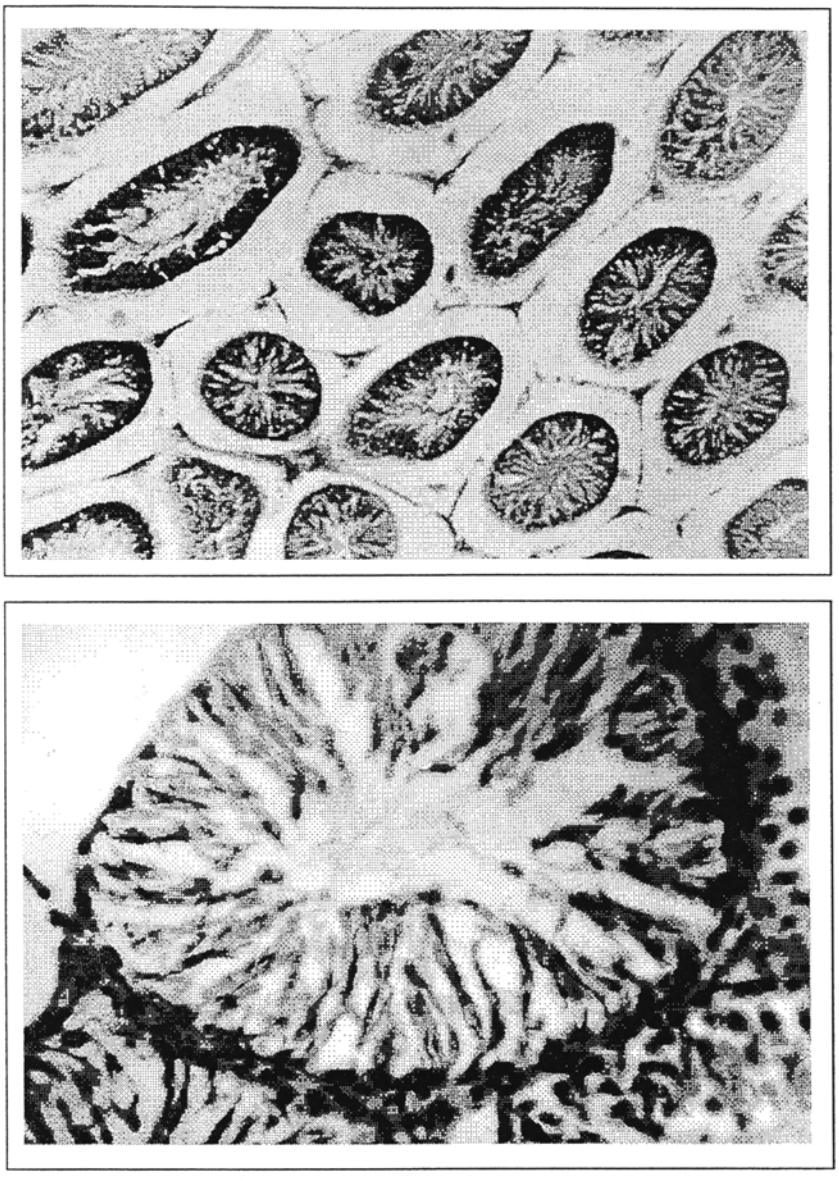

Fig. 2. Seminal canaliculus on day 5 after vepeside administration in maximum tolerance dose. Thinned spermatogenic epithelium. Spermatogonia alone on canalicular basal membrane.

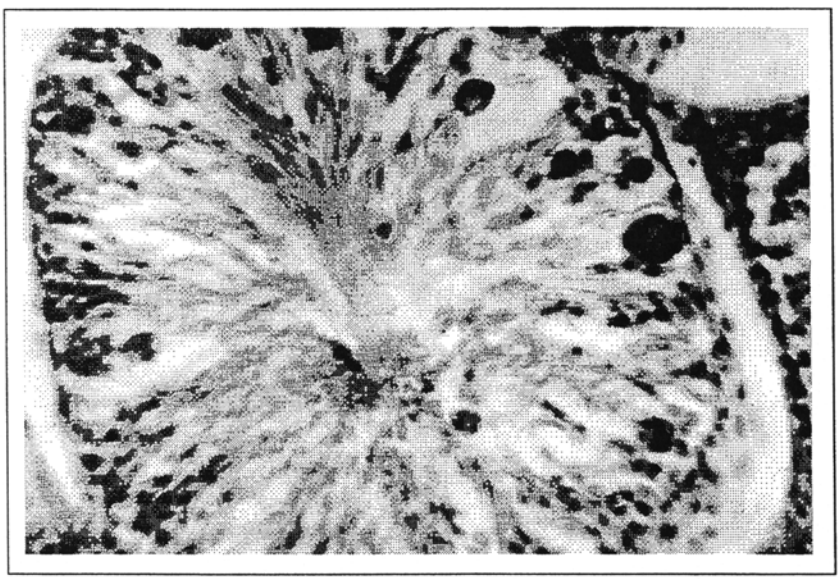

Fig. 3. Giant spermatogonia in twisted seminal canaliculi on day 14 after vepeside administration in maximum tolerance dose.

and prophase. DNA synthesis, preparation to mitosis, and initial stages of the first and second meiotic division take place in spermatogonia and in the first and second order spermatocytes. These cells are apparently the main target of the drug. On the other hand, the low total count of mature sex cells during all periods of the experiment is indicative of damaging effect of the drug on nondividing cells of spermatogenic epithelium, spermatides, and spermatozoa.

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Estimation of Na-Blocking Efficiency of Rihlocaine and Its Combinations with Low-Molecular-Weight Polymers on Isolated Rat Cardiomyocytes

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The effect of glucose-base polymers (dextrans with relative molecular weight 10, 40, and 70 kD) and vinylpyrrolidone (polyvinylpyrrolidone: 10, 24, and 40 kD) on changes in the sarcoplasmic Na^+ concentration in stimulated cardiomyocytes (1.0 Hz, 10 msec, 60 mV) were examined. In the concentration range of 1-100 μM, the polymer preparations produced cardioprotective effect on cells incubated under hypoxic conditions; the effect depended on the nature and molecular weight of the polymer. Rihlocaine (25 μM) inhibits by 42% elevation of intracellular Na^+ induced by plasma membrane depolarization. Dextran 40 is shown to significantly increase Na-blocking effect of rihlocaine.

Key Words: *dextran; polyvinylpyrrolidone; antiarrhythmics; cardiomyocytes*

To produce preparations with prolongated effects, both natural (carboxymethylcellulose, dextran) and synthetic (polyvinylpyrrolidone — PVP, polyvinyl alcohol) polymers with high biological compatibility and low toxicity have been used [3,6].

New preparations on the basis of traditional drugs immobilized on a carrier polymer or noncovalently bound to a polymer have the following advantages: enhanced pharmacological effectiveness, longer period of action, and lower side effects. Using the aconitine model of cardiac arrhythmia, we demonstrated antiarrhythmic activity of the sodium channel blocker rihlocaine (RHC) diluted in rheopolyglucin (10% solution of dextran 30/40) [2]. Under acute regional ischemia and reperfusion, the combination of RHC with dextran stabilized basic hemodynamic and cardiac indexes, thus demonstrating antiarrhythmic and antifibrillatory activities. At the same time, a certain decrease in the effectiveness of RHC was detected when it was combined with PVP.

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