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Studies on the Chemical Mutagen-induced DNA Repair Synthesis in Relation to Chromosome Exchanges

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突然變異誘發原에 의한 DNA回復合成과 染色體交換과의 聯關性에 관한 硏究

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摘 要

DNA 回復合成과 染色體交換과의 聯關性을 추구하기 위해 알킬화제 突然變異誘發原인 MMC, MNNG, MMS를 培養한 사람의 淋把球와 HeLa S_3 細胞에 處理하여 다음과 같은 結果를 얻었다.

1. 이들 알킬화제에 의해 誘發된 DNA 回復合成은 MMC, MNNG, MMS의 濃度가 각각 3×10^{-7}M, 1×10^{-6}M, 5×10^{-4}M에서 檢出되었다. 이는 MMC가 가장 强力한 DNA 回復合成 誘發原이며 다음이 MNNG 그 다음이 MMS임을 뜻한다. MMC 와 MNNG는 濃度增加에 따른 DNA回復合成率에 큰 차이를 보이지 않으나 MMS는 현저한 增加를 보인다.

2. MMC에 의한 染色體異常은 濃度가 增加함에 따라 그率에 현저한 增加를 보이나 染色體交換率에는 별차이가 없다. 그러나 MNNG는 染色體異常率에 차가 없고 染色體交換은 觀察되지 않았다. MMS는 染色體異常 및 染色體交換 모두 濃度의 增加에 따른 그率의 增加를 나타낸다.

이러한 結果는 突然變異誘發原에 의한 DNA回復合成이 染色體交換 및 染色體異常과 직접적인 연관성이 없음을 시사하는 것이다.

INTRODUCTION

DNA repair synthesis and chromosome aberrations have been repeatedly suggested that these two biological phenomena are closely related in the processes leading to mutagenesis and/or carcinogenesis (Bender *et al.*, 1974 ; Evans, 1975).

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Direct evidences support for this speculation, however, are still meager. Data accumulated on irradiated mammalian system revealed that there is no apparent correlation between DNA repair synthesis and chromosome aberrations (Park, 1972, 1975 ; Cleaver, 1974 ; Scott *et al.*, 1974). Recent studies indicate that damages induced in DNA is not correlated to those induced in chromosomes in chemical-treated mammalian cells (Cleaver, 1976 ; Wolff *et al.*, 1977).

The results reported here also strongly suggest that chemical-induced DNA repair synthesis seems not to be related to chromosome aberrations in cultured human cells.

MATERIALS AND METHODS

Materials used throughout this investigation were an established human cell line, HeLa S_3 and short-term cultures of human lymphocytes. Monolayer cultures of Hela S_3 cells were grown in milk dilution bottles (Kimax, 120 m*l*) as a stock culture using Eagle's minimum essential medium (MEM) with Earle's salts and 10% fetal calf serum and antibiotics (penicillin G, 100 units/m*l*; streptomycin, 100 μg/m*l*), and maintained in an exponential growth by routine passage using EDTA. For lymphocytes culture, 10 m*l* of venous blood from healthy donor were collected in a 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 supplemented with 20% fetal calf serum and antibiotics. The cultures were then stimulated with 0.1 m*l* of phytohemagglutinin-M (Difco) and incubated at 37°C.

The experiments involving chemical-induced DNA repair synthesis were carried out using monolayer cultures of HeLa S_3 cells. Chemical mutagens used were methyl methanesulfonate (MMS, Eastman Kodak), N-methyl-N'-nitro-N-nitrosoguanidine (MNNG, Sigma) and mitomycin-C (MMC, Sigma). These were dissolved in water, (MMS and MMC), and in phosphate buffered saline (PBS) at pH 7.2 (MNNG) as a 1 M stock solution and further diluted to various working concentrations with serum-free medium that were to be added to cells 10~15 minutes prior to use. The sterilization of the medium containing mutagen was accomplished by passage through a 0.45 μ pore size membrane filter inserted into Swinny hypodermic adaptor. For the induction of DNA repair synthesis, an appropriate number ($0.5\sim1.0\times10^6$ cells/m*l*) of monolayer cultures grown on Borosilicate cover slips (9×50 mm, Bellco) in Leighton culture tubes for 24 hours were treated to various concentrations of these mutagens for 1~3 hours at 37°C. After treatment, the cells were washed twice with medium and the cells were immediately followed by isotope labeling. ^{3}H-thymidine (Amersham/Searle) was incorporated to the cultures at a final concentra-

tion of 10 $\mu Ci/ml$ (specific activity, 40 Ci/mM) for 1~1½ hours at 37°C. The incorporation of ^{3}H-thymidine was terminated by washing the cells from the cultures three tims in cold PBS containing 100 $\mu g/ml$ of unlabeled thymidine. The cells were treated with hypotonic solution (0.075 M KCl) and fixed in 3 : 1 methanol acetic acid. Prior to autoradiography, the cover slips were stained with basic fuchsin staining for autoradiography (BFSA) and mounted on the slide glasses with the cells uppermost. Autoradiograms were prepared using autoradiographic stripping plate (Kodak AR-10). Silver grains over the nuclei of labeled cells were observed using oil immersion lens and DNA repair synthesizing cells were identified according to the criteria as described previously (Park, 1975).

For experiments involving chemical-induced chromosome aberrations, lymphocytes grown for 48 hours at 37°C were collected by centrifugation at 800 rpm for 5 minutes and exposed to mutagens by replacing with the serum-free medium containing various concentrations of these mutagens. The treatment was for a period of one hour at 37°C. After treatment with mutagens, the cultures were washed twice with the medium and the second washing was replaced with the original growth medium and return to incubator at 37°C. The cultivation was terminated at 72 hours after the initial inoculation. Colcemid (Gibco) was added to the cultures for final two and half hours of incubation at a final concentration of 0.06 $\mu g/ml$. The cells were harvested, treated with hypotonic solution for 25 minutes at 37°C and then fixed in 3 : 1 methanol-acetic acid. Chromosome preparations were made by air drying technique and the dried slides were stained with BFSA. Well spred metaphases were observed under oil immersion lens and the type of aberrations was scored according to the criteria of Bender *et al.* (1974).

RESULTS

Labeling indices and levels of excision repair induced in HeLa S_3 cells treated with various concentrations of chemical mutagens are shown in Table 1. Labeling index represents percentage of total labeled cells including both semiconservative (heavily labeled) and unscheduled DNA synthesizing cells (lightly and evenly labeled). Levels of excison repair represent percentage of unscheduled DNA synthesizing cells.

In control, 40% of HeLa S_3 cells were labeled in which majority were of semiconservative DNA synthesizing cells. The spontaneous unscheduled DNA synthesis was also observed although the proportion was negligible.

The induced DNA repair synthesis was detected in MMS, MNNG and MMC treated cells at the concentrations above 5×10^{-4}, $1\times10^{-6}$and 3×10^{-7}M, respectively. The labeling indices in MMS treated cells increased in direct proportion to dose increased, whereas no significant changes were found in both MNNG and MMC

treated cells. The increased percentage of labeling indices was found to be mainly due to increases of lightly labeled cells. The rates of semiconservative DNA synthesis (data not presented) were not significantly changed except at higher doses of MMS and MMC, which showed a slight decrease in percentage. This indicates that the higher concentration of these mutagens may inhibit the normal semiconservative DNA synthesis. The dose response of DNA repair synthesis revealed that the amounts of excision repair were proportional to dose in MMS treated group, while the levels were not changed in given doses of MNNG and MMC groups. Thus the labeling index and the levels of excision repair are shown to be related.

From the table it may be concluded that MMC is the most potent mutagen in inducing the DNA repair synthesis followed by MNNG and MMS is the least potent, and that DNA repair synthesis induced by MMS is dose-dependent, but not those by MNNG and MMC in given dose ranges.

Table 1. DNA repair synthesis fixed immediately following treatment with chemicals and ^{3}H-thymidine labeling.

Treatment		Labeling index[a]	Level of excision repair[b]
Mutagens	M	(%)	(%±S.E.)
Control	—	39.6	1.6±0.1
MMS	5×10^{-4}	50.6	14.0±1.4
	1×10^{-3}	61.6	18.3±3.6
	2×10^{-3}	84.3	27.2±4.7
MNNG	1×10^{-6}	45.0	4.6±0.9
	5×10^{-6}	46.8	7.2±1.1
	1×10^{-5}	47.8	7.3±1.1
MMC	1.5×10^{-7}	41.3	2.6±0.7
	3×10^{-7}	46.2	7.3±1.1
	6×10^{-7}	46.6	7.5±1.1
	1.5×10^{-6}	43.7	8.8±1.2

a: Based on 500 cells analyzed.
b: Percentage of lightly labeled cells among total labeled cells.

Table 2 shows chemical-induced chromosome aberrations in human lymphocytes. Chromosome preparations were made at 24 hours after treatment with chemicals in order to obtain various types of aberrations. Types of aberrations in metaphases were divided into two groups, i.e., chromatid- and chromosome type aberrations, and deletions and exchanges were scored in each type. Chromatid deletion includes both chromatid and isochromatid deletions and chromatid exchanges contain interarm symmetric and asymmetric exchanges, and dicentric and ring chromosomes are included in chromosome exchanges.

Table 2. Chemical-induced chromosome aberrations in human lymphocytes fixed at 24 hours after treatment with chemicals.

Treatment		Normal meta-phases (%)	Types of aberrations (%±S.E.)				Exchanges /cell	Total breaks /cell
Mutagens	M		Chromatid-type Deletion	Chromatid-type Exchange	Chromosome-type Deletion	Chromosome-type Exchange		
Control		97	3±1.7	—	—	—	—	0.03
MMS	5×10^{-4}	75	18±3.8	99±1.8	4±2.0	1±0.7	0.1	0.31
	1×10^{-3}	69	26±4.4	26±2.5	4±2.0	5±1.5	0.3	0.61
	2×10^{-3}	60	39±4.9	25±2.5	8±2.7	3±1.2	0.28	0.75
MNNG	1×10^{-6}	86	8±2.7	—	9±2.8	—	—	0.17
	5×10^{-6}	79	2±1.4	—	10±3.0	—	—	0.12
	1×10^{-5}	70	12±3.2	—	11±3.1	—	—	0.23
MMC	3×10^{-7}	78	11±3.1	4±1.9	4±1.9	6±2.3	0.1	0.25
	6×10^{-7}	71	23±4.2	8±2.7	6±2.3	1±0.9	0.09	0.38

In control 97% of lymphocytes showed normal metaphases and the rate of spontaneous aberration (breaks/cell) was 0.03 in which only chromatid deletions were observed.

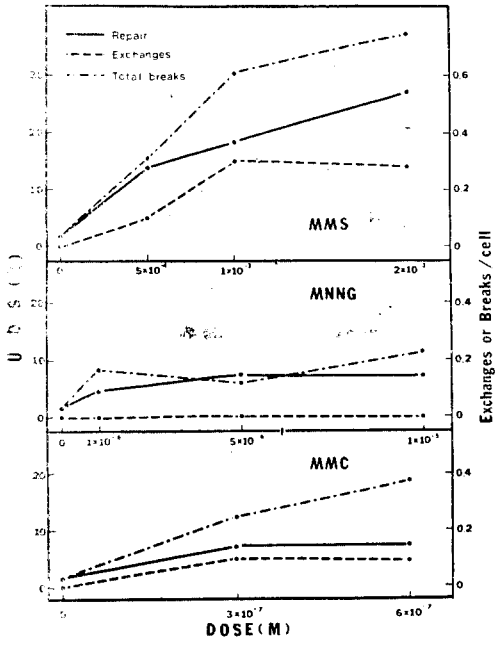

Fig. 1. Relationship between DNA repair synthesis, chromosome aberrations and exchanges in chemical—treated human cells.

In chemical treated groups, both chromatid and chromosome type aberrations were observed, and the percentage of normal metaphases decreased with dose increased. The majority of aberrations in MMS treated groups were of chromatid type, in which deletions increased with dose, but not exchanges at higher dose levels. In MNNG group, exchange type of aberrations were not observed and the aberration rate remained relatively unchanged in doses employed. The aberration rate in MMC group increased with dose and the increased aberration rate was mainly due to the increased frequency of chromatid deletions. These results indicate that alkylating agents

could cause not only chromatid-type but also chromosome-type aberrations, and that the induced aberration type and rate are different from different chemicals.

The relationship between DNA repair synthesis and chromosome aberrations is depicted in Fig. 1. In MMS treated group, rates of total aberration and DNA repair synthesis are proportional to dose increased, but the exchange rate remained unchanged at higher two dose levels. The aberration rate in MNNG group was marked at the initial dose and in latter two doses fluctuated. The rate of DNA repair synthesis was unchanged at higher two doses. In MMC treated group, the rates of DNA repair synthesis and exchanges remained constant, whereas the aberration rate was increased.

The overall results seem to suggest that DNA repair synthesis is not correlated with chromosome aberrations, although some parallel relations are shown in MMC treated group.

DISCUSSION

With regard to the possible involvement of primary damage induced in DNA and of its repair processes as the cause of chromosome aberration, there have been two divergent hypotheses so far proposed. The first affirmative postulation is that since DNA is the key substance in chromosome breakage and rejoining, essentially the same biological mechanisms are involved in the formation of chromosome aberration and in repair replication (Kihlman, 1971 ; Bender *et al.*, 1974) and post-replication repair (Evans, 1975). The second idea is that since the chromosome structure is quite different from the intact DNA molecule, the induced chromosome breaks and rejoining of chromosomes are the independent ability to repair processes of damaged DNA (Scott *et al.*, 1974 ; Cleaver, 1974).

Stich *et al.* (1973) and Sasaki (1973) reported, in Xeroderma pigmentosum (XP) cells, that normal excision of MMS or MNNG damage in DNA was correlated with normal levels of chromosome aberration and reduced repair of 4NQO damage was related to high levels of chromosome aberrations. These results suggest that unrepaired damage is correlated with high levels of chromosome aberrations. Recently, Wolff *et al.* (1977), however, showed that these chemical-induced repair replication was not correlated with the yield of sister chromatid exchanges in normal and XP cells. Although the mechanism involved in the formation of sister chromatid exchange is different from that leading to chromosome aberration, this may strongly indicate that chemical-induced damage in DNA and its repair is not correlated with damages in chromosomal level. Moreover, Cleaver (1976) suggested that sister chromatid exchange is closely correlated with the amount of unexcised damage in DNA. The speculation or the results of the other studies mentioned above are strongly supported by the data presented here which show that DNA repair synthesis may not be related to chromosome aberrations.

SUMMARY

DNA repair synthesis and chromosome aberrations induced by various concentrations of alkylating agents (MMS, MNNG, MMC) in cultured human lymphocytes and HeLa S_3 cells were studied to determine the possibility of correlation between these two types of biological phenomena, and the results obtained were as follows:

DNA repair synthesis was detected in MMC, MNNG and MMS treated HeLa S_3 cells at the concentrations of 3×10^{-7}M, 1×10^{-6}M, 5×10^{-4}M, respectively. These results indicate that MMC is the most potent mutagen followed by MNNG, and MMS is the least potent among these three types of alkylating agents. MMC and MNNG did not show any significant increases of DNA repair synthesis as dose increased, while MMS did.

Chromosome aberrations induced by MMC in human lymphocytes was increased as dose increased, but not chromosome exchanges. MNNG did not induce any significant amount of chromosome aberrations with doses, and exchanges were not observed in MNNG treated cells. MMS, however, induced both chromosome aberrations and exchanges, and their rates were increased as dose increased.

These results suggest that DNA repair synthesis induced by these alklating agents may not be directly related to the production of chromosome aberrations and exchanges.

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