The Repair of MNNG-Induced DNA Damage and Its Relation to Chromosome Aberrations in Mammalian Cells

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MNNG에 의한 DNA 회복합성과 염색체 이상과의 연관성에 관한 연구

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적   요

DNA 회복합성과 염색체이상과의 연관성여부를 추구하기 위하여 CHO 세포를 재료로 MNNG의 농도와 처리후 시간경과에 따른 절제회복율을 조사하고 이들 염색체 이상율과 비교하여 다음과 같은 결과를 얻었다.

1. MNNG에 의한 절제회복은 $0.5 \times 10^{-5}M$에서 $5.0 \times 10^{-5}M$ 까지 농도의 증가에 따른 절제회복율의 증가를 보인다. 또 $1 \times 10^{-5}M$ 처리후 0시간째는 절제회복율의 최고치를 보이다가 그후 점차 감소하여 24시간에는 0시간의 66%정도 나타난다.

2. MNNG에 의한 염색체이상은 $1 \times 10^{-5}M$ 처리후 6시간에 최대치를 보이며 이상형의 대부분은 염색분체 절단을 나타낸다. 그러나 시간이 경과함에 따라 염색분체절단은 감소하고 염색분체교환은 증가하여 24시간에는 두종류의 이상율이 비슷하게 이른다.

3. MNNG 처리후 시간경과에 따른 절제회복율은 전체이상율과 대체로 일치한다. 그러나 염색분체교환 또는 염색분체절단과는 어떤 비례관계도 보이지 않는다. 따라서 이같은 결과들은 MNNG에 의한 DNA 상해 및 그 회복은 염색체의 회복 현상과는 연관성이 없음을 암시하는 것이다.

INTRODUCTION

Ever since the DNA repair was postulated as one of repair processes of damaged DNA (Rasmussen and Painter, 1964), a number of investigator have attempted to shed some light on the relationship between DNA repair and other cellular recovery processes. Among these, studies on the possible involvement of primary damage induced in DNA as the cause of chromosome aberration have been a topic of repeated discussions (Kihlman, 1971; Evans, 1977; Sognier and Hittelman, 1979; Fornace et al., 1980).

It is considered that the DNA moiety of chromosome is the molecular species principally involved in the formation of chromosome aberrations (Kihlman, 1977). Evans (1966) was first to suggest that the primary lesions induced in chromosomes may be repaired by processes similar to those responsible for repair replication. This idea was later supported by other workers using mammalian cells exposed to radiations and chemicals (Stich et al., 1973; Sasaki, 1975).

Not all of the facts, however, fit this concept of the molecular mechanism of aberration formation. Wolff and Scott (1969) reported that cells from *Vicia faba,* xeroderma pigmentosum (XP) and Chinese hamster were able to produce chromosome aberrations, although they showed a reduced rate or absence of unscheduled DNA synthesis. These findings were subsequently confirmed in the same or other cells treated with radiations and chemicals (Painter and Wolff, 1973; Scott et al., 1974; Park et al., 1976). Therefore, this problem still remains an unsolved question.

Recent studies, however, seem to suggest there is no apparent relationship between the DNA repair and chromosome aberrations (Wolff et al., 1977; Soniger and Hittelman, 1979). The data reported here also indicate that excision repair induced by MNNG seems not to be related to chromosome aberrations.

MATERIALS AND METHODS

An established mammalian cell strain, Chinese hamster ovary (CHO) cells were used throughout this investigation. Monolayer cultures of this cell lines were grown in humidified 5% CO_2 incubator at 37°C using Eagle's minimum essential medium (MEM: Grand Biological Co.) supplemented with 15% fetal calf serum, penicillin G (100 units/ml) and streptomycin (100 μg/ml).

N-methyl-N-nitro-N-nitrosoguanidine (MNNG; Sigma Chemical Co.) was prepared as 1 M stock solution in Dulbecco's phosphate buffered saline (PBS) and further

diluted to various working concentrations with the serum-free medium prior to treatment. MNNG treatment was performed at 37°C for desired time.

For the determination of MNNG-induced chromosome aberrations, CHO cells grown in milk dilution bottles for more than 12 hours were treated to this chemical for an hour. The cultures were washed, replaced with fresh medium and then incubated for desired time. Colcemid ($0.06\ \mu g/ml$) was added during the final two hours of incubation. The mitotic cells were harvested by gentle shaking off the bottles, treated with hypotonic solution ($0.05\ M$ KCl) and then fixed in 3 : 1 methanol-glacial acetic acid. Chromosome preparations were made by the air-drying technique and stained with 4% Giemsa (Gurr's R66: Bio/medical Specialities). Chromosome aberrations were scored according to the criteria of Evans (1977).

For the determination of the excision repair ability, cells (5×10^4 cells/ml) were grown on cover slips (25×22 mm) in plastic petri dishes for 24 hours. ^{3}H-thymidine ($5\ \mu Ci/ml$, $40\sim60$ Ci/mM) was incorporated into the cells for an hour immediately after exposure to the chemical. Labeling with ^{3}H-thymidine was terminated by washing the cells three times in cold Hank's balanced salt solution (HBSS) containing 100 $\mu g/ml$ of unlabeled thymidine. Cells were fixed in 3 : 1 methanol-glacial acetic acid and soaked overnight in 4% perchloric acid (PCA) at 4°C. The cover slips were mounted on the slides with the cells uppermost. Autoradiograms were prepared using Kodak NTB liquid nuclear track emulsion (Eastman Kodak Co.). Silver grains over the nuclei in the lightly labeled cells were counted as described previously (Park et al., 1976).

RESULTS

These experiments were concerned with the determination of the possible relationship between the excision repair and chromosome aberrations induced in mammalian cells treated with MNNG.

Table 1 represents the dose response of DNA repair synthesis in CHO cells

Table 1. Dose response of DNA repair synthesis in CHO cells treated with MNNG

Treatment MNNG (M)		Average grains/cell* (mean±S.E.)
Control		4.1±0.5
MNNG	0.5×10^{-5}	27.4±1.5
	1.0×10^{-5}	30.0±4.3
	2.0×10^{-5}	37.2±5.1
	5.0×10^{-5}	40.5±1.8

* 50 cells were analyzed for grain counting. MNNG for 1 hour, ^{3}H-TdR for 1 hour and exposuring for 3 days.

treated with MNNG. The rates of DNA repair synthesis, represented as an average number of grains per cell, were dose-dependent and increased up to the maximum concentrations. This result indicates that MNNG is a potent DNA damaging agent which can induce base excision repair in CHO cells.

Table 2 shows the time dependence of DNA repair synthesis in CHO cells determined at various time intervals after exposure to MNNG. The maximum rate of DNA repair synthesis was occurred in the cells soon after the treatment. The excision repair was then gradually decreased with time and detected even at 24 hours after treatment with high frequency, appearing about 66% of 0 hour. This result suggest that the DNA damage induced by MNNG is long lasting damage to be repaired.

Table 2. Time dependence of DNA repair synthesis in CHO cells fixed at various times following treatment with MNNG and ^{3}H-thymidine.

Treatment MNNG (M)	Time after ^{3}H-TdR (hr)	Average grains/cell* (mean±S.E.)
—	0	4.9±2.8
1×10^{-5}M	0	50.3±3.5
1×10^{-5}M	0.5	48.2±2.6
1×10^{-5}M	2	46.5±3.7
1×10^{-5}M	6	43.3±2.4
1×10^{-5}M	12	35.3±2.7
1×10^{-5}M	24	33.1±2.2

* 50 cells were analyzed for grain counting. MNNG for 1 hour, ^{3}H-TdR for 1 hour and exposuring for 4days

Chromosome aberrations induced in CHO cells fixed at various times following the treatment with MNNG is shown in Table 3. In the control, 96% of the cells scored showed normal metaphases and the rate of spontaneous aberrations was 0.04 breaks/cell. In MNNG treated group, percentages of normal metaphases increased and the

Table 3. MNNG-induced chromosome aberrations in CHO cells fixed at various times after treatment with MNNG.

Treatment MNNG (M)	Times after treatment (hr)	Normal metaphase (%)	Type of aberrations				Total breakes per cell
			Chromatid-type		Chromosome-type		
			Deletion	Exchange	Deletion	Exchange	
Control	6	96	4± 1.0	—	—	—	0.04
MNNG 1×10^{-5}	6	47	127±29.2	15± 3.6	2±1.7	—	1.44
MNNG 1×10^{-5}	12	55	86± 3.0	47± 1.0	2±1.0	1±1.0	1.36
MNNG 1×10^{-5}	24	58	53±25.0	56±24.6	7±3.9	2±1.2	1.18

* Based in the 100 cells analyzed in each group.

total aberrations per cell decreased with time. The majority of aberrations were chromatid deletions in the cells fixed at six hours after the treatment. However, the amount of deletions decreased, whereas exchanges increased with time after MNNG treatment, resulting in about the same amounts of deletions and exchanges appeared at 24 hours. These results may indicate that the repair of chromosome aberrations were continued up to 24 hours by which broken ends were rejoined to

Table 4. Comparisons of chromosome aberrations and excision repair in CHO cells fixed at various times following treatment with MNNG.

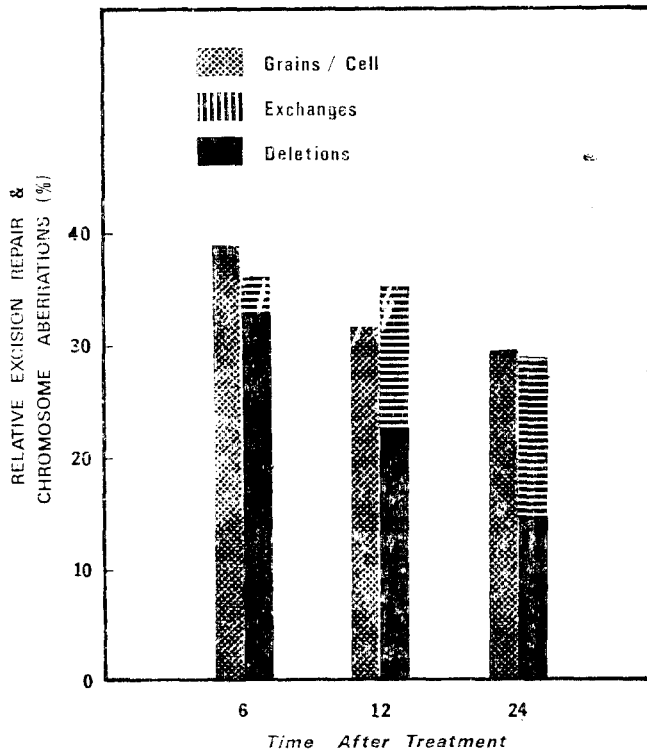| Time after	Relative excision repair	Total breaks	Deletions	Exchanges
	grains/cell±S.E. (%)	%	Total deletion % (fraction)	Total exchange % (fraction)
6	43.3±2.4 (38.8)	1.44 (36.0)	129 / 46.6 (90.0)	15 / 12.4 (10.0)
12	35.3±2.7 (31.6)	1.36 (34.5)	88 / 31.8 (64.7)	48 / 39.7 (35.3)
24	33.1±2.2 (29.6)	1.18 (29.5)	60 / 21.6 (50.8)	58 / 47.9 (49.2)

Fig. 1. Comparisons of excision repair and chromosome aberrations induced by MNNG in CHO cells.

form the exchange type of aberrations.

The relationship between excision repair and chromosome aberrations in summarized in Table 4 and Fig. 1. As shown in the table, the rates of excision repair do not show a correlation with either deletions or exchanges in each time group, although total aberration yields are related to the amount of excision repair. The overall results of these experiments suggest that the excision repair may not be related to chromosome aberrations in MNNG treated CHO cells.

DISCUSSION

DNA repair and chromosome aberrations have been repeatedly suggested that these two biological phenomena are closely related (Kihlman, 1977). One of the reasons supporting this idea is that agents which are known to induce DNA damage are also potent inducer of chromosome aberrations. Thus it was postulated that chromosome aberrations are produced by the misrepair of DNA damage (Bender et al., 1974). Sasaki (1973) reported that normal excision of MMS or MNNG damage in DNA was correlated with normal levels of chromosome aberrations and reduced repair of 4NQO damages was related to high levels of chromosome aberrations. These results indicated that unrepaired damage is correlated with high levels of chromosome aberrations. Wolff (1978), also suggested that the aberration yields are related to the amount of excision repair. The data presented here showed that the amount of excision repair seemed to be roughly related to the total aberrations yields, but not to be related to either deletion or exchange type of aberration.

There are several reasons why a relationship between excision repair and chromosome aberration does not seem to be likely. Among them, (1) damage to DNA alone would have minimal effects of the overall chromosome structure, whereas damage to protein would result in disruption of the chromosomes, thus breaks in DNA would be a secondary consequences of the protein damage (Cleaver, 1974), (2) cycloheximide, hydroxyurea and 5'-fluorodeoxyuridine, which produce chromosome damage by preventing repair, do not inhibit excision repair (Gautschi et al., 1973). Sognier and Hittelman (1979) recently found in bleomycin treated CHO cells that cycloheximide and streptovitacin A blocked chromosome repair but had little measurable effect on DNA repair. They suggested that the DNA lesions responsible for chromosome damage represent only a small portion for the total DNA lesions. The above results or suggestions strongly indicate that chromosome breaks and rejoining that lead to the formation of chromosome aberrations are not necessarily relatied to the excision repair of DNA damage.

However, it was reported that DNA damage may be circumvented by post-repli-

cation repair which is suggested to involve in DNA replication and recombination between duplexes (Lehmann et al., 1975). This repair mechanism was directly applied for the interpretation of chromosome aberration in which DNA damage and its repair are the molecular manifestation of chromosome aberrations. This interpretation was supported by observations that the frequency of chromosome aberrations induced by alkylating agents was markedly increased by caffeine, an inhibitor of post-replication repair in mammalian cells (Kihlman, 1977). Scott (1977) reported that Yoshida lymphosarcoma (YS) cells sensitive to sulphur mustard showed much more chromosome damage but less capacity for post-replication repair than resistant Yoshida tumor (YR) cells. From these observations he suggested that failure to perform the gap-filling process of post-replication repair results in chromosome aberrations which lead to cell death. Similar idea was also presented by Evans (1977) that the lesions produced by UV-light and alkylating agents develop into chromosomal aberrations as a result of error during replication.

Cleaver (1977), however, claimed that post-replication repair may not be involved in chromosome aberrations and sister chromatid exchanges based on the following two reasons. The first, this specific repair process in known as an early, transient response in replication (Lehmann et al., 1975), whereas chromosome aberrations can be generated long after lesions have been introduced into DNA. The second, XP-variant, which is defective in post-replication repair (Lehmann et al., 1975), shows normal levels of chromosome aberrations. Many other investigators have also maintained that DNA repair is the mechanism primarily involved in the formation of chromosome aberration in mammalian cells treated with metabolic inhibitors and chromosome damaging agents (Hartley-Asp, 1979; Preston, 1980).

Although there have been made an extensive studies on the relationship between DNA repair and chromosome aberrations, no convincing evidence for this relationship has been so far presented. Therefore it is not possible, at the present, to pinpoint any type of DNA lesion or specific repair process associated exclusively to chromosome aberrations.

ABSTRACT

The rates of excision repair at various doses and times after MNNG treatment in CHO cells were compared with the frequencies of chromosome aberrations to determine a possible relation between there two types of biological phenomena, and the results obtained were as follows:

1. The MNNG-induced excision repair was dose-dependent in the ranges between 0.5×10^{-5}M and 5.0×10^{-5}M. The maximum rate of excision repair was occurred in the cells soon after the treatment. The rates were then gradually decreased

and appeared about 66% of 0 hour at 24 hours.

2. The rates of chromosome aberrations induced by MNNG was the highest at 6 hours, in which majority were chromatid deletions. The rates of chromatid deletions decreased, whereas chromatid exchanges increased with time, resulting is about equal rates at 24 hours after treatment.

3. The rates of excision repair at different times after MNNG treatment were roughly related to the total breaks per cell. The rates, however, did not show any relation to either chromatid exchanges or deletions. These results may suggest that excision repair may not be directly related to chromosome aberrations in MNNG treated CHO cells.

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