STRUCTURAL CHANGES IN BLOOD NUCLEOID DNA AND DEVELOPMENT OF LATE SEQUELAE IN IRRADIATED RATS

S. D. Ivanov, I. V. Remizova, E. G. Kovan'ko, A. V. Gubareva,* and V. E. Komar

UDC 616-001.28-06-092:616.153:577.123.388]-092.9

KEY WORDS: AT segments of DNA; rat blood nucleoids; late sequelae of irradiation; fluorescence analysis.

The results of investigations of the spatial organization of DNA in chromosomes and data on the role of individual polynucleotide fragments in replication, transcription, and recombination are evidence that an important role in the structural and functional organization of the eukaryote genome is played by AT-rich segments [1]. It has been suggested that processes of oncogenesis may take place with their participation [1] and that loss of heterochromatin centromeric regions (where there are AT-sequences also) probably accompanies processes of aging [8]. Accordingly, in the investigation described below we studied changes in the structure of DNA due to AT-fragments in postirradiation reactions of peripheral blood leukocytes of animals and we attempted to discover a link between early changes in genome structure and certain late sequelae of irradiation. The following criteria of these sequelae were adopted: 1) the presence of changes in stained films of leukocytes 10-15 months after irradiation (shift of the formula to the left, hypersegmentosis, increased number of mononuclears); 2) shortening of the life span of the irradiated animals; 3) a pathological state of the internal organs 15 months after irradiation of the rats, associated with tumor development.

EXPERIMENTAL METHOD

Noninbred male albino rats were subjected to a single session of γ-ray irradiation (^{60}Co) in doses of 2 Gy (n = 30) and 4 Gy (n = 40), with a dose rate of 0.4 Gy/min. Blood samples from the caudal vein of the control (n = 30) or irradiated animals, 0.01 ml in volume, were treated with a lytic mixture under nucleoid isolating conditions, so that DNA was obtained in the near-native state [5]. A two-parameter criterion was used to characterize the nucleoid DNA: first, the content of AT-fragments and, second, the presence of a spiralized component. Accordingly, the structural state of the polynucleotide was evaluated with the aid of a coefficient of relative fluorescence (CRF), using as luminescent probes: 4',6-diamidino-2-phenylindole (DAPI), which binds specifically with the AT-tetranucleotide of DNA [7], and ethidium bromide, which intercalates into spiralized structures of the biopolymer [6]. The working concentration of DAPI was 0.1 μg/ml, and of ethidium bromide 3.8 μg/ml. The value of CRF was calculated by the equation:

$$CRF = K \times IF_{et}/IF_{DAPI},$$

where K is a coefficient allowing for dilution of the samples and of the standard, and also the intensity of luminescence of a reference DNA in the measuring cuvette, IF_{et} and IF_{DAPI} are the intensities of fluorescence of samples stained with ethidium bromide and DAPI, respectively. Measurements were made on a "650-40" instrument ("Hitachi," Japan). For an over-all evalua-

*Decreased.

Central Roentgeno-Radiologic Research Institute, Ministry of Health of the USSR, Leningrad. (Presented by Academician of the Academy of Medical Sciences of the USSR N. P. Napalkov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 109, No. 3, pp. 296-299, March, 1990. Original article submitted January 10, 1989.

0007-4888/90/0003-0385$12.50

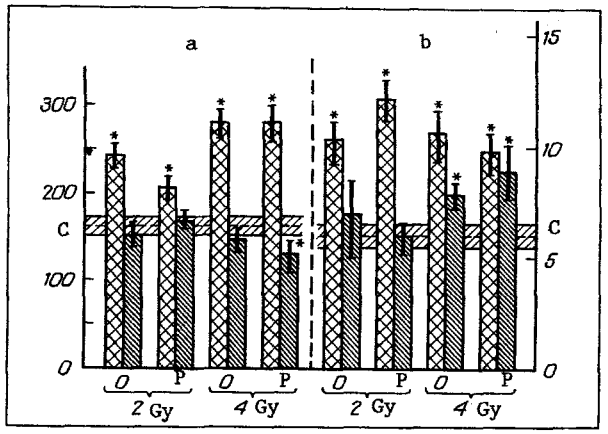

Fig. 1. Changes in structure of blood nucleoid DNA and DNA index of irradiated rats during development of late hematologic pathology. Abscissa, O) absence, P) development of pathological changes in groups of animals irradiated in doses of 2 and 4 Gy; ordinate, on left: values of CRF (in relative units), on right: values of DNA index (in conventional units). Explanation of symbols: a) changes in CRF, b) changes in DNA index: values of parameters 2 days (cross-hatched columns) and 30 days (obliquely shaded columns) after γ-ray irradiation. *) Values significantly ($p < 0.05$) differing from control (C).

tion of changes in ploidy of the leukocytes and their proliferative activity, we used the DNA index described previously [4], with some modifications. Blood cells of intact animals were used as standards, with close to the diploid (2C) DNA content. The value of the index for these cells was determined by dividing the DNA concentration of the blood nucleoids, measured with the aid of DAPI [2] by the number of leukocytes in 1 ml of the sample. The increase in the DNA index thus determined in the irradiated animals was associated mainly with the appearance of a fraction of leukocytes in the peripheral blood with altered ploidy (due to aneuploid cells or cells in the G_2-M phases) and/or proliferative activity was increased (cells in the S-phase of the cycle).

The cytologic characteristics of the blood samples also were determined, and in 30 animals which survived until the end of the experiment (15 months after irradiation) macro- and microscopic analyses of the internal organs were undertaken. The biochemical data on the state of DNA in the blood nucleoids 2 and 30 days after irradiation were grouped in accordance with the severity of the pathological changes, on the basis of the three criteria of late sequelae mentioned above.

EXPERIMENTAL RESULTS

Results grouped on the basis of hematologic parameters are given in Fig. 1. They show that molecular changes in the blood after 2 days are unable to reveal any specific response in the form of late hematologic changes linked with an increase in the DNA index or CRF. However, toward the 30th day, in rats irradiated in a dose of 2 Gy, values of the DNA index fell to control values, by contrast with animals irradiated in a dose of 4 Gy (Fig. 1b). Among this last group, changes in hematologic characteristics were recorded only in those rats which had significant deviations of the structure of their genetic material from the control (for values of CRF, see Fig. 1a), then only after 10-15 months. Thus only when significant disturbances of the nucleoid DNA structure of the blood leukocytes accompanied by an increase in ploidy and/or proliferative activity of the cells did late hematologic pathological changes develop on the 30th day after irradiation (in the case of a dose of 4 Gy), although in the absence of significant changes in these molecular parameters (in the case of a dose of 2 Gy) the pathological development of the blood leukocytes could take place. Assessment of the comparative contribution of the two components to the change in the value of CRF showed that it largely reflects ($r = 0.88$) damage to the structure of DNA connected with AT-fragments, whereas changes introduced by the component revealed by ethidium bromide amounted to 6-7%.

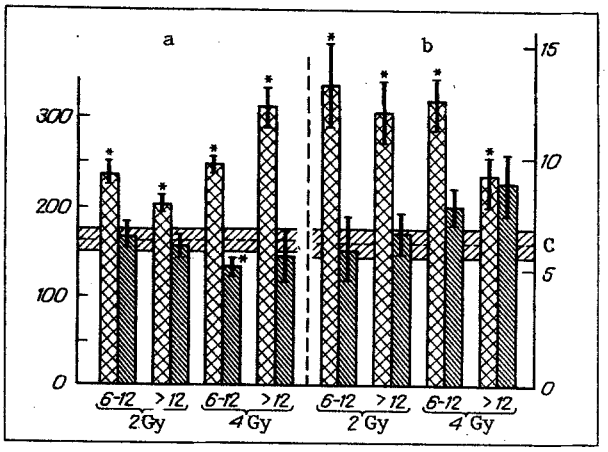

Fig. 2. Changes in structure of blood nucleoid DNA and DNA index of irradiated rats depending on life span. Abscissa, 6-12 and >12) life span of group of animals (in months) after irradiation in doses of 2 and 4 Gy. Remainder of legend as to Fig. 1.

Systematization of the data on the basis of life span of the rats in the 15-month period demonstrated the following. The time course of death of the animals irradiated in a dose of 2 Gy, with a mean life span (MLS) of 216 ± 32 days, was close to that of the control animals (MLS = 257 ± 38 days), but differed from that of death of rats exposed to irradiation in a dose of 4 Gy (MLS = 125 ± 19 days). Among animals surviving more than 12 months, differences were observed in the early biochemical responses to irradiation (Fig. 2a): irradiation in a dose of 4 Gy led to greater deviations in DNA structure than in a dose of 2 Gy. However, toward the 30th day the values of these parameters were restored to the control levels. In rats dying later (after 6-12 months) after irradiation in a dose of 4 Gy deviation from the normal DNA structure was observed, when measured after 30 days. Thus in rats with least ability to restore the structure of their nucleoid DNA by the 30th day after irradiation, shortening of the life span was observed. Changes in DNA structure of the blood nucleoids 2 days after irradiation did not allow the specificity of their damage to be correlated with the life span of the animals. Meanwhile, deviations of CRF of DNA from normal on the 30th day after γ-ray irradiation in a dose of 4 Gy were accompanied by shortening of the life span of the rats, but if these structural changes were absent (in the case of a dose of 2 Gy) mortality of the animals in the earlier period did not differ from the control. The parameter linked with DNA ploidy and proliferative activity of the leukocytes likewise did not reveal any regular pattern depending on the duration of survival of the animals (Fig. 2b).

Grouping of the results by comparison with the late sequelae associated with tumor formation showed that irradiation in a dose of 2 Gy led to a very small increase in relative proportion of tumor-bearing animals (25%) compared with the control group (17%), whereas γ-ray irradiation in a dose of 4 Gy increased it (to 67%). If the irradiated rats had pathological conditions which were not due to the development of malignant neoplasms (bronchiectasis, hyperplasia of lymphatic tissue, cardiosclerosis, etc.), despite the primary changes in CRF of DNA after 2 days, toward the 30th day the structure of the polynucleotide was restored to the control level (Fig. 3a). Meanwhile, in rats found to have tumors (squamous-cell carcinoma of the lung, lymphatic leukemia, hepatocellular carcinoma) by the 15th month after irradiation, deviations of DNA conformation connected with an increase in the number of probed AT-fragments compared with the group of control animals, were observed both after 2 and after 30 days. Thus comparison with tumor-bearing animals likewise did not reveal any specificity of the changes in CRF of DNA of the blood nucleoids on the 2nd day after γ-ray irradiation. Meanwhile, whereas on the 30th day after irradiation we recorded a normal structure of DNA, significant stimulation of tumor growth was not observed (irradiation in a dose of 2 Gy), but if significant changes in DNA structure of the nucleoids were present, marked activation of radiation-dependent oncogenesis took place (irradiation in a dose of 4 Gy). Data in the literature are evidence that one possible factor in activation of H-ras oncogenes may be disturbances of DNA conformation [2]. Under the influence of chemical carcinogens, changes of this kind are mediated through an increase in the relative proportion of AT-base pairs in the genome as a result of damage predominantly to guanine [9]. Thus changes in the conformation of DNA, linked with an increase in the number of AT-probed segments in it, which we found toward the 30th day after irradiation may be a reflection of a more general rule governing the process of oncogenesis, when induced by both chemical and radiational carcinogenic agents. On the other hand, no significant disturbances of the DNA

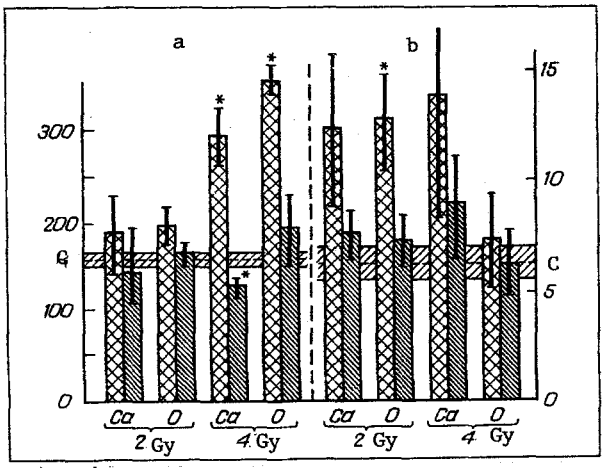

Fig. 3. Changes in DNA structure of blood nucleoids and DNA index of irradiated rats during tumor development. Abscissa, O) absence of tumors, Ca) development of tumors in groups of animals irradiated in doses of 2 and 4 Gy. Legend as to Fig. 1.

index could be found in our experiments to study initiation of tumors (Fig. 3b). These observations are in agreement with those of other workers [4] who found that most tumors induced by radiation in rats have a relatively small number of cells in the S- and G_2-M phases of the cycle, thereby demonstrating the low proliferative activity of these neoplasms.

Thus changes in DNA of the blood nucleoids 2 days after irradiation did not reveal specificity relative to late radiation pathology. After γ-ray irradiation in a dose of 2 Gy, no significant increase in the frequency of late pathological sequelae was observed in the rats compared with control animals, and no disturbances were found in the DNA parameters of nucleoids measured on the 30th day after irradiation. After γ-ray irradiation in a dose of 4 Gy, changes in the DNA index of the leukocytes (due to their ploidy and/or proliferative activity) among rats surviving the acute period (30 days) were accompanied by disturbances of the peripheral blood picture in the late stages, but these were not connected with tumor development or associated with shortening of the animals' life span. Late pathological, hematologic, gerontological, and oncologic sequelae of irradiation in the rats were associated with damage to the DNA structure of the leukocyte nucleoids, due to changes in their content of AT-fragments, and detected by methods used in the present investigation. It can be tentatively suggested that the course of postradiation repair and compensatory processes taking place in the animals from the 2nd through the 30th day, structural changes take place in the genetic material, and these are recorded in a certain group of rats as disturbances of DNA conformation, and on the 30th day after irradiation an increased number of AT-fragments is observed. It is in this group of animals that late sequelae of irradiation are subsequently manifested to a greater degree.

LITERATURE CITED

1. K. Scherrer and J. Monod, 16th FEBS Conference. Proceedings [in Russian], Vol. 1, Moscow (1987), pp. 305-315.
2. M. Barbacid, Ann. Rev. Biochem., **56**, 779 (1987).
3. C. F. Brunk, K. C. Jones, and T. W. James, Analyt. Biochem., **92**, 497 (1979).
4. K. Christov and I. Yanchev, Neoplasma, **32**, 335 (1985).
5. P. R. Cook and J. A. Brazell, Eur. J. Biochem., **84**, 465 (1978).
6. A. R. Morgan, J. S. Lee, D. E. Pulleyblank, et al., Nucleic Acids Res., **7**, 547 (1979).
7. J. Portugal and M. J. Waring, Biochim. Biophys. Acta, **949**, 158 (1988).
8. R. J. Schmookler, C. K. Lumpkin, J. R. McGill, et al., Nature, **301**, 394 (1983).
9. M. D. Topal, Carcinogenesis, **9**, 691 (1988).