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Effects of Ultraviolet Light on DNA Replication and Repair in Cultured Myoblast Cells of Chick Embryo*

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培養한 鷄胚筋細胞의 DNA複製 및 回復에 미치는 紫外線의 影響

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요 약

발생 12일째의 계배근세포를 약 7일간 배양하면서 자외선에 의한 절제회복, 광재활성 및 DNA 복제억제율을 조사하여 다음과 같은 결과를 얻었다.

자외선에 의한 절제회복은 계배근세포의 분화정도가 진행됨에 따라 감소하였으며, 이는 특히 자외선의 선량이 높을수록 뚜렷하였다.

DNA합성율은 분화가 진행된 세포일수록 현저히 감소하는 경향이었으며 각 분화단계에 따른 DNA 복제억제 현상은 배양 초기 세포인 경우 자외선 조사후 30분에서 1시간반 사이에서 가장 심하게 나타났고, 배양후기의 세포에서는 이러한 억제현상이 뚜렷하지 않았다.

또 배양 1일째 세포에서 광재활성에 의한 피리미딘 이량체의 감소율은 자외선 조사직후에 현저하였다가 그후 시간의 경과에 따른 차이는 보이지 않았다. 그러나, 절제회복만에 의한 이량체의 감소는 조사후 시간이 지남에 따라 서서히 감소하여 광재활성에 의한 이량체의 감소량과 비슷한 수준으로 접근하였다.

INTRODUCTION

The differentiation of embryonic skeletal muscle cells in culture has been extensively studied because this has been recognized as a suitable material for the studies of cell differentiation. It is characterized by the well-organized fibrillar structure containing myosin, actin and several specific proteins and enzymes (Potter, 1974; Ha *et al.*, 1979). Mononucleated myoblasts proliferate to form elongated myotubes containing many nuclei within a common cytoplasmic compartment under the favorable culture condition. These multinucleated

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muscle cells have never been shown to enter the DNA synthetic phase of the cell cycle (Stockdale and O'Neill, 1972; Cleaver, 1981).

Stockdale and O'Neill (1972), however, reported that after ultraviolet (UV) light irradiation every nuclei (99%) of differentiated muscle cells could incorporate ^{3}H-thymidine. Although it is not known whether any particular DNA polymerase is involved in the DNA repair of avian cells, it is assumed that excision repair does require a DNA polymerase to complete the repair of damaged DNA (Stockdale, 1969).

DNA repair is defined as the restoration of damage produced in DNA and thus two distinct biological features; an induced DNA damage by mutagens and carcinogens and a self protective enzymatic repair processes. Although DNA replication and excision repair have been extensively studied in various organisms, most of these studies have been performed in actively proliferating cells of mammalian system.

The purpose of the present investigation was, therefore, to determine the effects of UV-irradiation on DNA replication, excision repair and photoreactivation in the differentiating muscle cells of chick embryos to learn whether there is any relation between the process of cell differentiation and the abilities of DNA replication and repair.

MATERIALS AND METHODS

1. Muscle Cell Culture

Skeletal muscle cell cultures of developing chick embryo were prepared as described by O'Neill and Stockdale (1972) and Ha *et al.* (1979). The cultures were grown in a humidified 5% CO_2 incubator using medium 811 and 8102 (Ha *et al.*, 1979).

2. UV-irradiation

Prior to UV-irradiation, the growth medium was removed from the cultures and cells were washed twice with prewarmed phosphate buffered saline (PBS). The cells were exposed to 254 nm UV light from germicidal lamps at an incident dose rate of 1.42 J/m^2/sec as determined with the YSI Kettering No. 65 radiometer (Yellow Spring Instrument Co., Yellow Springs, Ohio) and incubated in fresh or radioactive medium.

3. Unscheduled DNA Synthesis Experiments

Cells grown on the collagen-coated coverslips were labeled with ^{3}H-thymidine (5μ Ci/ml, 53 Ci/mM), rinsed with PBS and fixed in 3 : 1 methanol glacial acetic acid and then soaked in 4% perchloric acid (PCA) overnight. The coverslips were mounted after dehydration. Autoradiograms were prepared by dipping the slide in Kodak NTB liquid nuclear track emulsion, exposed for 4 days, developed in D-19 developer and then stained with 4% Giemsa. Excision repair ability was determined by counting the silver grains in the uniformly and lightly labeled nuclei under oil immersion lens.

4. DNA Synthesis Experiments

Myoblasts prelabeled with ^{14}C-thymidine (0.01 μCi/ml, 52 Ci/mM) were rinsed with PBS

and exposed to UV light and incubated for desired times in the fresh medium. At various times after UV-irradiation, the cells were pulse-labeled with ^{3}H-thymidine (5μ Ci/ml) for 10 minutes and were rinsed in ice-cold saline citrate. The cells were then scraped off and collected on GF/A glass fibre filters. The filters were washed with 4% PCA, dehydrated with alcohol series, and then dried completely. The radioactivities of ^{3}H- and ^{14}C-thymidine incorporated into DNA were counted by a Packard Tri-Carb Liquid Scintillation Spectrometer. $^{3}H/^{14}C$ ratios were determined by liquid scintillation counting.

5. Photoreactivation and Excision of Pyrimidine Dimers

Cells labeled with ^{14}C-thymidine (0.2 μCi/ml) for 24 hours were rinsed with PBS and exposed to UV light. The cultures were then replenished with fresh medium and grown for desired times under the illumination of fluorescent lamp. At desired times, cells were washed with PBS and scraped off. After mixing with trichloroacetic acid (TCA, 10%), the suspensions were centrifuged and the supernatant was discarded. Cold formic acid (97%) was added to each tube and the tubes were sealed by O_2 flame and hydrolyzed at 180°C for one hour. The samples were frozen and the tubes were cut open. After evaporating the remaining solution, distilled water was added to each tube and the samples were spotted on silica gel G/UV_{254} plate (Polyram, Macherey-Nagal Co., Duren, Germany). The plate was dried and eluted in the solvent vapor-saturated chamber. The plate was dried and then sliced. Fraction of dimer content was calculated from radioactivities in dimer regions divided by those in monomers.

RESULTS

1. Unscheduled DNA Synthesis

The amounts of UV-induced excision repair in myoblast cells of chick embryos are shown in Table 1. The amount of unscheduled DNA synthesis induced by UV light was increased with UV dose. However, the relative UV repair, which is represented as mean number of grains produced by various doses of UV light at each experimental group subtracted by those of control, gradually decreased as increases of culture times, particularly at higher doses.

Fig. 1. shows the dose response of unscheduled DNA synthesis induced by UV light at various times after subculture of chick embryo muscle cells. The amount of UV-induced unscheduled DNA synthesis in one day cultures were sharply increased up to 40 J/m^2 and leveled off thereafter. The dose responses of unscheduled DNA synthesis in other cultures were decreased as increases of culture time, thus resulting in a saturation for about 20 J/m^2 in five and seven day cultures.

Fig. 2 shows the time dependence of unscheduled DNA synthesis induced by various doses of UV light in differentiating muscle cells of chick embryos. The amounts of unscheduled DNA synthesis induced by 40~50 J/m^2 were sharply decreased with time. However, these decreasing patterns were not remarkable at lower doses. The average

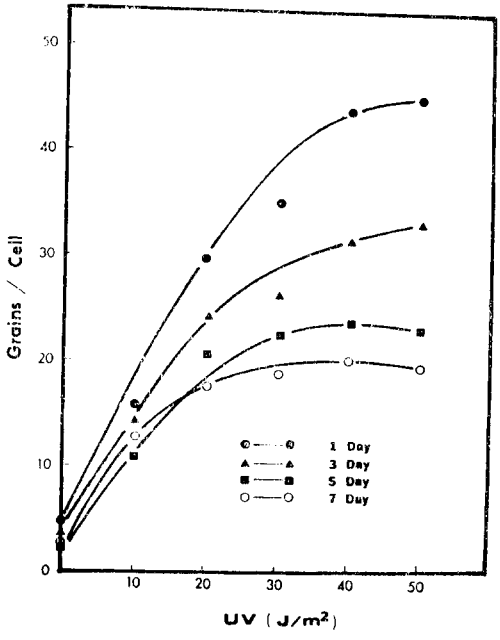

Fig. 1. Effects of UV light on the dose response of unscheduled DNA synthesis in cultured chick embryo myoblast cells.

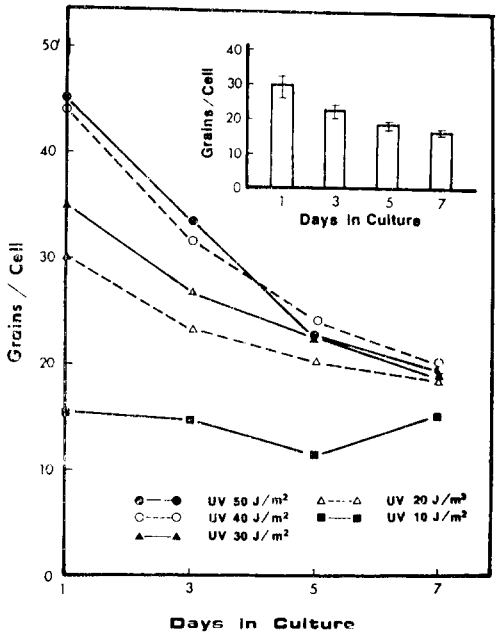

Fig. 2. Effects of UV light on the time dependence of unscheduled DNA synthesis in cultured chick embryo myoblast cells.

number of grains in one day cultures shown in the upper portion of Fig. 2 were about 30. This number was decreased to 55% at the seven day cultures.

These results indicate that the excision repair ability in differentiating muscle cells decreases with time, but retains its ability even in fully differentiated cells.

2. DNA Replication

Fig. 3 shows the rate of DNA replication in muscle cells of chick embryos at various culture periods. The DNA replication activity decreased as a function of time after culture, resulting in 0.2% of the original activity at seven day cultures. These results suggest that DNA replication activity is remarkably decreased in the course of differentiation.

The rate of DNA synthesis at various times after UV-irradiation is shown in Table 2 and Fig. 4. The results show that after UV-irradiation the ^{3}H-thymidine uptake was decreased within 30 to 90 minutes and the increasing pattern was not shown up to 3.5 hours. The pattern of inhibition of DNA replication by UV-irradiation showed two distinctly different tendencies. In one, two and three day cultures, the inhibition patterns were found to be essentially similar. But in five and seven day cultures, the patterns were quite different from those of earlier cultures. These differences might be due to the fluctuation caused by cell fusion during cell differentiation. In Fig. 4, the rates of DNA replication seem to be higher in five and seven day groups. But in consideration of

Table 1. Unscheduled DNA synthesis induced by UV in cultured chick embryo myoblast cells

Days in culture	Treatment UV(J/m²)	Average number of grains* per cell	Relative UV repair mean ± S.E.
1	0	4.6	
	10	15.8	
	20	29.9	
	30	35.0	29.4±5.39
	40	44.5	
	50	45.0	
3	0	3.7	
	10	14.9	
	20	23.6	
	30	26.5	22.3±3.3
	40	31.5	
	50	33.5	
5	0	2.1	
	10	11.7	
	20	20.8	
	30	22.9	18.3±2.2
	40	23.5	
	50	22.9	
7	0	2.2	
	10	13.2	
	20	18.7	
	30	19.0	16.3±0.9
	40	20.3	
	50	19.5	

* 50 cells were analyzed in each group.

Table 2. Effects of UV on DNA synthesis in cultured chick embryo myoblast cells

Days in culture	Treatment UV(J/m²)	% of control incorporation of ^{3}H-TdR at various time (hrs)			
		0	0.5	1.5	3.5
1	10	76.8	31.5	35.8	38.1
	20	55.6	31.7	21.7	26.6
	30	49.3	19.9	14.4	16.0
2	10	77.8	31.4	31.0	39.8
	20	59.1	20.3	30.9	26.2
	30	39.7	14.1	20.5	18.5
3	10	87.4	52.2	27.5	37.0
	20	45.0	35.6	22.4	21.5
	30	39.2	25.7	21.1	16.9
5	10	77.9	71.3	59.4	26.8
	20	76.2	65.8	45.9	26.5
	30	74.7	53.7	44.8	25.5
7	10	77.6	62.1	91.7	72.4
	20	65.4	50.8	55.2	64.1
	30	54.7	63.1	70.1	47.2

previous results (Fig. 3), it is assumed that absolute value would be far below compared to that of the earlier cultures.

3. Excision of Pyrimidine Dimer and Photoreactivation

Excision of pyrimidine dimers together with photoreactivation activity in one day cultures of chick embryo muscle cells at various times after UV-irradiation are shown in Table 3 and Fig. 5. Excision repair, i.e., excision of pyrimidine dimer, was found to be a slow and steady process while photoreactivation was rapid one in which monomerization only occurred soon after UV-irradiation.

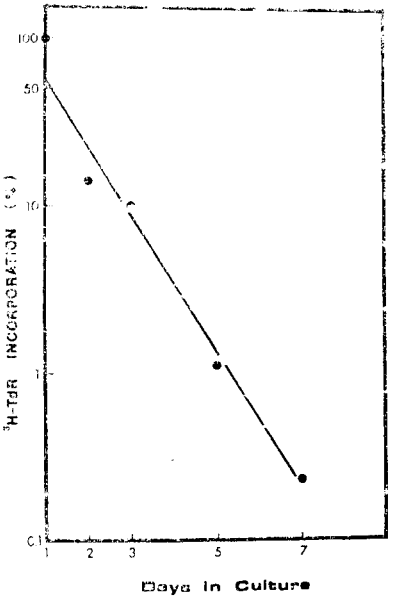

Fig. 3. Rates of DNA synthesis at various times after primary culture of chick embryo myoblast cells.

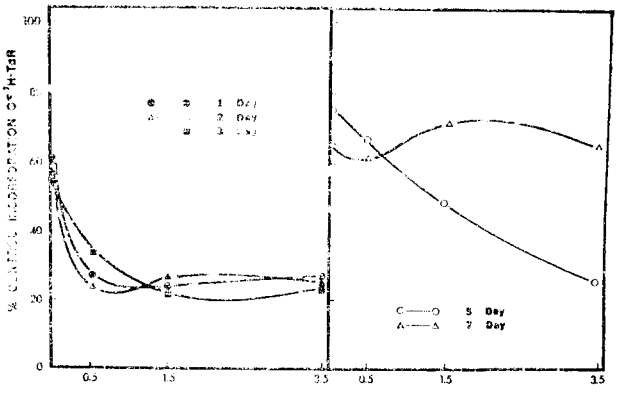

Fig. 4. Rates of DNA synthesis in cultured chick embryo myoblast cells irradiated with UV light.

Table 3. Photoreactivcation and excision of pyrimidine dimers at various times after UV-irradiation in cultured chick embryo myoblast cells

Treatment UV(J/m²)	Time after UV (hrs)	Time after P.R. (hrs)	*TT Content (TT/T)	TT fraction remained(%)
30	0	0	3.77×10^{-3}	100
30	1	0	3.24×10^{-3}	85.9
30	1	1	2.45×10^{-3}	65.0
30	4	0	3.16×10^{-3}	83.8
30	4	4	1.95×10^{-3}	66.3
30	12	0	2.94×10^{-3}	78.0
30	12	12	2.71×10^{-3}	71.9
30	24	0	2.17×10^{-3}	57.6
30	24	24	2.15×10^{-3}	57.0

** TT: Thymidine containing pyrimidine dimers.

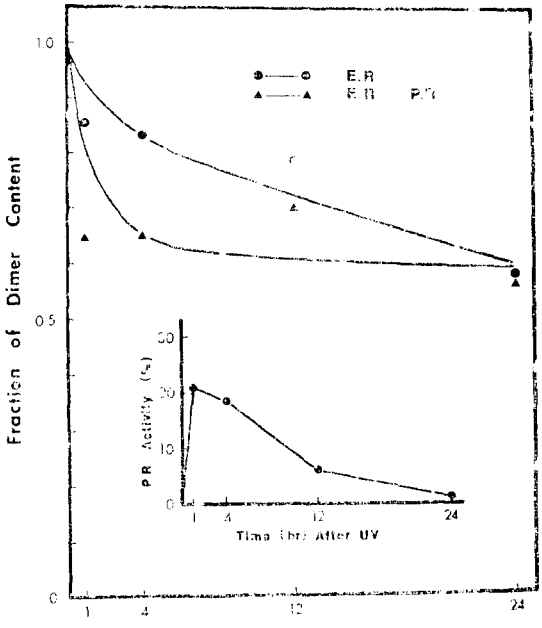

Fig. 5. Kinetics of loss of pyrimidine dimers induced by UV light in cultured chick embryo myoblast cells.

DISCUSSION

The present data indicate that differentiation is closely coupled with the cessation of normal DNA replication and the gradual reduction of unscheduled DNA synthesis. This result also suggests that despite the loss of DNA polymerase activity about 80~90%, muscle cells retain the ability to synthesize DNA of a repair type after UV-irradiation (Stockdale and O'Neill, 1972). The results are also consistent with the data using aging human diploid cells (WI-38) (Painter *et al.*, 1973). According to Painter *et al.* (1973), the senescent cell showed about 35% less repair replication. This is quite in agreement with that of the present results in which 40% of repair activity were lost.

Results of the replication inhibition by UV light showed that the recovery pattern according to the culture period was not clearly defined. These patterns are well corresponding with the results of UV-induced replication inhibition using HeLa S_3 cells (Painter, 1977). Photoreactivation has been investigated in many other organisms such as *E. coli* and green algae. This result has high coincidence with the results obtained using *Chlamydomonas reinhardi* in that it ocurrs rapidly (Small and Greimann, 1977).

ABSTRACT

DNA synthesis, unscheduled DNA synthesis, excision of pyrimidine dimers and photoreactivation were determined in UV-irradiated differentiating muscle cells at various times of primary culture of 12 day chick embryos and results obtained were as follows.

The rates of UV-induced unscheduled DNA synthesis were increased as increase of UV dose. And the rates were gradually decreased as the increase of time after culture, but at higher doses the decreasing tendency was remarkable. The patterns of DNA replication were changed drastically as a function of time so that in the seven day cultures the rate of ^{3}H-thymidine incorporation was found to be 0.2% of the original activity.

The pattern of inhibition of DNA replication by UV damage demonstrated that in cells of earlier stages there were no remarkable changes, but in cells of later stages there was significant fluctuation.

Photoreactivation and the excision of pyrimidine dimer in the one day cultures showed that photoreactivation occurred immediately after UV-irradiation, but excision of pyrimidine dimer was gradually and slowly occurred.

These results indicate that the differentiation of embryonic muscle cells accompanies the gradual reduction of DNA replication and unscheduled DNA synthesis, and that the photoreactivation is rapid process compared to excision repair.

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