CHANGES IN DNA AND SURFACE PROPERTIES OF PERIPHERAL BLOOD LEUKOCYTES IN MONKEYS AFTER WHOLE-BODY γ-IRRADIATION

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During the evalution of disturbances of DNA structure in irradiated cells great importance is attached to changes in spiralized and superspiralized components of the polynucleotide, responsible for activity of replication, transcription, and recombination enzyme [2, 5]. In recent years interest has grown in the study of the AT segments of DNA, which play an important role in the structural and functional organization of the genome, for they are located in its operator, intron, or satellite elements [6, 12]. However, there have been only sporadic studies of changes in AT-DNA after irradiation, and these on small laboratory animals [3, 4]. This paper describes a study of changes both in the AT component proper of DNA and the structural state of the whole genome, estimated from the ratio of spiralized structures and AT-sequences in DNA from blood nucleoids of irradiated monkeys. These characteristics of the genetic material also were studied parallel with an assessment of the state of the membranes in the leukocytes, which was tested on the basis of the adhesive properties of these cells. Data of this kind are essential for extrapolation of the experimental results obtained to man, within the context of deepening our understanding of the molecular and cellular mechanisms of radiation pathology.

EXPERIMENTAL METHOD

Experiments were carried out on male monkeys: *Macaca nemestrina* (n = 12) weighing 4-5 kg, and *Macaca rhesus* (n = 12), weighing 12-16 kg. Whole-body γ-irradiation of the animals of uniform intensity was carried out on a GUBÉ-4000 apparatus with a dose rate of 1.2 Gy/min and in doses of 6.2 Gy for *M. nemestrina* and 6.5 Gy for *M. rhesus*. Blood taken from a vein of the upper limb was analyzed 6 h and 1, 3, 4, and 5 days after irradiation. Leukocytes were then fractionated on the basis of differences in the adhesive properties of their membranes [11], as a result of which the total leukocyte population was divided into fractions of adhesive and nonadhesive cells. The total leukocyte population and the nonadhesive fraction were lysed under conditions leading to nucleoid formation [8] and the AT-DNA content in it was measured by a fluorescence method using 4′,6-diamidino-2-phenylindole (DAPI) [7], using rat thymus DNA, treated with ultrasound, as the standard. Structural changes in DNA were judged from changes in the coefficient of relative fluorescence, determined after staining the nucleotide of the blood cells by two fluorochromes: ethidium bromide (3.5 μg/ml) and DAPI (0.1 μg/ml). The first of these intercalates into spiral and superspiral structures of the polynucleotide [8], the second binds specifically with AT-pairs of the linear chain of the polymer [9]. Thus the coefficient of relative fluorescence reflected the degree of spiralization per unit length of AT-DNA.

EXPERIMENTAL RESULTS

The content of AT-DNA 6 h after irradiation was higher than initially, but by 24 h it had fallen on average by 40%. Later the DNA concentration in the nucleoids of the animals' blood continued to fall during the period of the investigation, and 4-5 days after irradiation it was down to 27-14% of the initial values. The dynamics of the fall of AT-DNA in the blood nucleoids and the depth of the leukocytopenia were similar in character in both species of irradiated monkeys. Estimation of the structural state of the DNA of the blood nucleoids with the aid of the two ___

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TABLE 1. Changes in AT-DNA Content of Blood Leukocyte Nucleoids of Irradiated *Macaca nemestrina*

Parameter	Total leukocytes		Fraction of adhesive leukocyte		Fraction of nonadhesive leukocytes	
	in all fractions	calculated per leukocyte	in all fractions	calculated per leukocyte	in all fractions	calculated per leukocyte
Initially	123,8±71,6	10,4±6,4	31,8±29,7	25,2±22,6	104,7±70,0	9,6±6,0
After irradiation for						
4 h	422,2±214,7	21,2±11,0	405,3±91,9	32,6±11,8	601±56,4	11,8±3,6
24 h	161,3±39,7	38,2±14,6	131,2±21,7	35,6±18,4	131,2±21,7	35,6±18,4

Legend. *$p < 0.05$ compared with initial value. Quantitative changes expressed in Table 1 relative to standard, as which rat thymus DNA treated with ultrasound was used.

fluorochromes showed that the principal changes in the polynucleotide took place between 6 and 24 h after irradiation. The coefficient of relative fluorescence continued to rise until the 5th day, but less sharply. These changes were similar in both species of monkeys although there were differences: 24 h after irradiation this increase in *M. rhesus* was less marked than in *M. nemestrina.* The time course of changes in the adhesive properties of the membranes of the total blood leukocytes is illustrated in Fig. 2b,* from which it can be concluded that membrane changes in the white blood cells after irradiation began earlier than structural changes in DNA, but they also disappeared faster. This conclusion is in agreement to some extent with data in the literature [10] on assessment of the state of the cell surface of the blood leukocytes with the aid of concanavalin A. Under these circumstances early (a few hours after x-ray irradiation) changes were found in the membranes, followed by their comparatively rapid normalization.

The results of quantitative analysis of AT-DNA in nucleoids of total leukocytes and of cells differing in their adhesive properties after irradiation, at a time when the most marked structural changes were taking place in the DNA (from 6 to 24 h) are given in Table 1. Changes in the AT-DNA concentration of nucleoids of the whole blood leukocyte population were mainly due to changes in the fraction of adhesive leukocytes (Table 1). However, a significant increase in nucleoid AT-DNA calculated per cell (Table 1) was found in the nonadhesive fraction, possibly indicating qualitative changes in the latter. Thus cells incapable of adhesion and with an increased content of detectable AT-DNA were found in the peripheral blood 24 h after irradiation. Gol'dberg et al. [1] found a large number of neutrophils with increased ploidy in the bone marrow of rats irradiated in lethal doses. These workers observed an increase in the number of these cells up to a maximum 24 h after irradiation. Our own results are in definite agreement with these data in the literature. It can be tentatively suggested that white blood cells with increased ploidy formed in the bone marrow of the monkeys were detectable in the present investigation at the periphery in the form of leukocytes with an increased AT-DNA content Also, the structure of DNA in the nucleoid, in which irradiation is followed by structural changes, as a result of which the accessibility of the AT segments of the nucleotide for binding with the fluorochrome is altered, can also be estimated, most probably with the aid of DAPI.

The experimental data suggest that 6 h after irradiation there is a compensatory release of a leukocyte population, consisting mainly of adhesive cells with a normal AT-DNA content, identical in structure with that observed initially, from the depots into the peripheral blood. Toward 24 h after irradiation the total number of white blood cells falls; moreover, the circulating nonadhesive leukocytes differ from the normal cells in the increased amount of AT-DNA detectable in them, against the background of a change in the initial structure of the polynucleotide.

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ADHESION OF LEUKOCYTES IN CHRONIC DIFFUSE LIVER DISEASES

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Histological changes arising in the liver in chronic diseases may be associated with disturbances of intercellular relations between hepatocytes. Electron-microscopic investigations have shown that during cholestasis in patients with primary biliary cirrhosis, partial or total separation of the hepatocytes takes place, and leads to an increase in the interhepatocytic space [1]. In addition, investigation of laboratory animals with experimentally induced cholestasis revealed considerable disturbances in the region of the tight junction, namely a decrease in the area of the contacting surfaces of the hepatocytes in animals with cholestasis by 50% compared with control animals [12]. Existing approach techniques enable some of the characteristics of intercellular interactions to be determined [3]. A special place among them is occupied by methods of estimation of the adhesive characteristics of intercellular junctions (IJ), determining their mechanical strength [4, 5]. In the present investigation one such method was used, based on determination of the number of separating whole cells and nuclei during a standard dispersion procedure [8, 10]. Several workers have shown that the number of single cells (hepatocytes, enterocytes) separated as a result of mechanical action on the tissue reflects the state of IJ: weakening of IJ leads to an increase in the number of cells separating from the tissue [3]. This approach technique was used successfully previously in a study of the macromolecular factors involved in the adhesive process [2, 6, 8-10].

The aim of this investigation was to study the adhesive properties of hepatocytes using biopsy material from patients with chronic diffuse liver diseases.

EXPERIMENTAL METHOD

The test object consisted of liver biopsy material from 72 patients, divided into eight groups on the basis of their final clinical diagnosis: 1) with primary biliary cirrhosis — PBC, including chronic cholestatic hepatitis — CCH, as the initial stage of PBC (11 patients), 2) with chronic hepatitis — CH, with moderate activity (five patients); 3) with chronic active hepatitis — CAH (four patients); 4) with chronic persistent hepatitis — CPH (11 patients); 5) with Gilbert's unconjugated hyperbilirubinemia — UH (11 patients); 6) with fatty degeneration of the liver — FD (11 patients); 7) with cirrhosis of the liver — CL (three patients); 8) with gastroenterologic diseases but without any histological changes in the liver: gastric and (or) duodenal ulcer, chronic gastritis, chronic pancreatitis, chronic cholecystitis, etc. — control (16 patients). Liver biopsy was carried out by Menghini's method with a needle 1.8 mm in diameter [2]. Liver biopsy tissue was cut into fragments measuring about 1 mm^3. Each tissue fragment was dispersed in 0.1 ml of 0.1% trypan blue in Ringer's solution, using a glass tissue disintegrator with 50-μ gap. Each fragment was dispersed under strictly standard conditions [8, 10]. Single hepatocytes (N_{sh}) and free cell nuclei (N_n) separated on dispersion were then counted in a Goryaev chamber. From these results the coefficient of dissociation (K_d) was calculated by the equation:

$$K_d = \frac{N_{sh}}{N_{sh} + N_n}.$$

At least five tissue fragments were counted for each patient and K_d was determined for each fragment. Changes in size of the cells were recorded by means of an ocular micrometer. The area of a hepatocyte from patients of the control group was chosen as the unit. The results were compared by Student's test.

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