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Effect of 3-Aminobenzamide on DNA Repair Synthesis and Chromosome Aberrations Induced by Mutagens in Synchronized Mammalian Cells

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The effect of 3-aminobenzamide (3AB), an inhibitor of poly (ADP-ribose) polymerase, on ethyl methanesulfonate (EMS)- or bleomycin (BLM)-induced DNA repair synthesis and chromosome aberrations was examined during the cell cycle of Chinese hamster ovary (CHO)-K_1 cells. The synchronized cells were obtained by using thymidine double block method and mitotic selection method. Three assays were employed in this study; unscheduled DNA synthesis, alkaline elution and chromosome aberrations. 3AB alone did not induce DNA repair and chromosome aberrations in all phases. The post-treatment with 3AB inhibited DNA repair synthesis induced by EMS or BLM in G_2 phase, whereas 3AB did not affect chromosome aberrations induced by EMS or BLM in all phases. These results suggest that 3AB aggravates the cell cycle disturbance which occur after DNA damage, and leads to an accumulation of cells at G_2 phase, and inhibits DNA repair synthesis, while the effect of 3AB on chromosome aberrations may need reevaluated.

INTRODUCTION

Poly (ADP-ribosyl)ation of a variety of nuclear proteins is an immediate response of most eukaryotic cells to DNA strand breaks, as induced by carcinogen treatment (Burkle *et al.*, 1990). Accumulating evidence suggests that poly (ADP-ribosyl)ation is involved, in some way, in DNA repair (Jacobson *et al.*, 1983; Wielchens *et al.*, 1983; Nomura *et al.*, 1984; Cleaver and Morgan, 1985; Cleaver and Morgan, 1987). Therefore, the role of poly (ADP-ribosyl)ation in DNA repair has been implied by extensive studies using poly (ADP-ribose) polymerase inhibitor (Creissen and Shall, 1982; Cleaver *et al.*, 1983; James and Lehmann, 1983; Walker *et al.*, 1984; Cleaver, 1985; Cleaver and Morgan, 1985; Cleaver *et al.*, 1985), and 3-aminobenzamide (3AB) is a representative inhibitor (Rankin *et al.*, 1989). This inhibitor prevents the rejoining of alkylating agent-induced DNA single-strand breaks

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to a greater extent in lymphoid cells than in other types of mammalian cells (Creissen and Shall, 1982; Cleaver *et al.*, 1983; Morgan and Cleaver, 1983; James and Lehmann, 1983). And also there is a report that chromosome aberrations induced by methyl methanesulfonate (MMS) was increased by 3AB (Matsuda *et al.*, 1989).

On the other hands, subsequent research into the cell-cycle dependence for metabolic process, chromosome aberration and DNA repair has been reported (Riddle and Hsie, 1978; Iijima and Morimoto, 1986; Moore *et al.*, 1988; Shafik *et al.*, 1988). It has been reported that there is a DNA repair system in G_2 cells which can be inhibited by the α-polymerase inhibitor aphidicolin, and that inhibition of repair leads to the production of chromosomal aberrations (Bender and Preston, 1982). And there is another report that the biological consequences of DNA damage and the efficiency of its repair probably depend upon the state of the chromatin in the respective domains (Leadon and Hanawalt, 1986).

The purpose of this study was to elucidate the role of poly (ADP-ribose) polymerase on chromosome aberrations and DNA repair process of DNA damage induced by ethyl methanesulfonate (EMS) or bleomycin (BLM) in relation to the cell cycle.

MATERIALS AND METHODS

Cell Culture

Chinese hamster ovary (CHO)-K1 cells were used throughout this investigation. Monolayer cultures of this cell line were grown at 37°C in humidified 5% CO_2 incubator using Eagle's minimum essential medium (Grand Island Biological Co., Grand Island, NY, U.S.A.) supplemented with 10% newborn calf serum and gentamycin (50 μg/ml).

Chemical Treatment

Ethyl methanesulfonate (EMS, Tokyo Kasei Co., Tokyo, Japan) or bleomycin (BLM, Nihon Kayaku, Japan) was dissolved in the serum-free medium prior to use and exposed to cells at 37°C for desired time. 3-Aminobenzamide (3AB, Sigma Chemical Co., St. Louis, MO, U.S.A.), an inhibitor of poly (ADP-ribose) polymerase, was dissolved in serum-free medium and further diluted to working concentrations. The cells were treated to this inhibitor for 1 hour.

Cell Synchronization

For the determination of the stage sensitivity of chromosome aberrations induced by chemicals during the progression of the cell cycle, the thymidine double block method as described by Aschihara and Baserga (1979) was used with minor modifications.

To determine DNA repair synthesis in relation to cell cycle, the cells were synchronized by mitotic selection method developed by Terasima and Tolmach (1963).

Unscheduled DNA Synthesis

The experiments involving unscheduled DNA synthesis were carried out as described by Cleaver and Thomas (1981) with minor modification. CHO cells grown on coverslips in plastic petridishes were exposed to chemicals. The cells were then labeled with 10

μCi/ml ^{3}H-thymidine for 1 hour after treatment with inhibitor. Labeling with ^{3}H-thymidine was terminated by washing the cells three times with cold Hank's balanced salt solution containing 100 μg/ml unlabeled thymidine. Autoradiograms were prepared by using Kodak NTB liquid nuclear track emulsion. Silver grains over nuclei of evenly and lightly labeled cells were counted.

Alkaline Elution Experiment

Alkaline elution was performed essentially according to Kohn *et al.* (1976) with minor modification. Cells were labeled with 0.02 μCi/ml of ^{3}H-thymidine for 24 hours and then exposed to chemicals. The cells were harvested with cold PBS-Merchant solution (150 mM NaCl, 4.28 mM K_2HPO_4, 0.71 mM KH_2PO_4), and filtered onto 2 μm pore size polycarbonate filter (Nuclepore Co., Pleasanton, CA), and lysed with lysing solution (2% SDS, 0.1 M Glycine, 0.025 M Na_2EDTA, pH 10.0). And then cells were eluted with eluting solution (30 mM tetrapropyl ammonium hydroxide, 0.02 M EDTA, 1% SDS, pH 12.1) at a flow rate of 0.035 ml/min in the dark. Fractions were collected at 90 minutes interval. The radioactivity remaining on filter was plotted against elution time.

Chromosome Aberrations

After treatment with chemicals, the cells were incubated at 37°C for desired time. Colchicine (0.04%, wt/vol) was added to the culture for final 2 hours of incubation. The mitotic cells were harvested by gentle shaking to dislodge the loosely attached mitotic cells. The cells were collected by centrifugation at 1,000 rpm for 10 minutes, and treated with hypotonic soln. (0.05 M KCl) for 5 minutes at 37°C and then fixed. Chromosome preparations were made by air-drying technique and satained with 6% Giemsa for 10 minutes. Chromosome aberrations were scored under the oil immersion lens according to the criteria of Evans (1977).

RESULTS

CHO cells synchronized by thymidine double block or mitotic selection method were used throughout this investigation.

The effect of 50 μM or 5 mM 3AB on unscheduled DNA synthesis induced by 5 mM EMS is shown in Fig. 1. The post-treatment with 3AB inhibited EMS-induced excision repair in G_2 phase, and resulted in the higher degree of unscheduled DNA synthesis than the group post-incubated without 3AB in G_2 phase. Fig. 2 represents the effects of 50 μM or 5 mM 3AB on unscheduled DNA synthesis induced by 40 μg/ml BLM. BLM-induced excision repair was also inhibited by 3AB in G_2 phase, while the inhibitory effect of 3AB was not shown in G_1 phase.

When 5 mM 3AB was added to cells damaged by EMS or BLM, there was an increment in single-strand break frequencies in synchronized CHO cells (Figs. 3 and 4). The post-treatment with 3AB inhibited the rejoining of DNA single-strand breaks by 120 mM EMS or 800 μg/ml BLM in G_2 phase, and resulted in the more accumulation of DNA single-strand breaks than that of the group post-incubated without 3AB. While the post-treatment with 3AB did not affect the rejoining of DNA single-strand breaks by EMS or BLM in G_1 phase, thus, the percentage of DNA remaining on filter after 9 hours elution was the same between the post-treated group with 3AB

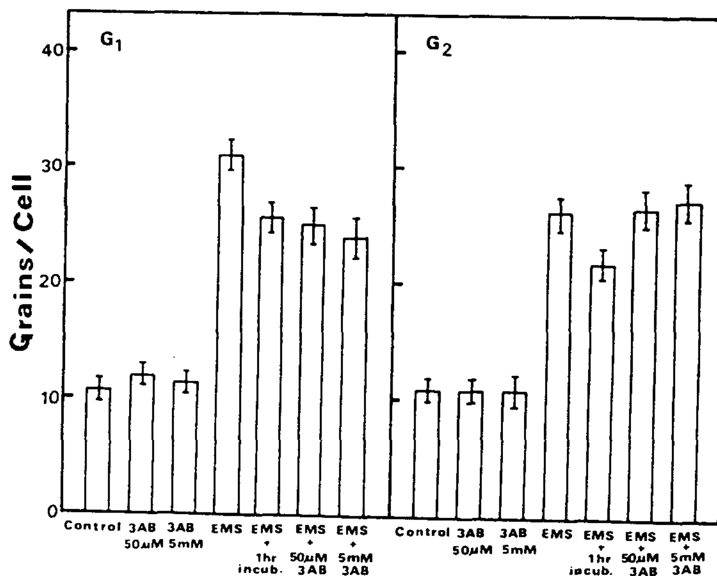

Fig. 1. Effect of 3AB on unscheduled DNA synthesis induced by 5 mM EMS in synchronized CHO cells.

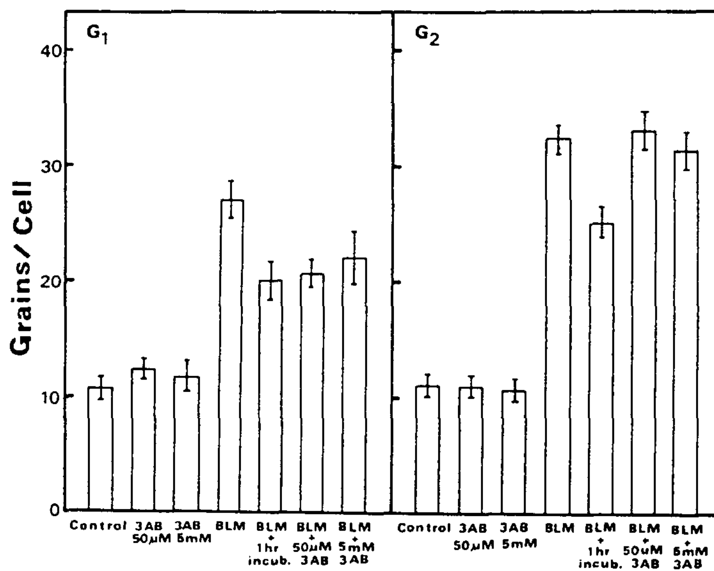

Fig. 2. Effect of 3AB on unscheduled DNA synthesis induced by 40 μg/ml BLM in synchronized CHO cells.

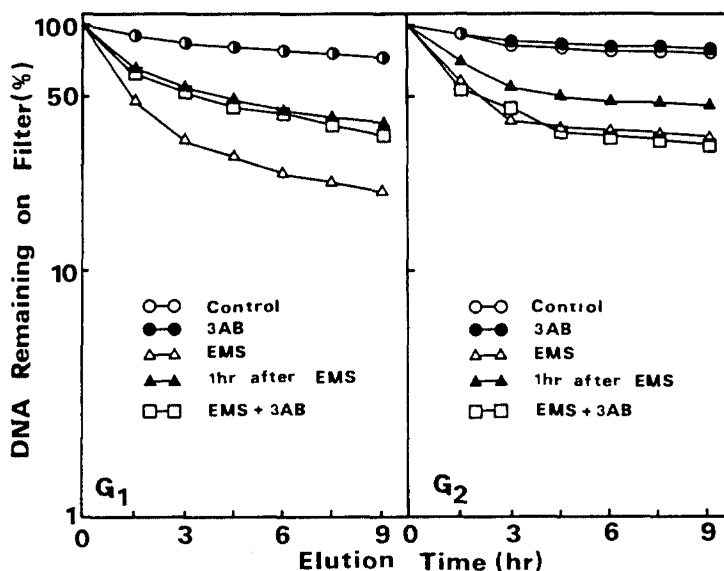

Fig. 3. Alkaline elution assay for EMS-induced DNA damage in synchronized CHO cells. Cells were treated with 120 mM EMS for 1 hour, and then treated with or without 5 mM 3AB for 1 hour.

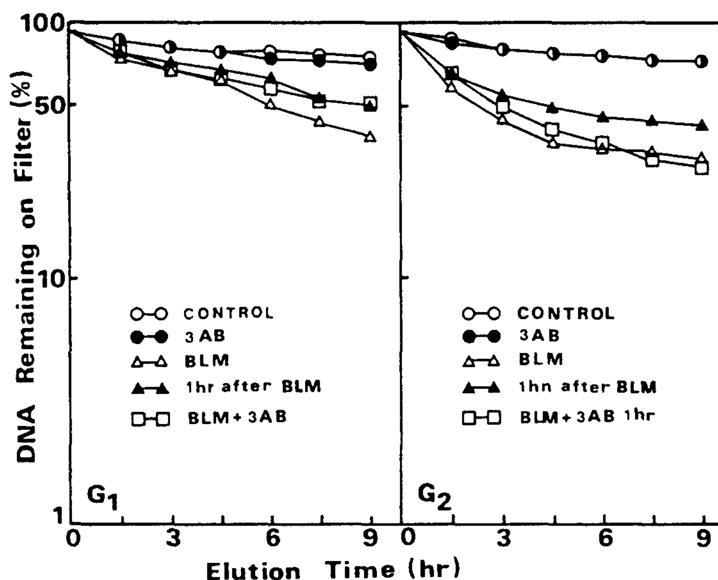

Fig. 4. Alkaline elution assay for BLM-induced DNA damage in synchronized CHO cells. Cells were treated with 800 μg/ml BLM for 1 hour, and then treated with or without 5 mM 3AB for 1 hour.

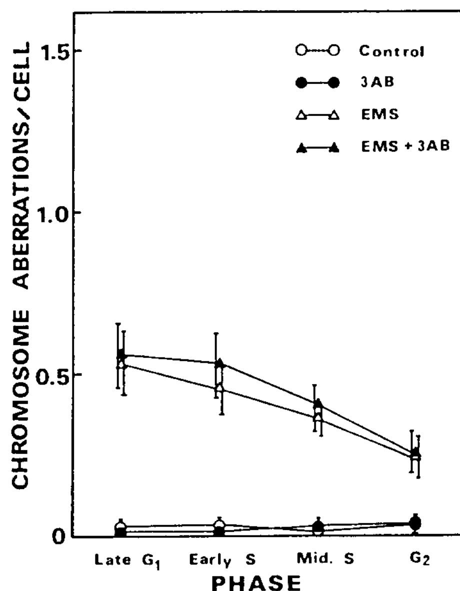

Fig. 5. **Effect of 5 mM 3AB on chromosome aberration induced by 40 mM EMS for 1 hour at different phases of the cell cycle.**

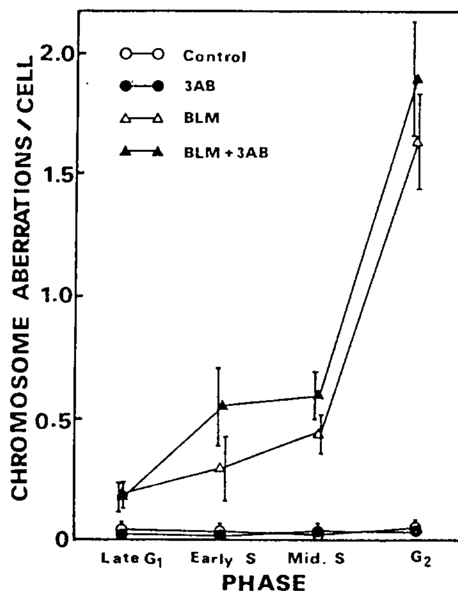

Fig. 6. **Effect of 5 mM 3AB on chromosome aberration induced by 100 μg/ml BLM for 1 hour at different phases of the cell cycle.**

and the group post-incubated without 3AB.

Figs. 5 and 6 show the effect of 5 mM 3AB on chromosome aberrations induced by 40 mM EMS or 100 μg/ml BLM in synchronized CHO cells. As shown in the figure, 3AB alone did not affect the frequency of chromosome aberrations over controls. And also the post-treatment with 3AB did not increase the chromosome aberrations induced by EMS or BLM in all phases of the cell cycle.

DICUSSION

The hypothesis that poly (ADP-ribosyl)ation is involved in the DNA ligase regulation (Creissen and Shall, 1982) has suggested that poly (ADP-ribosyl)ation is related to excision repair of DNA (Fujiwara, 1987). According to the hypothesis, DNA ligase is activated by DNA breakage-induced poly(ADP-ribosyl)ation and inhibited by 3AB or benzamide (Creissen and Shall, 1982). James and Lehmann (1983) observed a significantly retarded ligation by 5 mM 3AB of dimethyl sulfate (DMS)-induced DNA single-strand breaks in a normal human fibroblast strain. Such a ligase inhibition by high concentrations (5 mM) of 3AB following treatment with monoalkylating

agents has been more exemplified in lymphoid cells than fibroblasts (Borek *et al.*, 1984: Cleaver and Morgan, 1985: Cleaver *et al.*, 1985). Whereas, Fujiwara (1987) reported no inhibition of ligation of MMS-induced DNA single-strand breaks by 5 mM 3AB in human fibroblast NHSF6 cells and in the two other normal fibroblast strains. This contradiction is due to probably arise from differences in human fibroblast strains rather than in alkylating agents (DMS versus MMS) (Fujiwara, 1987). On the other hand, the ligation is strongly suggested to be independent of poly (ADP-ribosyl)ation (Morgan and Cleaver, 1983; Collins, 1985; Morgan and Ebisuzaki, 1985). Neither the delayed rate of ligation of DNA single-strand breaks nor longer repair patches in the presence of 3AB following treatment with alkylating agents of HeLa and WIL-2 cells has been reported (Collins, 1985; Morgan and Ebisuzaki, 1985). The apparent contradiction between early observations on the inhibitory effect of 3AB is due to the concentration of treated 3AB, and thus, the low concentration (50 μM) of 3AB accelerates the ligation of DNA repair patches (Cleaver and Morgan, 1987). However, our results show that 50 μM or 5 mM 3AB did not affect on DNA repair induced by EMS or BLM at G_1 stage.

Jacobson *et al.* (1985) reported that inhibitors of poly (ADP-ribosyl)-ation, 3AB, benzamide and benzoic acid, aggravate cell cycle disturbances which occur after DNA damage, leading to a prolongation of S-phase and to an accumulation of cells at the G_2-M boundary. 3AB also increase the population of G_2 cells in human fibroblasts treated with MMS (Boorstein and Pardee, 1984), and cause a large increase in the fraction of cells in the G_2 compartment in 5-hydroxymethyl-2'-deoxyuridine-treated CHO cells (Boorstein *et al.*, 1987). Our results obtained represent that the amount of unscheduled DNA synthesis and DNA single-strand breaks induced by EMS or BLM were affected by 3AB at G_2 phase. On the other hands, the others reported that poly(ADP-ribose) polymerase may regulate the activity of intracellular nuclease and other enzymes that can cause changes in chromatin structure (Cleaver and Morgan, 1985), and that the inhibition of poly(ADP-ribosyl)ation by 3AB increased the sister chromatid exchange frequencies (Morgan and Cleaver, 1982). While our results represented that chromosome aberrations induced by EMS or BLM were not affected by 3AB in all phases. Therefore, considering the others' reports and our results, the further studies are necessary to determine the detailed function of poly-(ADP-ribose) polymerase on DNA repair synthesis and chromosome aberration.

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동시화된 포유동물세포에서 돌연변이원에 의해 유발된 DNA 회복합성 및 염색체이상에 미치는 3-Aminobenzamide의 영향

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동시화시킨 CHO 세포의 각 주기동안 EMS 혹은 BLM에 의해 유발된 DNA 상해회복 및 염색체이상에 미치는 3-aminobenzamide(3AB)의 효과를 조사하였다. 동시화된 세포는 thymidine double block 방법과 mitotic selection 방법을 사용하여 얻었다. 본 연구에서는 비주기성 DNA합성법, 알칼리 유출법 및 염색체이상을 사용하였다. 3AB를 단독처리할 경우 모든 세포주기에서 DNA 회복합성 및 염색체이상이 유발되지 않았다. 3AB를 후처리하면 G_2 phase에서는 EMS 혹은 BLM에 의해 유발되는 DNA 회복합성이 저해되었다. 그러나 조사한 모든 주기에서 3AB의 후처리는 EMS 혹은 BLM에 의해 유발되는 염색체이상에는 영향을 미치지 않았다. 이러한 결과들은 poly (ADP-ribose) polymerase의 저해제인 3AB가 세포주기의 교란을 악화시켜 세포를 G_2 phase에 축적시키고, DNA회복합성을 저해하는 것으로 추측된다. 그러나 염색체이상에 대한 3AB의 효과는 재평가되어야 할 것이다.