PERSISTENCE OF RADIATION INJURIES TO CHROMOSOMES IN GENERATIONS OF IRRADIATED HUMAN DIPLOID CELLS

N. V. Chervonskaya

UDC 612.014.24.014.482 +616-018.15-001.28

The preservation of radiation injuries to chromosomes was studied during cultivation of irradiated human diploid cells. In the first subculture after irradiation the number of cells with chromosomal aberrations was 2.5 times greater than in the control culture. After two subcultures of the irradiated strain, on the eighth day after irradiation, cells bearing unstable chromosomal aberrations and tetraploid cells induced by irradiation disappeared from the population.

The study of injuries to genetic structures in irradiated cells and the fate of cells carrying these injuries is of considerable interest.

Investigation of circulating blood cells of irradiated persons has revealed chromosomal aberrations many months or years after irradiation [4, 7-11, 16, 17]. However, these investigators have not undertaken successive observations on chromosomal changes in the course of several cell generations after irradiation.

The investigation of this problem is facilitated by the use of cells of human diploid strains, which preserve their diploid karyotype and their initial properties for 50 ± 10 subcultures.

EXPERIMENTAL METHOD

Cells of diploid strains obtained from the lungs of 3-5-month human embryos in the Laboratory of Diploid Cells of the Institute were used in the investigation. Cells were grown on Eagle's medium with the addition of 0.5% lactalbumin hydrolysate solution (30%) and calf serum (10%).

The cells were irradiated 48 h after seeding on a type RUP-200 apparatus under the following conditions: 210 kV, 15 mA, 23.5 R/min; focus distance 60 cm. Filters: Al 0.75 mm and Cu 0.5 mm. Dose 50 R.

Chromosomal aberrations were counted in the metaphase stage. Specimens for cytogenetic analysis were prepared in the usual manner [15]. Cytogenetic analysis was carried out on cells of the first and second subcultures after irradiation. The first subculture after irradiation took place at two different times: 24 h after irradiation, before the first postradiation mitotic cycle was complete [2], and 4 days after irradiation. Cells were fixed 48 h after subculture, i.e., 3 and 6 days, respectively, after irradiation. The second subculture of the irradiated cells took place 5 days after the first, i.e., 6 days after irradiation, and these cells also were fixed 48 h after subculture (8 days after irradiation). Intravital staining of the cells was carried out by addition of an equal volume of 0.1% trypan blue solution to the cell suspension [6]. The φ method and Student's distribution were used for statistical analysis of the results [5]. Each test was repeated 3-5 times. No statistically significant difference was found between the results of identical tests, so that aggregated data are given in this paper.

Laboratory of Diploid Cells, Moscow Scientific-Research Institute of Virus Preparations, Ministry of Health of the USSR. (Presented by Academician of the Academy of Medical Sciences of the USSR N. A. Kraevskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 70, No. 12, pp. 89-92, December, 1970. Original article submitted June 8, 1970.

TABLE 1. Quality and Types of Chromosomal Aberrations in Human Diploid Cells in the First and Second Subcultures after Irradiation

No. of subculture after irradiation	Time after irradiation (in days)	No. of cells studied	Number of cells with chromosomal aberrations		Types of chromosomal aberrations											
					Total number of chromosomal aberrations		chromatid deletions		chromatid translocations		isochromatid and chromosomal deletions		asymmetrical chromosomal translocations (dicentrics)				No. of tetraploid cells (in %)
			abs.	%	abs.	%	abs.	%	abs.	%	abs.	%	with fragmentation		without fragmentation		
													abs.	%	abs.	%	
1	3	830	66	8,0±0,9	72	8,7±0,9	20	27,8±5,2	3	4,2±2,3	33	45,8±5,8	10	13,9±4,0	6	8,3±3,2	4,3±0,2
1	6	460	39	8,5±1,3	45	9,8±1,3	5	11,1±4,5	2	4,4±3,0	30	66,6±7,0	6	13,3±5,0	2	4,4±3,0	2,2±0,4
1	Control	718	23	3,2±0,6	23	3,2±0,6	14	60,9±10,1	1	4,3±4,2	4	17,4±7,9	2	8,7±5,8	2	8,7±5,8	2,3±0,4
2	8	306	6	2,0±0,8	6	2,0±0,8	3	50,0±20,4	—	—	2	33,3±19,2	—	—	1	16,6±15,1	1,8±0,6
2	Control	335	12	3,6±1,0	12	3,6±1,0	7	58,3±13,6	—	—	3	25,0±13,1	—	—	2	16,6±10,7	2,3±0,6

EXPERIMENTAL RESULTS

The number of cells with chromosomal aberrations in the first subculture 3 and 6 days after irradiation was approximately the same, namely 8.0-8.5%, compared with 3.2% of aberrant cells in the control culture. The difference is statistically significant ($P > 0.999$; Table 1). However, differences were found in the spectrum of chromosomal aberrations found at these times. In cells fixed 6 days after irradiation the number of terminal chromatid deletions was reduced, and there was a corresponding increase in the number of isochromatid and chromosomal deletions, predominantly chromosomal.

In the second subculture after irradiation the number of cells with chromosomal aberrations in the irradiated culture did not exceed the control figure. The spectrum of chromosomal aberrations in the irradiated cells likewise was similar to that in the control cells.

The increase in the number of tetraploid cells to 4.3% compared with 2.3% in the control was observed in culture 3 days after irradiation (Table 1). At other times their numbers in the irradiated and control cultures were the same.

By using the method of intravital staining of the cells, the number of living and dead cells in the control and irradiated cultures was determined after each subculture. Counts showed that at the first subculture 24 h after irradiation the number of dying cells in the irradiated culture averaged 36.8% compared with 5.1% in the control. At the first subculture 4 days after irradiation the number of dead cells in the irradiated and control cultures was 44.0 and 10.8% respectively, while at the second subculture, 6 days after irradiation, the number of dead cells in the irradiated culture was 26.5% and in the control 8.3%.

The results of this investigation indicate that human diploid cells containing chromosomal aberrations, as well as tetraploid cells induced by irradiation disappear from the population after two subcultures of the irradiated culture. This conclusion was confirmed by counting the number of dead cells after irradiation, showing that after the first subculture up to 44% of the irradiated cells had died compared with 10.8% of dead cells in the control culture.

The fate of some of these aberrations can be determined by analysis of the types of chromosomal abberrations in the cells at different times after irradiation. A decrease in the number of chromatid deletions and a parallel increase in the number of aberrations of the isochromatid and chromosomal deletion types in the cells 6 days after irradiation compared with cells at the previous time of fixation indicates reduplication of the acentric fragments. These fragments may be taken up in anaphase and, together with the separating chromosomes, they may enter the daughter nucleus [3], although most (about 70% of acentric fragments) are lost during the first postradiation mitosis [19].

The equal numbers of dicentrics with fragments 3 and 6 days after irradiation may be explained on the assumption that the cells carrying these injuries had not divided by the end of that period and were in the first postradiation mitosis. Cells containing dicentrics with fragments and chromatid translocations had disappeared completely from the population 8 days after irradiation. The number of residual aberrations was reduced to the control level.

A decrease in the number of aberrant cells in the irradiated culture too close to the control level was discovered by the study of cultures of human circulating blood leukocytes for 120 h after irradiation in vitro by γ rays in a dose of 100 R [1].

However, when the leukocytes of irradiated persons were investigated at various times after irradiation, a variety of changes was found among the chromosomes, such as dicentrics, rings, paired fragments (called "unstable aberrations" by Buckton et al. [11]), and also "stable changes" (to use these workers' terminology) in chromosomes: symmetrical translocations of chromosomes and pseudodiploid cells, which are much less common [7-11, 16, 17]. It is interesting to examine these findings in the light of recent studies of the life span of human lymphocytes. A hypothesis which has recently attracted great attention is that of Fitzgerald, which he put forward on the basis of the following facts [13, 14]. Unstable chromosomal aberrations are lost after one or several cell divisions. Their discovery in human leukocytes several months or years after irradiation must therefore indicate that the cells carrying these aberrations have not divided during that period, i.e., that they are long-living cells retaining their ability to divide.

Subsequent studies of the life span of human lymphocytes have shown that they can in fact be preserved in vivo without division for 530-1574, or even for as long as 6743 days, although along with these lymphocytes there are others whose life span is only a few days [12, 18, 19]. It is thus obvious that in the body of irradiated persons cells carrying unstable aberrations may persist for a long time whereas these aberrations do not survive in dividing blood cells. Stable chromosomal aberrations can persist for a longer time in dividing cells, since there is no loss of genetic material through the formation of these aberrations. Cells containing these changes do not die after division because of disturbance of the gene balance.

Treatment of lymphocytes with phytohemagglutinin or their cultivation in vitro stimulates their division. Elimination of cells with unstable chromosomal aberrations thus takes place more rapidly in vitro.

LITERATURE CITED

1. M. M. Antoshchina, V. M. Kozlov, and N. P. Bochkov, Genetika, No. 7, 114 (1969).
2. R. A. Gibadulin, L. V. Kuznetsova, and N. V. Chervonskaya, Byull. Éksperim. Biol. i Med., No. 4, 100 (1970).
3. Z. A. Dzhemilëv, Genetika, No. 5, 67 (1967).
4. N. D. Okladnikova, Genetika, No. 2, 152 (1970).
5. N. A. Plokhinskii, Biometry [in Russian], Novosibirsk (1961), p. 144.
6. R. I. Rapoport and I. I. Akopova, in: Theory and Practice of the Use of Cell Cultures in Virology [in Russian], Moscow (1965), p. 38.
7. M. A. Bender and P. C. Gooch, Radiat. Res., 16, 44 (1962).
8. M. A. Bender and P. C. Gooch, Radiat. Res., 18, 389 (1963).
9. A. D. Bloom, S. Neriishi, N. Kamada, et al., Lancet, 2, 672 (1966).
10. A. G. Bloom, S. Neriishi, and P. G. Archer, Lancet, 2, 10 (1968).
11. K. E. Buckton, P. A. Jacobs, W. M. Court Brown, et al., Lancet, 2, 676 (1962).
12. K. E. Buckton, P. G. Smith, and W. M. Court Brown, in: H. J. Evans et al. (editors), Human Radiation Cytogenetics (1967), p. 106.
13. P. H. Fitzgerald, J. Theoret. Biol., 6, 13 (1964).
14. P. H. Fitzgerald, in: H. J. Evans, et al. (editors), Human Radiation Cytogenetics (1967), p. 94.
15. P. S. Moorhead, P. C. Nowell, W. J. Mellman, et al., Exp. Cell Res., 20, 613 (1960).
16. C. E. Nasjleti, J. M. Walden, and H. H. Spencer, J. Nucl. Med., 7, 159 (1966).
17. A. Norman, M. S. Sasaki, R. E. Ottoman, et al., Radiat. Res., 23, 282 (1964).
18. A. Norman, M. S. Sasaki, R. E. Ottoman, et al., Blood, 27, 706 (1966).
19. M. S. Sasaki and A. Norman, Nature, 214, 502 (1967).