EXPERIMENTAL GENETICS

RADIOSENSITIVITY OF CHROMOSOMES OF CHILDREN OF CANCER PATIENTS TREATED BY ROENTGENOCHEMOTHERAPY

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The use of combined radiotherapy and chemotherapy in the treatment of cancer patients of reproductive age necessitates an evaluation of the state of health of children born to patients after such treatment. Information in the literature on this problem is limited in amount and contradictory in nature As a rule reproductive parameters, outcomes of pregnancies in surviving patients, and risk of carcinogenesis in the offspring have been studied [13, 14]. Cytogenetic studies of children born to irradiated parents have been concerned only with an assessment of the spontaneous level of chromosomal aberrations in their cells [9, 10]. There is virtually no information on the sensitivity of chromosomes of the children of irradiated parents to mutagenic action. Since correlation exists between stability of the genome and the risk of carcinogenesis, such information could be useful in predicting possible carcinogenic and genetic consequences of the action of these factors.

The aim of this investigation was to study sensitivity of chromosomes of lymphocytes, in children whose parents underwent radiotherapy and chemotherapy before conception, to irradiation in vitro.

EXPERIMENTAL METHOD

Cytogenetic tests were carried out on six children born to patients who had been treated for lymphogranulomatosis and 10 children of healthy parents. The antitumor treatment was given to the patients in accordance with standard schedules, depending on the type and stage of the disease, and it included courses of radiotherapy and chemotherapy. Children of the experimental groups were born 18, 19, 21, and 67 months after the end of treatment of the mothers, and 134 and 185 months after the end of treatment of the fathers. The age of the children of both groups at the time of the investigation was 5-13 years. The age of the parents at birth of the children was 22-35 years. At the time of investigation the children were healthy, and during the year preceding the investigation they had not undergone any diagnostic x-ray procedures.

Venous blood, taken in a volume of 0.3 ml, was transferred into plastic flasks containing 3 ml of culture medium: medium RPMI 80%, bovine serum 20%, penicillin, streptomycin, and phytohemagglutinin ("Gibco"). The blood in four flasks was irradiated at room temperature in doses of 0.25, 0.5, 1.0, and 1.5 Gy from a ^{60}Co γ-ray source, on an "Igur" apparatus (dose rate 0.36 Gy/min, error of measurement of dose not more than 4%). Flask 5 was not irradiated. The blood was cultured at 37°C for 52 h. Colcemid was added to the culture 2 h before removal. Metaphase preparations of lymphocytes were obtained by the standard method and examined under the microscope with magnification of 1350. Chromosomal and chromatid aberrations were counted during analysis of 100 metaphases for each dose and 150-200 cells in the unirradiated sample.

The results were subjected to statistical analysis on the PC AT-286 computer by Student's t test and by dispersion and regression analysis.

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TABLE 1. Cytogenetic Lesions in Irradiated Lymphocytes of Children Whose Parents Received Radiotherapy and Chemotherapy

Dose of irradiation of blood, Gy	Number of aberrant metaphases, %		Chromosomal aberrations per 100 cells						Chromatid aberrations per 100 cells	
			double fragments		dicentrics		rings			
	E	C	E	C	E	C	E	C	E	C
0	3.5	2,1	1,2	2,3	0	0	0	0	1,2	0,8
0.25	5,9	6,2	4,4	3,6	1,5	1,0	0,5	0,2	1,0	1,2
0,50	9,3	7,2	6,9	4,4	3,8	1,0	0,6	0,1	1,9	0,3
1,00	20,2	16,1	11,7	10,9	9,9	6,3	0,7	0,8	2,5	1,5
1,50	33,4	27,6	18,9	18,2	17,0	11,9	0,6	0,5	2,6	1,3

Legend. E) Children of patients; C) of healthy parents.

EXPERIMENTAL RESULTS

The results of cytogenetic analysis of lymphocytes irradiated in vitro in the G_0 stage in children of the experimental and control groups are given in Table 1.

Children of the groups compared did not differ significantly in the spontaneous frequency of chromosomal aberrations. A higher level of cytogenetic lesions compared with children of the control group was observed in children of the experimental group after irradiation of the lymphocytes in vitro. Two-factor dispersion analysis of the data, using parameters given in Table 1, revealed highly significant differences between the groups ($p = 0.01$), although the influence of the group factor was comparatively small ($\approx 4\%$) compared with the influence of the irradiation dose factor ($\approx 80\%$).

As regression analysis of the data showed, the dose dependence of the frequency of aberrant metaphases, the number of exchange aberrations (dicentrics and rings), and also the number of paired fragments could be approximated with a high degree of significance by a linear function Values of these parameters, for doses used in the experiments, agreed well with data obtained by other workers [1, 5].

The frequency of aberrations of chromatid type in the control group varied independently of the dose of irradiation. Meanwhile, in the experimental group the number of these aberrations was found to be a linear function of dose ($p < 0.05$). The transition from dose-independence of the frequency of chromatid aberrations in the region of small doses to a linear function in the region of high doses was observed by Saven'kaev [5], who explained it from the standpoint of the hypothesis that with low doses of irradiation the repair system reduces the number of primary lesions, which lie at the basis of formation of chromatid aberrations, whereas with high doses the repair system itself is damaged, leading to a dose-dependent increase in the number of chromatid aberrations. It can be tentatively suggested that in children of the experimental group the repair system was initially defective, and for that reason an increase in the number of chromatid aberrations was observed in these children with an increase in dose, whereas in children of the control group these lesions were repaired within the dose range from 0.25 to 1.5 Gy.

The level of stability of the hereditary structures of the body is known to be under genetic control. In man there are several hereditary syndromes in which increased fragility of the chromosomes is observed [8]. In so-called cancer families, the frequency both of spontaneous chromosomal aberrations and of those induced by the action of mutagens on cells in vitro is increased in patients and in their relatives compared with healthy blood donors [11]. Variability of the spontaneous level of cytogenetic disturbances also is characteristic of normal human populations [12].

The experimental results are evidence that different chromosomal and genomic rearrangements increase the probability of mutagenesis in cells [3, 7]. Artificial manipulations with genomes lead to the same effect: inbreeding, hybrid dysgenesis, introduction of foreign DNA.

In 1973 one of us (I.E.V.) postulated and confirmed experimentally that irradiation of parents can induce instability of the offspring's chromosomes [2]. It has been shown that somatic cells of the offspring of male rats irradiated in a dose of 4.2 Gy are more sensitive to the mutagenic action of radiation than those of the offspring of nonirradiated parents [3]. Increased mutability of the sex cells of the offspring of irradiated males also has been demonstrated on *Drosophila* [7].

There is evidence that cytogenetic radiosensitivity is increased in directly irradiated animals and man in the late periods after exposure to radiation [4, 14]. A similar phenomenon has been described for irradiated cells in vitro [6]. Our results are evidence that the radiosensitivity of chromosomes of children whose parents received antitumor radiotherapy and chemotherapy is higher than that of children of the control group.

The fact that children of the experimental group, while not differing significantly from the control in the spontaneous level of chromosomal aberrations, were more sensitive to the action of mutagens, indicates a latent defect of their cells, detectable only in response to additional procedures. A similar phenomenon also has been observed in relation to the offspring of irradiated animals [3].

Evidently children of cancer patients, born after they have received combination antitumor therapy, constitute a group with increased mutagenic (carcinogenic) risk, requiring long-term monitoring and preventive measures aimed at reducing this risk. The small size of the experimental sample does not allow this conclusion to be unequivocally confirmed. The role of factors such as the disease itself, the age of the parents, the time from the end of treatment until conception, and the dose of irradiation and chemotherapy must also be analyzed.

At the present time, when quite large groups of people are exposed to radiation the phenomenon of induced hypersensitivity to mutagens must be taken into consideration when standard levels of radiation damage are determined, especially if it is combined with exposure to other factors with a toxic action on the gene.

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