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Effect of Mitomycin C on Chinese Hamster Cells

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培養한 Chinese Hamster 細胞에 미치는 Mitomycin C의 影響

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摘 要

*Streptomyces caespitosus*에서 分離된 抗生物質인 Mitomycin C를 Chinese hamster, *Cricetulus griseus* (2n=22) euploid 細胞株인 22Emb. (♀, 20일된 embryo에서 培養한 것)과 5PSP (♂, polyoma transformed subclone)에 分量과 時間을 달리해서 處理했을 경우 일어나게 되는 特異한 染色體 異常을 觀察하였다.

Mitomycin C의 分量과 處理時間 그리고 細胞株에 따라서 染色體에 미치는 影響이 한결같지 않지만 第1, 第2 染色體의 region 5와 7, 그리고 X 染色體의 第2 收縮環(secondary constriction)에 보다 甚한 染色體 切斷을 보여 주었다.

이것은 Mitomycin C가 特히 染色體의 仁形成體(nucleolar-organizar)에 關聯되어 일어나는 現象이 아닌가 생각된다.

INTRODUCTION

There has been some interest in the effects of chemical agents on mammalian chromosomes as a means to arriving at an understanding of the molecular architecture of the chromosome (Somer and Hsu 1962).

Mitomycin C is a chemically reactive antibiotics derived from *Streptomyces caespitosus*. The drug selectively inhibits DNA synthesis and degrades cellular DNA but does not affect the synthesis of RNA or protein (Shiba et al. 1959). In tissue culture systems, Mitomycin C inhibits mitosis, reduces cell viability, and produces nuclear disorganization and giant cells.

Cohen and Shaw (1965) and Nowell (1964) described that Mitomycin C inhibits

mitosis and causes breaks and exchanges in chromosomes of cultured human leukocytes. They reported that the distribution of breaks were nonrandom with a marked excess of breaks in the secondary constriction regions of chromosomes numbers 1, 9 and 16. Again Shaw and Cohen (1965) showed that a process analogous to somatic crossing over as well as reciprocal translocation between nonhomologous autosomes have been induced by Mitomycin C. Specially they noted the breaks and rearrangements in the proximal heterochromatin of all the chromosomes and in the secondary constriction of autosomes numbers 1 and 9.

In this study, the Chinese hamster, *Cricetulus griseus* (2n=22) was used for somatic cell genetics. This species features (a) low chromosome numbers, (b) all autosomes and sex chromosome type can be identified easily, (c) retention of euploidy among normal, malignant and virus transformed cells, (d) rapid proliferation of continuous euploid cells, (e) breakage of chromosomes by viruses and chemical agents, which are localized at specific sites, and (f) normal cultured cells do transform when exposed to neoplastic agents (Yerganian et al. 1964).

The present study was undertaken in order to carry out analysis of the chromosome aberrations induced by Mitomycin C in the Chinese hamster cells.

MATERIALS AND METHODS

The cell lines employed were 22Emb. ♀, originated from a 20 day-old embryo, and 5PSP ♂, a subclone of a polyoma-transformed male cell lines, which is euploides with duplicated secondary constriction of X chromosomes. Both cell lines were in the about 20th passages at the time of using. Modified Puck's medium supplemented with 15% fetal calf serum was used throughout this experiment. Conditioned media were taken from the culture bottle 5 hours after adding fresh media to the bottle. Mitomycin C was obtained from Sigma Chemical Co., St. Louis, Missouri.

Approximately 50,000 cells were seeded in each cytology tube, in which contains a cover slip, and grown for 48 hours and then treated with 5μg, 3μg, 1μg, and 0.5μg per ml of media of Mitomycin C. After 30 minutes, 1 hour, and 2 hours of treatment, samples of tubes were washed twice with conditioned media and kept conditioned media for 15 to 22 hours, including 3 to 4 hours of colchicine treatment (0.2 ml of 2.5×10^{-6} M per ml of media=96.25 μg/ml). The tubes were fixed with a solution of 3 parts of 95% alcohol and one part of glacial acetic acid, after 5 minutes hypotonicity. The day after fixing, cover slips were stained with propiono-carmin and slides were made by a squashing technique. Slides were examined under a phase microscope, and microphotographs were made.

RESULTS AND DISCUSSION

Concentrations of 3 μg and 5 μg per ml of Mitomycin C inhibited mitosis greatly and caused multiple chromosomes and chromatid breaks involving all chromosomes. More detailed observation was made with concentrations of 0.5μg and 1μg per ml. The mean length of each chromosome was used for the calculation of the expected frequencies of chromosome aberrations (Table 1). Chromosome length measurements were obtained from Hsu and Zenzes (1964) who had estimated the length of each chromosome type as a percentage of the total length of chromosomes in a diploid cell. The breaks were random, however, contrary to the randomness of breaks induced by x-irradiation, chromosomes of 1, 2 and X chromosome sustained more breaks in proportion to their length. Concentration of 1 μg of Mitomycin C did not give marked excess of breaks at the secondary constriction of 5 PSP cells. Yet low concentration of 0.5 μg of Mitomycin C showed double amount of breaks in modified X chromosomes. This suggests that either a concentration of 1 μg fails to result in combining the drug to the sites of modified X chromosome, or the modified X chromosome is highly sensitive to the high concentration of the drug and affected cells are inviable and are eliminated.

Figures 1 and 2 are diagrammatic representation of chromosomes 1, 2 and X of 22Emb. ♀ and also modified X chromosome of 5PSP ♂, respectively, showing the

Table 1. Frequencies of chromosome and chromatid breaks among 5 PSP ♂ and 22 Emb. ♀ cell lines

Chromosome	Aberration expected (%)	5 PSP ♂ Aberration observed (%)				22 Emb. ♀ Aberration observed(%)	
		0.5μg/ml 1 hr	1μg/ml 30 min	1μg/ml 1 hr	1μg/ml 2 hrs	0.5μg/ml 30 min	0.5μg/ml 1 hr
1	22.2	21.7	37.5	41.8	29.5	21.8	30.0
2	16.6	15.2	19.6	20.9	19.3	14.5	13.3
X_1	4.8	17.4	19.6	19.4	14.3	18.2	23.4
X_1^{p1}	—	15.2	1.8	6.0	2.5	—	—
X_1^{d2}	—	13.0	1.8	6.0	7.7	—	—
X_2	4.8	—	—	—	—	14.6	23.3
Y	4.2	0	0	0	0.8	0	0
3	10.2	8.7	7.1	3.0	8.4	9.1	3.3
4	9.0	0	1.8	3.0	6.7	3.6	0
5. 6. 7	20.6	8.7	10.8	10.5	10.0	18.2	6.7
8. 9.10	12.4	0	0	1.5	0.8	0	0
Total cell number		100	380	330	345	100	100
Average breaks/cell		0.46	0.15	0.20	0.34	0.55	0.30

1=Proximal secondary constriction of modified X_1.
2=Distal secondary constriction of modified X_1.

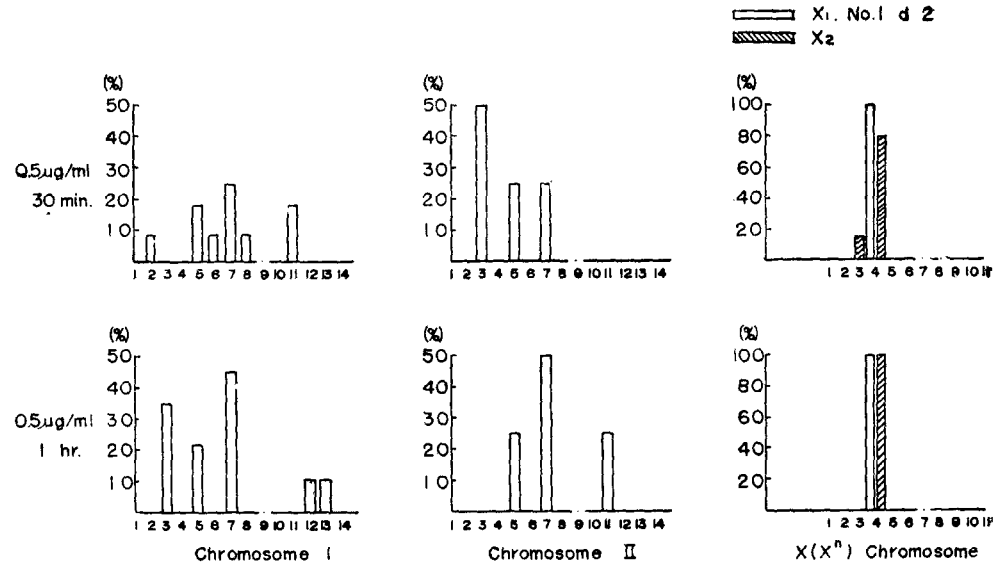

Fig. 1. The relative frequency of breaks for each chromosome region in percent of total breaks of 22 Emb. ♀.

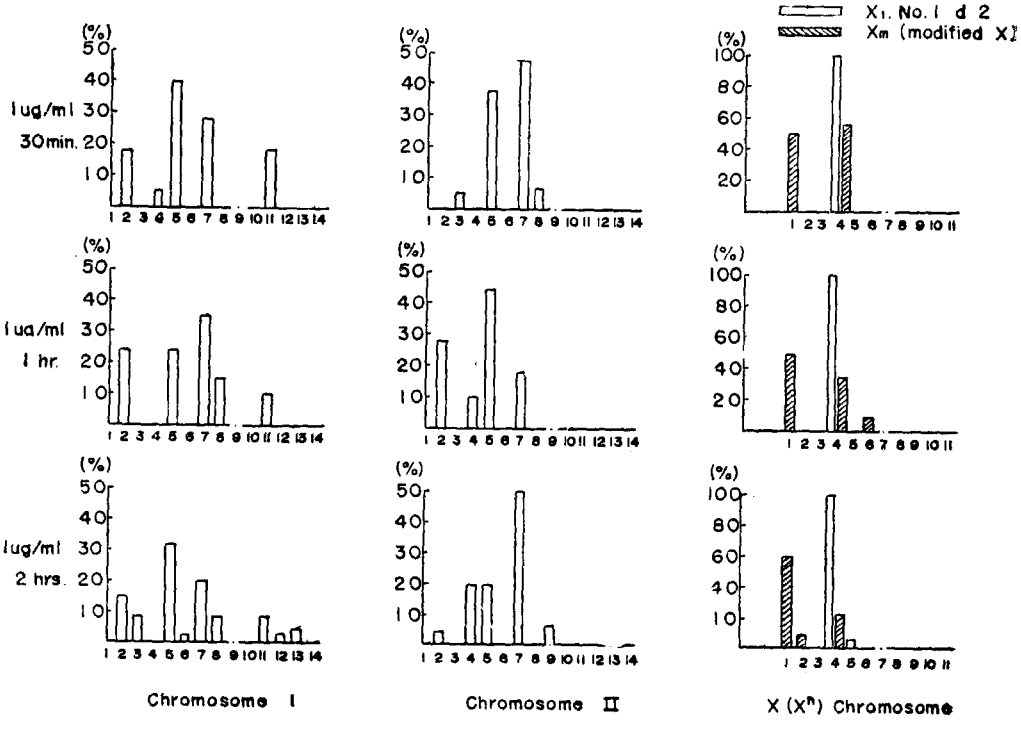

Fig. 2. The relative frequency of breaks for each chromosome region in per cent of total breaks of 5 PSP ♂.

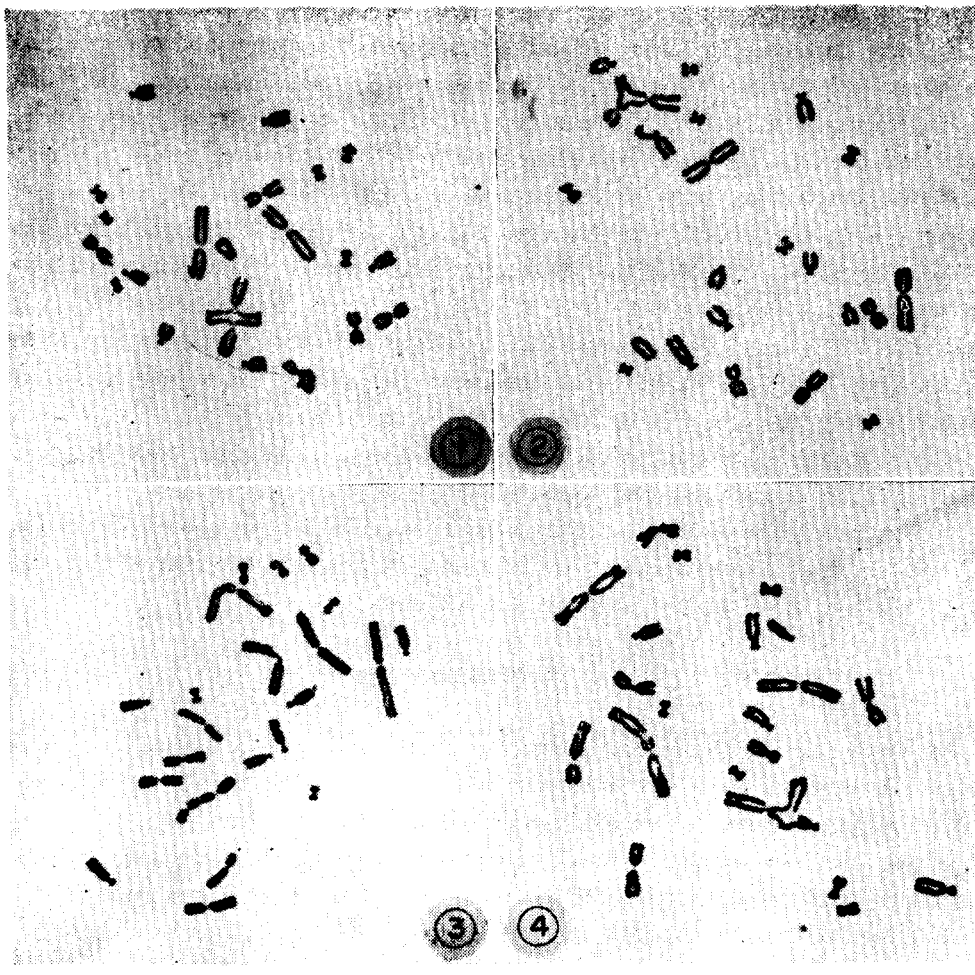

Fig. 3. (1) Translocation between chromosome 2 at centromeres of 5PSP ♂.
(2) Translocation between region 5 of chromosomes 1 and X of 5PSP ♂.
Chromosome breakge at region 7 of chromosome 1. Chromatid breakage at chromosome 3. (3) Chromatid breakage at proximal secondary constriction of modified X of 5 PSP ♂. (4) Translocation between region 7 of chromosmes 1 and 8 of 5 PSP ♂.

relative frequency of breaks according to each chromosome region following treatment of cells for different time periods with Mitomycin C. This indicates that regions 5 and 7 of chromosomes 1 and 2, and the secondary constriction of chromosome X show the most striking effect of Mitomycin C, although region 2 of chromosomes 1 and 2 has a relatively higher frequency of breakage. These regions are also effected by other agents such as BUdR (Hsu and Somers 1961,

Ho et al. 1963). Palmer (1970) also reported that treatment of BUdR induced significantly increased frequency of all secondary constrictions and chromosomal abnormalities on human leukocytes. The reason why these agents are mostly effecting specifically secondary constriction is difficult to explain. However, some evidence has been presented indicating that the chromosome regions bearing secondary constriction may have structural characteristics making them more susceptible to the action of various agents (Hsu and Somers 1961; Somers and Hsu 1962). This might be probable that these agents are either incorporated into nucleolar organizing regions during the course of active DNA metabolism (Doi et al. 1967; Kodama 1967), or possibly accumulated in or upon nucleoli, thereby leading to an incomplete dissolution of nucleolar substance and hence, the residual remnants result in disturbance of the distal portion of heterochromatin.

Figure 3 shows that Mitomycin C also induces a few reciprocal translocations, besides chromosome breakages, between nonhomologous chromosomes.

In conclusion, the results of this experiment suggest that dosage of Mitomycin C and length of treatments are very important factors for induction of chromosomal abnormalities, which vary with the cell lines.

SUMMARY

Mitomycin C, chemically reactive antibiotics derived from *Streptomyces caespitosus*, was introduced to Chinese hamster, *Cricetulus griseus*, (2n=22) cells (22Emb. ♀, 5PSP ♂) to carry out an analysis of the chromosome aberration.

It was found that regions 5 and 7 of chromosomes 1 and 2, and secondary constriction of chromosome X showed the most striking effect of Mitomycin C.

The relationship between Mitomycin C and secondary contriction was discussed.

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