ONCOLOGY

Effects of Etoposide and Fludarabine in Subtoxic Doses on Karyotype of Human Malignant Lymphoid Namalwa Cells

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We studied changes in the karyotype of transplanted Namalwa cells induced by DNA-damaging antitumor preparations etoposide and fludarabine in subtoxic doses. The relative number of cells containing increased number of chromosomes and the incidence of chromatid aberrations with primary damage to chromosomes 2, 5, 11, 16, and 17 increased. Cytogenetic changes developed even after short-term incubation of cells with antitumor preparations and were observed during further culturing in a medium not containing etoposide or fludarabine.

Key Words: *lymphoid cells; chemotherapy; subtoxic doses; modal chromosome number; chromosome aberrations*

Optimization of chemotherapy providing maximum apoptotic death of tumor cells plays an important role in the treatment of patients with malignant lymphomas and leukemias [1]. Although antitumor preparations differ in the mechanisms of action and chemical structure, most of them directly or indirectly impair DNA synthesis and replication [2]. Published data show that topoisomerase II inhibitors act as mutagens producing chromosome deletions, unequal crossing-over, and aneuploidy [10]. Purine nucleotide analogues with antimetabolic activity also induce chromosome deletions [11].

Preparations of these groups, including etoposide and fludarabine phosphate (fludarabine), are used for the therapy of leukemias and lymphomas. Etoposide is a synthetic glycoside whose cytotoxic effects are related to inhibition of topoisomerase II and stabilization of the DNA-topoisomerase complex [6]. Fludarabine belongs to the group of purine nucleosides, acts as a metabolic inhibitor of DNA-synthesizing enzymes ribonucleotide reductase, DNA polymerase, DNA primase, and DNA ligase, and blocks DNA synthesis. Fludarabine also partially inhibits RNA polymerase II and suppresses protein synthesis [1].

It was shown that etoposide and fludarabine therapy can produce secondary leukemias [7,13]. Leukemogenic activity of etoposide is related to its site-specific effect on leukemia-associated genes [8]. Changes in the karyotype of malignant cells after long-term culturing with chemotherapeutic agents and the formation of resistant clones were studied [4,12]. Little is known about cytogenetic changes produced by short-term exposure to chemotherapeutic agents in concentrations not causing immediate cell death.

Here we studied changes in the karyotype of transplanted lymphoid Namalwa cells produced by DNA-damaging antitumor preparations etoposide and fludarabine in subtoxic doses that differ in the mechanism of action.

MATERIALS AND METHODS

Experiments were performed on transplanted human lymphoid Namalwa cells (B cell line) obtained from the collection of cell cultures (R. E. Kavetskii Institute of Experimental Pathology, Oncology, and Radiobiology). The suspension of cells was cultured in RPMI-

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1640 medium containing 10% fetal bovine serum, 2 mmol/liter L-glutamine, and 40 mg/ml gentamicin at 37°C. Colchicine in a concentration of 0.03-0.05 mg/ml was added to cultured cells in the phase of active growth (48 h after passage) for 40 min to obtain metaphase plates. The cells were treated with hypotonic 0.55% KCl for 15 min. Metaphase chromosomes were obtained routinely [3]. Preparations of chromosomes were stained with 2% Romanovsky—Giemsa dye in phosphate buffer (pH 6.8) or treated differentially for visualization of G- and C-bands [14,15].

Etoposide (Brystol-Myers Squibb SpA) and fludarabine phosphate (Schering AG) were used in subtoxic doses for Namalwa cells [6,9]. Changes in cell karyotype were studied immediately after incubation with etoposide in a dose of 0.2 mg/ml for 24 h and treatment with etoposide in doses of 0.3 and 1.0 mg/ml for 4 h followed by 14-day incubation in a drug-free medium. The effects of fludarabine were evaluated 72 h after its addition to the culture medium in a dose of 0.3 mg/ml.

Cytogenetic analysis of Namalwa cells included evaluation of the number of chromosomes and structural chromosome aberrations in metaphase plates, the type of aberrations, and marker chromosomes. For evaluation of the modal number of chromosomes not

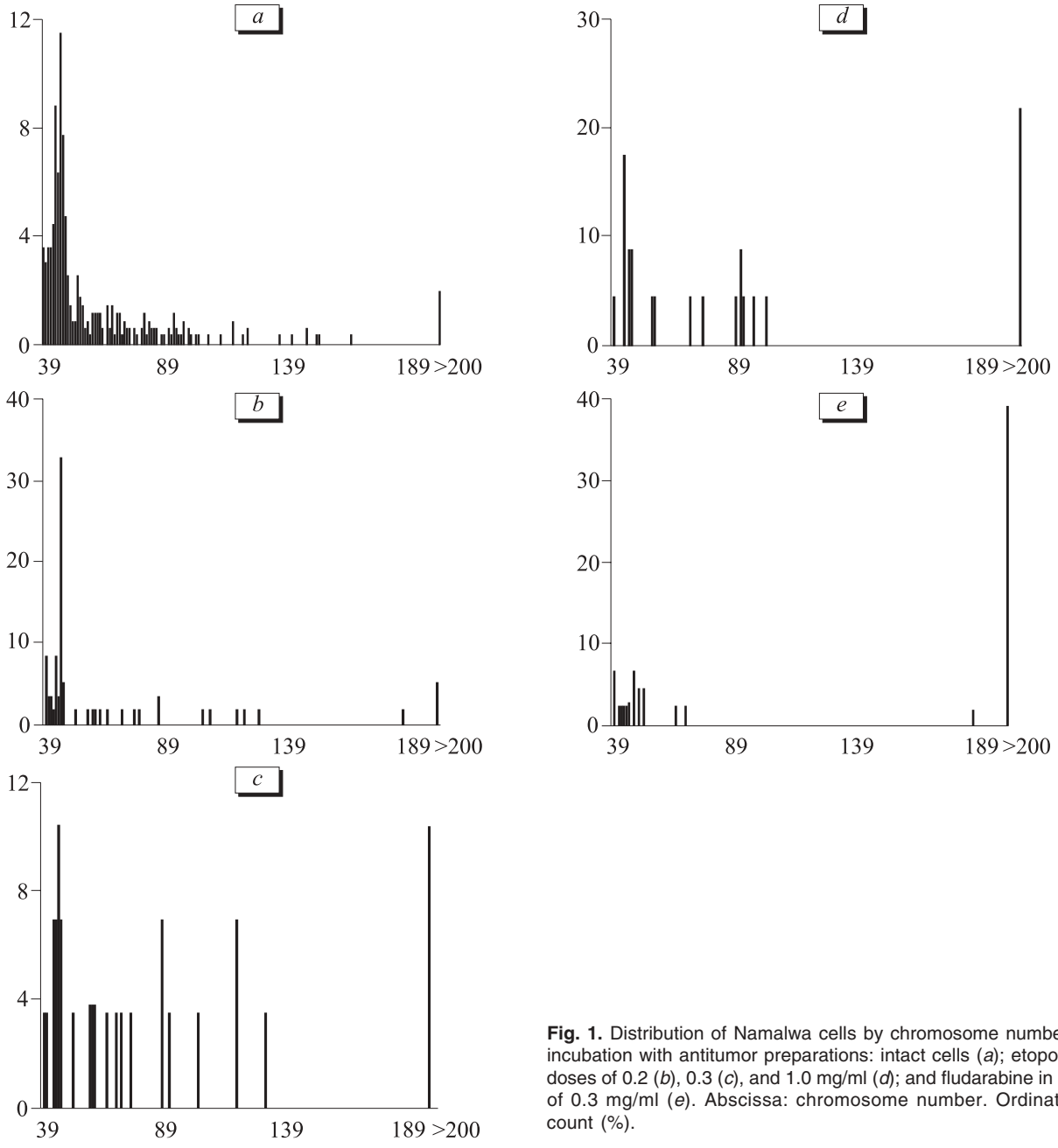

Fig. 1. Distribution of Namalwa cells by chromosome number after incubation with antitumor preparations: intact cells (*a*); etoposide in doses of 0.2 (*b*), 0.3 (*c*), and 1.0 mg/ml (*d*); and fludarabine in a dose of 0.3 mg/ml (*e*). Abscissa: chromosome number. Ordinate: cell count (%).

less than 100 routinely stained metaphase plates were examined. Karyotype analysis of the cell culture was performed on metaphases differentially stained by the G-method (30-100 cells). We examined 700 metaphases (control, *n*=380; incubation with antitumor preparations, *n*=320). The results were analyzed by methods of variational statistics.

RESULTS

Cytogenetic analysis showed that intact Namalwa cells contain 40-280 chromosomes. The modal number of chromosomes corresponded to the near-diploid range: 45-47 chromosomes (Fig. 1). Y chromosome was absent. Translocation *t* (8, 14) with the formation of a marker chromosome $14q^+$ was found in 56.7±3.2% cells. Chromosome aberrations were present in 16.1±0.6% cells. The mean number of aberrations in aberrant cells was 1.5 (most of them were chromosome aberrations). Paired acentric fragments and dicentric chromosomes were revealed in 56.7 and 33.3% cells, respectively. Chromatid aberrations were presented by single acentric chromosome fragments (Table 1). Apart from aberrations in chromosomes 3 (3p12) and 6 (6p22) typical of these cells, we revealed damage to chromosomes 1, 2, 4, 5, 10, 12, and 13. C-staining showed that 70% cells contained a large heterochromatin fragment on derivative chromosome 2 (2q22,24). This fragment serves as a marker of Namalwa cells [3].

Incubation of Namalwa cells with etoposide and fludarabine in subtoxic doses induced changes in the number of chromosomes and chromosome aberrations. After 24-h culturing in a medium with 0.2 mg/ml etoposide the number of metaphase chromosomes was the same (40-200, Fig. 1), while the incidence of chromosome aberrations increased by more than 2 times (39.8±1.7%) due to a rise in the count of chromatid aberrations (Table 1). We revealed a considerable number of chromatid breaks with single fragments and new types of aberrations (interchromosome chromatid-chromatid exchanges and ring chromosomes). Damage to chromosomes 1, 2, 5, 6, 11, 13, 16, and 17 developed most frequently.

The number of near-tetraploid and polyploid cells increased, while diploid modal chromosome number remained unchanged after incubation of Namalwa cells with etoposide in a dose of 0.3 mg/ml for 4 h and culturing in a medium not containing this preparation for 14 days (Fig. 1). The ratio of cells with chromosome aberrations remained high and 2-fold surpassed the control. We observed high incidence of chromatid aberrations (Table 1). Aberrations in chromosome 7 and D chromosomes were found most frequently. After treatment of cells with etoposide in a higher dose (1.0 mg/m, 4 h) and culturing for 14 days the number of polyploid cells (triploid, tetraploid, and polyploid) was higher than in the control. The number of aberrant cells slightly decreased, but surpassed the control. The ratio between chromosome and chromatid aberrations changed: with increasing the dose of etoposide the number of chromosome aberrations decreased, while the number of chromatid aberrations increased 2-fold. Aberrations were revealed in chromosomes 2 and 5 and D chromosomes 13 and 14.

Changes in the karyotype of Namalwa cells were most pronounced after culturing with fludarabine in a dose of 0.3 mg/ml for 72 h. The number of metaphase chromosomes was similar (40-200 or higher). We revealed 2 classes of diploid and polyploid modal chromosome numbers (Fig. 1). The number of structural

TABLE 1. Incidence and Type of Chromosome Aberrations in Namalwa Cells Treated with Etoposide and Fludarabine

Parameter	Control	Etoposide, mg/ml			Fludarabine, 0.3 mg/ml
		0.2	0.3	1.0	
Ratio of cells with aberrations (per 100 cells)	16.1±0.6	39.8±1.7	32.1±2.8	26.1±2.9	57.1±2.9
Types of aberrations, %					
chromosome					
paired acentric fragments	56.7	24.0	42.1	11.1	30.0
dicentric chromosomes	33.3	7.5	15.8	11.1	3.3
ring chromosomes	—	11.1	5.3	11.1	3.3
translocations	—	11.1	—	11.1	3.3
Total	90.0	53.7	73.7	44.4	40.0
chromatid					
single acentric fragments	10.0	40.7	26.3	55.6	60.0
chromatid-chromatid exchanges	—	5.6	—	—	—
Total	10.0	46.3	26.3	55.6	60.0

chromosome aberrations increased. Chromatid aberrations were predominant. Aberrations were found in chromosomes 2, 4, 5, 7, 11, 14, 16, and 17.

Our results indicate that etoposide and fludarabine in subtoxic doses produce cytogenetic changes in transplanted lymphoid Namalwa cells. These changes were most pronounced immediately after treatment with the test preparations. It should be emphasized that changes in the karyotype did not block cell proliferation (at least for several generations). Further culturing of cells was accompanied by disappearance of some chromosome aberrations, and their total number decreased. Increasing the dose of etoposide was followed by an increase in the number of chromatid aberrations typical of chemical mutagenesis.

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