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Studies on Chromosome Aberrations Induced by N-ethyl-N-nitrosourea and N-methyl-N-nitrosourea in CHO cells

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N-ethyl-N-nitrsourea와 N-methyl-N-nitrosourea에 의한 CHO 세포의 염색체 이상에 관한 연구

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적 요

CHO 세포를 재료로 ENU와 MNU를 여러농도로 처리한 후 시간 경과에 따른 염색체 이상율을 조사하여 다음과 같은 결과를 얻었다.

(1) ENU와 MNU에 의한 염색체 이상 빈도는 처리 후 시간의 경과와 농도에 따라 상이한 현상을 나타냈다.

(2) ENU 처리군에서는 염색체 이상형은 염색분체 절단이 대부분이었으나 고농도군 (10^{-3} M)에서는 처리 후 24시간에 염색분체 교환이 염색분체 절단을 능가하였다.

(3) MNU 처리군에서는 염색체 이상형은 염색분체 절단이 대부분이었으나 10^{-4} M 이상에서는 처리후 12시간부터 염색분체 교환이 염색분체 절단을 능가하였다.

INTRODUCTION

The effects of chemicals on chromosomes have been studied for several decades, but only during recent years the significance of this research have been emphasized. Because chromosomes represent the vehicles of genetic material, studying the chemical effects on chromosomes has important aspects; (1) identifying toxic agents and their degree of damage, and (2) interpreting the structure and function of chromosomes by chemical

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action (Wolff and Scott, 1969; Bender *et al.*, 1974; Park *et al.*, 1976; Hsu and Au, 1979).

Since N-nitroso compounds were reported as potent mutagenic carcinogen, many investigators have attempted to study the role of these chemicals on mutagenesis and/or carcinogenesis by using various systems at various levels of inquiry (Walker *et al.*, 1973; Goth *et al.*, 1974; Cleaver *et al.*, 1977; Thust *et al.*, 1980). However, the effect of these compounds on chromosome levels has not been well established.

The purpose of the present investigation was, threfore, to learn the kinetics of chromosome aberration induced by some N-nitroso compounds at various doses and times after treatment.

MATERIALS AND METHODS

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 as stock cultures using Eagle's minimum essential medium (MEM: Grand Island Biological Co.) supplemented with 10% fetal calf serum, penicillin G (100 units/ml) and streptomycin (100 ug/ml).

N-ethyl-N-nitrosourea (ENU) and N-methyl-N-nitrourea (MNU) were dissolved as 0.1 M stock solution in acetone and further diluted to various working concentrations with the serum-free medium prior to treatment. Final concentration of acetone did not exceed 0.2%. Since previous studies showed that both chemicals were rapidly decomposed, a fresh stock solution was prepared for each experiment.

For the determination of chromosome aberrations CHO cells grown in milk dilution bottles for more than 12 hours were incubated with the serum-free medium containing ENU or MNU for 30 minutes, respectively. After treatment with chemicals, the cells were washed, replaced with growth medium and then incubated for desired time. Colcemid was added during the final two hours of incubation. The mitotic cells were harvested by gentle shaking off the bottles, treated with hypotonic solution (0.075 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 R 66: Bio/medical Specialities). Well spread metaphases were observed using oil immersion lens and the type of chromosome aberrations was scored according to the criteria of Evans (1977).

RESULTS

The present study was concerned with the chromosome aberration induced by ENU and MNU at various doses and times after treatment with these chemicals. During these experiments it became obvious that the frequency of chromosomal aberrations drastically

Table 1. ENU-induced chromosome aberrations in CHO cells fixed at various times and doses after treatment with ENU*

Treatment ENU (M)	Time after treatment (hr)	Aberrant metaphase (%)	Type of Aberrations: Chromatid type deletion	Chromatid type exchange	Chromosome type deletion	Chromosome type exchange	break/cell
control	6	5	7	1	—	—	0, 05
0. 2% Acetone only	6	7	7	1	—	—	0. 07
10^{-5}	6	20 ±1	18. 3± 0. 41	2. 0±0	4. 5±0. 5	0	0. 25
10^{-5}	12	18 ±1	14 ± 1. 0	4 ±1. 0	1 ±0	2 ±0	0. 21
10^{-5}	24	15 ±3	13. 5± 5. 5	—	3. 5±0. 5	2 ±0	0. 19
10^{-4}	6	22. 5±2. 5	20 ± 2. 0	2. 0±0	4. 5±2. 5	—	0. 27
10^{-4}	12	18 ±1. 0	13. 1± 0. 5	—	4. 5±0. 5	6. 0±1. 0	0. 24
10^{-4}	24	19 ±1. 0	17. 5± 0. 5	—	3. 5±2. 5	2. 0±1. 0	0. 23
10^{-3}	6	43 ±4. 0	44. 5± 6. 5	10 ±1. 0	5 ±2	5 ±1. 0	0. 65
10^{-3}	12	30 ±2. 5	21 ± 2. 5	12 ±2. 0	11 ±2. 0	4 ±1. 0	0. 48
10^{-3}	24	79. 3±4. 70	61. 3±10. 5	96. 3±4. 1	10. 7±1. 5	16. 3±2. 4	1. 85

* Based in the 300 cells analyzed in each group.

Table 2. MNU-induced chromosome aberrations in CHO cells fixed at various times and doses after treatment with MNU

Treatment MNU (M)	Time after treatment (hr)	Aberrant metaphase (%)	Type of aberrations: chromatid type deletion	chromatid type exchange	chromosome type deletion	chromosome type exchange	break/cell
10^{-5}	6	14. 33±1. 86	9 ± 2. 08	2 ± 1. 0	5 ±1. 73	—	0. 16
10^{-5}	12	15 ±1. 0	17 ± 2. 0	—	2 ±1. 0	1. 0±0. 5	0. 20
10^{-5}	24	13. 17±1. 62	10. 83± 1. 82	2. 34±0. 65	3. 5 ±0. 92	1. 7±0. 48	0. 18
10^{-4}	6	22. 75±4. 79	12. 75± 2. 32	3. 5 ±1. 18	3. 75±1. 03	4 ±1. 0	0. 24
10^{-4}	12	24 ±1. 0	24 ± 1. 0	4. 5 ±1. 0	3 ±1. 0	—	0. 32
10^{-4}	24	27 ±7. 87	21. 75± 5. 56	6 ±2. 04	9. 75±3. 50	2. 5±0. 95	0. 40
10^{-3}	6	38. 33±6. 97	40. 33± 9. 9	13. 0 ±2. 19	7. 17±3. 97	3. 0±0. 55	0. 64
10^{-3}	12	43. 5 ±2. 50	24. 5 ± 5. 50	52 ±6. 0	6. 5 ±1. 5	3. 0±0. 5	0. 86
10^{-3}	24	74. 5 ±2. 75	67. 25±12. 44	112. 5 ±19. 9	24. 75±5. 40	6. 5±1. 32	2. 11
10^{-4}	48*	39	27	12	17	2. 5±0. 5	0. 59
10^{-3}	48*	99	**				

* Based in the 100 cells analyzed in each group.
** Uncountable due to the increased number of aberrations

depends on the length of the post-treatment period.

Chromosome aberrations induced in CHO cells fixed at various times following the treatment with ENU are shown in Table 1 and 2. In control group, 5% of the cells

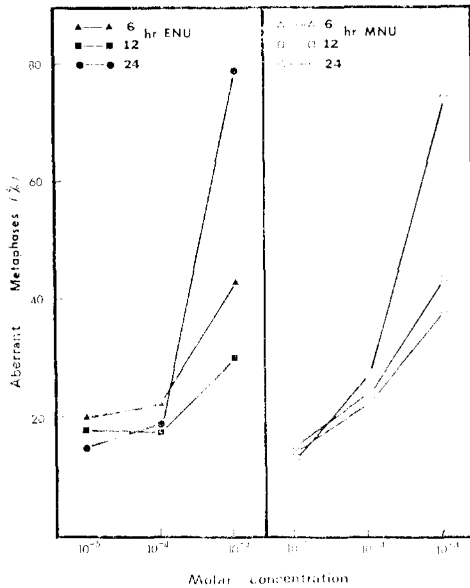

Fig. 1. Comparisons of aberrant metaphases induced by ENU and MNU, respectively.

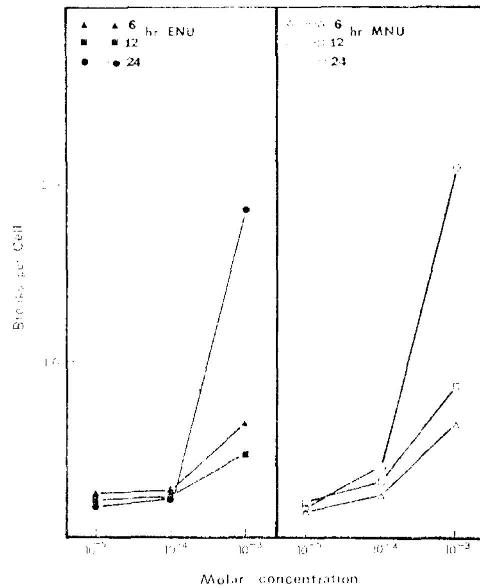

Fig. 2. Comparisons of chromosome breaks per cell exposed to ENU and MNU.

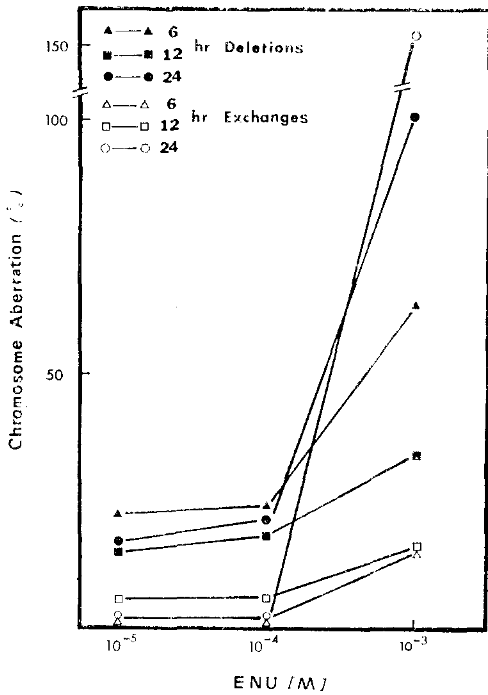

Fig. 3. Comparison of chromosomal deletions and exchanges induced by ENU in CHO cells

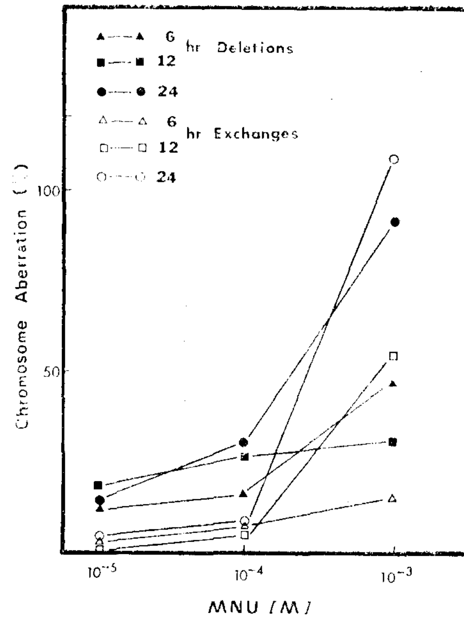

Fig. 4. Comparison of chromosomal deletions and exchanges induced by MNU in CHO cells

showed aberrant metaphases and the rate of spontaneous aberrations was 0.05 break per cell. The frequency of chromosome aberrations observed in the cells treated with 0.2% acetone was 7%, suggesting that there are no significant increases in numbers of chromosome aberrations after treatment with this solvent alone. Because frequencies of aberrant metaphases induced by ENU and MNU were increased up to 24 hours after treatment, the pilot studies with MNU were investigated. These pilot studies showed that frequencies of aberrant metaphases induced by MNU were increased until 48 hours after treatment with 10^{-4} and 10^{-3} M MNU. As shown in Table 2, the frequency of aberrant metaphases treated with 10^{-3} M MNU was uncountable due to the increased number of aberrations at 48 hours after treatment. In this group, the majority of aberration was chromatid exchanges (Fig. 5C).

The percentages of aberrant metaphases and the rates of chromosome aberration induced by ENU and MNU depicted in Fig. 1 and 2. The frequencies of chromosome aberrations induced by each chemical were found to be dose dependent, but each compound showed a characteristic time course aberration induction. ENU was more effective for induction of chromosome aberrations than MNU in earlier samples, whereas MNU was rather more effective in later samples.

The comparison of deletions and exchanges induced by ENU and MNU was shown in Fig. 3 and 4. The major type of aberrations induced by ENU was chromatid deletions except 10^{-3} M group at 24 hours after treatment, but chromatid exchanges were predominant in 10^{-3} M group at 24 hours. In MNU-treated groups, the majority of aberrations was chromatid deletions in both 10^{-5} and 10^{-4} M groups, but chromatid exchanges were predominant in 10^{-3} M group after 12 hours. ENU was found to be more effective than MNU for induction of chromosome aberrations in CHO cells in 10^{-5} M group, whereas MNU was more effective than ENU in 10^{-4} and 10^{-3} M groups.

The above results may suggest that the repair of chromosome aberrations in CHO cells were continued more than 24 hours.

DISCUSSION

The present results indicate that chromosome aberrations induced by ENU and MNU in CHO cells seem to be drastically dependent on the duration of the post-treatment period, and that the aberration kinetics of these closely related compounds may be different.

Alkylation or methylation in O^6-position of guanine base has recently been received much attention as highly mutagenic and potentially carcinogenic lesions. Suter *et al.* (1980) suggested that CHO cells can excise O^6-methyl- but not O^6-ethylguanine from DNA. This may indicate that in CHO cells ethylating agents seem to be more effective in mutagenic action than methylating agents. The present data showed that ENU was more effective for induction of chromosome aberrations than MNU in earlier samples, whereas MNU

was more effective in later samples. Ishidate *et al.* (1977) reported that N-nitrosourea derivatives produced more aberrations at 48 hours, and that N-nitrosoguanidine derivatives gave more aberrations at 24 hours than 48 hours after treatment. The present results and other published data (Kim and Lee, 1980) are good accordance with this investigation.

Thust *et al.* (1980) reported that for all nitrosamides tested with Y79-E cells, chromatid exchanges were the most frequent type of chromosome aberration followed by chromatid breaks, and that no sequence of aberration types, i.e., chromatid breaks, isochromatid breaks, depending on the length of the post-treatment period, was observed. However, derived aberrations such as dicentric and ring chromosomes were usually occured in later samples. The present data revealed that deletions occured in earlier, but exchanges in later samples.

Thust *et al.* (1980) suggested that the 24 hours schedule in clastogenicity test may give a coarse clue for mutagen and/or carcinogen activity, resulting in misleading conclusion. The sampling time, therefore, is essential role in obtaining reliable data for clastogenicity study and this has been repeatedly emphasized for nitroso compounds (Soukup *et al.*, 1976; Sanger *et al.*, 1976; Ishidate *et al.*, 1977).

The present data showed that CHO cells had already undergone 2-3 cell cycles when maximal aberration rates occurred. This result may indicate that chromosomal defects were determined immediately during treatment but the lesions occurred several cell cycles later. If chromosomal aberrations are a consequence of misrepair or mispairing of alkylated sites, this would imply that DNA may replicate repeatedly despite the presence of alkylated base. This is to be expected from the data on the persistence of alkyl derivatives in nucleic acids (Singer, 1979).

ABSTRACT

Chromosome aberrations induced by ENU and MNU were investigated in CHO cellls at various doses and times after treatment. The results obtained were as follows: The frequency of chromosome aberrations induced by ENU and MNU drastically depends on the length of the post-treatment period and the concentration of these chemicals. In ENU-treated groups, the major type of aberration was chromatid deletions in earlier samples but the frequency of chromatid exchanges increased with time, revealing, predominant type at 24 hours after treatment with 10^{-3} M. In MNU-treated groups, chromatid deletions were also major type but frequency of chromatid exchanges were predominant from 12 hours after treatment with 10^{-4} and 10^{-5} M.

REFERENCES

Bender, M.A., H.G. Griggs and J.S. Bedford., 1974. Mechanism of chromosomal aberration. III.

Chemicals and ionizing radiation. *Mutation Res.* **23**:197-212.

Cleaver, J.E., 1977. DNA repair mechanisms and the generation of sister chromatid exchange in human cell lines from xeroderma pigmentosum patients. *In*: Molecular human cytogenetics (R.S. Sparkers *et al.*, editors). Academic press. pp. 341-354.

Evans, H.J., 1977. Molecular mechanism in the induction of chromosome aberrations. *In*: Progress in Genetic Toxicity (D. Scott, B.A. Bridges and F.H. Sobels, editors). Elsevier/North Holland. pp. 24-51.

Goth, R. and M.F. Rajewsky, 1974. Persistence of ethylguanine in rat brain DNA: Correlation with nervous system-specific carcinogenesis by ethylnitrosourea. *Proc. Nat. Acad. Sci.* **71**:639-643.

Ishidate, M.J., and S. Odashima, 1977. Chromosome tests with 134 compounds on Chinese hamster cells *in vitro*, A screening for chemical carcinogens, *Mutation Res.* **48**:337-354.

Hsu, T.C. and W. Au., 1979. Effects of chemical mutagens on chromosomes. *In*: Effects of drugs on the cell nucleus (Busch, H., S.T. Crooke and Y. Daskal, editors). Academic press. pp. 315-332.

Kim, C.K. and C.B. Lee., 1980. The repair of MNNG-induced DNA damage and its relation to chromosome aberrations in mammalian cells. *Korean J. Zool.* **23**(3):115-123.

Park, S.D., K.I. Um and K.H. Choi., 1976. Studies on chemical mutation-induced DNA repair synthesis in relation to chromosome exchange. *Korean J. Zool.* **19**: 179-186.

Sanger, W.G. and J.D. Eisen., 1976. Clastogenic effects of methylnitrosourea and ethylnitrosourea on chromosome from human fibroblast cell lines. *Mutation Res.* **34**:415-426.

Singer, B., 1979. N-Nitroso alkylation agents; Formation and persistence of alkyl derivatives in mammalian nucleic acids as contributing factors in carcingenesis. *J. Hatl. Cancer Inst.* **62**:1329-1339.

Soukup, S.W., W. Au., 1975. The effects of ethylnitrosourea on chromosome aberrations *in vitro* and *in vivo. Humangenetic.* **29**:319-328.

Suter, W., J. Brennand, S. MoMillen, and M. Fox, 1980. Relative mutagenicity of antineoplastic drugs and other alkylating agents in V 79 Chinese hamster cells, in dependence of cytotoxic and mutagenic reponses. Elsevier/North-Holland Biomedical press. pp. 171-181.

Thust, R., J. Mendel, H. Schwarz, and R. Warzok., 1980 Nitrosated urea pesticide metabolites and other nitrosamides activity in clastogenicity and SCE assays, and aberration kinetics in Chiness hamster V 79-E cells. *Mutation Res.* **79**:239-248.

Walker, I.G. and D.F. Ewant, 1973. Repair synthesis of DNA in HeLa and L-cells following treatment with methylnitrsourea or ultraviolet light. *Can. J. Biochem.* **51**: 148-157.

Wolff, S. and D. Scott., 1969. Repair of radiation-induced damage to chromosomes. *Exp. Cell Res.* **55**:9-16.