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Effects of Thymidine Analogs on Mitomycin C Induced DNA Repair Synthesis

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Mitomycin C에 의한 DNA 回復合成에 미치는 Thymidine 相似體의 影響

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적 요

HeLa S_3 세포에서 MMC에 의해 유발된 DNA회복합성은 농도(0.05~0.5μg/m*l*)에 따른 증가를 보이지 않고 그 율도 비교적 낮아 0.1~0.5μg/m*l* 농도에서 조사한 전 세포의 7~9%를 나타내고 있다. 시간 변화에 따른 실험에서는 MMC를 제거한 후 24시간까지 거의 비슷한 율로 DNA회복합성이 계속되고 있다. thymidine 상사체중 BUdR을 전처리한 군에서만이 MMC에 의한 DNA회복합성을 증가시켰다. 그러나 BUdR 또는 IUdR과 MMC를 복합처리 할 경우 시간경과에 따라 정상 DNA합성은 감소된다.

이들 결과는 MMC에 의해 유발된 DNA손상은 빠르고 느린 두단계로 회복됨을 암시하는 것이라 생각된다.

INTRODUCTION

Mitomycin C (MMC) has been shown to cause a specific block in DNA synthesis by virtue of crosslinking of compementary polynucleotide chains (Makino and Okada, 1974). Covalent linkages between the compementary strands of DNA have also been reported to be induced by psolalen in conjunction with light, and nitrogen or sulfur mustards (Cole, 1973).

The repair mechanism of crosslinks produced by nitrogen mustards in bacterial

system has been suggested to be similar to that involved in the repair of ultraviolet (UV) light damage to DNA (Lawley and Brooks, 1968). Cole (1973) subsequently reported that the repair of crosslinks induced in DNA by psoralen follows a two-step process which includes an excision of one side of interstrand crosslink, followed by exchange between homologous DNA. Sasaki (1975), however, postulated that crosslinks caused by MMC in mammalian cells might be repaired by a process independent of the excision ability responsible for the repair of damage induced by UV light. Although such an exchange mechanism has been suggested to be operative for the removal of crosslinks (Latt, 1974), the repair processes of crosslinks in mammalian cells are not fully understood.

Thymidine analogs, particularly 5-bromodeoxyuridine (BUdR) have been shown to have sensitization effects on radiation or chemical-induced DNA repair synthesis (Lohman *et al.*, 1972; Park and Um, 1975). To date, no one has yet attempted to study the effects of base analogs on DNA repair synthesis induced by crosslinking agents.

The present studies were therefore undertaken to characterize the MMC-induced DNA damages and their repair processes in terms of excision repair and to determine whether base analogs would lead to an enhancing effect on MMC-induced DNA repair synthesis or not.

MATERIALS AND METHODS

HeLa S_3 were used throughout this investigation. Monolayer cultures of this cell line were grown at 37°C using Eagle's minimum essential medium (MEM) (Gibco) supplemented with 10% fetal calf serum and antibiotics (Penicillin G, 100 units/ml; Streptomycin, 100 μg/m*l*; Kanamycin, 50 μg/m*l*).

Mitomycin C (Sigma), for laboratory use, was dissolved in phosphate buffered saline (PBS) as 1 mg/m*l* stock solution and further diluted to various working concentrations in the growth medium without serum prior to treatment. For the induction of excision repair of DNA, an appropriate number ($1.0-5.0\times10^6$ cells/m*l*) of monolayer cultures grown on cover slips (9×50mm, Bellco) for 48 hours in Leighton culture tubes were treated to MMC.

For the determination of the effects of thymidine analogs on MMC-induced unscheduled DNA synthesis, the cells grown for 24 hours were exposed to 5-bromodeoxyuridine (BUdR) or 5-iododeoxyuridine (IUdR) (Sigma) for 24 hours at a final concentration of 0.2 mM prior to the MMC treatment. The experiments involving DNA repair synthesis were carried out two different procedures, dose response and time dependence. For the dose response experiment, ^{3}H-thymidine (Amersham/Searle) was incorporated to the cultures at a final concentration of

10 μCi/ml (specific activity, 40-60 Ci/mM) for an hour immediately after treatment with various concentrations of MMC for two hours.

For the time dependence study, cells were labeled with ^{3}H-thymidine for an hour from 0 to 24 hours after treatment with MMC. Labeling with ^{3}H-thymidine was terminated by washing the cells three times in PBS containing 100 μg/ml of unlabeled thymidine. The cells were treated with hypotonic solution, fixed and then stained. Autoradiograms were prepared using autoradiographic stripping plate (Kodak AR-10), and the DNA repair synthesis was analyzed according to the criteria as described previously (Park and Um, 1975).

RESULTS

The dose response for the MMC-induced DNA repair synthesis (UDS) is shown in Table 1. The rate of semiconservative DNA synthesis (NDS) remained unchanged except in the highest concentration (0.5 μg/ml). Lightly labeled cells (UDS) increased up to 7.3% in 0.1 μg/ml group, but this level did not show to increase as the concentraions increased. These results suggest that DNA repair synthesis induced by MMC may not be dose dependent, and that NDS is suppressed at relatively higher concentrations of MMC.

Table 1. Dose response of DNA repair synthesis in HeLa S_3 cells fixed immediately following treatments with Mitomycin C and ^{3}H-thymidine labeling

Treatments* MMC (μg/ml)	Labeling index** (%)	Labeling pattern (%±S.E.)***	
		Heavily labeled(NDS)	Lightly labeled(UDS)
—	39.8	39.4±2.1	0.4±0.2
0.05	41.3	38.7±2.1	2.6±0.7
0.10	46.2	38.9±2.1	7.3±1.1
0.20	46.6	39.1±2.1	7.5±1.1
0.50	43.7	34.9±2.1	8.8±1.2

* Mitomycin C (MMC) for 2 hours and ^{3}H-TdR for 1 hour.
** Labeling index was based on the analysis of 500 cells.
*** NDS: Semiconservative DNA synthesis.
UDS: Unscheduled DNA synthesis.

Table 2 shows the effects of thymidine analogs on MMC-induced DNA repair synthesis. In the combined treatment with BUdR and MMC, the rates of UDS were shown to increase in all dose ranges as compared with the corresponding single treatment with MMC. However, in IUdR pretreated group, there was no increase in the rate of UDS. These results indicate that only BUdR shows to have sensitization effect on DNA repair synthesis induced by MMC.

Table 2. Effects of thymidine analogs on Mitomycin C-induced DNA repair synthesis in HeLa S_3 cells

Treatments*		Labeling index (%)	Labeling pattern (%±S.E.)	
BUdR or IUdR (mM)	MMC (μg/ml)		Heavily labeled (NDS)	Lightly labeled (UDS)
—	—	39.8	39.4±2.1	0.4±0.2
BUdR 0.2	—	46.2	42.8±2.2	3.4±0.8
BUdR 0.2	0.05	48.6	42.4±2.2	6.2±1.0
BUdR 0.2	0.10	54.0	43.9±2.2	10.1±1.3
BUdR 0.2	0.20	53.6	41.9±2.0	11.7±1.3
BUdR 0.2	0.50	53.9	41.5±2.1	12.4±1.4
IUdR 0.2	—	38.9	37.3±2.1	1.6±0.5
IUdR 0.2	0.05	39.5	35.0±2.1	4.5±0.9
IUdR 0.2	0.10	43.1	36.4±2.1	6.7±1.1
IUdR 0.2	0.20	43.5	36.0±2.0	7.5±1.1
IUdR 0.2	0.50	43.0	35.4±2.1	7.6±1.1

* BUdR: 5-bromodeoxyuridine or IUdR: 5-iododeoxyuridine for 24 hours.

Table 3 represents the time dependent study of DNA repair synthesis. As shown in the table, MMC-induced excision repair occurs for as long as 24 hours after removal of MMC, and the rate of UDS showed relatively constant at all time courses. These data may suggest that the majority of damages induced in DNA by MMC might be irrepairable damages or those to take longer time to be repaired.

Table 3. Time dependence of DNA repair synthesis in HeLa S_3 cells fixed at various time intervals following treatments with Mitomycin C and ^{3}H-thymidine labeling

Treatment* MMC (μg/ml)	Time after ^{3}H-TdR (hrs)	Labeling index (%)	Labeling pattern (%±S.E.)	
			Heavily labeled(NDS)	Lightly labeled(UDS)
0.1	0	44.7	38.9±2.1	5.8±1.1
0.1	2	45.7	39.6±2.1	6.1±1.0
0.1	4	45.1	39.0±2.1	6.1±1.0
0.1	8	45.3	38.9±2.1	6.4±1.0
0.1	13	46.0	39.1±2.1	6.9±1.1
0.1	17	40.8	36.4±2.1	4.4±0.9
0.1	24	40.9	36.4±2.1	4.5±0.9

* MMC for 1 hour.

Fig. 1 shows the effects of thymidine analogs on the time dependent study. DNA repair synthesis in the combined treatment groups occurs for as long as 24 hours likewise the single MMC group. NDS was found to be reduced remarkably

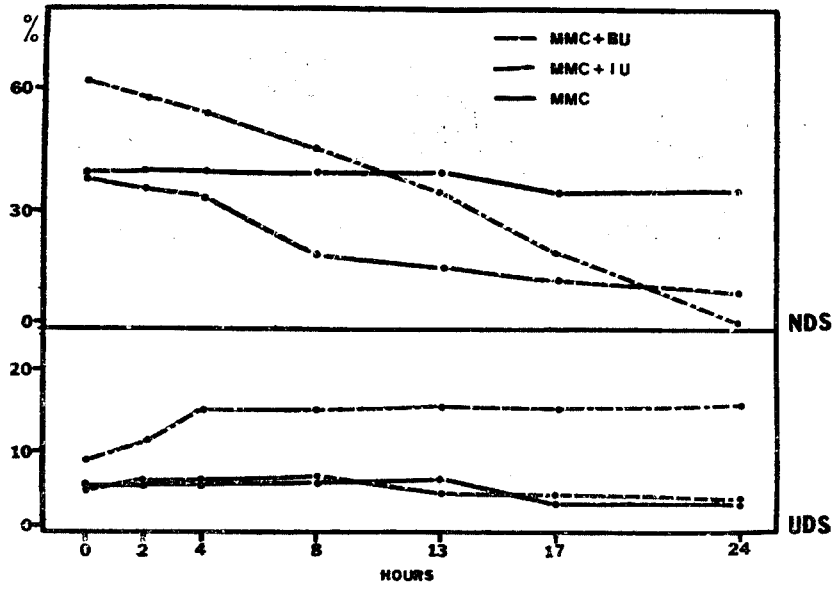

Figure 1. Time dependence of UDS and NDS in MMC-treated HeLa S_3 cells pretreated with thymidine analogs. BUdR or IUdR+MMC groups show gradual decrease in NDS, while MMC alone does not. BUdR+MMC increases the rate of UDS up to 4 hours and then levels off, while other two groups remain unchanged.

with time in both BUdR and IUdR pretreated group.

These results seem to suggest that MMC may not be an effective chemical for the induction of DNA repair synthesis, and that damages induced in DNA by MMC take quite longer time to be repaired.

DISCUSSION

It has been demonstrated that crosslinking agents could cause not only crosslinks but also various other type of damages to DNA, mono and diadducts. Consequently, a variety of repair processes may be expected to be occurred in response to their damages induced in DNA.

Reid and Walker (1969) showed that crosslinks produced by sulfur mustard were cut in two hours, but diguaninyl sulfur mustard moiety was released about 20 hours later. Howard-Flanders and Cole (1973) suggested that the gaps formed by the excision of monoadducts produced by psoralen with light are presumably repaired quickly, but gaps caused by the actions of excision enzymes on crosslinks remain open for longer periods. The data presented here showed that the amounts of DNA repair synthesis induced by MMC are relatively lower and it occurs for as long as 24 hours with similar incidences. These and other accumulated data may suggest that MMC could cause monoadducts within intrastrand as

well as crosslinks between interstrands of DNA. From the point of view, it is tempting to conclude that slow repair processes might be involved in the repair of crosslinks and that fast repair may be associated with damages other than crosslinks. However, the mechanism involved in how the substituted base with BU interacts with DNA resulting in the sensitization effects of MMC-induced DNA repair synthesis is unknown.

Further studies with dose protraction and varying time of treatment combined with these base analogs may be expected to provide more useful information on the sensitization effects of these or MMC-induced DNA repair synthesis.

SUMMARY

Dose response for DNA repair synthesis induced by various concentrations of MMC (0.05~0.5 $\mu g/ml$) in HeLa S_3 cells was not dose-dependent and the amounts of it were relatively lower, representing 7~9% of total DNA synthesizing cells in 0.1~0.5 $\mu g/ml$ concentrations. Time dependence study showed that MMC-induced DNA repair synthesis occurred as long as for 24 hours with similar incidences in all time courses. Pretreatment with BUdR was found to have a sensitization effect on MMC-induced DNA repair synthesis, but that with IUdR was not. Combined treatment with BUdR or IUdR and MMC suppressed remarkably the semiconservative DNA synthesis especially at later time course.

These results seem to suggest that damages induced in DNA by MMC might be repaired by both fast and slow excision processes.

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