GENETICS

Damaging Effect of Taxol on Mouse Spermatogenesis

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Taxol produced a specific effect on mouse spermatocytes I and on stem spermatogonia, which leads to overall degeneration of spermatocytes I and long lasting disorders of spermatogenesis. Breaks of the axial and lateral elements of the synaptonemal complex, aneuploidies in spermatocytes in early, middle, and late pachytene, and accumulation of cells with associations of sex chromosomes and autosomes were observed, which attested to blockade of spermatogenesis in late pachytene and diplotene of meiosis prophase I. Mesna, a chemoprotective agent, reduced total toxicity and lethal effects of taxol, but did not prevent destruction of the testes.

Key Words: *taxol; spermatogenesis; mouse testicles; mesna*

Studies of side effects of antitumor drugs are mainly focused on the so-called limiting toxicity, which limits the use of these drugs. At the same time, realization of the mechanisms and approaches to reducing other life-compatible toxicities deteriorating quality of life receive less attention. One of such manifestations of toxicity is damaging effect of cytostatics on normal rapidly dividing cells. Spermatogenesis maintained by multiple mitotic divisions of spermatocytes I and II is the most sensitive target for antitumor drugs.

The efficiency of taxol (plant alkaloid) in the treatment of various malignant tumors, including breast, lung, prostatic, and ovarian cancer, was recently demonstrated [12]. The antitumor effect of taxol is based on its capacity to bind formed microtubules (preferably tubulin β-subunit) and induce their polymerization [3]. Taxol inhibits DNA, RNA, and protein synthesis and arrests cells in the G_2-M phases of the cell cycle, which results in the formation of genetically abnormal aneuploid cells [4,6]. The aim of the present study was to investigate its effect on the specific structure of meiotic chromosomes and synaptonemal complex (SC) in spermatocytes and on spermatogenesis in general.

We also investigated the antitoxic effect of a thiol preparation mesna: whether it neutralized the effect of taxol on spermatogenic cells. Previous studies showed that combined treatment with taxol and mesna reduced the lethal effect and hematological toxicity of taxol [1].

MATERIALS AND METHODS

Experiments were carried out on male CBA mice aged 2-4 months (22-28 g) from Stolbovaya Breeding Center of Russian Academy of Medical Sciences. The animals were divided into 3 groups, 6 animals each.

Group 1 animals were intraperitoneally injected with taxol (Bristol Mayers Squibb) in a single dose of 20 mg/kg (LD_{50}). Group 2 mice were intraperitoneally injected with 200 mg/kg mesna (Asta Medica) according to the following protocol [1]: 4 injections with 25, 20, and 15-min intervals. Group 3 animals were injected with mesna (200 mg/kg) 30 and 5 min before and 15 and 30 min after taxol.

The drugs were dissolved in 0.9% NaCl immediately before injections (0.1 ml per animal).

Six intact animals served as the control.

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The animals were sacrificed by cervical dislocation at various terms after drug injections (days 5-160).

For histological examination, the whole testis was fixed in Bouins fixative, 5-μ paraffin sections were stained with hematoxylin and eosin.

For electron microscopy, tissue fragments were fixed in glutaraldehyde and OsO_4 and contrasted with lead uranyl acetate. Ultrathin sections were contrasted with aqueous solution of lead citrate.

Total preparations of SC of flattened nuclei of mouse spermatocytes I were prepared using a modified method [8]. The preparations were fixed in 4% paraformaldehyde in 0.1 M sucrose (pH 8.4-8.6), contrasted in 50% $AgNO_3$ (pH 3.5-5.5), and examined under a JEM 100B electron microscope.

RESULTS

The walls of seminal tubules of intact mice were represented by Sertoli cells and all types of differentiating sex cells: spermatogonia, spermatocytes, spermatides, and spermatozoa (Fig. 1, *a*). Electron microscopy of spermatocytes I showed SC structure typical of these cells and consisting of two lateral elements (LE) and one central element surrounded with chromatin (Fig. 2, *a*).

The total preparations of flattened nuclei SC of intact mouse spermatocytes I contained 19 autosomal bivalent SC and a sex bivalent (Fig. 3, *a*). Early, middle, and late pachytene were identified by morphology of axial elements (AE) of sex chromosomes and synapses between them.

Analysis of histological and ultrathin sections of the testes on day 5 after taxol injection revealed no essential deviations from the control. However, the total preparations of spermatocyte I contained cells with impaired SC structure: breaks and terminal deletions of autosomal SC lateral elements and fragments of pachytene sex chromosome AE (Fig. 3, *b, c*; Table 1). This indicates that taxol induced chromosome fragmentation both in early pachytene cells and in spermatogonia which did not yet enter meiosis. Induction of DNA fragmentation in somatic cell nuclei with taxol was previously reported [4].

Increased number of cells with associations between sex chromosome AE and autosomal SC lateral elements was seen in total SC preparations at the stages of middle and late pachytene (Fig. 3, *d*; Table 2). These associations result from inactivation of normally inactive X chromosome and prevent the formation of sex body, which leads to spermatocyte degeneration [5].

Pachytene spermatocytes contained an aneuploid set of SC: 19+XY±1-2 univalent or bivalent autosomal and sex chromosome SC (Fig. 3, *e*; Table 3). The appearance of such cells probably suggests that taxol induced irregular chromosome segregation at anaphase during the last mitotic division of spermatogonia. Further differentiation of these spermatocytes could lead to the appearance of genetically deficient progeny, because aneuploid spermatocytes are not selected during spermatogenesis, fertilization, or the first cycle of zygote development [6,7].

Examination of histological sections on days 22, 36, and 50 after taxol injection showed empty seminal tubules with Sertoli cells and solitary spermatogonia, spermatocytes I, and spermatides at the testis periphery (Fig. 1, *b*).

Electron microscopy of ultrathin sections showed invagination of the nuclear membrane and intranuclear vacuoles in leptotene and zygotene spermatocytes I (Fig. 2, *b*), and local dilatations of the perinuclear space paralleled by destruction of the nuclear pore complex, nuclear and then cell membranes in pachytene cells (Fig. 2, *c*). Fragments of degenerating spermatocytes were seen in vacuolated cytoplasm of Sertoli cells.

In group 2 no essential differences from the control were seen, which suggests that mesna did not damage spermatogenic cells.

Examinations of histological and ultrathin sections on days 5-50 after combined treatment with ta-

TABLE 1. Number of Spermatocyte Nuclei (%) with Breaks of AE and LE SC of Autosomes and Sex Chromosomes in the Control and on Day 5 after Taxol Injection (20 mg/kg) and Mesna (200 mg/kg)

Group of mice	Pachytene					
	early		middle		late	
	autosomes	XY	autosomes	XY	autosomes	XY
Intact (*n*=57)	—	—	—	—	7.1	—
1 (*n*=64)	23.5	5.9	3.8	—	19.0	9.5
2 (*n*=81)	—	—	8.3	—	1.8	—
3 (*n*=66)	40.0	10.0	34.6	11.5	40.0	10.0

Note. Here and in Tables 2, 3,: *n*: cell number.

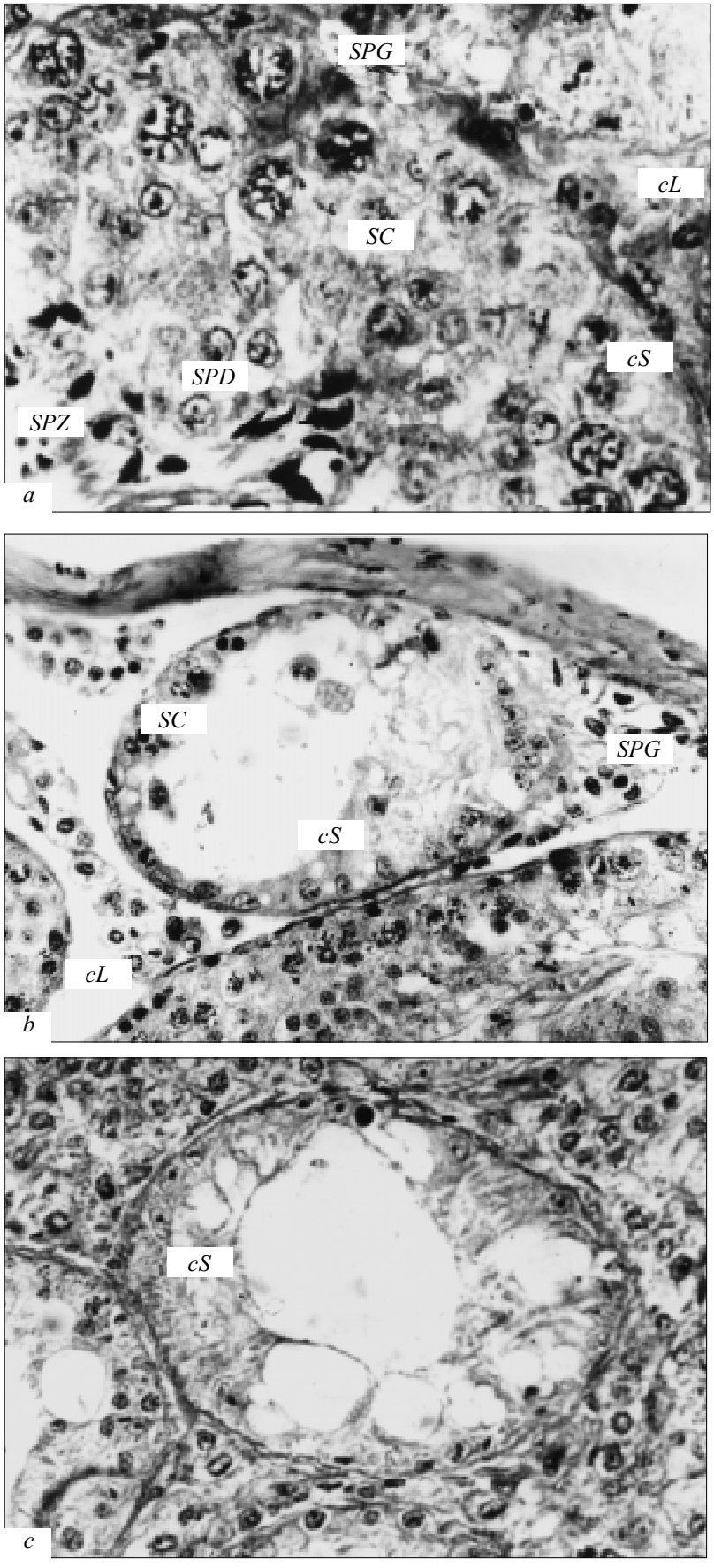
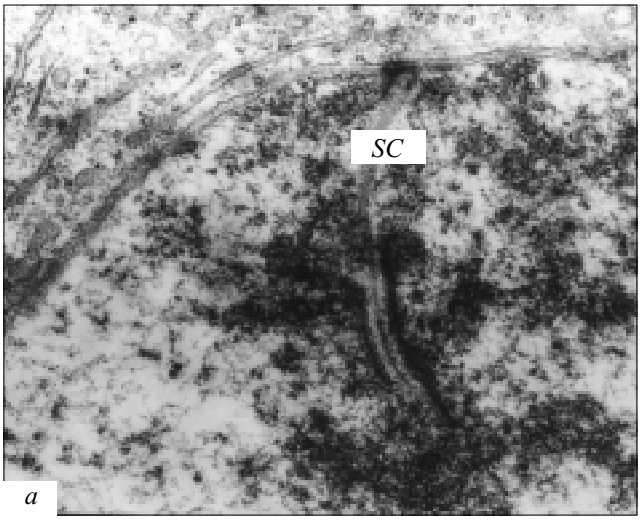
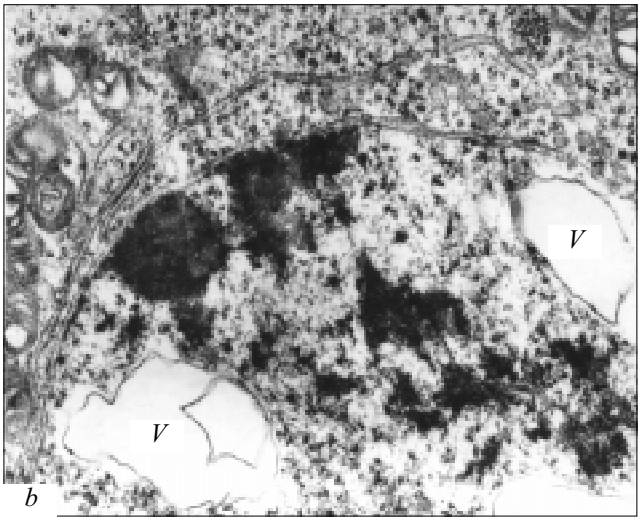
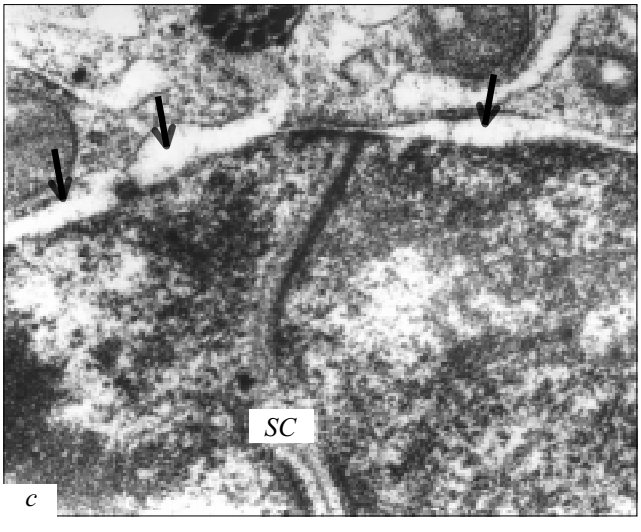


Fig. 1. Histological sections of mouse testis. *a*) control. Testicular tubule walls are represented by Sertoli cells (*cS*), spermatocytes (*SC*), spermatogonia (*SPG*), spermatides (*SPD*), and spermatozoa (*SPZ*). Leidig cells (*cL*) are seen in the interstitium. ×720; *b*) degeneration of seminal tubule at the periphery of testis on day 22 after taxol injection. Tubular wall is represented by Sertoli cells, individual spermatogonia and spermatocytes, ×420; *c*) seminal tubule degeneration and interstitial cell hyperplasia on day 160 after injection of taxol with mesna. Tubular walls contain only Sertoli cells with vacuolated cytoplasm, solitary spermatogonia, spermatocytes, and macrophages, ×420.







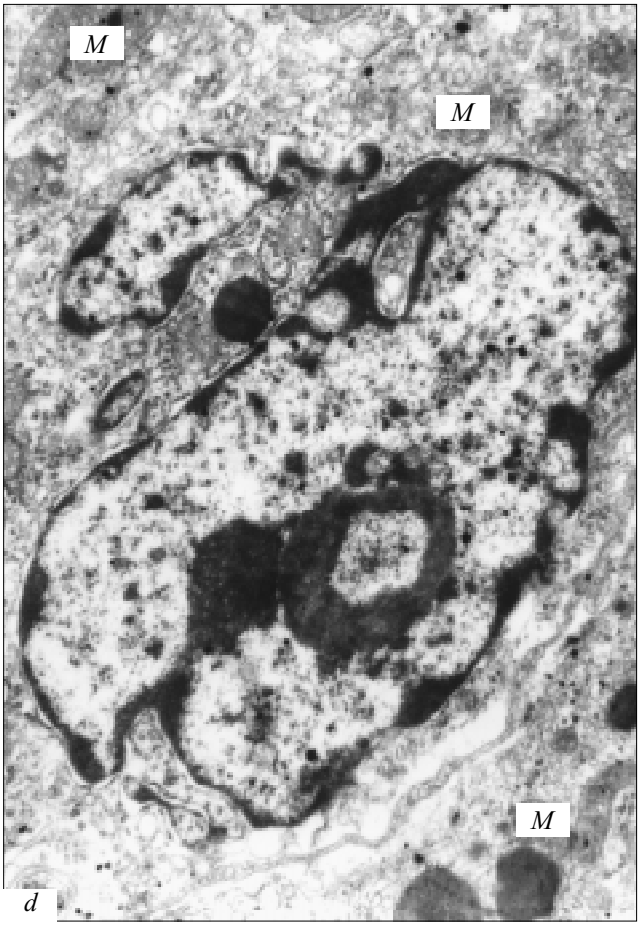


Fig. 2. Ultrathin sections of mouse testis. *a*) control. Spermatocyte I at the pachytene stage. Synaptonemal complex (*SC*), ×25,000; *b, c*) degeneration of spermatocytes I on day 22 after taxol injection: *b*) leptotene. Invagination of internal nuclear membrane with formation of intranuclear vacuoles (*V*), ×20,000; *c*) pachytene. Zones of local dilatation of perinuclear space (arrow) and destruction of nuclear membrane. Synaptonemal complex. Destruction of porous complex elements, ×20,000; *d*) macrophages infiltration of seminal tubule wall on day 160 after combined treatment with taxol and mesna. Irregularly shaped macrophagal nucleus with histochromatin at the periphery. Basophilic cytoplasm with numerous mitochondria (*M*), ×15,000.

xol and mesna showed no deviations from the normal. However, analysis of total SC preparations at pachytene showed all disorders in SC structure observed after taxol injection (Tables 1-3). Increased number of such disorders in comparison with group 1 indicates that mesna did not attenuate the effect of taxol on spermatocytes and even potentiated its mutagenic effect.

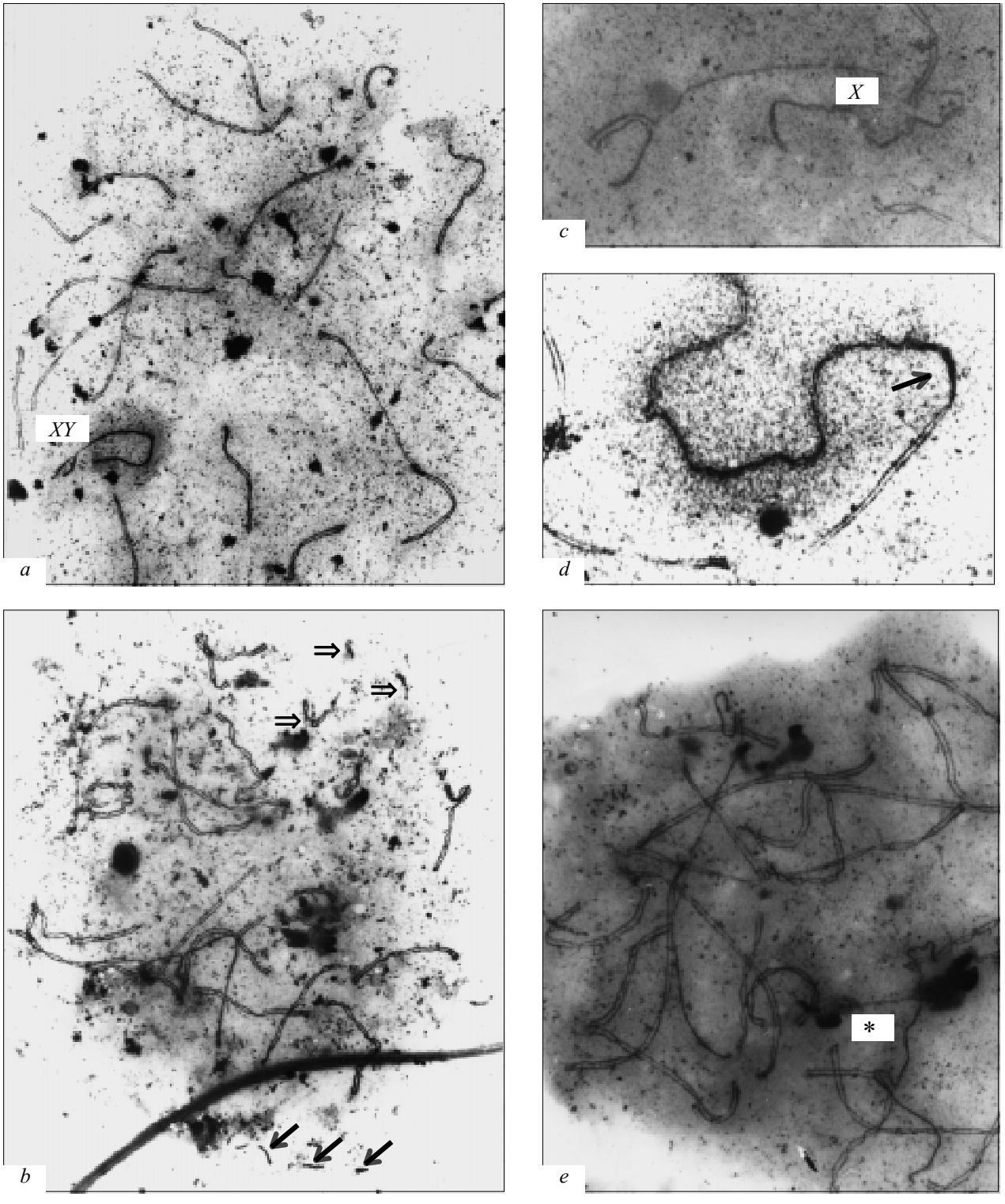


Fig. 3. Total preparations of spermatocytes I (SC) in the control (*a*) and on day 5 after taxol injection (*b*-*e*) at early (*e*) and middle pachytene stages (*a*-*d*). *a*) autosomal bivalent 19 SC and synapted AE of sex chromosomes (*XY*), ×4500; *b*) fragments of sex chromosome AE (arrow) and autosomal SC LE (double arrow), ×5400; *c*) fragments of X chromosome AE (*X*), ×9000; *d*) association of X chromosome AE with autosomal SC LE (arrow), ×11,000; *e*) aneuploid number of SC, "odd" AE (asterisk), sex bivalent (*XY*), ×6300.

Fifty days after injections of taxol with mesna the relative count of late pachytene and diplotene spermatocytes increased (while at early stages of prophase I they were almost completely absent), which can result from spermatogonia selection and partial blockade of spermatogenesis at these stages.

TABLE 2. Percentage of Spermatocyte Nuclei with Associations of X and Y Chromosomes with AE and Autosomal SC LE in Control and after Injections or Taxol (20 mg/kg) and Mesna (200 mg/kg)

Mouse group	Term, days	Pachytene			
		middle		late	
		X autosome	Y autosome	X autosome	Y autosome
Intact (*n*=57)	—	3.8	—	3.8	—
1 (*n*=64)	5	19.20	3.8	9.5	—
2 (*n*=69)	5	—	—	5.3	—
3 (*n*=66)	5	15.45	3.8	5.0	—
4 (*n*=42)	22	—	—	13.8	2.8

TABLE 3. Percentage of Spermatocyte Nuclei with Aneuploid Number of Autosomal SC in Control and on Day 5 after Injection of Taxol (20 mg/kg) and Mesna (200 mg/kg)

Mouse group	Pachytene		
	early	middle	late
Intact (*n*=57)	—	—	—
1 (*n*=64)	11.7	7.7	9.5
2 (*n*=81)	—	—	—
3 (*n*=66)	15.0	15.4	15.0

On day 160 group 3 mice remained alive in contrast to group 1. However, most seminal tubules were atrophic (Fig. 1, *c*) and contained only vacuolated Sertoli cells, solitary spermatogenic cells, and macrophage infiltrate (Fig. 2, *d*). The absence of differentiating sex cells in total SC preparations indicated irreversible disorders of spermatogenesis, induced by taxol at the level of stem spermatogonia, which is in line with published reports [9,10]. Hence, injections of mesna decreased some toxic effects of taxol but did not prevent the destruction of testis caused by these drugs.

Ultrastructure of degenerating spermatogenic epithelium cells and inflammatory infiltration (macrophages) in tubular walls corresponded to the picture of developing autoimmune response [2]. Autoantigens which are intolerable for males first appear in spermatocytes at the pachytene stage [11]. In addition, the formation of the hematotesticular barrier is over by the first appearance of pachytene cells in the testis. These facts suggest that destruction of the nuclear and cell membranes and subsequent release of SC antigens from spermatocytes I, caused by taxol can induce an autoimmune process leading to destructive disorders in the testicular tissue.

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