CHROMOSOMAL INJURIES IN SOME CELL GENERATIONS AFTER TREATMENT OF HUMAN CELL CULTURES WITH RUBOMYCIN C*

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(Rubonycinum)

Metaphase chromosomes were investigated in cultures of human embryonic fibroblasts after treatment with the antitumor antibiotic rubomycin C (daunomycin, rubidomycin). Two effects were found: polyploid cells and chromosomal aberrations. During subculture the number of injured cells fell to the control level by the fourth to sixth passage. On further subculture, however, chromatid exchanges and marker chromosomes were found in some cells until the fifteenth passage.

The dynamics of the chromosomal injuries in human cell cultures after exposure to the action of viruses [10, 12], temperature shock [9], and radioactive radiations [3, 8] has been investigated. However, the duration of persistence of chromosomal aberrations in human cell cultures after exposure to chemical mutagens has received little study.

The object of this investigation was to determine chromosomal aberrations in a series of cell generations after treatment of cultures of human embryonic fibroblasts with the antitumor antibiotic rubomycin C. The cytogenetic effect of this compound on human cell cultures has been described previously [6, 11].

EXPERIMENTAL

Human embryonic fibroblasts were cultivated by the method described in [4]. A suspension of embryonic cells obtained from the muscle tissues of 6- to 9-week human embryos with the aid of 0.25% sterile trypsin solution was incubated at 37°C. The optimal concentration of cells was 250,000 per mm^3 nutrient medium containing 50% Eagle's medium, 30% lactalbumin hydrolysate solution, and 20% bovine serum. Rubomycin C was added to the culture of dividing cells for 2 h in concentrations of 0.05 μg/ml (series I) and 0.02 μg/ml (series II and III). The same volume of Hanks' solution was added to the control culture. Twenty-four hours after removal of the rubomycin, one-half of the cells was treated for 6 h with colchicine in a concentration of 0.3-0.5 μg/ml, then subjected to hypotonic treatment, and fixed with methanol-acetic acid fixative. The other half of the cells from the experimental and control cultures was subcultured by the method of Hayflock and Moorhead [7]. Chromosome preparations were made 48 h after subculture. They were stained with azure-eosin by Romanowsky's method and examined under oil immersion with a magnification of 1350×.

EXPERIMENTAL RESULTS

Series I. Chromosome preparations were obtained in eight passages after treatment of the original culture with rubomycin C in a concentration of 0.05 μg/ml. No dividing cells were found at the first pas-

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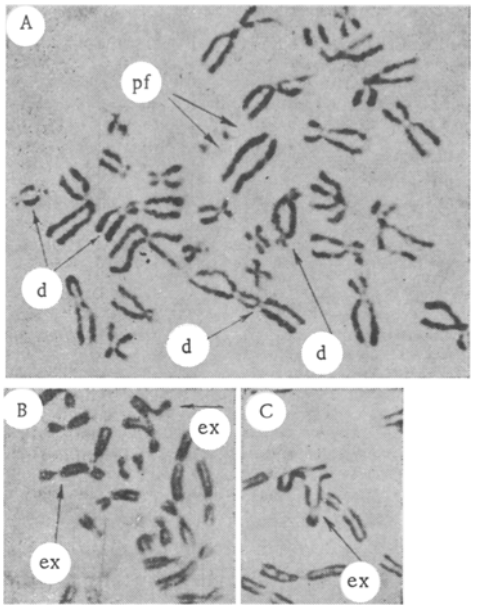

Fig. 1. Fragments of metaphase plates in culture of human embryonic fibroblasts at second (A), seventh (B), and fifteenth (C) passages after treatment with rubomycin C in a concentration of 0.02 μg/ml. pf) Paired fragments; ex) chromatid exchange; d) dicentric chromosome. Romanowsky's azure-eosin, 1350 ×.

sage, solitary mitoses were found at the second, while at the third passage 16 metaphase plates were counted, including four with aberrations (paired fragments) and ten tetraploid. At the fourth passage, among 100 metaphase plates examined eight had chromosomal aberrations and eight were tetraploid. At the fifth and sixth passages 3% and 4% of cells, respectively, were found with chromosomal aberrations, but there were no polyploid cells. At the seventh (Fig. 1B) and eighth passages the frequency of cells with chromosomal aberrations was no higher than in the control cultures, i.e., 0-1%. The mitotic activity of the cells from the fourth passage after treatment with rubomycin was greater than the mitotic activity in the control culture, and the activities in the experimental and control cultures did not become equal until the eighth passage.

Series II. Of the metaphase plates examined 24 h after treatment with rubomycin C in a concentration of 0.02 μg/ml 29.4% contained chromosomal aberrations. At the second passage (Fig. 1A) 49.5% of cells contained aberrant chromosomes and 30% of cells had a triploid set of chromosomes. After the fourth passage the cells in the experimental culture degenerated.

Series III. After the first fixation the number of cells with aberrations was 35.8%, at the second passage 13.6%, and at the third 3.5%. By contrast with the two previous series of investigations there was no increase in the frequency of polyploid cells. At the eighth passage there were two aberrations in 100 cells: one chromatid fragment and one chromosomal F/G translocation. At the fifteenth passage two cells with chromosomal aberrations were found: one chromatid rupture and one chromatid exchange (Fig. 1C).

It is uncertain whether induced chromosomal injuries can persist in cell cultures. For instance, after infection of human cell cultures with SV40 virus dicentric chromosomes were found on the fifth day, while on the twelfth day more than half of all cells studied were near-tetraploid and contained at least one dicentric chromosome [11, 13]. Kuliev [2] observed polyploid cells with chromosomal aberrations over a series of passages in a culture of embryonic fibroblasts in which virus infection was presumed to have occurred. After treatment of human cell cultures with temperature shock, polyploid cells were found during subculture for several months [9]. Polyploidization was also observed after treatment of human cells with ionizing radiation [8] and with N-nitroso-N-methylurea [5].

Polyploidization is evidently a nonspecific reaction of cells to several types of agent. The appearance of tetraploid cells in the experiments of series I and II can be attributed to the action of rubomycin C on the achromatic part of the division spindle. As a result, endomitosis takes place, followed by the formation of tetraploid cells with aberrations of chromosomal type. By the fourth to sixth passage after treatment with rubomycin the frequency of cells with chromosomal aberrations was no longer higher than the control. However, at the seventh passage, solitary chromatid exchanges were still found, indicating the presence of potential or subchromatid ruptures [1]. The discovery of a chromosomal translocation at the fifteenth passage may indicate that induced cell clones with stable marker chromosomes can be formed.

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