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Effects of Thymidine Analogs on Methyl Methanesulfonate Induced Chromosome Aberrations in Human Lymphocytes *in Vitro*

Yung Sun Kang, Sang Dai Park and Kyung Hee Choi
(Dept. of Zoology, Seoul National University)

Methyl Methanesulfonate에 의한 사람의 培養細胞 染色體에 미치는 Thymidine 相似體의 影響

姜 永 善·朴 相 大·崔 慶 喜
(서울大 自然大 動物學科)

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摘 要

染色體의 構造的인 異常에 미치는 Methyl methanesulfonate (MMS)와 thymidine 相似體 (BUdR, IUdR)의 영향을 究明하기 위해 사람의 白血球 培養細胞에 2.0 mM의 BUdR 또는 IUdR을 24時間 前 처리하고 이어 농도를 달리한 MMS (0.0~2.0 mM)를 1時間 처리한 뒤 24時間째 染色體標本을 작성하여 아래와 같은 결과를 얻었다.

1. MMS를 단독 처리한 對照區에서는 관찰된 染色體 異常型의 대부분이 예상외로 染色分體 異常型을 나타냈다.
2. BUdR 또는 IUdR의 단독처리도 染色體異常을 유발한다. 또한 MMS와 이중 처리한 경우에는 단독 처리보다 染色體異常型이 훨씬 증가한다. 따라서 thymidine 相似體는 MMS에 의한 染色體 異常型을 變化시켜 주는 感受性物質로 作用함이 판명되었다.

INTRODUCTION

It has been demonstrated that the treatment with alkylating agent to cells at various stages of the cell cycle induces different types and frequencies of chromosome aberrations (Kihlman, 1971). Evans and Scott (1969) reported that only chromatid type aberrations were scored at the first and second mitosis in *Vicia faba* cells following treatment with nitrogen mustard (HN_2). Chu (1969) working with *in vivo* cells of Ehrlich ascites tumor and rat bone marrow,

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showed that the chromatid type aberrations were occurred as late as 60 hours after ethyl- and methyl-methanesulfonate (EMS and MMS) treatment. Sasaki (1973) found in human lymphocytes that MMS-induced chromosome aberrations were essentially of the chromatid type and that S-stage cells were the most sensitive to MMS. Other workers, however, maintained that the chromosome type aberrations were also observed in human and plant cells after treatment with other chemicals (Nawar *et al.*, 1971 ; Bochkov and Kuleshov, 1972 ; Brooks and Greggar, 1973). It is, therefore, assumed that the sensitivities to alkylating agent in mammalian cells are not well established yet.

Although the mechanism involved in the induction of chromosome aberrations remains to be elucidated, the molecular action of MMS on DNA has been proposed. It first methylates DNA, primarily on N-7 of guanine and N-3 of adenine bases, the DNA then undergoes depurination and single strand breaks (Fox and Fox, 1973). Clarkson and Evans (1972) reported that DNA damage caused by MMS seems to be repaired by processes similar to those involved in the repair replication by ionizing radiation, which is associated with single strand breaks. This may strongly indicate that MMS-induced DNA damage is largely the same one as induced by ionizing radiation.

An incorporation of thymidine analogs, particularly 5-bromodeoxyuridine (BUdR), into DNA of mammalian cells has been suggested to increase the chromosome aberration and single strand breaks of DNA in irradiated cells following to these compounds (Dewey *et al.*, 1966 ; Lohman *et al.*, 1972). It is postulated that the substitution of bromouracil (BU) in the place of thymine enhances an increment of the primary lesions in DNA leading to single strand breaks (Sawada and Okada, 1972). Legator and Flamm (1973) suggested that these thymidine analogs would lead to base substitution resulted in an increase of purine bases by transition mutation. If so, the substituted DNA with these base analogs may provide a large proportion of the action site to be attacked by MMS. Park and Um (1975) recently confirmed that BUdR and 5-iododeoxyuridine (IUdR) were found to be potent chemical sensitizers on MMS-induced DNA repair synthesis, which is associated with the single strand breaks of DNA and their repair.

Though there has been made of chromosome aberrations induced by alkylating agent, quantitative data concerning the characterization of MMS-induced chromosome aberration have scarcely been reported. Moreover no one has yet attempted to study the effects of base analogs on chemical induced chromosome aberration in mammalian cells.

The present experiments were therefore carried out to determine the rate and type of chromosome aberrations and to elucidate whether thymidine analogs

would lead to a cumulative or enhancing effect on MMS-induced chromosome aberrations in human cells.

MATERIALS AND METHODS

1. Cell Culture

Materials used throughout the present investigation were short-term cultures of human lymphocytes. One hundred m*l* of venous blood obtained from healthy man were collected in heparinized syringe, transferred into blood separation vial (Difco) and allowed to stand for 30 minutes at 4°C. Leucocyte-containing upper plasma layer was drawn off with a Pasteur pipette and added to the growth medium in a ratio of 1 m*l* of plasma with leucocytes to 4 m*l* of T.C. medium 199 (Gibco) supplemented with 20% inactivated fetal calf serum and antibiotics (penicillin G, 100 units/m*l*; streptomycin, 100 μg/m*l*). The cultures were then stimulated with 0.1 m*l* of phytohemagglutinin-M (Difco) and incubated at 37°C.

2. Methyl methanesulfonate (MMS) Treatment

MMS (Eastman Kodak) was dissolved in phosphate buffered saline (PBS) as 1 M stock solution and further diluted to various working concentrations in the serum-free medium immediately prior to use. For the induction of chromosome aberration, the cultures grown for 48 hours were treated to MMS from 0.5 mM to 2.0 mM (Park and Um, 1975) for one hour at 37°C. After treatment with MMS, the cultures were washed twice with the medium and the second washing was replaced with the original growth medium and returned to incubator at 37°C.

3. Incorporation of Thymidine Analogs

5-bromodeoxyuridine (BUdR) and 5-iododeoxyuridine (IUdR) (Sigma) were prepared as 1 M stock solution in growth medium and further diluted to 2.0 mM working concentration prior to use. For the determination of the effects of these base analogs on MMS-induced chromosome aberrations, the cultures grown for 24 hours were exposed to BUdR or IUdR for 24 hours at 37°C. Immediately after treatment with BUdR or IUdR, the cultures were washed twice with growth medium and then followed by the treatment with MMS as described previously. The sterilization of the medium containing base analog or MMS was accomplished by passage through 0.45μ pore size membrane filter inserted into a Swinny hypodermic adapter.

4. Chromosome Preparation

The cultivation was terminated at 72 hours after initial incubation. Colcemid (Gibco) was treated to cultures for final two and half hours of incubation at a final concentration of 0.06 μg/m*l*. The cells were harvested, treated with

hypotonic solution (0.075M KCl) for 25 minutes at 37°C and then fixed in glacial acetic acid-ethanol(1 : 3). Chromosome preparations were made by air-drying technique and the dried slides were stained with the basic fuchsin staining for autoradiography (BFSA). Well spred metaphases were observed under oil immersion lens and the type of chromosome aberrations was scored according to the criteria of Kihlman (1971).

RESULTS

MMS-induced chromosome aberrations in human lymphocytes is shown in Table 1. Chromosome preparations were made at 24 hours after treatment with MMS in order to obtain chromosome type aberrations. Types of aberration in metaphases were divided into two groups, i.e., chromatid- and chromosome type aberrations, and deletions and exchanges were scored in each type of aberrations. As stated, chromatid deletions include gap, break and isochromatid break and chromatid exchanges contain interarm symmetric- and asymmetric exchanges, and dicentric and ring chromosomes are included in chromosome exchanges.

Table 1. Frequency of MMS-induced chromosome aberrations in human lymphocytes fixed at 24 hours after treatment.

Treatment	Total cells scored in mitosis	Normal metaphases (%)	Type of aberrations (%±S.E.)				Breaks*** /cell
			Chromatid-type		Chromosome-type		
MMS (mM)			Deletions*	Exchanges**	Deletions	Exchanges	
Control	100	97	3±1.7	–	–	–	0.03
0.5	100	75	18±3.8	17±3.8	4±2.0	2±1.4	0.41
1.0	100	69	26±4.4	51±5.0	4±2.0	9±2.9	0.90
2.0	100	60	39±4.9	50±5.0	8±2.7	6±2.4	1.02

*Chromatid deletions include gap, break and isochromatid break.
**Chromatid exchanges include interarm symmetric and asymmetric exchanges.
***Total breaks=deletions+2 (exchanges).

In the control, 97% of the cells showed normal metaphases and the rate of spontaneous aberration (breaks/cell) was 0.03 in which only chromatid deletions were observed. In MMS treated group, the percentage of normal metaphases was decreased with dose increased and the majority of aberrations were of chromatid type. However, chromosome type aberrations were also scored in a proportion of about 1/7 of total aberrations in all dose levels. In 0.5 mM MMS group, chromatid deletions and exchanges were found to be predominant aberration type with equal frequencies. However, in 1.0 mM group, chromatid exchanges were remarkably increased, and the aberration rate was about 2 folds than that of 0.5 mM group. The increased aberration rate was mainly

due to the increased frequency of chromatid exchanges. In 2.0 mM, deletions of both types were increased, whereas exchanges remained unchanged. The aberration rate of this group was slightly increased but this value was not marked comparing to the previous dose ranges.

These results indicate that MMS could cause not only chromatid-type but also chromosome-type aberrations, and that the aberration rates seem not to be dose dependent. These findings are not to be demonstrated in the previous workers.

Table 2 represents the effects of thymidine analogs on MMS-induced chromosome aberrations in human lymphocytes. As shown in the table, the single treatment with either of these base analogs also induces chromosome aberrations including both chromatid and chromosome-types. IUdR was found to be more effective chromosome breaking agent. In the combined treatment with BUdR+MMS, the percentages of abnormal metaphases were not changed and even the aberration rates of the latter dose levels were lower than those of corresponding single MMS treated group. However, the distribution of aberration type was quite different. In BUdR+0.5 mM MMS group, the aberration of chromosome type was about half value of total aberrations. But the rates were sharply increased at the latter two groups. In IUdR+MMS treated group, the percentage of abnormal metaphases and the aberration rate were slightly increased comparing to BUdR treated group. Chromosome type aberrations in both deletions and exchanges were markedly increased at 2.0 mM group.

Table 2. Effects of thymidine analogs on MMS-induced chromosome aberrations in human lymphocytes fixed at 24 hours after MMS treatment.

Treatments		Total cells scored in mitosis	Normal metaphases (%)	Type of aberrations (%±S.E.)				Breaks /cell
				Chromatid-type		Chromosome-type		
BUdR or IUdR+MMS				Deletions	Exchanges	Deletions	Exchanges	
BUdR 2.0	–	100	93	3±1.7	–	4±2.0	–	0.07
BUdR 2.0	0.5	100	75	19±3.9	13±3.4	7±2.6	10±3.0	0.49
BUdR 2.0	1.0	100	68	23±4.2	12±3.3	20±4.0	8±2.7	0.63
BUdR 2.0	2.0	61	56	25±5.5	8±3.5	23±5.4	16±4.7	0.72
IUdR 2.0	–	97	86	10±3.0	–	7±2.6	–	0.17
IUdR 2.0	0.5	98	66	25±4.4	21±4.1	5±2.2	4±2.2	0.55
IUdR 2.0	1.0	97	62	21±4.1	20±4.1	14±3.5	10±3.0	0.65
IUdR 2.0	2.0	57	54	19±5.2	12±4.3	26±5.8	18±5.1	0.75

Table 3 summarises the effects of thymidine analogs on MMS-induced chromosome aberrations. The percentage of abnormal metaphases was the highest in the IUdR+MMS group followed by BUdR+MMS and single MMS groups, respectively. These results do not seem to indicate that the single treatment

with MMS induces more chromosome aberrations than the combined treatment, because the percentages of abnormal metaphases were higher in the combined treated groups. Rather these may be due to the increased occurrence of multi-chromatid exchanges in the single treatment with 1.0~2.0 mM MMS treated groups. The occurrence of chromosome aberrations seems to be different in three experimental groups. Chromatid type aberrations were much more occurred in the single treatment with MMS, whereas both chromatid and chromosome-type aberrations were observed in the combined treatment with about equal frequencies.

Table 3. Comparisons of the induction of chromosome aberrations in three experimental groups.

Treatments	Normal meta-phases (%)	Average breaks/cell	Type of aberrations (%)	
			Chromatid-type	Chromosome-type
MMS	68	0.776	85.8	14.2
BUdR+MMS	66	0.613	54.3	45.7
IUdR+MMS	61	0.650	60.5	39.5

From the results of this investigation it may be concluded that thymidine analogs, BUdR and IUdR, were found to be sensitizers enhancing MMS-induced chromosome type aberrations in human cells.

DISCUSSION

The induced chromosome aberration is divided into two groups, chromatid and chromosome type aberrations. The former is characterized by a single break in one of two sister chromatids when cells are treated with chemical mutagen during or after DNA synthesis. The latter is represented by breakages in both chromatids at the same locus, which are occurred before DNA synthesis of the treated cells (Kihlman, 1971).

The present result showed that both chromatid and chromosome type aberrations were observed, although the arrested metaphases were expected to be in the pre-DNA synthetic stage when MMS treated. These data are consistent with those of published data (Nawar *et al.*, 1971 ; Bochkov and Kuleshov, 1972 ; Brooks and Greggar, 1973), which studied with other chemicals. Scott *et al.* (1974) recently suggested that alkylating agents require DNA synthesis for the formation of chromosome aberrations. Thus it seems likely that the aberration production induced by MMS is not direct but rather delayed effect.

Thymidine analogs have been demonstrated to increase the radiosensitivity of mammalian cells by virtue of their incorporation into DNA in place of

thymine (Sawada and Okada, 1972 ; Bender *et al.*, 1974). The radiosensitization effect by BUdR has been proposed that the substituted base enhances an increment of single strand breaks attributed to a random increase in energy absorption of the BU-containing DNA strand after irradiation (Lohman *et al.*, 1972). Park and Um(1975) reported that BUdR and IUdR were found to be potent chemical sensitizers enhancing MMS-induced single strand breaks of DNA and its repair. The present results also indicate that BUdR or IUdR affect to increase the chromosome-type aberration in MMS-treated cells. This may strongly suggest that these base analogs would enhance and potentiate the delayed effect of MMS to become a direct effect on chromosomes.

SUMMARY

Chromosome aberration induced by methyl methanesulfonate (MMS) and the effects of thymidine analogs (BUdR or IUdR) on MMS-induced chromosome aberration were studied in human lymphocyte cultures. Single treatment with MMS to lymphocytes induces both chromatid and chromosome type aberrations with high frequency of chromatid type. The combined treatment of BUdR or IUdR with MMS was found to be more effective in increasing the rate of chromosome type aberrations.

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