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Effects of Ara-C on UV and MMS-induced Excision Repair, Chromosome Aberrations, Sister Chromatid Exchanges and Replication Inhibition

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자외선과 MMS에 의한 절제회복, 염색체이상, 자매염색분체 교환 및 복제억제 현상에 미치는 Ara-C의 영향

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적 요

DNA회복합성과 염색체이상, 자매염색분체 교환 및 복제억제 현상과의 연관성을 추구하기 위해서 HF_1, CHO 및HeLa S_3 세포를 재료로 자외선 또는 MMS를 처리하기전 또는 후에 ara-C를 처리하여 그 효과를 비교 검토하였다.

(1) Ara-C는 자외선 및 MMS에 의한 DNA회복합성을 억제하였으며 이 억제효과는 ara-C를 후 처리한 경우 더욱 현저하였다.

(2) Ara-C는 자외선이나 MMS에 의한 염색체 이상율을 증가시켰다. 특히 MMS 처리후 ara-C를 처리한 실험군에서는 염색체이상율이 상승효과를 보였는데 이는 염색분체 절단이 증가된 때문이었다.

(3) Ara-C는 염색체이상에서와는 달리 자외선이나 MMS에 의한 자매염색분체 교환을을 증가시키지 않았다. 이는 특히 MMS군에서 전처리한 경우에 뚜렷하였다.

(4) Ara-C를 처리하면 DNA합성율이 즉시 감소했다가 회복되었다. 그러나 ara-C와 자외선 또는 MMS를 복합처리하면 DNA 합성양상이 처음에는 ara-C의 영향처럼 보이다가 뒤에는 자외선 또는 MMS에 의한 반응과 같이 나타났다.

이같은 결과들은 ara-C가 DNA 상해요인이 아님에도 염색체이상 또는 염색분체교환 유발요인으로 작용함을 나타내며, DNA 회복기작이 염색체이상, 자매염색분체교환 및 복제억제 현상과 직접적인 상관성이 없음을 시사하는 것이다.

INTRODUCTION

The possible involvement of damage induced in DNA and its repair processes in the development of chromosome aberrations, sister chromatid exchanges and replication inhibition has been a subject of much discussion (Park *et al.*, 1976; Kihlman, 1977; Wolff, 1978; Preston, 1980).

There have been two divergent hypotheses so far proposed. The first postulation is that since DNA is the key substance in chromosome breakage and rejoining, essentially the same biological mechanisms are involved in DNA repair and in the formation of chromosome aberrations and sister chromatid exchanges (Kihlman, 1971, 1977; Bender *et al.*, 1974; Evans, 1977; Taylor, 1978). The second idea is that since the structural configuration of chromosome is different from that of the intact DNA molecule, the induced chromosome breaks and rejoining of chromosome aberrations are the independent ability to repair the damaged DNA(Wolff and Scott, 1969; Wolff and Cleaver, 1973; Cleaver, 1974; Scott *et al.*, 1974).

Irrespective of these controversial hypotheses, one clear fact on this problem is that DNA molecule is a principal target for the mutagen action and is responsible for the mutation involving chromosome aberrations, sister chromatid exchanges, replication inhibition and others. Therefore, investigation on the action mechanism of a known DNA inhibitor on these different biological phenomena induced by mutagen may provide a useful information for this relationship.

1-β-D-arabinofuranosylcytosine (ara-C) is a nucleoside analog of 2'-deoxycytidine in which the deoxyribose is replaced by arabinose. This drug has been shown to cause an inhibition of DNA replication (Jones *et al.*, 1976; Wist *et al.*, 1976; Fridland, 1977; Bell and Fridland, 1980) and of excision repair (Stenstrom *et al.*, 1974; Hiss and Preston, 1977; Dunn and Regan, 1979), chromosome aberrations (Benedict *et al.*, 1970; Wobus, 1976; Preston, 1980) and sister chromatid exchanges (Raposa, 1978).

The predominant effect of ara-C is a strong inhibition of DNA replication. Fridland (1977) and Bell and Fridland (1980) have recently reported that the replication inhibition by ara-C is due to the profound blocking of the replicon initiation followed by minor inhibition of DNA chain elongation. Hiss and Preston (1977) and Dunn and Regan (1979) have also reported that ara-C inhibits UV-induced nucleotide excision repair, thus resulting in an accumulation of single strand breaks.

There is a similarity between this effect of inhibitors on repair and their corresponding effects on normal replication in which Okazaki fragments continue to be synthesized but sealing of gaps between them is blocked (Magnusson, 1973;

Laipis and Levine, 1973). This effect can be seen at the chromosome level by demonstration of decondensation of chromosomes with the accumulation of unsealed excision breaks at high doses of UV-light (Collins *et al.*, 1977). Preston (1980) recently reported that ara-C showed a synergistic effect of X-ray induced chromosome deletion, and postulated that the large increase in deletion by ara-C could be a result of the inhibition of repair, which directly or indirectly leads to double strand breaks responsible for exchange type of aberration. These results seem to indicate some inverse correlation between excision repair and chromosome aberrations.

So far no one has yet attempted to investigate the effects of ara-C on alkylating agent-induced base excision repair, chromosome aberrations and sister chromatid exchanges. Since MMS appears to be different from UV-light in inducing damage in DNA and its corresponding repair mechanism, it is conceivable that the apparent variations in the inhibitory effects of ara-C are expected to occur.

The purpose of the present investigation is, therefore, to determine the effects of ara-C on UV-light or MMS induced excision repair, replication inhibition, chromosome aberrations and sister chromatid exchanges to elucidate if any possible relationship exists.

MATERIALS AND METHODS

1. Cell Culture:

Three established mammalian cell strains, chinese hamster ovary (CHO), human cervical carcinoma (HeLa S_3) and normal human fibroblasts (HF_1), were used throughout this investigation. Monolayer cultures of these cell strains were grown in humidified 5% CO_2 incubator at 37°C using Eagle's minimum essential medium (MEM: Grand Island Biological Co., Grand Island, N.Y.) supplemented with 15% fetal calf serum, penicillin G (100units/ml) and streptomycin (10μg/ml).

2. UV-Irradiation:

Prior to UV-irradiation, the growth medium was removed and cells were washed once with Puck's phosphate buffered saline (PBS). They were then exposed to various fluences of 254nm UV-light from mercury germicidal lamps with an incident dose rate of 1.4J/m^2/sec, determined with a YSI-Kettering No.65 radiometer (Yellow Springs Instrument Co., Yellow Springs, Ohio).

3. Methyl Methanesulfonate Treatment:

Methyl methanesulfonate (MMS: Eastman Kodak Ltd., Rochester., N.Y.) was dissolved to 1M stock solution in PBS and further diluted to various working concentrations with the serum-free medium prior to treatment. MMS treatment was performed at 37°C for desired time. After treatment with MMS, the cells

were washed and replaced with fresh growth medium and then incubated.

4. 1-β-D-Arabinofuranosylcytosine Treatment:

1-β-D-arabinofuranosylcytosine (Sigma Chemical Co., St. Louis, Missouri) was dissolved in PBS as 1mg/ml stock solution and further diluted to the working concentration (10μg/ml) in the serum free medium prior to use. Prior to ara-C treatment, the growthmedium was removed from the cultures, and the cells were treated with ara-C for 30 minutes before or after UV-light or MMS treatment.

5. Unscheduled DNA Synthesis:

For the determination of the excision repair ability, HF_1 cells (5×10^4cells/ml) were grown on cover slips (25×22mm) in plastic petri dishes for 24 hours. ^{3}H-thymidine (Amersham/Searle, Corp., Arlington Heights, Illinois. sp. act., 40—60Ci/mM) was incorporated into the cells at a final concentration of 10μCi/ml for an hour immediately after combined treatments with ara-C and UV-light or MMS. Labeling with ^{3}H-thymidine was terminated by washing the cells three times in cold Hank's balanced salt solution (HBSS) containing 100μg/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 cells grown on cover glasses were mounted on the slides with the cells uppermost. Autoradiograms were prepared using Kodak NTB liquid nuclear track emulsion. Sliver grains over the nuclei in the lightly labeled cells were counted for the determination of the unscheduled DNA synthesis.

6. Chromosome Aberrations:

Ara-C was treated to CHO cells grown in milk dilution bottles immediately before or after treatment with UV-light or MMS. The cultures were then incubated for additional six hours. Colcemid (0.06μg/ml) was added during the final two hours of incubation. The mitotic cells were then harvested by gentle shaking off the bottles, treated with hypotonic solution (0.05M 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). Chromosome aberrations were scored according to the criteria of Evans (1977).

7. Sister Chromatid Exchanges:

CHO cells treated with ara-C and UV-light or MMS were grown for 24 hours (2 rounds of replication) in the presence of 10μM BUdR to produce "harlequin chromosomes". Chromosome preparations were made as described previously. Differential staining of chromatids was produced by the technique of Perry and Wolff (1974). The slides were stained in Hoechst 33258 (0.5μg/ml) for 12 minutes and washed briefly in distilled water. The slides were then mounted in Sörensen buffer (pH 6.8) with coverslips and exposed to fluorescent light for two hours. After removing coverslips the slides were rinsed, dried thoroughly and subsequently

stained with 4% Giemsa (Gurr's R66, pH 6.8) for 10—15 minutes. The number of sister chromatid exchanges per cell was scored.

8. DNA Replication Inhibition:

An appropriate number ($0.5—1.0\times10^7$ cells/ml) of HeLa S_3 cells were grown for 24 hours in 35mm plastic petri dishes containing 0.01μCi/ml of ^{14}C-thymidine (52m Ci/mM). The cells were then washed and exposed to MMS or UV-light. Immediately before or after exposure to these mutagens, ara-C was treated. At various times cultures were pulse labeled with 20μCi/ml of ^{3}H-thymidine (40—60Ci/mM) for 10 minutes. Afterlabeling, the radioactive medium was thoroughly removed and the cells were quickly washed with ice-cold 0.15M sodium chloride-0.015M sodium citrate (SSC) three times.

The cells were then scrapped off in SSC and collected on Whatman GF/C glass fiber filter that had been presoaked with cold 4% PCA and dehydrated by stepwise treatment with 75%, 95% and 100% ethanol and dried completely. The filters were placed in scintillation vials and to which were added 10ml of the scintillation fluid which consisted of 4g of Omnifluor (New England Nuclear, Boston, Massachusetts) per liter of toluene. The radioactivities of the incorporated ^{3}H-thymidine and ^{14}C-thymidine were determined using a Packard Tri-carb scintillation spectrometer.

RESULTS

The effects of pre-and post-treatment with ara-C on UV-induced unscheduled DNA synthesis in normal human fibroblasts HF_1 are shown in Table 1. The rate of unscheduled DNA synthesis induced by UV-light, represented as an average number of grains, was increased up to 20J/m^2 and reached almost a plateau thereafter. Ara-C at a concentration of 10μg/ml does not seem to induce unscheduled DNA synthesis, suggesting that it is not DNA damaging agent. Treatment with ara-C prior to UV-irradiation appeared to inhibit UV-induced excision repair. This inhibitory effect was more prominent in the post-treated group.

The inhibitory effect of ara-C on UV-induced excision repair in terms of both net UV repair and relative UV repair indicate that pre-or post-treatment with ara-C inhibited 18% and 32% of UV-induced unscheduled DNA synthesis, respectively. These results indicate that ara-C inhibits UV-induced nucleotide excision repair and that the inhibitory effect is much prominent in its post-treatment.

Table 2 represents the effects of ara-C on MMS-induced unscheduled DNA synthesis. The data show that MMS-induced unscheduled DNA synthese is dose dependent and that pre- or post-treatment with ara-C inhibits MMS-induced unscheduled DNA synthesis. Particularly, post-treatment with ara-C suppresses MMS-induced excision repair at the same level in all dose ranges. The inhibitory effects of ara-C on MMS-induced excision repair show that 26% and 59% of MMS-induced

Table 1. The effects of ara-C on UV-induced unscheduled DNA synthesis in normal human fibroblasts HF_1.

Treatments		Average No. of grains* per cell±S.E.	Net UV repair	Relative UV repair	
Ara-C (μg/ml)	UV (J/m^2)			%	mean±S.E.
0	0	4.7±1.1	0	100	
0	10	29.8±1.9	25.1	100	100
0	20	35.7±0.8	31.0	100	
0	30	37.2±0.7	32.5	100	
10	0	6.4±0.7	0	0	
10	10	25.7±1.3	19.3	76.9	
10	20	32.4±1.4	26.0	83.9	82.2±2.71
10	30	34.3±1.4	27.9	85.8	
**10	0	6.2±0.7	0	0	
10	10	21.3±2.4	15.1	60.2	
10	20	28.7±0.9	22.5	72.6	68.0±3.91
10	30	29.3±1.1	23.1	71.1	

* 50 cells were analyzed for grain counting.
** Ara-C treatment after UV-irradiation.

Table 2. The effects of ara-C on MMS-induced unscheduled DNA synthesis in normal human fibroblasts HF_1.

Treatments		Average No. of grains* per cell±S.E.	Net MMS repair	Relative MMS repair	
Ara-C (μg/ml)	MMS (mM)			%	mean±S.E.
0	0	4.1±0.5	0	100	
0	0.5	14.2±1.9	10.1	100	
0	2.0	25.1±2.1	21.0	100	100
0	4.0	32.7±2.4	28.6	100	
10	0	6.3±1.1	0	0	
10	0.5	13.7±3.7	7.4	73.3	
10	2.0	21.3±1.7	15.0	71.4	74.3±2.06
10	4.0	28.7±2.8	22.4	78.3	
**10	0	6.8±0.9	0	0	
10	0.5	11.5±0.7	4.7	46.5	
10	2.0	15.4±0.9	8.6	41.0	41.2±3.03
10	4.0	17.1±1.1	10.3	36.0	

* 50 cells were analyzed for grain counting.
** MMS treatment before exposure to ara-C.

unscheduled DNA synthesis were inhibited by pre- or post-treatment with ara-C, indicating more remarkable effects in post-treated. group. These results strongly

suggest that ara-C also inhibits MMS-induced base excision repair, and that this inhibitory effect seems to be greater than that observed in UV-induced nucleotide excision repair.

Table 3. The effects of ara-C on UV-induced chromosome aberrations in CHO cells fixed at 6 hours after treatment.

Treatment		Normal metaphases (%)	Type of aberrations*				Breaks per cell
			chromatid-type		chromosome-type		
UV (14J/m²) or MMS (2mM) +	Ara-C (10μg/ml)		deletions	exchanges	deletions	exchanges	
—	—	96	4±0.9	—	—	—	0.04
UV	—	27	144±3.9	17±2.1	1±0.7	—	1.62
—	Ara-C	71	29±1.7	—	—	—	0.29
UV	Ara-C	17	184±2.7	15±1.7	—	—	1.99
**Ara-C	UV	21	164±4.5	15±2.0	—	—	1.77
MMS	—	31	151±2.9	4±1.0	4±1.5	—	1.59
MMS	Ara-C	20	214±3.7	3±1.0	—	—	2.17
**Ara-C	MMS	28	172±4.2	1±0.7	—	—	1.73

* Based on the 100 cells scored
** Ara-C pretreatment.

Table 3 shows the effect of ara-C on the frequency of chromosome aberration induced by UV-light or MMS in CHO cells fixed at six hours after the teatment. As expected, the type of aberrations was mostly chromatid-type aberrations. In the control, 96% of the cells scored showed normal metaphases and the rate of spontaneous aberration was 0.04 breaks per cell. Ara-C was found to be a chromosome breaking agent which induced 29% of abnormal metaphases comprising of 0.29 breaks/cell at treatment of 10μg/ml for 30 minutes treatment. In 14J/m² UV-irradiated group, the percentage of normal metaphases decreased to 27%. Most of aberrations were of the chromatid-type in which majority were deletions.

In both ara-C and UV treated groups, percentages of normal metaphases were decreased and the aberration rates were increased as compared to the single UV-irradiated group. The increased rate of chromosome aberrations was found to be due to the increase of deletions, but not exchanges. The enhancing effects by ara-C on UV-induced chromosome aberrations were remarkable in its post-treat ment. The aberration rate of post-treatment was 1.99 breaks per cell, which is higher than the sum of the rates produced by UV-light and by ara-C separately. In pretreated group, however, the rate of aberration was higher than that of single UV group, but lower than sum. These results indicate that posttreatment with ara-C is more productive for inducing chromosome aberrations by UV-light, and that ara-C appears to increase the frequency of chromatid deletion, but not

exchange type aberration.

Treatment with 2.0mM MMS decreased the normal metaphases to 31% and induced chromosome aberrations at the rate of 1.59 breaks per cell. Treatment with ara-C followed by MMS exposure to cells raised the aberration rate much higher than those of each agent independently. From the results, it may be concluded that the post-treatment with ara-C exhibits a synergistic effect on MMS-induced chromosome aberration, and that this synergistic effect is mainly due to the increase of chromatid deletion.

The effects of ara-C on the induction of sister chromatid exchanges in CHO cells irradiated with UV-light are shown in Fig 1. In the control which is only BUdR substituted cells, the number of sister chromatid exchanges per cell and per chromosomes were 8.1 and 0.38, respectively (data are not shown). This value is regarded as a spontaneous occurrence due to BUdR treatment. Ara-C at the concentration of 10μg/ml appeared to induce sister chromatid exchanges about 1.5 times of control value.

In 10J/m^2 UV-irradiated group, the rates of sister chromatid exchanges per chromosome and per cell were 0.91 and 19.7, respectively, which represent more than

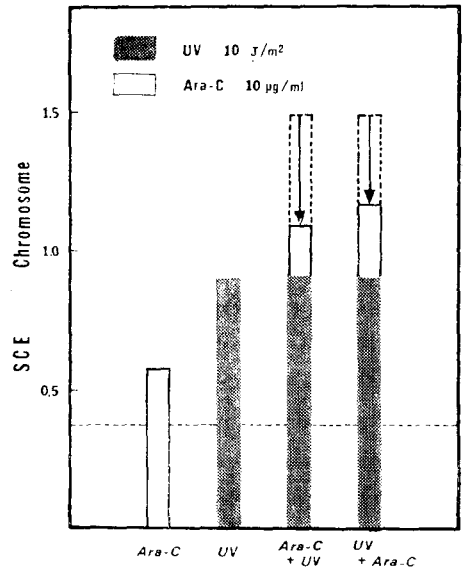

Fig. 1. Histogram showing the effects of ara-C on UV-induced ister chromatid exchanges (SCE) in CHO cells. SCEs per chromosome induced by both ara-C and UV-light were less than the sum of those induced by ara-C and UV independently, regardless of the order of treatment.

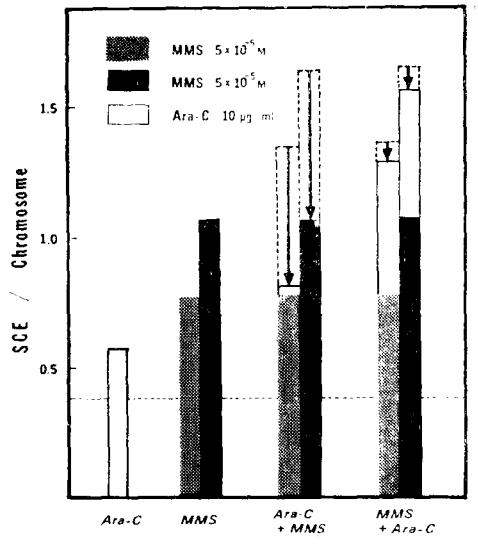

Fig. 2. Histogram showing effects of ara-C on MMS-induced sister chromatid exchanges (SCE) in CHO cells. SCEs per chromosome in the pretreatment with ara-C were almost the same as the single MMS treatment, while those in the posttreatment showed an additive effect.

twice of the control. Pre- or post-treatment with ara-C increased the rates of sister chromatid exchanges induced by UV-light, but the rates were far less than those of each agent independently. This result indicates that ara-C does not show any enhancing effects of UV-induced sister chromatid exchanges, even in the case of its post-treatment after UV-irradiation. These effects are quite different from those of chromosome aberrations.

Fig. 2 shows the effects of ara-C on MMS-induced sister chromatid exchanges (Fig. 2). In the group treated with 5×10^{-6} and 5×10^{-5}M MMS, the rates of sister chromatid exchanges per chromosome were 0.77 and 1.07, respectively. It is believed that MMS is very effective agent in inducing sister crhromatid exchanges even at lower concentrations. Treatment with ara-C prior to MMS-treatment does not show any enhancing effect on MMS-induced sister chromatid exchanges, and the rate is almost the same as the corresponding single treatment with MMS. Post-treatment with ara-C showed almost the same value as the sum of the sister chromatid exchanges seen in cells exposed to MMS or ara-C alone, indicating an additive effect. The pre-treatment with ara-C, however, showed the same value of sister chromatid exchanges per chromosome as the single treatment with MMS.

The above results strongly indicate that action mechanism of ara-C in UV-or MMS-induced sister chromatid exchanges are different from that in UV-or MMS-induced chromosome aberration, suggesting that different mechanisms are involved in the production of chromosome aberrations and of sister chromatid exchanges.

Fig. 3 shows the effects of ara-C on the rate of DNA synthesis in HeLa S_3 cells at various times after irradiation with UV-light. Ara-C treatment to replicating HeLa S_3 caused an immediate decline and recovery of the rate of DNA synthesis. The rate of DNA synthesis reached minimum at 0.5 hour in the level of 18% of control, and then sharply recovered and showed a control level at 4.5 hours after ara-C treatment. This result indicates that ara-C appears to be an inhibitor of DNA replication, but not a DNA damaging agent.

In 14 J/m^2 UV-irradiated group, the rates of DNA synthesis were decreased with time until 4.5 hours to the level of 20% of control. In combined treatment with ara-C and UV-light, the pattern of DNA synthesis was different from that of single exposure to UV-light, so that the initial response was similar to that of ara-C until 1.5 hours, but the later response was similar to that of UV-irradiated group. The rate of DNA synthesis was significantly decreased and showed a minimum at 0.5 hour in the level of 15% of control. At later times the rates were slightly increased up to 20% at 1.5 hours and then kept until 4.5 hours after irradiation. This tendency of DNA synthesis was similar in both pre- and post-treatment with ara-C, but more severe effects were found in the post-treatment.

Fig. 4 represents the effects of ara-C on the rate of DNA synthesis in HeLa S_3 cells

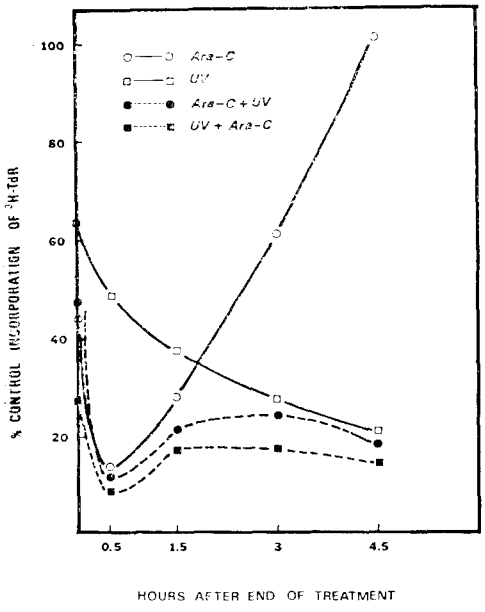

Fig. 3. Effects of ara-C on UV-induced replication inhibition in HeLa S_3 cells. Ara-C cause an immediate decline and then increase in the rates of DNA synthesis. UV-light decreased the rates of DNA synthesis with time up to 4.5 hours after irradiation. Treatment with ara-C before or after UV-irradiation showed similar responses to ara-C at early periods, but later the responses were similar to those of UV-irradiated group.

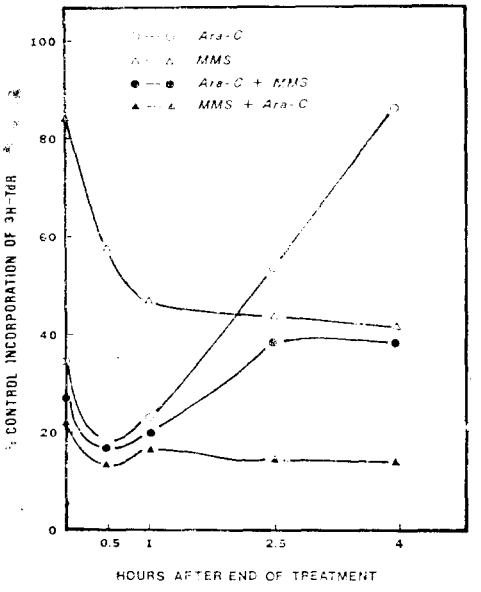

Fig. 4. Effects of ara-C on MMS-induced replication inhibition in HeLa S_3 cells. The rates of DNA synthesis in MMS treated cells were decreased until 1 hour and then remained unchanged until 4 hours after MMS treatment. In post ara-C treated cells, the initial inhibited rate of DNA synthesis did not show to recover until 4 hours, although pre-treated cells appeared to recover to the level of MMS treated cells 2.5 and 4 hours after treatment.

treated with MMS. In the 2 mM MMS treated group, the rates of DNA synthesis were decreased with time until 1 hour and then remained unchanged until 4 hours after MMS treatment. In the ara-C and MMS treated group, the rates of DNA synthesis were about 20% of control at 0.5—1 hour, and then recovered to about 40%, but still below that of MMS alone group. In post- ara-C treated group, the initial inhibitory rate of DNA synthesis did not show to recover until 4 hours after MMS treatment. These results suggest that ara-C affects the DNA replication at an early period of times after UV or MMS treatment.

The overall results of this study seem to indicate that the action mechanism of ara-C is different in different biological systems, since the effects of ara-C on UV- or MMS-induced excision repair, chromosome aberrations, sister chromatid exchanges and replication inhibition are different.

DISCUSSION

The present results on the effects of ara-C on UV-light or MMS induced unscheduled DNA synthesis, chromosome aberrations, sister chromatid exchanges, and replication inhibition show that these four biological phenomena in mammalian cells seem not to be related one another.

Ara-C appeared to inhibit the unscheduled DNA synthesis induced by UV-light as well as MMS, and the inhibitory effects were remarkable in its post-treatment. Hiss and Preston (1977) have reported that ara-C inhibits DNA repair in human cells exposed to UV-light, 4-nitroquinoline-1-oxide (4NQO), mitomycin-C, N-methyl-N-nitrosoguanidine (MNG) and 8-hydroxyquinoline (HQ). Dunn and Regan (1979) have subsequently shown that ara-C produces an inhibition of the pyrimidine dimer excision in human cells. The present results are in good accord with those of previous investigators. These results and other published data strongly suggest that the inhibitory action of ara-C on excision repair seems to be involved in the polymerization-ligation step, possibly by incorporation of several ara-C molecules into repair-replicating DNA, distorting the helix and thus preventing the polymerase from functioning (Hiss and Preston, 1977; Dunn and Regan, 1979; Preston, 1980).

It has been demonstrated that agents which are known to induce DNA damage are also potent inducer of chromosome aberrations and that chromosome aberrations are produced by the misrepair of DNA damage (Bender *et al.*, 1974; Wolff, 1978; Preston, 1980). However, the relationship between DNA damage and chromosome aberrations is not well understood. One approach to understanding how DNA lesions can be translated into chromosome aberrations is to compare the rate of repair of both types of lesions after induction of damage.

The present results show that ara-C exhibits the synergistic effect on MMS or UV-induced chromosome aberrations, mainly by increases of chromatid deletions. These results seem to indicate that chromosome aberration is inversely related to excision repair, but the mode of action of ara-C in inducing chromosome aberrations is quite different from that of excision repair. Preston (1980) has recently shown that ara-C increases X-ray induced chromatid deletions, but not exchange type of aberrations. From these results he postulated that ara-C inhibits the repair of damages that lead to the formation of exchange aberrations. It is, therefore, presumed that ara-C inhibits the repair during the polymerization-ligation step, thus resulting in an accumulation of single strand breaks as a result of incomplete repair synthesis. The breaks accumulated in the presence of ara-C may interact to give aberrations.

Induction of sister chromatid exchanges by ara-C was first described by Raposa (1978). He found that human lymphocytes had two-fold increase in sister chromatid

exchanges after phytohaemaglutinin stimulation and exposure to 2μg/ml of ara-C. The present results also show that ara-C is a potent agent for the induction of sister chromatid exchanges.

Although the molecular mechanism responsible for the production of sister chromatid exchanges is not known, ara-C might produce sister chromatid exchanges by incorporation into DNA as a fraudulent nucleoside at the replicating form with subsequent distortion of the DNA helix. The present results show that ara-C does not increase the rate of sister chromatid exchanges, particularly in the pretreatment with MMS. These results strongly indicate that action mechanisms of ara-C on UV-or MMS-induced sister chromatid exchanges are different from those on UV-or MMS-induced chromosome aberrations, suggesting that different mechanisms are involved in the production of chromosome aberrations and sister chromatid exchanges (Wolff *et al.*, 1977; Wolff, 1978).

The inhibitory action of ara-C on DNA replication has been postulated to involve two distinct steps; a block of the formation of new replicons followed by the inhibition of DNA chain elongation (Bell and Fridland, 1980). Reichard *et al.* (1978) have also obtained an evidence with *E. coli* that DNA primase, required for DNA chain initiation, is the primary target for this drug. Thus, it is tempting to speculate that the effect of ara-C on DNA replication is a specific inhibition of an enzyme involved in the initiation of replicon formation in mammalian cells. Park and Cleaver (1979a.b) have recently shown that DNA replication in UV damaged human cells depends on excision repair ability of the cells and that recovery from UV damage involves changes in the number of actively synthesizing replicons and in the sizes of DNA made. The results presented here concerning DNA replication show that ara-C causes an immediate decline followed by a recovery of the rate of DNA synthesis indicating that ara-C is not DNA damaging agent. These results are in good agreement with those of unscheduled DNA synthesis experiments.

The overall results in this study strongly indicate that DNA damage and its repair processes are not necessarily related to chromosome aberrations and sister chromatid exchanges.

ABSTRACT

Unscheduled DNA synthesis, chromosome aberrations, sister chromatid exchanges and DNA replication inhibition induced by the combined treatments with ara-C and UV-light or MMS in HF_1, CHO and HeLa S_3 cells were studied, and the results obtained were as follows:

(1) Ara-C was found to inhibit UV-or MMS-induced unscheduled DNA synthesis and the inhibitory effect of ara-C was more remarkable in its post-treatment. (2) Ara-C enhanced the rate of chromosome aberrations induced by MMS or UV-light. Post-treatment with ara-C exhibited the synergistic effect on MMS-induced chro-

mosome aberrations mainly by increases of chromatid deletions. (3) Contrarily, ara-C did not increase the rate of sister chromatid exchanges, particularly in the pre-treatment with MMS, although it was found to induce sister chromatid exchanges. (4) The rate of DNA synthesis was declined immediately after ara-C treatment and then recovered. The combined treatments with ara-C and UV-light or MMS showed that the initial response on replication inhibition was similar to that of ara-C, but later responses were similar to that of UV-light or MMS treated group.

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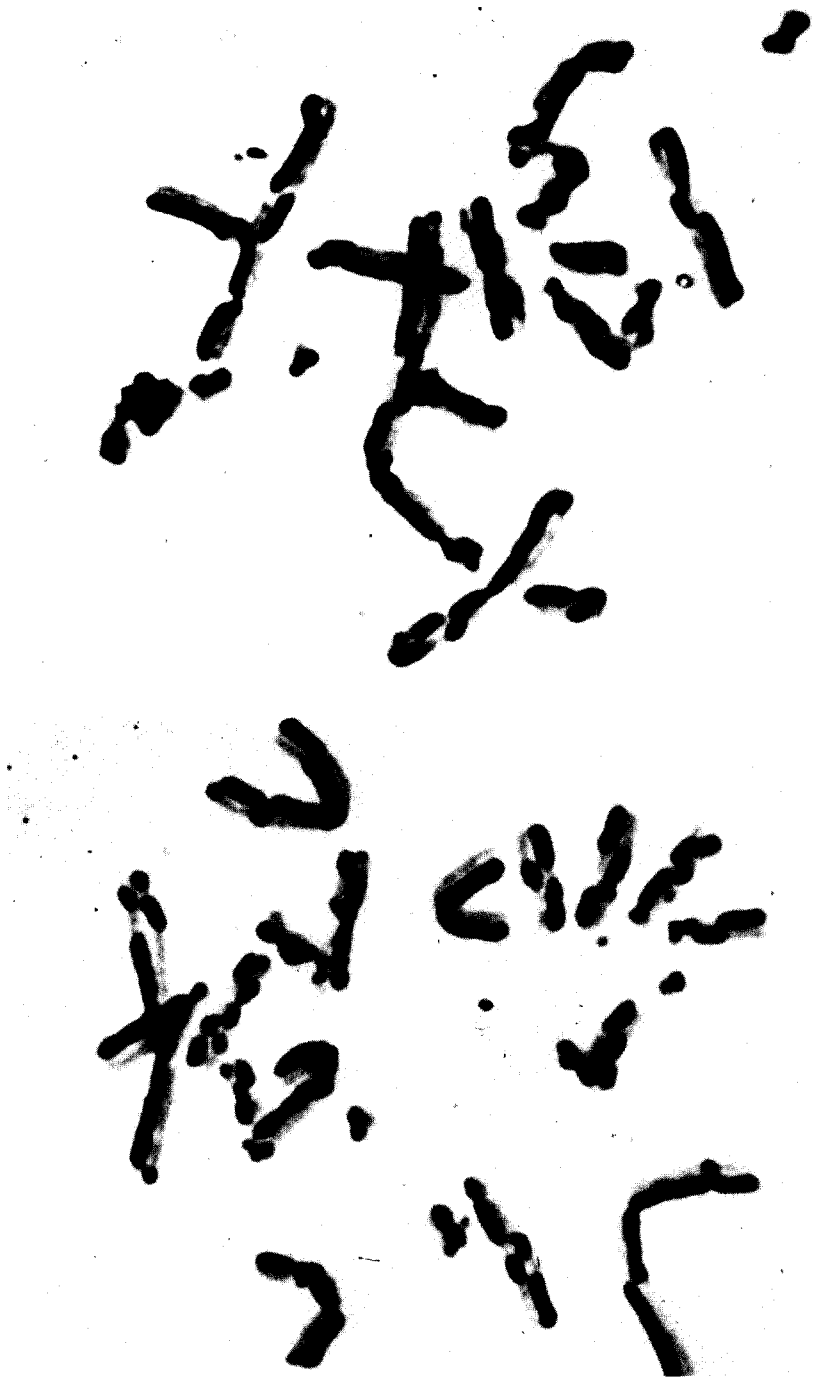

Fig. 5. Metaphase plates showing sister chromatid exchanges (SCE) in CHO cells. Note the high incidences of SCE in the cells obtained by the treatment with 5×10^{-5} MMS (A) and 5×10^{-5} MMS plus 10 μg/ml ara-C (B).