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CELL CYCLE-DEPENDENT POTENTIATION OF X-RAY-INDUCED CHROMOSOMAL ABERRATIONS BY 3-AMINOBENZAMIDE

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The poly(ADP-ribose) polymerase inhibitor 3-aminobenzamide had dramatically different effects on X-ray-induced cytogenetic damage in human lymphocytes depending on the stage of the cell cycle in which cells were irradiated. 3-Aminobenzamide (0.08 - 3.00 mM) potentiated the frequency of chromosomal aberrations when lymphocytes were irradiated in G_1, S, or late G_2. No effect was observed, however, when lymphocytes were irradiated in G_0 or at the S/G_2 boundary 6 h before termination of culture. These results indicate that poly(ADP-ribose) polymerase may be involved in chromosomal repair of radiation damage only during specific stages of the cell cycle.

The activity of poly(ADP-ribose) polymerase is stimulated by DNA strand breaks produced either directly by ionizing radiation or indirectly by various chemicals (1). The role of ADP-ribosylation in the response of cells to induced DNA breakage is still unclear (2-6), but the results of experiments using poly(ADP-ribose) polymerase inhibitors, e.g., 3-aminobenzamide (3AB), suggest that ADP-ribosylation is involved in some aspects of the recovery of cells from damage induced by radiation. A number of these experiments, however, have yielded conflicting results. One of the areas of controversy concerns the effect of poly(ADP-ribose) polymerase inhibitors on the frequency of X-ray-induced chromosome aberrations. Natarajan et al. (7) found an increase in the frequencies of dicentrics and rings, as well as an increase in the frequency of chromosome breaks, after unstimulated G_0 human lymphocytes were irradiated and incubated with 3AB before phytohemagglutinin (PHA) stimulation. Three other reports failed to substantiate this observation (8-10). On the other hand, there is evidence that inhibition of ADP-ribosylation does enhance cytogenetic damage after mitogen stimulation (7,8).

Poly(ADP-ribose) polymerase activity in human lymphocytes increases after mitogen stimulation (11) and fluctuates as lymphocytes progress through the cell cycle (11-13). Because poly(ADP-ribose) activity is obligatory in mitogen-induced activation of human lymphocytes (12), it seemed possible that the inhibition of ADP-ribosylation after radiation damage could differ in stimulated and unstimulated lymphocytes and could vary at different stages of the cell cycle. To investigate these possibilities, we examined the effect of 3AB on

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X-ray-induced chromosome damage at different stages of the cell cycle of normal human lymphocytes.

Materials and Methods

Peripheral blood lymphocytes obtained by venipuncture from normal, healthy individuals were incubated in RPMI 1640 tissue culture medium containing 10% fetal calf serum, antibiotics, 2 mM L-glutamine, and 2% PHA (GIBCO). Lymphocytes were irradiated with 0.4 or 2 Gy from a Philips RT100 X ray machine (250 kVp; half-value layer, 1.06 mm Cu; exposure rate, 1 Gy/min). Cells irradiated in G_0 were incubated at 37°C for 6 h after X-ray exposure and then stimulated with PHA. G_0 cells treated with 3AB were incubated with the chemical for 30 min before irradiation and for 6 h after irradiation. Before mitogen stimulation, cells were washed to remove 3AB. To test the effects of 3AB on stimulated lymphocytes, the drug was added immediately after PHA stimulation and cells were treated continuously throughout the experiment.

To analyze chromosomal damage in cells irradiated during the G_1, S, and G_2 phases of the cell cycle, cultures were irradiated at different times during the experiment. The specific timing of irradiation and subsequent collection of mitotic cells for cytogenetic analysis was based on previous autoradiographic studies of mitotic cells from lymphocyte cultures that had been exposed to [^{3}H]thymidine, which labels cells undergoing DNA synthesis (14,15). Under the experimental conditions used in this study, lymphocytes enter S phase 24 h after the addition of PHA. Consequently, for irradiations in G_1, cultures were irradiated 21 h after the addition of PHA and cells were harvested at 48 h. When PHA-stimulated lymphocyte cultures are irradiated with 2 Gy at 48 h of culture and cells are harvested 10 h later, all the mitotic cells collected are derived from cells that were undergoing DNA synthesis at the time of X-ray exposure. Therefore, for S-phase experiments cells were harvested at 58 h. When cultures irradiated at 48 h are harvested 6 h later, approximately 60% of the mitotic cells are derived from cells that were in late S phase at the time of irradiation, and the remaining 40%, which do not show incorporation of [^{3}H]thymidine, are derived from cells that were in the early G_2 phase when irradiated. Thus, for late-S-to-early-G_2 experiments, cells were harvested at 54 h. None of the mitotic cells collected 3 h after 0.4 Gy of X rays at 48 h are labeled, and thus all cells were in late G_2 when irradiated. Therefore, to analyze chromosomal damage in populations of late-G_2 cells, cultures were irradiated at 48 h and mitotic cells were collected at 51 h. Colcemid (2×10^{-7} M final concentration) was added for the last 2 h of culture except in late-G_2 experiments, in which it was added for the last 3 h of culture.

Chromosome preparations were made by swelling cells in a hypotonic solution of 0.075 M KCl for 10 min, fixing in two changes of methanol:acetic acid (3:1), and dropping onto glass microscope slides. Slides were stained with 5% Giemsa for 5 min, rinsed, and mounted with coverslips. Two hundred metaphase cells were analyzed for each point. Chromosomal aberrations were categorized as chromatid breaks, exchanges, or chromosome aberrations (dicentric plus ring chromosomes). Gaps or achromatic lesions less than the width of the chromatid were not included. Student's t-test was used for statistical analysis.

Results and Discussion

The effect of increasing concentrations of 3AB on X-ray-induced aberrations at various stages of the lymphocyte cell cycle is summarized in Table 1. In agreement with our earlier findings (9,10), and those of Heartlein and Preston (8), 3AB did not affect aberration yield in unstimulated G_0 lymphocytes. Lymphocytes irradiated in G_1, S, or late G_2 all showed more cytogenetic damage in the presence of 3AB, irrespective of the 3AB concentration, than in the absence of 3AB. 3AB also potentiated X-ray-induced breaks when cells were irradiated in late G_2 even when the drug was added after the X-ray treatments (data not shown). Thus, the coclastogenic effects of 3AB in late G_2 cannot be attributed to perturbations in lymphocyte proliferation kinetics, which could have resulted

Table 1

THE EFFECT OF 3-AMINOBENZAMIDE ON ABERRATIONS INDUCED BY X RAYS AT VARIOUS STAGES OF THE LYMPHOCYTE CELL CYCLE

Cell cycle stage	No. of subjects[a]	3AB conc. (mM)	X-ray dose (Gy)	Chromatid aberrations (%)		Chromosome aberrations (%)
				Breaks	Exchanges	
Controls (unirradiated)	2	0	0	1.3	0	0
	1	0.08	0	0.5	0	0
	1	0.30	0	1.0	0	0
	1	3.00	0	1.0	0	0
G_0	2	0	2	8.3	0.8	56.5
	1	0.08	2	4.0	0	69.5
	1	0.30	2	3.5	0	69.5
	1	3.00	2	14.5	0	52.0
G_1	2	0	2	10.0	3.0	61.0
	1	0.08	2	3.0	1.5	84.0[b]
	1	0.30	2	15.0	2.0	98.5[b]
	1	3.00	2	15.5	4.5	95.0[b]
S	2	0	2	43.3	12.3	3.0
	1	0.08	2	81.5[b]	9.5	4.0
	1	0.30	2	82.5[b]	25.5[b]	3.0
	1	3.00	2	62.5[b]	23.5[b]	1.0
S/G_2	2	0	2	35.3	4.0	0
	1	0.30	2	31.5	2.5	0
	1	3.00	2	31.5	4.5	0
	1	5.00	2	32.0	3.0	0
G_2	2	0	0.4[c]	53.8	3.5	0
	1	0.30	0.4	103.0[b]	12.5	0
	1	3.00	0.4	70.5[b]	6.5	0

[a] 200 cells per subject were analyzed.
[b] Significantly greater yield of aberrations compared to X rays alone ($p < 0.01$; Student's t-test).
[c] A lower dose was used for G_2 lymphocytes because of their radiosensitivity.

in the sampling of cells irradiated at different portions of G_2, where chromosomal radiosensitivity changes (16). Curiously, however, 3AB, even at concentrations as high as 5 mM, did not increase the yield of chromosome damage when lymphocytes were irradiated at the S/G_2 boundary, 6 h before cultures were terminated. At this time approximately 40% of the lymphocytes were replicating DNA, as deduced from parallel cultures pulse-labeled with [^{3}H]thymidine for 30 min immediately before X-irradiation (data not shown). X-irradiation at the S/G_2 boundary has been repeated in a number of subsequent experiments, and in no instance has 3AB been found to potentiate chromatid aberration frequencies (15).

The reason 3AB enhances cytogenetic damage when cells are X-irradiated 10 h or 3 h before termination of culture but not 6 h before is not known. Our data suggest that poly(ADP-ribose) is important after X-irradiation in G_1, S, and late G_2, but that there is a period late in S and early in G_2 when the inhibition of poly(ADP-ribosyl)ation does not affect the formation of chromosomal aberrations. It is of interest that Heartlein and

Preston (8) found that, although 3AB potentiated chromosome damage in G_1, no potentiation of damage was induced at the G_1/S boundary. It has been suggested that ADP-ribosylation may be crucial at certain stages of the cell cycle to permit normal cell proliferation after DNA damage (17,18). Our data indicate that ADP-ribosylation may be involved in chromosomal repair only at specific times during the cell cycle. The G_1/S boundary and the G_2/S boundary are probably not important periods for ADP-ribosylation. At these boundaries there are gross changes in chromatin structure, e.g., when chromatin decondenses to permit DNA synthesis and when chromatin structure stabilizes after replication and before mitotic condensation.

Inhibitors of poly(ADP-ribose) polymerase elicit a number of effects within a cell after ionizing radiation. There is a delay in single-strand-break rejoining (9,19), an increase in cytotoxicity (20,21), and a reduction in potentially lethal damage repair (2,22), but no effect on sister chromatid exchange frequency (9,23) or mutagenesis at either the Na/K ATPase or the hypoxanthine guanine phosphoribosyl transferase locus (24). We now show that 3AB potentiates X-ray-induced chromosome aberrations in PHA-stimulated lymphocytes when irradiation occurs in G_1, S, or late G_2, but not at the S/G_2 boundary or in unstimulated G_0 lymphocytes.

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