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Environmental Toxic Agents on Genetic Material and Cellular Activity V. The Roles of DNA Polymerases on Mutagen-Induced DNA Repair Synthesis in Relation to Cell Cycle in Chinese Hamster Ovary Cells

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Chinese hamster ovary (CHO)-K_1 cells exhibited a differential sensitivity in the process of DNA repair synthesis induced by ethyl methanesulfonate (EMS) or bleomycin (BLM) in relation to cell cycle. Two assays were employed in this study: alkaline elution and unscheduled DNA synthesis. The post-treatment with aphidicolin (APC), an inhibitor of DNA polymerase α, inhibited DNA repair synthesis induced by EMS in G_2 phase, while APC did not show any effect on BLM-induced DNA repair synthesis in all phases. On the other hands, the 2', 3'-dideoxythymidine (ddTTP), an inhibitor of DNA polymerase β, inhibited DNA repair synthesis induced by EMS or BLM in both of G_1 and G_2 phases. These results suggested that the involvement of DNA polymerase α and β in DNA repair was dependent on cell stage or used chemical agent.

INTRODUCTION

The enzymatic pathways involved in DNA repair vary with the types of damage, but the most general mechanism is excision repair (Bohr *et al.*, 1987), and several enzymes concerned with excision and polymerization of DNA have also been identified (Collins and Johnson, 1984: Collins *et al.*, 1984: Downes *et al.*, 1985: Mattern *et al.*, 1982).

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Many conclusions concerning the functions at these enzymes can be obtained using enzyme inhibitors. Aphidicolin (APC) is the specific inhibitor of DNA polymerase α, which is largely responsible for replicating nuclear DNA (Cleaver, 1984: Th'ng and Walker, 1985). And 2′, 3′-dideoxythymidine 5′-triphosphate (ddTTP) is the inhibitor of DNA polymerase β, which has been postulated to have a role in the repair of DNA (Collins and Johnson, 1984: Dresler and Kimbro, 1987: Th'ng and Walker, 1985: Um *et al.*, 1988: Yamada *et al.*, 1985).

On the other hands, it has been recognized that cells at various stages of cell cycle exhibit differential sensitivities to radiation and chemicals (Watanabe and Horikawa, 1980). The sensitivity is usually expressed in terms of chromosome damage, inhibition of DNA synthesis, mitotic delay and survival after treatment with mutagens at different phases of the cell cycle (Giulotto *et al.*, 1978). All these effects are the results of combined factors operating directly on the DNA molecule or on the cellular components during and after treatment with mutagens (Giulotto *et al.*, 1978).

The purpose of this study was to elucidate the roles of these specific enzymes, DNA polymerase α and β on DNA repair synthesis induced by ethyl methanesulfonate (EMS) or bleomycin (BLM) in relation to cell cycle in CHO cells.

MATERIALS AND METHODS

Cell Culture

Chinese hamster ovary (CHO)–K_1 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 (MEM; Grand Island Biological Co., Grand Island, N.Y.) 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. Aphidicolin (APC, Sigma Chemical Co., St. Louis, MO), an inhibitor of DNA polymerase α, was dissolved in dimethyl sulfoxide. And 2′, 3′-dideoxythymidine 5′-triphosphate (ddTTP, Sigma Chemical Co., St. Louis, MO), an inhibitor of DNA polymerase β, was dissolved in distilled water and further diluted to working concentrations. The cells were treated to these inhibitors for 1 hour.

Cell Synchronization

Monolayer cultures of CHO cells were synchronized with a slight modification of the method developed by Terasima and Tolmach (1963) using mitotic selection. The degree of synchronization achieved was determined by the rate of DNA synthesis by autoradiography. For this purpose, the cells were pulse labeled with ^{3}H-thymidine (Specific activity; 77.9 Ci/mM, Amersham Co., England) for 10 minutes at a final concentration of 1 μCi/ml. Labeling with ^{3}H-thymidine was terminated by washing the cells three times with PBS containing 100 μg/ml unlabeled thymidine. Autoradiograms were prepared and the degree of synchronization was measured by the labeling index of synchronized cell population.

Alkaline Elution Experiments

Alakline elution was performed essentially according to Kohn *et al.* (1976) with minor modification. Cells were labeled with 0.2 μCi/ml of ^{3}H-thymidine for 24 hours and then exposed to chemicals. The cells 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_2-EDTA, pH 10.0). Cells were eluted in the dark with eluting solution (30 mM tetrapropylammonium hydroxide, 0.02 M EDTA, 1% SDS, pH 12.1) at a flow rate of 0.035 ml/min. Fractions were collected at 90 minutes interval. The radioactivity remaining on filter were plotted against elution time.

Unscheduled DNA Synthesis

The experiments involving unscheduled DNA synthesis were carried out 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 for three times in cold Hank's balanced salt solution (HBSS) containing 100 μg/ml of 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.

RESULTS

Chinese hamster ovary (CHO) cells synchronized by mitotic selection method were used in this experiment. The generation time of CHO cells were 16 hours: durations of G_1, S, G_2 or M-phase of CHO cells occupy about 4, 9, 2 or 1 hour, respectively (data not shown).

Fig. 1 shows the effect of 5 μg/ml APC on DNA single-strand breaks induced by 120 mM EMS for 1 hour in synchronized CHO cells. DNA single-strand breaks induced by 120 mM EMS were not affected by APC, thus, the percentage of DNA remaining on filter after 9 hr elution of post-treated group with APC was the same as the group post-incubated without APC in G_1 phase, while the post-treatment with APC decreased the rejoining of DNA single-strand breaks by EMS and resulted in the much more accumulation of DNA single-strand breaks than that of the group post-incubated without APC in G_2 phase.

The effect of 5 μg/ml APC on DNA single-strand breaks induced by 800 μg/ml BLM for 1 hour in synchronized CHO cells is shown in Fig. 2. The percentage of DNA remaining on filter of post-treated group with APC was the same as the group post-incubated without APC in G_1 and G_2 phases, respectively. This result suggests that APC does not affect the rejoining process of DNA single-strand breaks induced by BLM in G_1 and G_2 phases.

The effect of 200 μM ddTTP on DNA single-strand breaks induced by 120 mM EMS or 800 μg/ml BLM in synchronized CHO cells is shown in Figs. 3 and 4. As shown in the figures, the post-treatment with ddTTP was resulted in the much more accumulation of DNA single-strand breaks as compared with the group post-incubated without ddTTP in G_1 and G_2 phases, respectively.

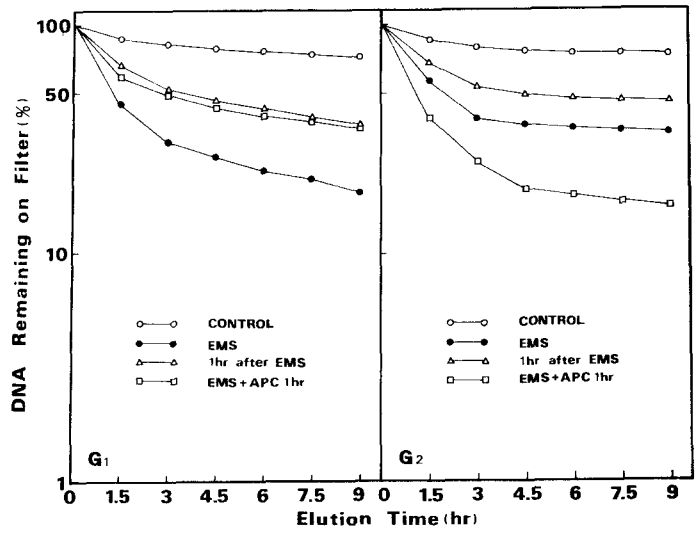

Fig. 1. Effect of 5 μg/ml APC on DNA single-strand breaks induced by 120 mM EMS for 1 hour in synchronized CHO cells.

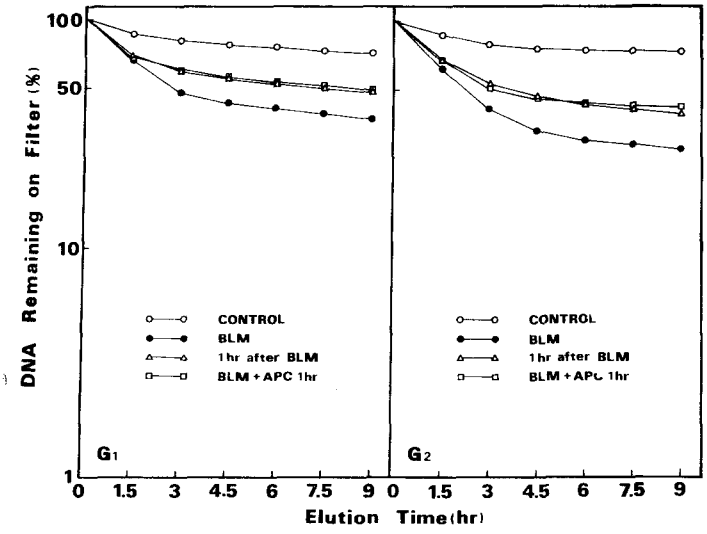

Fig. 2. Effect of 5 μg/ml APC on DNA single-strand breaks induced by 800 μg/ml BLM for 1 hour in synchronized CHO cells.

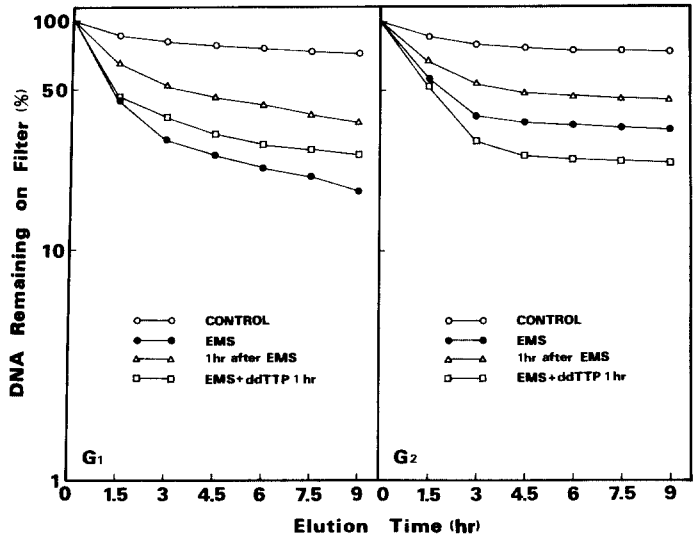

Fig. 3. Effect of 200 μM ddTTP on DNA single-strand breaks induced by 120 mM EMS for 1 hour in synchronized CHO cells.

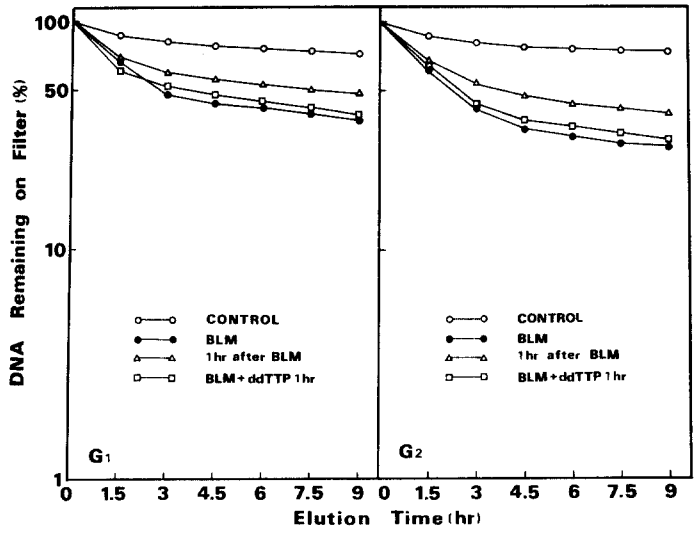

Fig. 4. Effect of 200 μM ddTTP on DNA single-strand breaks induced by 800 μg/ml BLM for 1 hour in synchronized CHO cells.

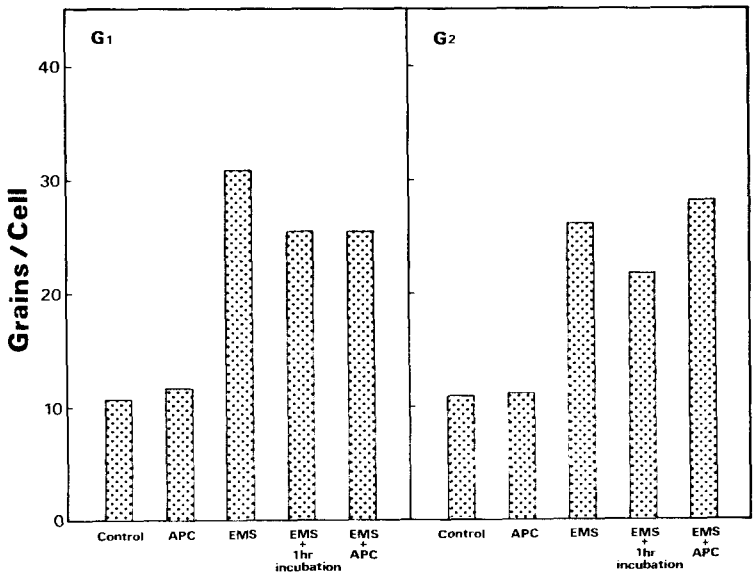

Fig. 5. Effect of 5 μg/ml APC on unscheduled DNA synthesis induced by 5 mM EMS for 1 hour in synchronized CHO cells.

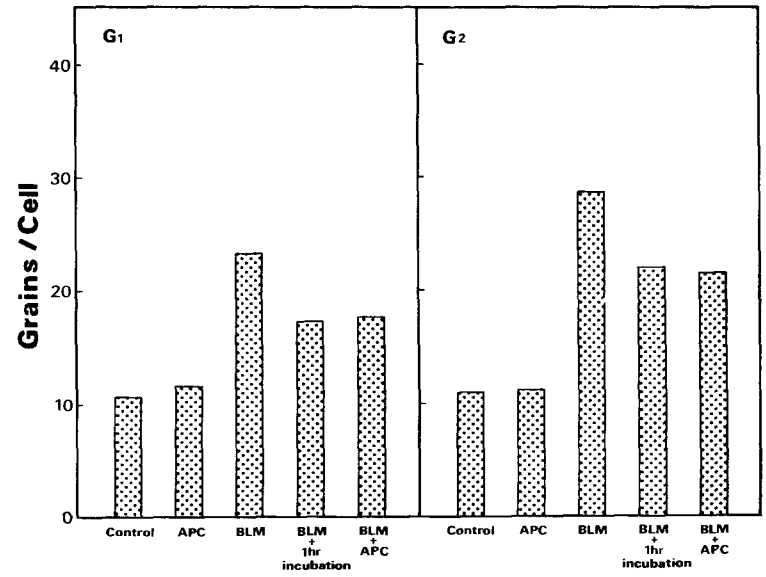

Fig. 6. Effect of 5 μg/ml APC on unscheduled DNA synthesis induced by 40 μg/ml BLM for 1 hour in synchronized CHO cells.

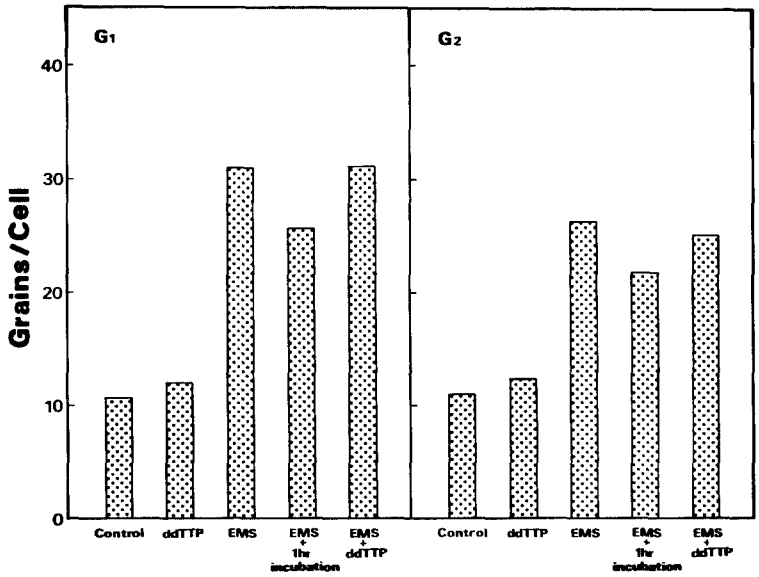

Fig. 7. Effect of 200 μM ddTTP on unscheduled DNA synthesis induced by 5 mM EMS for 1 hour in synchronized CHO cells.

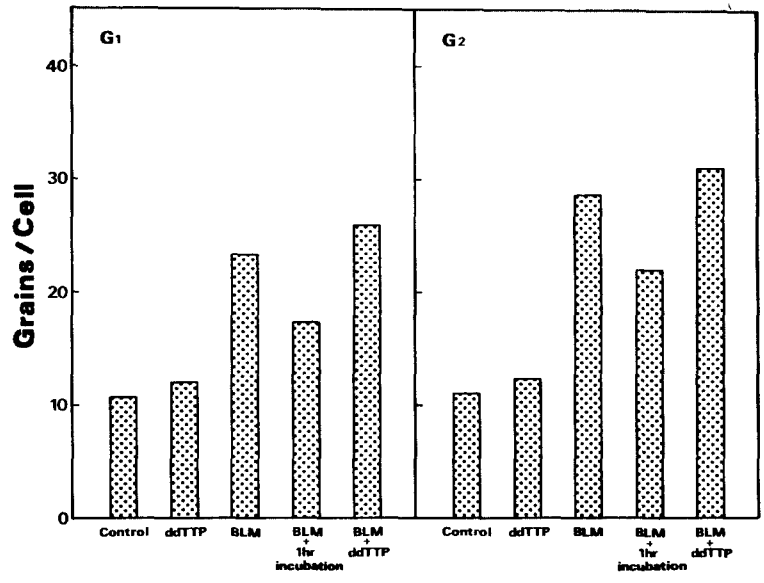

Fig. 8. Effect of 200 μM ddTTP on unscheduled DNA synthesis induced by 40 μg/ml BLM for 1 hour in synchronized CHO cells.

The effect of 5 μg/ml APC on unscheduled DNA synthesis induced by 5 mM EMS for 1 hour in synchronized CHO cells is shown in Fig. 5. In G_2 phase, the post-treatment with APC inhibited EMS-induced excision repair and was resulted in the higher degree of unscheduled DNA synthesis than the group post-incubated without APC, but no inhibition effect of APC was shown in G_1 phase.

Fig. 6 shows the effect of 5 μg/ml APC on unshceduled DNA synthesis induced by 40 μg/ml BLM for 1 hour in G_1 and G_2 phases, As shown in the figures, APC did not inhibit BLM-induced excision repair in G_1 and G_2 phases.

The effect of 200 μM ddTTP on unscheduled DNA synthesis induced by 5 mM EMS or 40 μg/ml BLM for 1 hour in synchronized CHO cells is shown in Figs. 7 and 8. The amount of unscheduled DNA synthesis was increased in the group post-treated with ddTTP comparing with the group post-incubated without ddTTP in G_1 and G_2 phases. These results indicate that ddTTP is effective inhibitor on the repair process of DNA damage induced by EMS or BLM.

DISCUSSION

There were reports that DNA polymerase α was required a larger gaps for initiating DNA synthesis, whereas polymerase β required a smaller gap (Grossman, 1981: Mosbaugh and Linn, 1984). By using DNA polymerase α inhibitor APC and polymerase β inhibitor ddTTP, we already reported the roles of these enzymes on DNA repair synthesis induced by EMS or BLM in asynchronous CHO cells (Um *et al.*, 1988). The results represented that EMS-induced unscheduled DNA synthesis and DNA single-strand breaks were inhibited by APC and ddTTP, whereas BLM-induced DNA repair synthesis were inhibited by only ddTTP in asynchronous CHO cells. On the other hands, many properties of DNA polymerase α and β have been well studied in cell extract, and their activities with respect to cell cycle phase have been examined using synchronous baby hamster kidney cells (Castellot, *et al.*, 1979). Bender and Preston (1982) reported that there was a DNA repair system in G_2 cells which could be inhibited by the polymerase α inhibitor APC. van Zeeland *et al.* (1982) also reported that lesions induced by UV and alkylating agents were susceptible to inhibition of G_2 repair. Moore *et al.* (1986) subsequently reported that much of the effect of the repair inhibitors seemed to be exerted in early G_2. Reidy (1987) recently reported that the presence of 20 μM APC or Ara C during G_2 phase increased falate-and deoxyuridine-sensitive chromosome breakage. Our results obtained represented that EMS-induced DNA single-strand breaks and unscheduled DNA synthesis in CHO cells were inhibited by APC in G_2 phase and were not affected by APC in G_1 phase. These results were generally consistent with others' reports.

Considering above others' reports, a possible explanation on our results was that the involvement of DNA polymerase α and β in DNA repair was dependent on cell stage or used chemical agent.

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환경성 유해요인이 유전물질과 세포활성에 미치는 영향
V. CHO세포에서 세포주기에 따라 돌연변이원에 의해 유발된 DNA회복합성에 미치는 DNA중합효소의 역할

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세포주기에 따라 emthyl methanesulfonate (EMS)와 bleomycin (BLM)이 유발한 DNA 회복합성 과정에 미치는 DNA 중합효소의 역할을 알아보기 위하여 동시화시킨 Chinese hamster ovary (CHO)세포를 재료로하여 알칼리 유출법과 비주기성 DNA 합성법으로 조사한 결과는 다음과 같다. DNA 중합효소 α의 저해제인 aphidicolin(APC)을 후처리할 경우, G_2기에서 EMS에 의해 유발된 DNA 회복합성을 저해한 반면, BLM에 의해 유발된 DNA 회복합성은 저해하지 않았다. 한편 DNA 중합효소 β의 저해제인 2', 3'-dideoxythymidine 5'-triphosphate (ddTTP)는 세포의 G_1과 G_2기 모두에서 EMS와 BLM에 의해 유발된 DNA 회복합성을 저해하였다. 이상의 결과들은 DNA 회복합성 과정중에 DNA 중합효소 α와 β의 참여유무는 세포주기와 돌연변이원의 종류에 따라 다른것으로 추측된다.