KOREAN JOURNAL OF ZOOLOGY
Vol. 14, No. 4, 175~180 (1971)

DNA Synthesis and Radiosensitivity in Synchronized Human Kidney Cells in Vitro

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동시화시킨 사람의 신장세포에 있어서의 DNA 합성과 방사선감수성

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(Received November 5, 1971)

적 요

5-AU (5-aminouracil)를 처리하므로써 세포분열의 동시화를 촉진시킨 사람의 신장세포의 분열활동, 염색체 이상 및 DNA복제 양상에 미치는 X-선의 영향을 조직배양 및 자기방사법 (autoradiography)을 통하여 추구하였다.

5-AU처리구에서 분열활동의 최고점은 5-AU를 처리한 뒤 10시간에서 나타나며, 대조구에 비해서 6배나 높음을 보여준다. 5-AU 처리후 100R의 X-선을 조사한 실험구에서는 X-선의 영향은 주로 세포분열을 지연시키고 분열활동을 저해시킬 뿐 아니라 분열활동의 최고점을 보여주는 시간을 불규칙하게 한다.

대조구에서 세포당 염색체이상율은 0.030에 불과하나 5-AU를 처리할 경우는 0.147로 높아진다. 한편 5-AU+100R 및 5AU+200R의 X-선 처리구에서의 세포당 염색체 이상율은 각각 0.583 및 0.669로 보다 높아짐을 보겠다. 한편 세포당 1R당 평균 염색체 이상율은 0.0035가 된다. 본 실험결과를 통해 보면 5-AU가 표지된 분열상의 출현빈도 및 표지강도를 높이고 있음을 알겠는데, 그것은 5-AU가 세포주기중 S기에 놓인 세포를 축적시키는 힘이 있기 때문이라고 보겠다. 이와는 반대로 X-선은 세포의 표지강도와 표지된 분열상의 출현빈도를 저하시킨다.

INTRODUCTION

Recently many investigators in cytology and molecular cytogenetics fields have been greatly interested in mitotic synchronization using chemicals. In 1963, Smith et al. succeeded in partial cell synchrony of plant cells by the treatment of 5-AU (5-aminouracil). Reiter and Littlefield (1964) obtained synchronized mouse fibroblasts by the use of 5-fluorodeoxyuridine (FUdR). Steffen and Stolzmann (1969) reported that addition of amethopterin with adenosine, FUdR or thymidine in high concentrations to phytohaemagglutinin stimulated human lymphocytes caused synchronization of cells. Lavronsky (1969) obtained synchronous cell population

in *Crepis capillaris* by the treatment with 5-AU.

But the use of chemicals for the purpose of synchronization is limited, as severe temporal distortions may result by the chemical stresses imposed on cells (Kim and Perez, 1965). Olivieri and Brewen (1966) observed chromosome aberrations in 5-AU synchronized human leucocyte cells. Kang et al. (1970) suggested that synchronizing agents induced chromosome aberrations in the cultured human kidney cells and HeLa cells. Some other authors have achieved the studies on chromosome aberration and radiosensitivity of synchronized cells in vitro. However, the synchronization effects of agents in connection with the stage radiosensitivity and DNA synthetic pattern of synchronized cells are still to be more studied.

The present experiment was designed to clarify the mechanism of cell synchrony and its relation to radiosensivity by studying the effect of 5-AU on DNA synthetic pattern of chromosome together with its effect on the mitotic activity and the frequency of aberrations in 5-AU synchronized cultured cells.

MATERIALS AND METHODS

Materials for this study were kidney tissues obtained from 4 fetuses (male: 2, female: 2) aborted artificially at 5 to 6 months of pregnancy.

In accorance with the method of Chu (1965), 5-AU (final concentration: 3mM) was added to the 24- hour cultured cells for 24 hours. Growth medium was TC media 199 supplemented with 10% bovine serum. 100 and 200R of X-irradiations were performed with an X-ray generator, General Electric Maxitron 250-III operated at 230 kv and 10 mA with Th 2 filter. Tritiated thymidine was treated at a final concentration of 0.5 μCi/ml medium and autoradiographic stripping film (Kodak AR-10) was applied. The detailed procedures on air-drying and autoradiography were performed as described previously (Kang and Park, 1969).

RESULUS

As shown in Fig. 1, cells were observed every two hours after removal from 5-AU

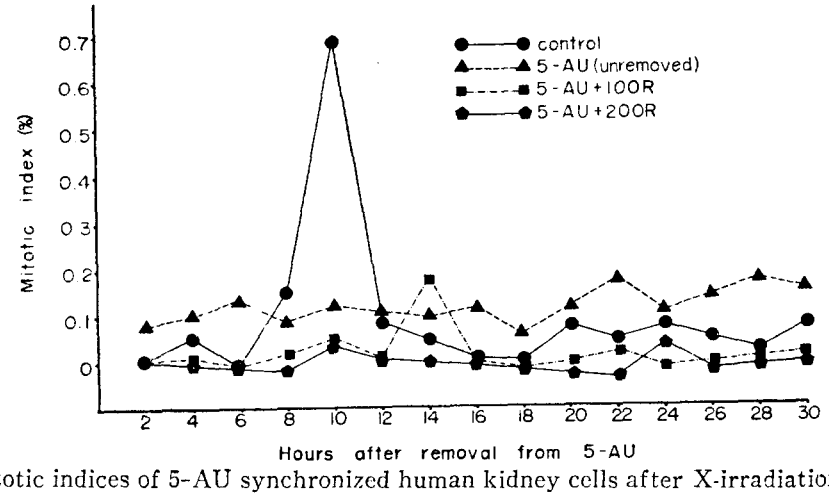

Fig. 1. Mitotic indices of 5-AU synchronized human kidney cells after X-irradiation in vitro.

up to 30 hours in synchronized human kidney cells. In control group, any significant difference of mitotic activity with time was not observed. However, experimental group (5-AU) showed mitotic peak (0.7) at 10 hours after treatment.

Table 1. Frequency of chromosome aberrations in synchronized human kidney cells after X-irradiation.

Experimental groups	Cells scored	Types of aberrations (%)				Total breaks per cell	Breaks per cell per roentgen
		Chromatid-type		Chromosome-type			
		CD	ICTD	CE	Rings and dicentrics		
Control	100	2.0	1.0	—		0.030	
5-AU	103	7.9	1.9	4.9		0.147	
5-AU-100R	102	43.7	6.8	7.8	None	0.583	0.0035
5-AU+200R	98	51.0	8.1	7.8		0.669	

CD; chromatid deletions
ICTD; isochromatid deletions
CE; chromatid exchanges
Total breaks; chromatid deletions+isochromatid deletions+2 (chromatid exchanges+dicetrics+rings)

In 5-AU+100R group, the mitotic peak (0.2) appeared at 14 hours, whereas in 5-AU+200R group the mitotic activity was remarkably reduced to show mitotic peaks 0.06 and 0.07 at 10 and 24 hours after treatment respectively. From the above results, it can be assumed that partial synchronization is occurred by 5-AU. In other words, 5-AU group showed mitotic peak 6 times higher than that of control group and X-irradiation caused the mitotic delay as well as mitotic inhibition.

Table I shows the frequencies of chromosome aberration in each experimental group observed at 10 hours after removal from 5-AU. In control group, the chromosome aberrations per cell were 0.030, which consists of 2% chromatid deletion and 1% isochromatid deletion, while those of 5-AU treated group was 0.147 showing 7.9% of chromatid deletion, 1.9% of isochromatid deletion and 4.9% chromatid exchange. In 5-AU+100R groups, the chromosome aberrations per cell were 0.583 (chromatid deletion: 43.7%, isochromatid deletion: 6.8%, chromatid exchange: 7.8%) and 0.669 (chr-

Table 2. Frequency of labeled metaphases in synchronized human kidney cells after X-irradiation.

Experimental groups	Metaphases scored	Labeling intensity (%)*			Total labeled metaphases (%)
		LL	ML	HL	
Control	892	10.3	32.6	24.3	67.2
5-AU	921	11.2	18.7	52.4	82.3
5-AU+100R	865	5.6	18.5	35.7	59.8
5-AU+200R	853	3.7	20.1	29.8	53.6

*Labeling intensity; LL (lightly labeled) ML (moderately labeled) HL (heavily labeled).

omatid deletion: 51.0%, isochromatid deletion: 8.1%, chromatid exchange: 7.8%). Chromosome aberrations per cell per R removed those by 5-AU is 0.035.

Table II shows the frequencies of labeled metaphases and the effect of X-ray on the labeling intensity in synchronized cells observed from 2 to 30 hours after labeling. In control group, 67.2% of 892 cells were labeled, and this comprised lightly labeled metaphases (10.3%), moderately labeled ones (32.6%) and heavily labeled ones (24.8%). In 5-AU treated group, the frequencies of heavily,moderately and lightly labeled metaphases were 52.4%, 18.7% and 11.2% respectively to show labeled metaphases, 82.4% of 921 cells observed. In 5-AU+100R and 5-AU+200R groups, 59.8% and 53.6% were labeled respectively, in which heavily, moderately and lightly labeled metaphases were 35.7%, 18.5% and 5.6% in the former and 29.8%, and 3.7% in the latter respectively. From the above results, it can be assumed that 5-AU accumulates the labeled cells, whereas X-irradiation reduces both the frequencies of labeled metaphase and the labeling intensity.

DISCUSSION

Smith et al. (1963) was the first to carry out a chromosome work in synchronized cells, and they succeeded in obtaining high mitotic peak (up to 62.5%) at 14 hours after 24-hour treatment of 5-AU (700 ppm) in *Vicia faba*. On the mechanism of mitotic synchronization of 5-AU, Smith et al. (1963) and Prensky and Smith (1965) reported that 5-AU blocked DNA synthesis of the cells at S stage, but did not block the activites of the cells at other stages of the cell cycle to produce cell-accumulation at S stage, so the partial cell synchronization occurs when 5-AU is removed. Chu (1965) obtained partial cell synchrony (27%), at 7 hours after 24-hour treatment of 5-AU in 24 hour cultured Chinese hamster cell line, and made announcement that 5-AU inhibited the DNA synthesis of chromosome and mitotic activities. Brewen and Olivieri (1966) also observed that 5-AU caused mitotic delay. However, Jakob and Trosko (1965) and Resch and Schroeter (1969) suggested that the action of 5-AU on cell synchronization might be mediated through the accumulation of G_2 cell. This suggestion was somewhat supported by the observation that 5-AU did not block DNA synthesis.

Futhermore Socher and Davision (1971) suggested that treatment of *Vicia faba* with 5-AU indicated that cells were stopped at the point of S-G_2 transition. So above all mechanisms proposed are rather contradictory each other.

It is a well established fact that chromosome-type aberrations (rings, dicentrics, etc) are induced only in G_1 stage, but chromatid-types (deletions, exchanges) are produced in S or G_2 (Brewen and Christie, 1967; Kang and Park, 1969). Hence, the facts that only chromatid type aberrations were observed in the present experiment suggest that 5-AU act in G_2 or S stage of cell cycle. Furthermore, our autoradiographic data showing increase of labeled metaphases in 5-AU treated group indicated that action of 5-AU may be of the nature of accumulation of cells at S stage (Table 1 and 2). So our results from the present experiments, although preliminary and incomplete, tend to offer su-

port to the view of Chu (1965) and Prensky and Smith (1965). But the difference in mitotic activity with their experiments is thought to be due to the difference in culture conditions or in the material used.

DNA synthesis is usually inhibited by X-irradiation (Wong, 1968). Kang and Park (1969) reported that radiation inhibited DNA synthesis and mitotic activity, expecially those of G_2 cells in study on the stage radiosensitivity of cultured human kidney cells. In our present data the decrease of labeled metaphase and the mitotic index observed in X-irradiated groups in spite of removal of 5-AU are thought to be due to the fact that DNA synthesis is blocked and mitotic activity is inhibited by X-irradiation as shown in the result of Eckstein et al. (1967).

The more detailed study on the stage radiosensitivity and the pattern and time sequence of the DNA synthesis in synchronized cells is expected to contribute to find out the relations between stage radiosensitivity and the mechanism of cell synchronization.

SUMMARY

The effects of X-irradiation on the mitotic activity, the chromosome aberration and the DNA synthetic pattern in synchronized human kidney cells treated with 5-AU were measured in the present experiment.

When 5-AU was added, mitotic activity was markedly suppressed. After removal of the cells from the chemical, its activity proceeded synchronouly and reached peaks at hours 10. In 5-AU+100R groups, it was observed that X-ray caused mitotic delay, the irregularity of the time when mitotic peak appeared and the inhibition of mitotic activity. In the control group, chromosome aberrations per cell was 0.030, whereas 0.147 in 5-AU treated group. In 5-AU+100R and 5-AU+200 R groups, chromosome aberrations per cell were 0.583 and 0.669 respectively and the average chromosome aberrations per cell per R was 0.0035. 5-AU increased the frequency of labeled metaphases together with labeling intensity, and this is thought to be due to the accumulation of cells by 5-AU at S stage. On the contrary, X-ray decreased the labeling intensity and the frequency of labeled metaphases.

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