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The Effects of Inhibitors of DNA Polymerases and Topoisomerase on Chromosome Aberrations Induced by Mutagens in Synchronized Mammalian Cells

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The effects of aphidicolin (APC), 2', 3'-dideoxythymidine 5'-triphosphate (ddTTP), and novobiocin (NOV) on the frequencies of chromosome aberrations induced by ethyl methanesulfonate (EMS) or bleomycin (BLM) were examined in synchronized Chinese hamster ovary (CHO)-K_1 cells. The cells were synchronized by the thymidine double block method. APC, ddTTP and NOV alone did not affect the frequencies of chromosome aberrations. The cells in late G_1 and early S phases were sensitive to the induction of chromosome aberrations by EMS, whereas cells in G_2 phase were most sensitive to chromosme aberration by BLM. The post-treatment with APC after EMS treatment increased the frequencies of chromosome aberrations in late G_1 and S phases. The post-treatment with ddTTP or NOV did not affect the frequencies of chromosome aberrations induced by EMS or BLM at all phases. When the cells were pre-treated with NOV before EMS plus NOV treatment, an increment of chromosome aberrations was observed at late G_1 and early S phases. These results suggest that the different mutagens have different cell cycle responses, and that the action of enzymes involved in chromosome aberrations is also different.

INTRODUCTION

The several enzymes concerned with excision and polymerization have been identified (Mattern *et al.*, 1982; Collins and Johnson, 1984; Downes *et al.*, 1985). Among these, it has been reported that DNA polymerase α is largely responsible for replicating nuclear DNA (Cleaver, 1984; Th'ng and Walker, 1985), and that DNA polymerase β has a role in the repair of DNA (Collins and Johnson, 1984; Th'ng and Walker, 1985; Yamada *et al.*, 1985; Dresler and Kimbro, 1987; Um *et al.*, 1988). Moreover DNA topoisomerase

has been known to function in DNA replication (Jazwinski and Edelman, 1984) and repair (Mattern *et al.*, 1982). The functions of these enzymes can be studied using the specific enzyme inhibitors. Among them, it has been known that aphidicolin (APC) is an inhibitor of DNA polymerase α (Smith and Paterson, 1983; Th'ng and Walker, 1985), that 2', 3'-dideoxy-thymidine 5'-triphosphate (ddTTP) is an inhibitor of polymerase β (Th'ng and Walker, 1985; Yamada *et al.*, 1985; Dresler and Kimbro, 1987), and that novobiocin (NOV) is an inhibitor of DNA topoisomerase II (Liu *et al.*, 1980; Mattern and Scudiero, 1981).

On the other hand, during the last several years, post-treatment with DNA polymerase inhibitors after an exposure of mammalian cells to a variety of agents have been proved to be a useful tool for the study of the mechanism involved in the induction of chromosome aberrations (Ishii and Bender, 1980; Nishi *et al.*, 1981). During progression of the cell cycle, chromosomes undergo many structural changes to accomodate their functional needs. These structural changes are mediated and facilitated, in part, by a group of enzymes called DNA topoisomerase, and thus, the enzyme is also required for chromosome condensation in early mitosis (Uemura *et al.*, 1987). In recent studies, it was suggested that DNA topoisomerase was present not only in the mitotic phase of cell cycle but also in G_1 and S phases of cell cycle (Markovits *et al.*, 1987).

The purposes of this study were to elucidate the role of DNA polymerase and DNA topoisomerase on chromosome aberrations induced by ethyl methanesulfonate (EMS) or bleomycin (BLM) in relation to different phases of the cell cycle in synchronized Chinese hamster ovary (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 1 hour. Aphidicolin (APC, Sigma Chemical Co., St. Louis, MO), an inhibitor of DNA polymerase α, was dissolved in dimethyl sulfoxide. The 2', 3'-dideoxy-thymidine 5'-triphosphate (ddTTP, Sigma Chemical Co., St. Louis, MO), an inhibitor of DNA polymerase β, and novobiocin (NOV, Sigma Chemical Co., St. Louis, MO), an inhibitor af DNA topoisomerase II, were dissolved in distilled water and further diluted to working concentrations. The cells were treated to these inhibitors for 1 h after treatment with mutagens.

Cell Synchronization

For the determination of the stage sensitivity of chromosome aberrations induced by EMS or BLM during the progression of the cell cycle, monolayer cultures of CHO cells were synchronized by thymidine double block method as described by Aschihara and Baserga (1979) with minor modification. CHO cells cultured in exponential growth were treated for 12 hours with 3 mM thymidine. They were then grown for 7 hours in regular medium and then again for 9 hours in medium containing 3 mM thymidine. After release from thymidine, cells were labeled with 1 μCi/ml ^{3}H-thymidine (specific activity: 77.9 Ci/mM, Amersham Co., England) for 10 minutes. Labeling with ^{3}H-thymidine was terminated by washing the cells three times with PBS containing 100 μg/ml unlabeled thymidine. The coverslips were treated with hypotonic solution, fixed, dried and mounted on slide. Autoradiograms were prepared by using Kodak NTB liquid nuclear emulsion. The degree of synchronization was measured by the labeling index of synchronized cell population.

Chromosome Aberrations

After treatment with EMS or BLM for 1 hour in the different phases of the cell cycle, the cells were washed twice with PBS and then incubated at 37°C after treatment with APC adn ddTTP for 1 hour, respectively. Colchicine (0.04%, wt/vol) was added to the culture for final 2 hours of incubation. The mitotic cells were then harvested by gentle shaking to dislodge the loosely attached mitotic cells at 11 hours after thymidine release. The cells were collected by centrifugation at 1000 rpm for 10 minutes, and treated with hypotonic solution (0.05M KCl) for 9 minutes at 37°C and then fixed. Chromosome preparations were made by the air-drying technique and stained with 4% Giemsa for 10 minutes. Chromosome aberrations were scored under the oil immersion lens according to the criteria of Evans (1977).

RESULTS

Chinese hamster ovary (CHO) cells synchronized by thymidine double block method were used throughout this investigation. The degree of synchronization was established in terms of the labeling index in DNA synthesis. The generation time of CHO cells is 16 hours: the durations of G_1, S, G_2, and M-phases of CHO cells occupy about 4, 9, 2 and 1 hours, respectively (data not shown).

Fig. 1 shows the effect of 10 μg/ml APC or 40 μM ddTTP on chromosome aberrations induced by 40 mM EMS at different phases of the cell cycle. APC or ddTTP alone did not induce chromosome aberrations. The chromosome aberrations of the cells were shown to be most sensitive to EMS in early S phase. And the post-treatment with APC increased the chromosome aberrations induced by EMS in late G_1 and early S phase, while the group with ddTTP did not increase the chromosome aberrations induced by EMS in any phases.

The effect of APC or ddTTP on chromosome aberrations induced by 100 μg/ml BLM at different phases of the cell cycle are shown in fig. 2. As shown in the figure, the cells in G_2 phase were most sensitive to BLM, whereas the cells in late G_1 and early S phases were less sensitive than mid. S and G_2 phases to BLM. And there was no increment of the frequencies

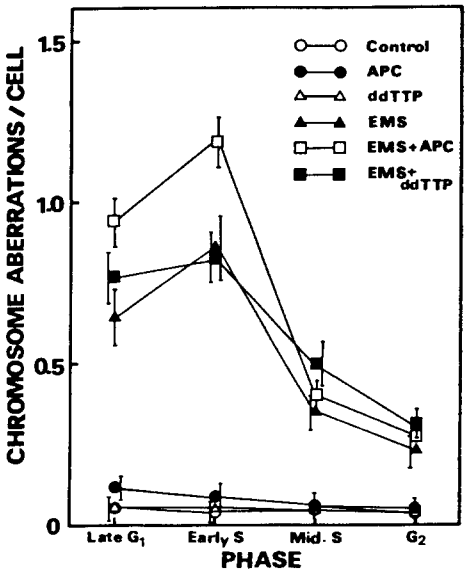

Fig. 1. Effect of 10 μg/ml APC or 40 μM ddTTP on chromosome aberrations induced by 40 mM EMS for 1 hour at different phases of the cell cycle.

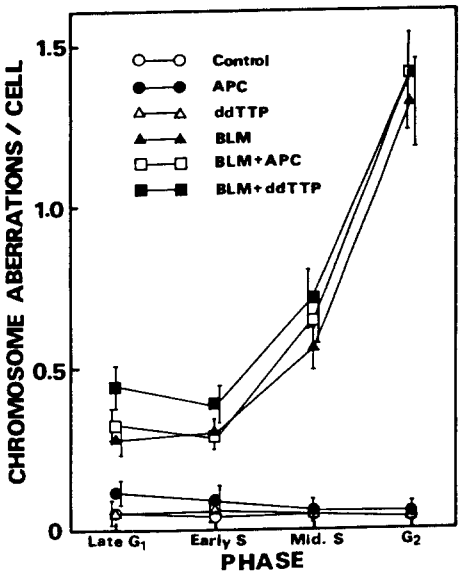

Fig. 2. Effect of 10 μg/ml APC or 40 μM ddTTP on chromosome aberrations induced by 100 μg/ml BLM for 1 hour at different phases of the cell cycle.

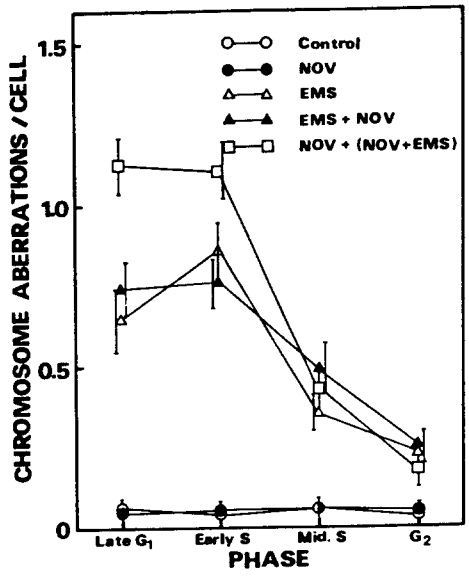

Fig. 3. Effect of 400 μM NOV on chromosome aberrations induced by 40 mM EMS for 1 hour at different phases of the cell cycle.

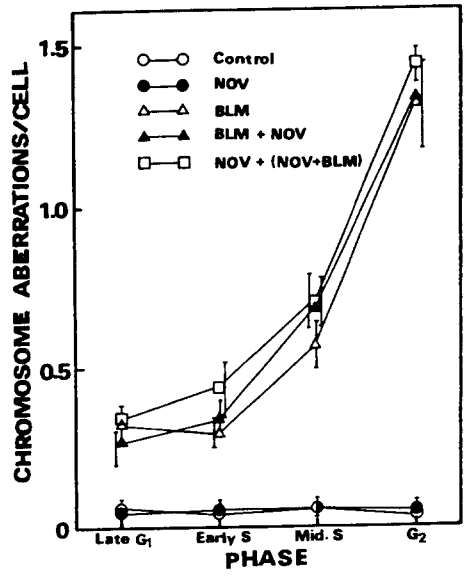

Fig. 4. Effect of 400 μM NOV on chromosome aberrations induced by 100 μg/ml BLM for 1 hour at different phases of the cell cycle.

of the chromosome aberrations in post-treated group with APC or ddTTP compared with BLM alone group. These results suggest that post-treatment with APC or ddTTP do not affect chromosome aberrations induced by BLM in any phases of the cell cycle.

Fig. 3 represents the effect of 400 μM NOV on chromosome aberrations induced by 40 mM EMS at different phases of the cell cycle. NOV alone did not induce the chromosome aberrations. As shown in the figure, the post-treatment with NOV did not affect chromosome aberrations induced by EMS, whereas pretreatment with NOV showed increased effect on chromosome aberrations induced by EMS at late G_1 and early S phases.

Fig. 4 shows the effect of 400 μM NOV on chromosome aberrations induced by 100 μg/ml BLM at different phases of the cell cycle. In the figure, pre- or post-treatment with NOV did not show any statistically significant effect on chromosome aberrations induced by BLM, and thus, the chromosome aberrations were not increased in pre- or post-treatment with NOV compared with BLM alone group in any phases.

DISCUSSION

Many environmentally physical and chemical agents are known to produce DNA lesions that ultimately result in chromosome aberrations (Sognier and Hittelman, 1986). There were the results concerning the effect of DNA polymerases on chromosome aberrations. van Zeeland *et al.*, (1982) reported that APC increased the chromosome aberrations induced by X-ray in G_1 of human peripheral lymphocytes, whereas APC does not have a significant effect on chromosome aberrations by X-rays in the G_2 phase of CHO cells. He also reported that the CHO cells exposed to UV irradiation or alkylating agents in the G_1 phase and challenged with APC in G_2 just before fixation have higher frequencies of aberrations than cells treated with the mutagens alone. Above results indicate that it is due to an inhibition of rejoining of DNA-strand breaks by APC (Natarajan *et al.*, 1980; Waters, 1981). One would expect that a delay in rejoining of strand breaks will increase the possibility for misrepair leading to increased frequencies of chromosome aberrations (Bender and Preston, 1981). van Zeeland *et al.*, (1982) suggested that the cell types and the conditions of the cells strongly influence the biological response to APC. Reidy (1987) recently reported that the presence of 20 μM APC or Ara C during G_2 phase increased folate- and deoxyuridine-sensitive chromosome breakage. These results and other published data suggest that the biological response to APC is dependent on the cell types or chemical agents used.

Tricoli *et al.*, (1985) reported that DNA topoisomerase II specific activity showed no detectable change during G_1, S and M phases of the cell cycle in mouse embryo fibroblasts cells. Hsiang *et al.* (1988) demonstrated that the topoisomerase II level in HeLa cells synchronized by a double thymidine

block remained relatively constant throughout the late G_1, S, G_2 and M phases of the cell cycle. On the other hand, early replicating and late-replicating chromatins differ in their chromatin structures and functions (Uemura *et al.*, 1987), and it was known that topoisomerase II was continuously required for chromosome structural changes (Holm *et al.*, 1985; Uemura *et al.*, 1987). The use of specific inhibitor of the enzyme can be useful tool to elucidate the role of enzyme. Deaven *et al.* (1978) observed that CHO cells were most sensitive to chromosome damage by 4'-(9-Acridinylamino) methanesulfon-m-anisidide, an inhibitor of topoisomerase II, at G_1-S border. Uemura *et al.* (1987) reported that post-treatment with NOV of the γ-irradiated cells resulted in a significant increase of the aberrations. Kishi (1988) demonstrated cytogenetically the existence of novobiocin-sensitive process to chromosome recombination in G_1 lymphocytes. However, the present results showed that the pre-treatment with NOV increased EMS-induced chromosome aberrations in late G_1 and early S phases, whereas there was no effect of NOV on chromosome aberrations induced by BLM at any phases.

Considering our overall results and others' reports, we suggest that the action of DNA polymerases and DNA topoisomerase involved in the chromosome aberrations is different during cell cycle.

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동시화된 포유동물 세포에서 돌연변이원에 의해 유발된 염색체 이상에 미치는 DNA 중합효소와 DNA위상이성질화효소의 저해제의 효과

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동시화시킨 CHO세포를 재료로하여 EMS 혹은 BLM에 의해 유발된 염색체 이상에 미치는 APC, ddTTP, 그리고 NOV의 효과를 조사하였다. 세포의 동시화는 thymidine block방법을 사용하였다. APC ddTTP, 그리고 NOV의 단독처리는 염색체 이상을 유발하지 않았다. EMS에 의해 유발된 염색체 이상은 late G_1과 early S기에 높은 감수성을 나타내었고, BLM에 의해 유발된 염색체 이상은 G_2기에 가장 높은 감수성을 나타내었다. APC를 후처리할 경우 late G_1기와 early S기에서 EMS에 의해 유발된 염색체이상을 증가시켰다. 그러나 ddTTP나 NOV를 후처리할 경우 EMS나 BLM에 의한 염색체 이상에 영향을 미치지 않았다. EMS와 NOV를 복합처리하기전 NOV를 전처리하면 late G_1기와 early S기에서의 염색체 이상이 증가되었다. 이런 결과들은 여러가지 돌연변이원에 따라 각기 다른 세포내 반응이 유발되며 염색체 이상에서의 각 효소들의 활성 또한 다른 것으로 추측된다.