γ-Radiation-Induced Irreparable Damage to DNA of HeLa Cells

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Exposure of HeLa cells to γ-radiation at 0.1 Gy and then at 5 Gy reduces their ability to repair double-strand DNA breaks to a greater extent than irradiation with a single dose of 5 Gy. Modifying effects of 0.1 Gy on double-strand DNA breaks and on cell survival are observed after irradiation during logarithmic but not stationary phase of growth. Primary γ-induced irreparable double-strand breaks correlates with cell survival regardless the irradiation regime. It is suggested that such a damage is primarily responsible for reproductive death of HeLa cells.

Key Words: *γ-radiation; DNA damage; HeLa cells*

Primary mechanisms underlying irreparable damage to the chromatin DNA of eukaryotic cells occur and leading to their death have been extensively investigated [2,14,15]. After irradiation in lethal doses, not only single-strand (SS) DNA breaks but also the bulk of double-strand (DS) breaks can be repaired [2, 14,15]. Lethal damage is that after which no less than 1% of the DS breaks is not repaired [15]. Since nuclear DNA is organized into regular superhelical structural units contacting the nuclear membrane [1], radiation may lead to the release of large DNA fragments by destroying these contacts: two direct DS breaks yield a DNA fragment of 10^8 D. It has been shown [6] that such DS breaks are not readily repaired. DNA repair in the logarithmic (log) phase of cell growth differs from that in the stationary (stat) phase [6,8]. Exposure of HeLa cells to γ-radiation in a low dose (0.1 Gy) sensitizes them to subsequent sublethal dose (5 Gy), judging from several cellular and biochemical parameters (survival rate, growth kinetics, proliferative activity, ATP activity, and DNA synthesis) [3-5]. We decided to compare at cellular and molecular levels the effects of irradiation in two regimes (exposure to 5 Gy and 0.1 Gy+5 Gy) on cells in the log and stat phases. To this end, rotational viscosimetry was used to study the formation and repair of primary DNA injuries (SS breaks, DS breaks, and damage to nucleoid superhelical DNA) in HeLa cells after their irradiation in the log or stat phase of cell growth.

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MATERIALS AND METHODS

HeLa cells were grown by the standard methods in medium 199 supplemented with 10% bovine serum, penicillin (100 U/ml), and streptomycin (100 U/ml) and passaged every 5-7 days. The monolayer was detached with 0.02% EDTA at 37°C, and single cells were obtained by pipetting. Cells were counted in Goryaev's chamber. Cell suspension was diluted with the culture medium to 7×10^4 cells/ml and dispensed into culture flasks (3 ml per flask). The culture had the following characteristics: 24-26 h lag phase, 2-7 days log phase, and 7-12 days stat phase. Cells in the monolayer were irradiated (^{137}Cs) at room temperature in the following regimes: 1) 0.1 Gy (dose rate, 0.67 Gy/min), 2) 2.5, 5, or 10 Gy (dose rate, 7.5 Gy/min), or 3) 0.1 Gy and after 3 min 5 Gy. The effects of radiation in the log phase (day 4) and stat phase (day 10) were then compared. Intact cells served as controls and were examined at the same times as the irradiated cells.

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The effects produced by radiation were estimated by changes in specific viscosity (η_{sp}) of cell lysates under neutral (2 M NaCl, 0.1 M Na_2-EDTA, 0.5% Triton X-100; pH 8.0, 24 h, 24°C [10]) and alkaline (1 M NaCl, 0.01 M Na_2-EDTA, 0.1 M NaOH; pH 12, 1 h, 24°C [9]) conditions. This parameter was measured in a Zimm rotational viscosimeter at 25°C in 0.1 ml of cell suspension (1.35×10^6 cells) plus 3.9 ml of the lysing neutral or alkaline solution. Since the viscosity of each sample gave a particular bell-shaped curve depending on the number of rotations, the maximum viscosity (the mean of three values) was considered as a reliable criterion. In each experiment, two tests were performed, and each variant was the mean of 6-10 experiments. As a rule, cell lysis was started 10 min after irradiation. In experiments with postirradiation DNA repair, experimental and control cells were placed in "starvation" medium 199 for 2 h at 37°C.

Severity of the radiation-induced damage was evaluated from the ability of the cells to produce visible colonies using our modification [3] of a previously described method [13].

RESULTS

Rotational viscosimetry allowed us to study SS and DS breaks in DNA of HeLa cells in relation to the radiation dose. Alkaline lysates correspond to "alkaline DNA complexes" (ADC) according to the terminology [9], and reduction in their viscosity is associated predominantly with accumulation of SS breaks in the DNA. Neutral lysates, or "neutral DNA complexes" (NDC), correspond to maximally relaxed nucleoids [10]. Low radiation doses increase viscosity by causing SS breaks with the resultant relaxation of DNA superhelices, whereas higher doses reduce viscosity through accumulation of DS breaks.

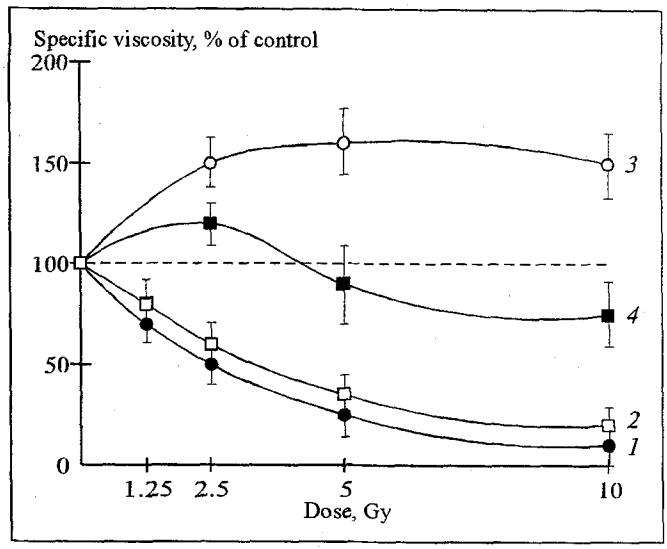

Fig. 1. Specific viscosity of "alkaline" (*1* and *2*) and "neutral" (*3* and *4*) DNA complexes in HeLa cells as a function of γ-radiation dose and cell growth phase. Cells were irradiated during logarithmic (*1* and *4*) and stationary (*2* and *3*) phases.

Figure 1 shows the dose-effect curves for specific viscosities of ADC and NDC immediately after irradiation of HeLa cells in the log and stat phases of cell growth. It can be seen that h_{sp} of ADC depends on radiation dose in the 1.25-10 Gy range practically without differences between the log and stat phases. This indicates that SS breaks have been accumulated in these phases at equal rates. By contrast, the dose-effect curves for these phases are different in the case

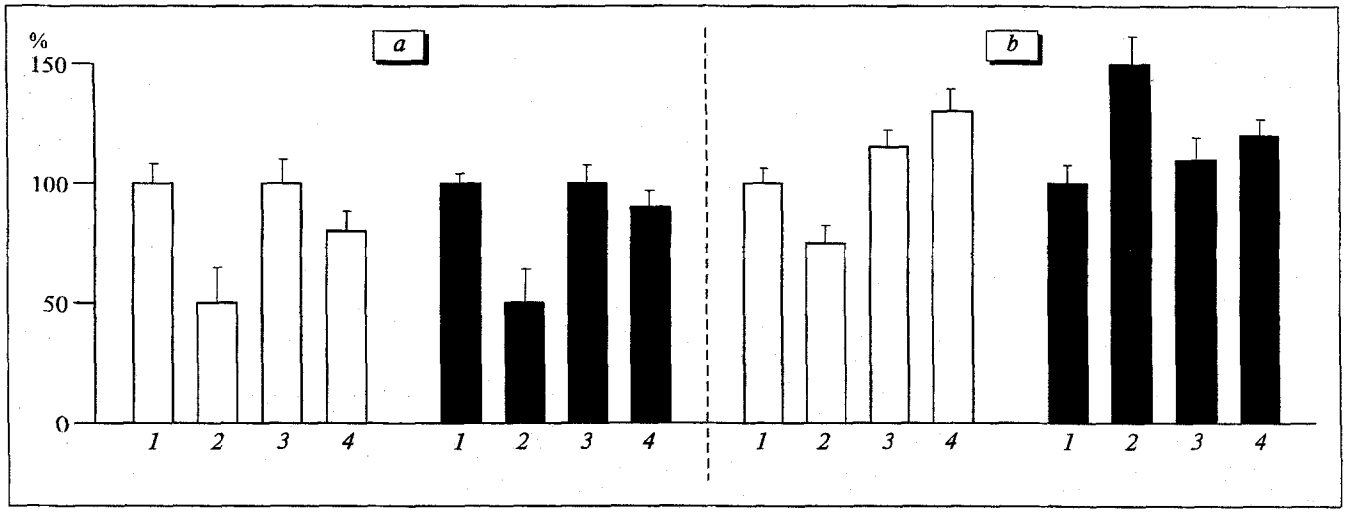

Fig. 2. Repair of single-strand (*a*) and double-strand (*b*) DNA breaks (viscosity of "alkaline" and "neutral" DNA complexes in *a* and *b*, respectively) in HeLa cells as a function of γ-radiation dose and cell growth phase. White bars: logarithmic phase; shaded bars: stationary phase. Repair conditions: "starvation" medium 199, 2 h at 37°C. *1*) nonirradiated cells (100%); *2*) 10 min after 5 Gy; *3*) 2 h in the "starvation" medium after 5 Gy; *4*) same as in *3* but after 0.1 Gy+3 min+5 Gy.

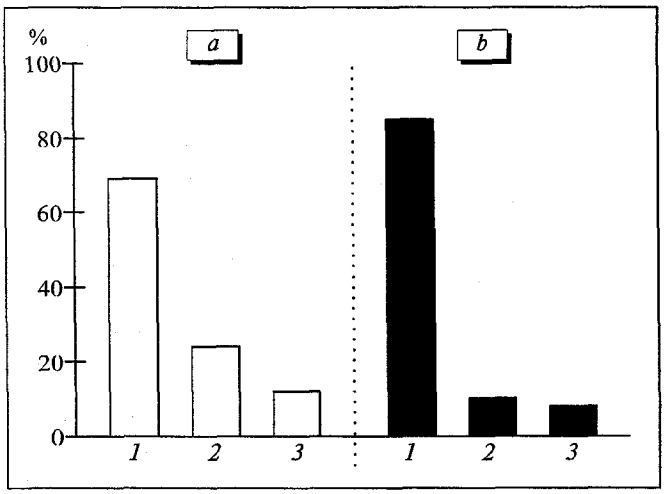

Fig. 3. Percentage survival of clonogenic HeLa cells after their irradiation in the logarithmic (*a*) and stationary (*b*) phases. *1*) nonirradiated cells; *2*) 5 Gy; *3*) 0.1 Gy+3 min+5 Gy.

of NDC: the stat phase is characterized by a monotonous nucleoid relaxation which reaches the maximum at 5 Gy and plateaues at 10 Gy, the relaxation effect (as shown by increased viscosity) at 2.5 Gy in the log phase is followed at 5 Gy by degradation (decreased viscosity) which increases at 10 Gy, indicating that more DS breaks had been accumulated.

Figure 2 shows the repair of γ-induced breaks in chromatin DNA of HeLa cells in the log and stat phases. The SS and DS breaks were repaired almost completely during these phases after 5 Gy. However, after 0.1 Gy+5 Gy, when they were radiosensitized [4,5], SS breaks were not be completely repaired (as indicated by ADC viscosity) and even greater unrepaired injuries were detected at the level of NDC (residual nucleoid relaxation), particularly in the log phase. Cells exposed to 0.1 Gy prior to irradiation with 5 Gy were much less capable of repairing SS and DS breaks at the level of "alkaline" and especially "neutral" DNA complexes than they were after exposure to only 5 Gy.

Objective evaluation of γ-induced SS and DS breaks strongly depends on the chosen methodological approach. In order to identify the most labile and metabolically active chromatin fraction in which irreparable DS breaks are produced by radiation, we reduced the ionic strength of lysing solution by 50% and shortened the time of lysis to 30 min (1 M NaCl, 0.05 M Na_2-EDTA, 0.25% Triton X-100; pH 8.0, 24°C). Exposure of cells in the log phase to 0.1 Gy resulted in a significant nucleoid relaxation (the viscosity increased by 21%), if the cells were in the log phase and had little or no effect if they were in the stat phase at the time of irradiation (Table 1). Higher doses (2.5-10 Gy) led to increased DNA degradation (a decrease in viscosity), which was greater in the log than the stat phase. It should be stressed that DNA breaks were not repaired after 5 or 10 Gy in either of these phases. The 0.1+5 Gy regime caused a greater damage to the nucleoid DNA in the log phase with no evidence of repair. Cells irradiated with 0.1 Gy were more susceptible to subsequent irradiation with 5 Gy. These results correlate with the survival rates of total (clonogenic+ nonclonogenic) HeLa cells irradiated according to each scheme [4]: thus, survival rate in the log phase was lower than in the stat phase (45-40% *vs.* 50%). If the survival rates only of clonogenic cells are considered, the exposure to 5 Gy decreased the survival rate by 3-fold and that to 0.1+5 Gy by 6-fold in the log phase, and both exposure regimes had equal effects on the survival of cells in the stat phase (Fig. 3). In other words, the dose of 0.1 Gy influenced the survival of clonogenic cells only when it was applied in the log phase.

TABLE 1. Radiation-Induced Damage and Repair of Nucleoid DNA in HeLa Cells (η_{sp}, % Relative to Nonirradiated Control Cells)

Radiation dose, Gy	Log phase	Stat phase
0.1	121±6	101±4
0.5	102±5	98±4
2.5	90±3	95±3
5.0	68±4	82±3
5.0*	60±2	76±2
0.1+3 min+5.0	51±3	80±3
0.1+3 min+5.0*	46±2	71±4
10.0	64±3	73±3
10.0*	61±4	66±4

Note. The values are means of 5 tests. *Repair in "starvation" medium 199, 2 h, 37°C, without serum. Lysing mixture: 1 M NaCl, 0.05 M Na_2-EDTA, 0.25 Triton X-100; pH 8.0, 30 min, 24°C.

The different responses of HeLa nucleoid in the two growth phases may be attributed to differences in the conformation of its DNA between the log and stat phases. In fact, calculations showed that the nucleoid DNA in the log phase is 2 times more relaxed (η_{sp}=0.68±0.02) than in the stat phase (η_{sp}= 0.35±0.01). This means that the "radiosensitive volume," i.e., the radiation target, is increased in the log phase through DNA decompaction and relaxation in the metabolically active chromatin fraction. The exposure to 0.1 Gy expanded the target and blocked the process after 5 Gy. In this way, the number of irreparable DS breaks increases.

We have hypothesized [7] that chromosomal DNA contains hyperradiosensitive "hot spots:" specific lipoprotein linkers that determine of the folding of high-polymer DNA duplexes and serve to anchor

DNA coils to the nuclear matrix. Lipid peroxides formed in these linkers by chain mechanism, as indicated by the high chemical yield (10 molecules/100 eV), may amplify radiation effects at the DNA level. The location of DNA topoisomerase II at the base of the DNA coil suggests that this enzyme is involved in the production of DS breaks in these hot spots. This hypothesis is supported by the relationship between the ability of cell to repair γ-induced DS breaks and its sensitivity to the topoisomerase II inhibitors [11] and by the occurrence of DS breaks occur in anchor DNA [12].

Primary irreparable DS breaks induced in DNA by γ-radiation in biological doses correlate with cell survival in the log and stat phases of cell growth, suggesting that DNA damage is responsible for the reproductive death of HeLa cells.

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