Restoration of Chromosome Aberrations of Irradiated Tumor Cells by DNA and its Precursors*

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放射線誘発染色体異常に対する DNA 及びその先駆体の修復効果

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^{60}Co γ 線による Ehrlich 腹水腫瘍細胞の誘発染色体異常に対するＤＮＡ及びその先駆体の 500 R照射5時間後投与による修復効果を，染色体及び娘染色体切断を指標に各細胞令について検討した．最も効果の著しいのは dGMP で，特に G_1 細胞の染色体切断が$^1/_3$以下に減少した．ＤＮＡ及びdAMPによつてもやや効果がみられた．dCMP及びＴＭＰはいずれの細胞令についても異常頻度は照射のみの場合と殆んど同様であつた．

Kobayashi (1973) showed that chromosome aberrations of tumor cells after sublethal irradiation could be slightly restored by addition of adrenochrome. Petrovic *et al.* (1963, 1966, 1970) reported that DNA precursors may be of considerable improtance in restoration of damage in L-cells *in vitro* after a lethal dose of irradiation. It was therefore of considerable interest to determine whether treatment with DNA and its precursors had a similar restorative effect on chromosome aberrations induced by radiation.

This paper reports results indicating that DNA and its precursors do in fact have this effect.

Materials and Methods

Ehrlich ascites carcinoma cells were used. Six days after inoculation of tumor cells, mice were ir-

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radiated with 500 R from a ^{60}Co gamma-ray source at a dose rate of 50 R/min. Radiation dose was measured with a Victreen Radocon dosimeter probe placed in the card-board box containing animal. Calf thymus DNA (Worthington Biochemical Co.) or one of its precursors (Sigma Chemical Co.) was injected at a dose of 2.5 mg/25 g body weight 5 hours after irradiation. The five hours mean the first time that DNA synthesis and mitoses of this tumor cells were disappeared.

The following DNA precursors were used: deoxyadenosine-5′-monophosphate (dAMP), deoxyguanosine-5′-monophosphate (dGMP), deoxycytidine-5′-monophosphate (dCMP) and thymidine-5′-monophosphate (TMP). They were dissolved in physiological saline (buffered at pH 7.2) and injected into mice intraperitoneally.

To study the effects of these compounds on cells of different ages, chromosome aberrations were examined on cells at various stages in the cell cycle. The latter was determined by labeling the cells with tritiated thymidine. For labeling studies, 5 μCi of ^{3}H-thymidine (Radiochemical Centre, Amersham) were injected into the peritoneal cavity of tumor bearing mice, weighing 25 g. In *in vivo* experiments labeling was terminated by removing cells from the peritoneal cavity and washing them with 20 μg/ml of unlabeled thymidine, as described in the previous paper (1966). Smear preparations were made at 2-hour intervals for 35 hours after irradiation. Preparations were then fixed with acetic alcohol. Then liquid emulsion (Sakura NR-M_2) was added according to dipping method, and after exposure (2 weeks), development, and staining, the percentage of labeled cells in anaphase was recorded.

For chromosomal analysis, the preparations were made by squashing the cells with acetic dahlia. Frequencies of chromosome aberrations were calculated from results on 50 cells in metaphase examined 18 (G_2), 24 (S) and 30 (G_1) hours after irradiation.

Results

Cell age

The generation time of normal Ehrlich tumor cells is approximately 17 hours: the S period lasts for 7 hours and the G_2 period 3 to 4 hours (Fig. 1, A). A dose of 500 R caused a prolongation of the cell cycle (Fig. 1, B). Changes in the mitotic index of cells labeled with tritiated thymidine, as a function of time after irradiation were found to be similar to that reported in our previous papers (1968a, b). Preparations for chromosome study made 18 hours after irradiation consisted mainly of G_2 cells, those

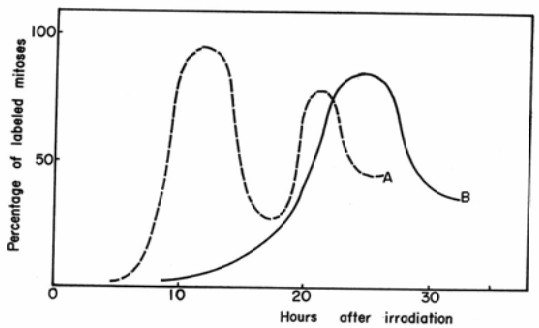

Fig. 1. Percentage of labeled mitoses (anaphasic cells) in Ehrlich ascites tumor cells before (A) and after (B) irradiation with 500 R.

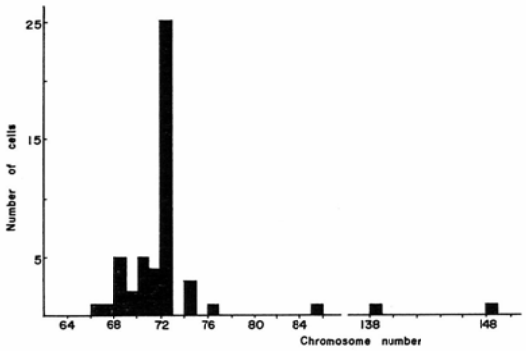

Fig. 2. Distribution of chromosome numbers in Ehrlich tumor cells. Cells with 72 chromosomes were the most frequent. These cells are stem cells of this tumor.

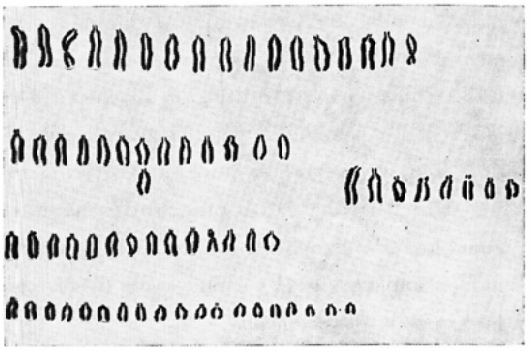

Fig. 3. Karyotype of a normal Ehrlich carcinoma cell with 72 chromosomes (stem cell).

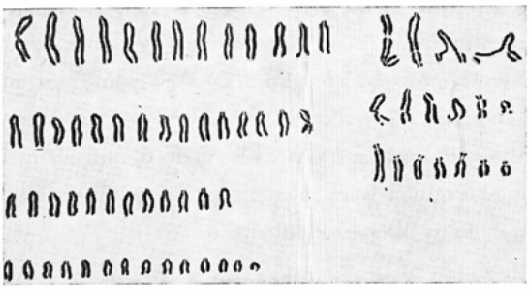

Fig. 4. Karyotype of an Ehrlich tumor cell 24 hours after irradiation with 500 R. Many chromatid and chromosome-type breaks and a complex chromatid exchange are seen.

after 24 hours consisted of S cells and those after 30 hours of G_1 cells.

Chromosome number

Figure 2 shows the distribution of chromosome numbers in Ehrlich ascites tumor cells. The stem cells of this tumor had 72 rod-shaped chromosomes (Fig. 3).

Chromosome aberrations induced by radiation

Radiation induced some chromosome aberrations, as shown in Fig. 4. Aberrations were analyzed by measuring the frequency of chromosome or chromatid type breaks.

In samples (G_2 cells) taken 18 hours after irradiation with 500 R, 80% of the metaphasic cells had chromosome aberrations, 60% of which were chromatid breaks and the rest being other chromosome breaks. 70% of S cells (24 hours samples) showed chromosome aberration, 50% of which were chromatid breaks. 60% of G_1 cells (30 hours samples) showed chromosome aberration, 30% of which were chromatid breaks (Fig. 5 A).

Restorative effects of DNA and its precursors

After irradiation with 500 R chromosome aberrations in samples treated with DNA or one of its precursors were compared with those in untreated samples.

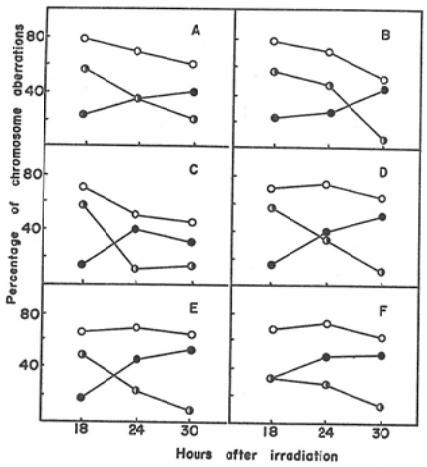

Fig. 5. A - F. Frequency of chromosome aberrations 18 (G_2), 24 (S) and 30 (G_1) hours after irradiation. Cells were treated with DNA or one of its precursors 5 hours after irradiation.

—○—: Total chromosome aberrations
—◑—: Chromatid breaks
—●—: Chromosome breaks

A: No addition. B: with DNA. C: with dGMP. D: with dAMP. E: with dCMP. F: with TMP.

Addition of DNA reduced chromosome breaks of G_1 cells, but G_2 and S cells were only slightly affected by DNA (Fig. 5 B). The frequency of chromosome breaks was 28.6% in G_2, 35.0% in S and 90% in G_1 cells. The frequency of chromatid breaks was 71.4% in G_2, 65.0% in S and 10% in G_1 cells.

Addition of dGMP considerably decreased the frequency of chromosome aberrations, from those of cells without dGMP. The frequency of chromosome aberrations was 70% in G_2, 50% in S and 45% in G_1 cells. Chromatid breaks in S cells and chromosome breaks in G_2 cells were markedly reduced (Fig. 5 C).

On addition of dAMP, the frequency of chromosome aberrations was 70% in G_2, 75% in S and 65% in G^1 cells (Fig. 5 D). Chromatid-type breaks in G_2 cells were slightly less than in cells without dAMP, but the total frequency of chromosome aberrations was similar to that in cells without dAMP.

On addition of TMP, the frequency of chromosome aberrations was 65% in G_2, 70% in S and 65% in G_1 cells. These values were almost the same as in cells without dCMP (Fig. 5 E). The frequency chromatid breaks was 76.1% in G_2, 35.9% in S and 15.4% in G_1 cells.

On addition of TMP, the frequency of chromosome aberrations was 70% in G_2, 75% in S and 65% in G_1 cells. The frequency of chromosome breaks was 50% in G_2, 60% in S and 87.9% in G_1 cells. The frequency of chromosome breaks in G_2 cells was slightly less than in cells without TMP (Fig. 5 F).

Discussion

Recovery of an animal from radiation injury seems to depend on various factors, such as repair of the injured cells and recovery of the cell population with proliferation of undamaged cells. The present study was on the recovery of cells treated with substances related to nucleic acids at the cellular level.

DNA (calf thymus) or one of its precursors was injected into the tumor bearing mice 5 hours after irradiation with 500 R and the numbers of chromosome aberrations (chromosome and chromatid breaks) in tumor cells treated with these materials were compared with those in cells treated with physiological saline. The greatest reduction in chromosome and chromatid breaks was observed in samples treated with dGMP.

Djordvic *et al.* (1962) concluded that the restorative effect of such compounds can be seen in restoration of the reproductive capacity and other disturbed functions of the cell. DNA and its precursors contain some components capable of restoring damage in chromosomes induced by radiation, and this corresponds to a restoration of the reproductive capacity of cells. But it is not possible to say that the reduction of chromosome aberrations induced by these substances is related to a restoration of the normal structure of the chromosome.

The mechanism of restoration by DNA and its precursors seems to relate the size of the molecule which reaches the nucleus of the irradiated cell (Petrovic *et al.* 1970) and a source of these compounds in the cells (Smets *et al.* 1967). But its mechanism is still far from clear.

Summary

DNA and its precursors were injected into mice bearing Ehrlich ascites tumors 5 hours after whole-body irradiation with 500 R. Their injection resulted in a significant reduction in the chromosome and chromatid breaks in the irradiated tumor cells. The most pronounced reduction in chromosome aberrations was observed in cells treated with dGMP.

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