Bimodal induction of sister-chromatid exchanges by luminol, an inhibitor of poly(ADP-ribose) synthetase, during the S-phase of the cell cycle

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Abstract. The cell cycle dependence of sister chromatid exchanges (SCEs) induced by luminol, a new potent inhibitor of poly(ADP-ribose) synthetase, was studied in Chinese hamster V79 cells. Continuous treatment with luminol during two whole cell cycles in the presence of 5-bromo-2'-deoxyuridine (BrdUrd), or in the first or second cycle induced SCEs very efficiently in a linear dose-dependent manner. However, no enhancement of SCE levels was observed after luminol treatment in a cycle preceding BrdUrd treatment, in contrast to results found with other strong SCE inducers such as cis-diammine-dichloroplatinum (II) (CDDP) and mitomycin C (MMC). Luminol was about ten times as effective in inducing SCEs as 3-aminobenzamide (3AB), an inhibitor of the NAD$^+$ site of poly(ADP-ribose) synthetase. The induction of SCEs by luminol was restricted to the S-phase of the cell cycle with peaks at an early and a late stage, corresponding to the biphasic replication of DNA. The mechanism of SCE appears to be the same at the early and late stages of S-phase for luminol-induced SCE formation.

Introduction

The ubiquitous phenomenon of sister chromatid exchange (SCE) is the exchange of two homologous DNA helices between sister chromatids visualized graphically in metaphase chromosomes (Taylor 1958). Although SCE analysis is widely used as a sensitive measure of the genotoxic effects of environmental mutagens or carcinogens, the molecular mechanisms by which chromosomal DNA lesions result in SCEs are still unknown (Latt 1981; Ikushima 1989). SCE-inducing agents are not necessarily restricted to agents that react directly with DNA; there are rather strong SCE inducers among agents that modify the metabolism of chromatin. A family of inhibitors of poly(ADP-ribose) synthetase is especially interesting because poly(ADP-ribose)ylation has been implicated in DNA repair (e.g. Durkacz et al. 1980), DNA replication (Tanuma and Kanai 1982) and recombination (Natarajan et al. 1981). It has also been reported that there is a positive correlation between the inhibitory activity of the agents on the enzyme and their effects in inducing SCEs (Oikawa et al. 1980). Inhibition of poly(ADP-ribosylation) has been recently reported to cause the efficient and specific loss of transfected, amplified oncogenes from NIH 3T3 transformants (Nakayasu et al. 1988), and of the amplified MYC gene in HL-60 cells (Shima et al. 1989).

In the present study it was found that luminol, a new strong inhibitor of poly(ADP-ribose) synthetase (Banasik et al. 1989), induced SCEs very efficiently in a linear dose-dependent manner in cultured Chinese hamster cells and that the induction of SCEs was restricted to the S-phase of the cell cycle in the presence of luminol. Luminol gave rise to a bimodal increase in SCEs in cells treated acutely at different times during the S-phase, corresponding to the biphasic DNA replication in S-phase (Goldman et al. 1984).

Materials and methods

Cells. Chinese hamster V79 cells were cultured in α-modified Eagle's minimum essential medium (Sigma) with 10% fetal bovine serum (M.A. Bioproducts) and penicillin-streptomycin. The pH was maintained by incubation at 37°C in a humidified 5% CO$_2$ atmosphere and by addition of 0.02 M Hepes to the medium.

SCE analysis. Cells (4 x 10$^5$) were inoculated into 25 cm$^2$ plastic tissue culture flasks (Falcon) and 5 μM 5-bromo-2'-deoxyuridine (BrdUrd) was added to the culture the next day. Cytosine (0.2 μg/ml; Gibco) was added 24 h after BrdUrd addition. After 1 h, cells were harvested, detached with 0.25% (w/v) trypsin, centrifuged, treated hypotonically with 0.075 M KCl for 18 min at room temperature and fixed in 3:1 methanol: acetic acid. Slides were stained by a modified fluorescence-Giemsa technique (Perry and Wolff 1974; Ikushima 1977): 0.5 μg/ml Hoechst 33258 (20 min), illuminated by black light for 30 min while mounted in 2×SSC, and stained with 2% Giemsa in Sorressen's phosphate buffer (pH 6.8) for 15 min. SCEs were scored in 50 metaphases for each experimental point.
Treatment with luminol and other chemicals. Treatment with 0.25, 0.5 and 1 mM luminol was carried out during the first or second cell cycle (for 12 h), or the two whole cell cycles (for 24 h). For some experiments luminol treatment was conducted for one cell cycle (12 h) prior to BrdUrd labeling. Acute luminol treatments (0.5 mM) were carried out for 1 h at different times during the second cycle, BrdUrd labeling being initiated 12 h before the first acute treatment.

Treatments with 3-aminobenzamide (3AB), one of the most popular inhibitors of poly(ADP-ribose) synthetase, were also carried out at concentrations of 1, 2 and 5 mM in the second cell cycle. For comparison of the persistence of SCE inducibility, cells were treated with two other frequently used SCE inducers, mitomycin C (MMC) and cis-diaminedichloroplatinum (II) (CDDP), for 2 h during the preceding and the first cell cycle at concentrations of 50 and 1 μg/ml, respectively.

Cell cycle analysis. The durations of the component phases of the cell cycle were determined from the changes in the percentage of labeled mitoses after BrdUrd pulse labeling. A series of flasks of logarithmically growing cells were pulse labeled with 10 μM BrdUrd for 20 min, and samples were taken at 1 h intervals after the pulse. The BrdUrd incorporated into nuclear DNA was immunocytochemically detected using monoclonal antibody to BrdUrd (Cell proliferation kit; Amersham UK). Duplicate parallel cultures were treated with 0.5 mM luminol for 1 h following the BrdUrd pulse.

Results

Chinese hamster V79 cells were cultured in the presence of BrdUrd for two contiguous cell cycles and treated with luminol in the same medium during the first or second cycle of the whole of the two cycles. Another aliquot of the cells was treated first with luminol for one cell cycle and then, cultured in BrdUrd medium for two cell cycles. Figure 1 shows that luminol induced surprisingly many SCEs at rather low concentrations, the frequency of induced SCEs being greater than 100 SCEs/cell after a two cycle treatment at 1 mM. A picture of a typical metaphase with a large number of SCEs after luminol treatment is given in Fig. 2. Little difference in the induced SCE frequency was observed between first-cycle and second-cycle treatment, suggesting no potentiation by incorporated BrdUrd of SCE induction. There was also no enhancement of SCE induction in cells treated during the cycle prior to BrdUrd labeling.

As shown in Fig. 3, luminol can induce ten times more SCEs per cell than 3AB, indicating a correlation between SCE inducibility and inhibitory activity against poly(ADP-ribose) synthetase. SCE induction by luminol is restricted to the cell cycle during which luminol is present in the medium (Fig. 4; see also Fig. 1), and thus differs from that found with other SCE-inducing agents such as MMC and CDDP.

Figure 5 shows the effects of 1 h treatments with 0.5 mM luminol at various times during the second cell cycle. The results indicate that the frequency of induced SCEs varies bimodally with two peaks over the time...