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Effects of 3-Aminobenzamide on DNA Strand Breaks and Excision Repair in CHO Cells Exposed to Methyl Methanesulfonate and Ultraviolet-light

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MMS와 자외선을 처리한 CHO세포에 있어서 DNA사 절단과 절제회복에 미치는 3-aminobenzamide의 영향

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요 약

MMS와 자외선에 의한 DNA의 절제회복과 단사절단에 미치는 poly(ADP-ribose) polymerase의 저해제인 3-aminobenzamide의 영향을 CHO 세포를 재료로 조사하여 다음과 같은 결과를 얻었다.

1. MMS에 의한 비주기성 DNA합성률과 DNA단사 절단률은 이 저해제에 의해 모두 증가하였다. 이는 poly (ADP-ribose) polymerase가 MMS에 의해 유발된 염기 절제회복의 incision step을 억제하는 결과라 생각된다.
2. 자외선에 의한 비주기성 DNA합성률과 DNA단사 절단률은 이 저해제에 의해 모두 감소하였다. 이는 poly (ADP-ribose) polymerase가 자외선에 의해 유발된 nucleotide 절제회복의 incision step을 돕는 작용을 하는 결과로 생각된다.
3. MMS와 자외선을 복합처리한 실험군에서는, DNA 단사 절단률은 이 저해제에 의해 영향을 받지 않았으며, 비주기성 DNA 합성률은 자외선 단독 처리군의 수준으로 증가되었다. 이는, 이 저해제가 MMS와 자외선으로 유발된 절제회복의 incision step에는 독립적으로 작용하며, 그 이후의 단계에서, MMS에 의해 부분적으로 불활성화 되었던 pyrimidine dimers의 절제를 완전하게 해주는 것으로 해석된다.

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INTRODUCTION

Poly (ADP-ribose), a unique biopolymer synthesized from NAD^+ by an eukaryotic nuclear enzyme, has been suggested to be involved in DNA replication (Hilz and Stone, 1976; Edwards and Taylor, 1980), DNA repair (Durkacz *et al.*, 1980; Shall *et al.*, 1981), and some regulatory processes in gene expression (Hayaishi and Ueda, 1977; Poirier *et al.*, 1982) through alteration of the chromatin structure. Evidences for a direct of poly (ADP-ribose) in the recovery process from DNA damage have been obtained by using inhibitors of poly (ADP-ribose) polymerase. It has been shown that repair replication (Miwa *et al.*, Sims *et al.*, 1981), sister chromatid exchanges (SCE) (Hori, 1981; Morgan and Cleaver, 1982), mutation frequency (Morgan and Schwartz, 1982) and strand breaks of DNA (Shall *et al.*, 1981; Park *et al.*, 1983a) were enhanced when cells were treated with DNA damaging agents in the presence of the inhibitors of poly (ADP-ribose) polymerase.

Durkacz *et al.* (1980), however, reported that inhibitors of poly (ADP-ribose) polymerase inhibited the repair of alkylated damage in mouse lymphoma cells. Cleaver *et al.* (1982) also showed that these inhibitors have no or little effect on repair replication and single strand breaks after exposure to ultraviolet (UV) light or methyl methanesulfonate (MMS). Morgan and Cleaver (1982) found that the inhibitor increased SCE in human and Chinese hamster ovary (CHO) cells exposed to MMS but not to UV-light. Moreover, Bohr and Klenow (1981) reported that the inhibitor stimulated DNA repair in human cells while inhibiting it in mouse cell lines. These observations indicate that the role of poly (ADP-ribose) formation in relation to DNA damage and repair has not been fully clarified.

Recent studies demonstrated the amounts of excision repair in human and Chinese hamster cells irradiated with UV-light were inhibited by alkylating agents (Park *et al.*, 1981; Park and Cleaver, 1982). Strand breaks induced by the combined treatment with these two agents, however, were found to be additive in the amount (Park *et al.*, 1983b). These results suggest that mechanisms involved in the interactions of DNA damages induced by two different DNA damaging agents and their repair process are very complicated, and might involve multiple factors controlling these processes.

The present investigation was, therefore, determined the effects of 3-aminobenzamide on excision repair and strand breaks in CHO cells exposed to single or combined treatment with UV-light and MMS in order to elucidate which step of repair process is involved in the formation of poly (ADP-ribose).

MATERIALS AND METHODS

Cell Culture

Chinese hamster ovary (CHO) 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% calf serum, penicillin G (100 unit/ml) and streptomycin (100 μg/ml).

UV-Irradiation

Cells were exposed to various fluences of 254 nm UV-light from germicidal lamps at an incident dose rate of 1.42 J/m²/sec. and determined with a YSI-Kettering radiometer (Yellow Springs Instrument Co., Yellow Springs, Ohio).

Chemical Treatment

Methyl Methanesulfonate (MMS, Eastman Kodak Ltd., Rochester, N.Y.) was dissolved in the serum-free medium prior to use and exposed to cells at 37°C for an hour. 3-Aminobenzamide (3-AB; Sigma Chemical Co., St. Louis, Mo.), was dissolved in the serum-free medium. Cells were incubated with the inhibitor for desired time prior to, during or after MMS treatment and UV-irradiation. After UV-irradiation, cells were incubated for two hours in the presence of 2 mM of hydroxyurea (HU; Sigma) and 10 μM of 1-β-D-arabinofuranosyl cytosine (ara-C; Sigma) to accumulate DNA single strand breaks induced by UV-light.

Alkaline Elution Experiment

DNA single strand breaks induced by MMS and UV-light were measured by alkaline elution technique as described by Kohn *et al.* (1976) with a minor modification. Cells were labeled with 0.2 μCi/ml of ^{3}H-thymidine (sp. act., 53 Ci/mM; The Radiochemical Centre Ltd., Amersham, England) for two days, and then exposed to MMS and UV-light or both in the presence or absence of the inhibitor. Cells were harvested with cold PBS-Merchant solution (NaCl 150 mM, K_2HPO_4 4.28 mM, KH_2PO_4 0.71 mM), filtered onto 2 μm pore size polyvinyl chloride filter (Millipore Corp., Bedford, Mass.) and lysed with lysing solution (0.2% sodium laurylsarcosine, 2 M NaCl, 0.04 M Na_2EDTA, pH 10.0). The filters were then washed with 10.02 M Na_2EDTA (pH 10.3) and eluted in the dark with a solution consisting of 0.02 M H_4EDTA plus tetrapropylammonium hydroxide (10% in water) added in the amount required to give the final pH 12.1~12.2 at a flow rate of 0.05 ml/minute. Fractions were collected at 60 minutes intervals. The radioactivities remaining on filters were counted using a Packard Tri-Carb liquid scintillation spectrometer after treating them with 1 N HCl at 60°C for one hour followed by 0.4 N NaOH at room temperature for 30 minutes. Fractions of radioactivity remaining on filter were plotted against elution time.

Unscheduled DNA Synthesis Experiment

CHO cells grown on cover slips in plastic petri dishes were exposed to MMS, UV-light or both. The cells were then labeled with ^{3}H-thymidine (10 μCi/ml) for an hour in the presence or absence of the inhibitor. ^{3}H-thymidine incorporation was terminated by washing the cells three times with phosphate buffered saline (PBS) containing 100 μg/ml of unlabeled thymidine, followed by incubation in the growth medium containing unlabeled

thymidine for 30 minutes. Cells were then fixed in glacial acetic acid: methanol (1:3) and soaked overnight in 4% perchloric acid and dehydrated. Autoradiograms were prepared by using Kodak NTB liquid nuclear track emulsion. Silver grains over the nuclei of evenly and lightly labeled cells were counted.

RESULTS

1. *Effects of 3-AB on Excision Repair*

The synergistic effect of 3-AB on MMS-induced unscheduled DNA synthesis (UDS) is shown in Fig. 1. The dose response of UDS induced by MMS was increased up to 5 mM. Five mM 3-AB induced only a few grains per cell, suggesting that it may not be a DNA damaging agent as we expected. Treatment with MMS in the presence of 3-AB, however, resulted in a greater increases in the amount of UDS than expected if the effects of both agents were independent and additive. These results suggest that 3-AB enhances MMS-induced base excision repair.

Fig. 2 shows the inhibitory effect of 3-AB on UV-induced UDS. Contrary to its effect on MMS-induced UDS, 3-AB inhibited the UV-induced excision repair at all doses employed. The amount of UDS induced by 30 J/m^2 UV-light was significantly decreased as the concentration of 3-AB was increased from 0.5 mM to 10 mM (Fig. 3). These results suggest that 3-AB inhibits UV-induced nucleotide excision repair.

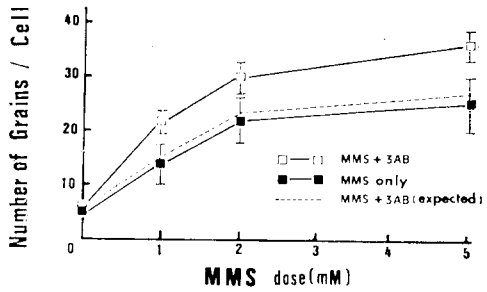

Fig. 1. The effects of 3-AB on MMS-induced unscheduled DNA synthesis in CHO cells.

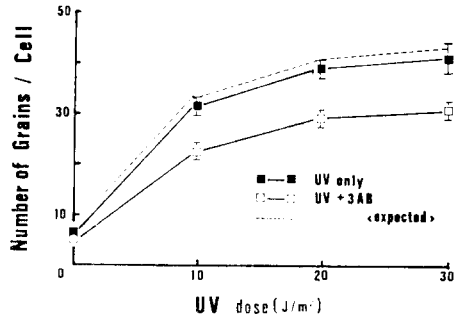

Fig. 2. The effects of 3-AB on UV-induced unscheduled DNA synthesis in CHO cells.

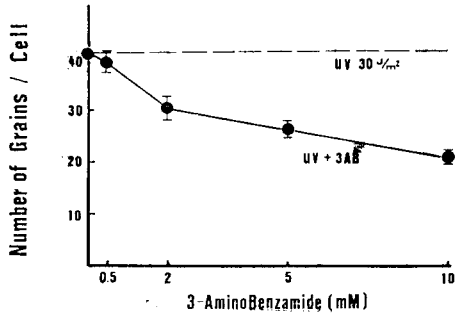

Fig. 3. The effects of increasing concentrations of 3-AB on UV-induced unscheduld DNA synthesis in CHO cells.

Fig. 4 represents of 3-AB on the combined treatment with MMS and UV-light. Amounts of UDS induced by both MMS and UV-light in the presence of 3-AB were almost the same as that induced by UV alone. These results indicate that 3-AB may alleviate the inhibitory effect of MMS on UV-induced excision repair. This effect of 3-AB was also confirmed in the MMS post-treatment group.

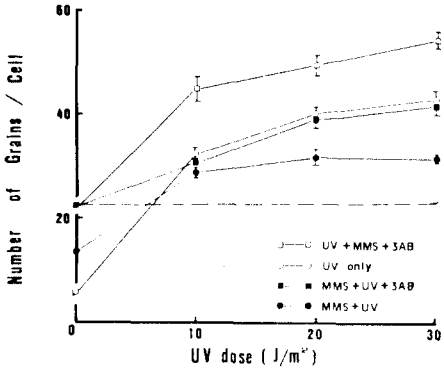

Fig. 4. The effects of 3-AB on unscheduled DNA synthesis induced by MMS and UV-light in CHO cells.

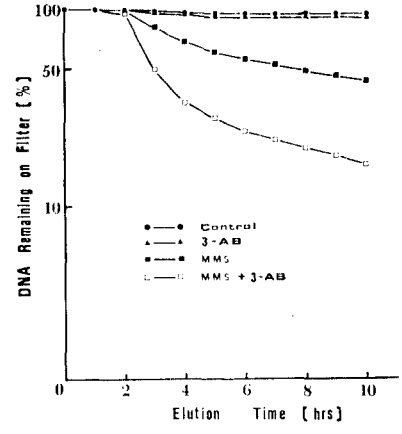

Fig. 5. The effects of 3-AB on the DNA single strand breaks formation induced by MMS in CHO cells.

2. *DNA Fragmentation Effects*

The amounts of DNA single strand breaks induced by 2 mM MMS were in the presence of 5 mM 3-AB (Fig. 5). However, 5 mM 3-AB reduced amounts of strand breaks in cells

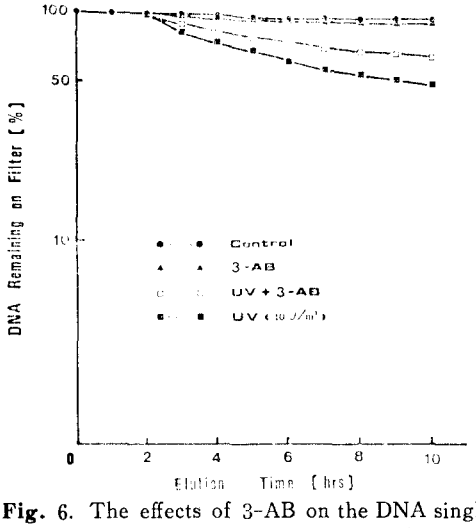

Fig. 6. The effects of 3-AB on the DNA single strand breaks formation induced by UV-light in CHO cells.

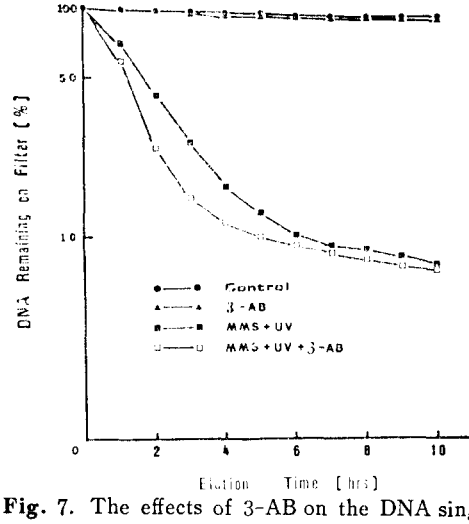

Fig. 7. The effects of 3-AB on the DNA single strand breaks formation induced by MMS and UV-light in CHO cells.

irradiated with 10 J/m^2 UV-light plus are-C and hydroxyurea (Fig. 6). The effect of 3-AB on the amounts of strand breaks in cells treated with MMS and UV-light simultaneously are shown in Fig. 7. As shown in the figure, 3-AB did not change the pattern and slop of elution profile. It seems that 3-AB increased the single strand breaks induced by MMS but decreased those induced by UV-light. The same elution profile may be resulted from the countervailing action of the independpnt effect of 3-AB on MMS-and UV-induced repair. Therefore, these results indicate that 3-AB exerts its effect independently on the incision step of MMS-and UV-induced excision repair in the combined treatment.

The overall results of the present experiments suggest that poly (ADP-ribose) polymerase exerts its effect significantly on the lesion produced by UV-light and MMS in the single treatment. In the case of combined treatment of MMS and UV-light, this polymer affects the incision step of base and nucleotide excision repair independently and exerts an alleviating effect of inhibition of UV-induced repair by MMS.

DISCUSSION

The results obtained from this study strongly suggest that poly (ADP-ribose) polymerase seems to be involved in excision repair with different modes of actions depening on the kinds of damage and its repair pathway. Nomura *et al.* (1981) reported that poly (ADP-ribose)-sensitive endonuclease participates in DNA metabolism in the cells exposed to methyl-nitrosourea (MNU). They also suggested that DNA breakage induced by DNA damaging agents alone might be reduced as a consequence of the inactivation of the endonuclease, but in the presence of poly (ADP-ribose) synthesis inhibitor, the fragmentation of DNA by the endonuclease might continue at a rate higher than the rates of DNA repair.

It was reported that the enhancement of UDS or SCE by inhibitors of poly (ADP-ribose) polymerase was resulted from the increase of DNA strand breaks induced indirectly by the activation of Ca^{++}, Mg^{++}-dependent endonuclease in consequence of inactivation of poly (ADP-ribose) polymerase (Miwa *et al.*, 1981). Durkacz *et al.* (1980) proposed that poly (ADP-ribose) formation is involved in the rejoining process other than incision step. However, the present results indicate that inhibitor of poly (ADP-ribose) polymerase influences the incision step of the excision repair induced by both MMS and UV-light.

Recently it was reported that 3-AB synergistically increased SCE in CHO and GM 637 cells exposed to MMS but not to UV-light (Morgan and Cleaver, 1982). Cohen and Berger (1981) suggested that stimulation of poly (ADP-ribose) polymerase actity by UV-irradiation of normal cells could be attributed to the strand breaks induced by the DNA repair process. They illustrated that the failure of UV-irradiation to stimulate poly (ADP-ribose) polymerase activity in xeroderma pigmentosum cells could be attributed to their inability to perform the incision step required for the repair of UV-induced DNA damage. Berger

et al. (1979) reported that the synthesis of DNA and poly (ADP-ribose) were increased abruptly in response to UV-irradiation. And the lowest concentration of added DNase, which effectively stimulate poly (ADP-ribose) synthesis, also resulted in concurrent stimulation of DNA repair synthesis. Moreover, Bohr and Klenow (1981) reported an inhibitory effect of 3-AB on UDS in L 1210 and Sarcoma 180 cells irradiated with UV-light.

In this report, we showed that poly (ADP-ribose) polymerase acts negatively on the MMS-induced excision repair while influencing positively on the UV-induced excision repair. These different effects on repair process of the inhibitor was also shown by Althaus *et al.* (1982). They reported that isonicotinamide, the ADP-ribosyltransferase inhibitor, enhanced DNA repair synthesis treated with various carcinogens involving MMS but did not affect that induced by other kinds of carcinogens involving 4-nitroquinolin-N-oxide (4NQO). They suggested that the interaction between ADP-ribosylation and DNA repair processes apparently is dependent on the chemical nature of the repair-stimulating agent.

Park *et al.* (1981) and Park and Cleaver (1982) demonstrated that exposure of UV-damaged human cells to MMS or MNNG markedly inhibited UV-induced excision repair. Park *et al.* (1983b), however, recently found that single strand breaks were additive in the amount when cells were exposed to MMS and UV-light concomitantly. They suggested that the large protein complex required for cleaving either side of pyrimidine dimers may be partially inactivated by alkylation with result that only one cleavage instead of two may occur. The present results are in good accordance with their suggestion. Therefore, it can be suggested that the inhibitor may potentiate the both side cleavage of pyrimidine dimer induced by UV-light, which repair process inhibited by MMS resulting in one side cleavage in cells exposed to MMS and UV-light. This phenomenon may increase the amount of grains but not change the profile of alkaline elution. Accumulated evidencies also indicate that poly (ADP-ribose) polymerase is closely related to DNA repair and exerts its effects on DNA damages with variable capacity depending on the type of DNA-damaging agents.

ABSTRACT

Amounts of DNA single strand breaks and unscheduled DNA synthesis in CHO cells exposed to MMS were increased in the presence of 3-aminobenzamide, a potent inhibitor of poly (ADP-ribose) polymerase. However, those in cells irradiated with UV-light were decreased. These results suggest that poly (ADP-ribose) polymerase acts negatively on the MMS-induced base excision repair but positively on the UV-induced nucleotide excision repair. In the combined treatment with MMS and UV-light in the presence of this inhibitor, amounts of strand breaks were just the same as those in the absence of the inhibitor. But those of unscheduled DNA synthesis were increased up to the amount induced by UV-light alone. These results may suggest that poly (ADP-ribose) polymerase affects the

incision step of excision repair induced by MMS and UV-light independently, and that it may potentiate the complete cleaving of UV-induced pyrimidine dimers possibly by the repair enzymes which might have been partially inactivated by MMS.

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