GENETICS

Study of Mouse Synaptonemal Complex after Injection of Camptotecin

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Total synaptonemal complexes of male mouse meiotic chromosomes were examined under electron microscope at different periods after a single injection of the antitumor drug camptotecin. At the stage of meiosis prophase I, the drug induced sharp decondensation of chromatin, distention of chromosomes and elements of synaptonemal complex, and blocked desynapsis of homologous chromosomes at the diplotene stage, thus "arresting" damaged cells. The effect of camptotecin lasted 34 days after injection and involved 15 to 90% cells in different periods.

Key Words: *camptotecin; meiosis prophase I; synaptonemal complex; chromosome desynapsis blocking*

Camptotecin (CT) is a plant alkaloid used as an antitumor drug [14]. Its effect was for a long time believed to be due to inhibition of DNA-topoisomerase I [8]. This drug reversibly stabilizes covalent protein-DNA complexes in the S phase, thus damaging DNA molecules, increasing the number of chromosome aberrations and sister chromatid exchanges, and leading to cell "arrest" by blocking them in the G_2 period [5,9]. Recent studies demonstrated a wider spectrum of CT effects. It affects nuclear matrix proteins by causing chromatin restructuring and cell degeneration [15].

Male reproductive tissues are characterized by a high level of proliferation. During chemotherapy they may become a side target for CT-induced apoptosis. This prompted us to study the effect of CT on spermatogenic cells. Electron microscopic analysis of the synaptonemal complex (SC) at the stage of meiosis prophase I is the most effective cytogenetic test of inter- and intrachromosomal aberrations of meiotic chromosomes [3,4,12]. SC is a structure whose timely formation and normal function at the prophase I leptotene-diplotene stages is obligatory for strictly homologous synapsis of meiotic chromosomes [1,2, 11]. It was shown in animal experiments that cells with disordered SC structure and abnormal chromosome synapsis or desynapsis are selected and do not pass through prophase I, which disorders spermatogenesis and results in temporary sterility of males [2,3].

Using electron microscopy, we analyzed the structure and behavior of meiotic chromosome SC during the meiosis prophase I at various periods after a single injection of CT.

MATERIALS AND METHODS

Experiments were carried out on 12 male (CBA× C57BL) F_1 mice. Camptotecin in dimethylsulfoxide (DMSO) was injected intraperitoneally in a dose of 15 mg/kg to 6 animals. An equivalent volume of DMSO was injected to 4 control mice, and 2 mice were intact. All animals were kept under standard conditions. Experimental mice were sacrificed by dislocation of cervical vertebrae 3 h and on days 2,

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7, 9, 13, and 34 after CT injection; controls were sacrificed 3 h and on days 7, 13, and 34 after DMSO injection; intact controls were sacrificed on days 9 and 34.

Total preparations of meiotic chromosome SC were made as described elsewhere [13]. Testicles were removed, cleansed from fat and tunica, crushed in 2-3 drops of Eagle's medium (37°C), and centrifuged for 10 min at 1500 rpm, after which the supernatant was discarded and the precipitate was homogenized in Eagle's medium. Drops of the resultant spermatocyte suspension were layered onto drops of hypotonic solution (0.5% NaCl or 0.2 M sucrose solutions) and after 0.5-1 min were transferred onto glass coated with a film (Falcon plastic solution in chloroform). The preparations were fixed in 4% paraformaldehyde in 0.1 M sucrose solution (pH 8.4-8.6), contrasted in 50% $AgNO_3$ (pH 3.5-5.5), and examined under a JEM-100M electron microscope. Morphologic changes of spermatocyte sex bivalent were criteria for determining the stages of meiosis prophase I.

RESULTS

Three hours after CT injection intense, an $AgNO_3$ staining of chromatin from both sides of the lateral elements of formed SC was observed in spermatocyte nuclei of experimental animals. Three hours and on days 2 and 7 after CT, at the stages of late pachytene-diplotene we observed nuclei whose morphology markedly differed from the control (Fig. 1, *a-c*). Their area was 3-7 times larger than in intact controls, the configuration of X and Y chromosomes corresponded to late pachytene-diplotene, but the process of chromosome desynapsis was blocked (Fig. 2, *a-c*). The

Fig. 1. Total preparations of synaptonemal complexes (SC) of spread mouse spermatocytes under electron microscope. Control. $AgNO_3$ staining. ×5000. *a*) late pachytene; 19 autosomal SC bivalents; sex bivalent (XY) at the periphery of nucleus; *chromocenter zones. *b*) scheme of chromocenter (*) formed by peritelomere sites of heterochromatin of two autosomes. *c*) diplotene; arrows show desynapsis of axial elements of autosomal bivalents.

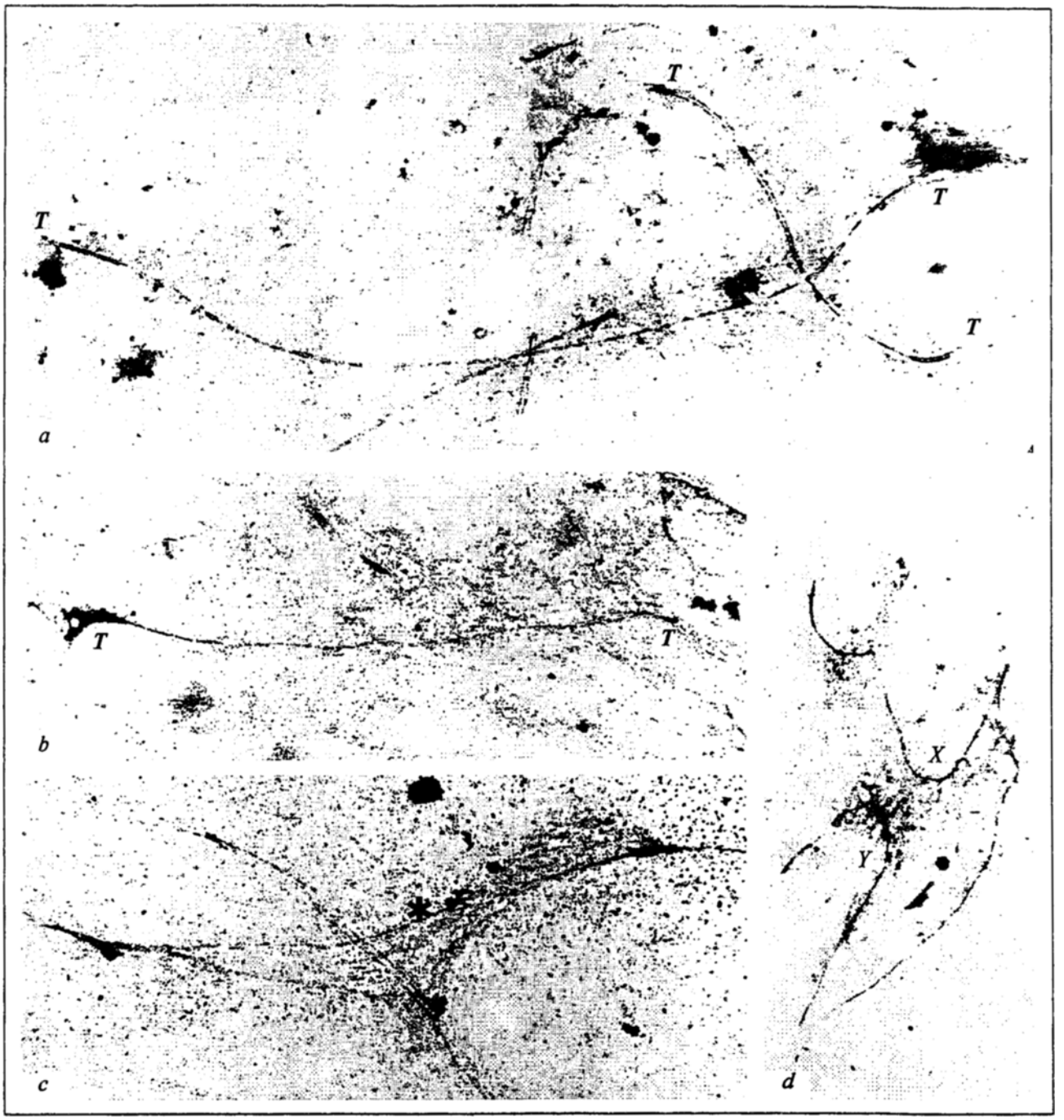

Fig. 2. Comparative morphology of synaptonemal complex (SC) after a single injection of camptotecin (15 mg/kg). Late pachytene-diplotene. Day 7 after camptotecin injection. $AgNO_3$ staining. ×5000. *a*, *b*) autosomal SC stretching in the absence of their desynapsis, chromatin decondensation.c) chromatin stretching and decondensation in the chromocenter (*). T) autosomal bivalent SC telomeres. *d*) sex bivalent (XY) at the nucleus periphery; axial elements of chromosomes are stretched, chromatin decondensed.

number of enlarged nuclei after 3 h was 15% and on day 2, 16% of the total number of examined nuclei (Fig. 3). Lateral elements of autosomes (Fig. 2, *a-c*) and axial elements of sex chromosomes (Fig. 2, *d*) were extended, unevenly thickened, and fragmented. Chromatin was decondensed and formed electron-dense zones round SC elements. Chromocenters normally represented by small electron-dense zones formed by SC heterochromatin telomeres [7] (Fig. 1, *a*, *b*) were similarly stretched with clearly seen fibrils of decondensed chromatin and electron-dense SC telomeres (Fig. 1, *d*). On day 7, the number of

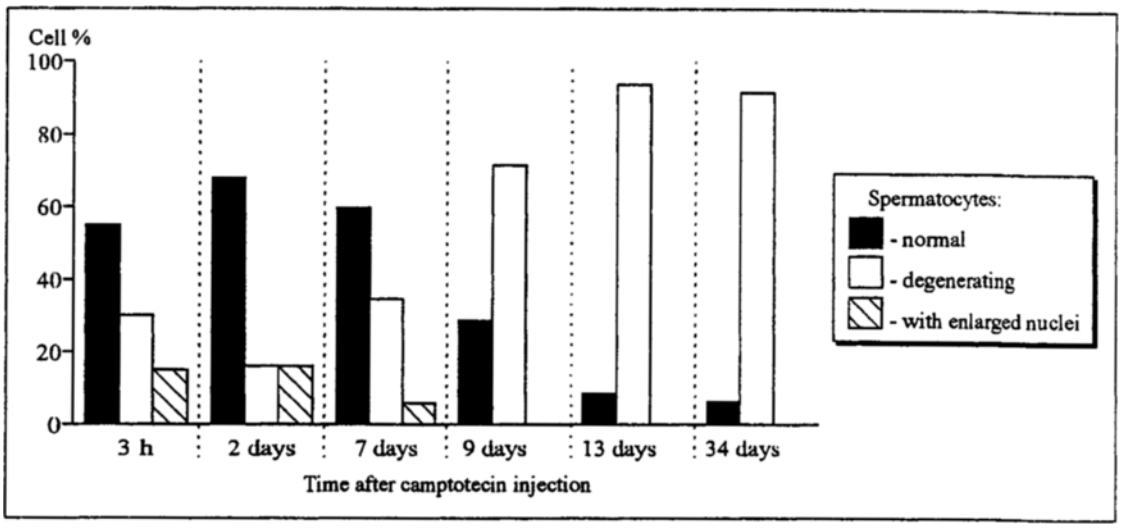

Fig. 3. Ratio of normal to damaged spermatocytes of the first order at various terms after a single injection of camptotecin.

nuclei with stretched SC decreased to 5.8%; in addition, there were fragments of degenerating spermatocyte nuclei with homogenous chromatin on the preparations (34,6% of the total number of examined nuclei, Fig. 3). Fragments of lateral elements and telomeres of degenerating SC were clearly seen (Fig. 4, *a, b*). On days 9, 13, and 34 after CT injection there were no enlarged nuclei with stretched SC and decondensed chromatin, and the number of nuclei in intact spermatocytes decreased from 28.6% on day 9 to 6.3 and 4.3% on days 13 and 34, respectively. At the same time, the number of degenerating nuclei of irregular shape increased in comparison with the previous term to 71.4, 93.7, and 91.4% on days 9, 13, and 34, respectively (Fig. 3). The abundance of such structures and almost complete absence of normal nuclei suggest that CT impairs actively dividing early spermatogonia. Therefore, on day 34 after a single injection of the drug its effect was still observed, and spermatogenesis did not recover.

The structure of spermatocyte nuclei SC in control mice to which the solvent was injected differed from that of intact mice.

Our results differ from published reports describing experiments with mutagens of different classes. Generally, mutagens induce local fractures and translocations in early spermatogonia but not chromatin decondensation. Disorders of SC structure, nonhomologous synapsis of SC elements, and formation of multivalent SC were observed during prophase I

Fig. 4. Degeneration of spermatocytes of the first order after a single injection of camptotecin (15 mg/kg) on days 9 (*a*) and 13 (*b*) after injection. $AgNO_3$ staining. ×11,000. T) autosomal synaptonemal complexes telomeres. Arrow shows degenerating elements of synaptonemal complex.

[3,4,12]. Later, damaged cells were "arrested" during pachytene and did not complete meiosis prophase I. A characteristic feature of spermatocyte "arrest" during pachytene was association of aberrant chromosomes with sex bivalent in the center of the nucleus [3].

In HL-60 culture CT led to active degradation of chromatin, manifesting by its "pulverization" as early as after 2 h of incubation [6]. The changes involved 30-40% of cells. After 6-8 h, nuclear organelles were destroyed and completely disintegrated, and the cells died. Incubation of L1210 cells with CT did not cause fragmentation of nuclear chromatin but increased the nuclei [6]. These data are in line with reports about nonspecific effect of CT on nuclear matrix proteins in the S phase; the agent caused rearrangement and degradation of chromatin, leading to cell death [15].

We demonstrated the mechanism of CT effect on the structure of SC and meiotic chromatin, mediating the selection of cells during diplotene. It is noteworthy that during diplotene, in contrast to pachytene, spermatocytes are arrested during normal formation of a sex vesicle and its transposition to the periphery of the nucleus. Injection of CT leads to chromatin decondensation, increase of the nucleus, elongation of SC elements, block of homologous chromosome desynapsis, as well as to cell degeneration and death. The drug effect persisted for more than 34 days after a single injection and involved in different periods from 15 to 90% of cells, which should be borne in mind when planning chemotherapy for subjects of a reproductive age.

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