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Induction of Chromosome Shattering and Micronuclei by Ultraviolet Light and Caffeine. I. Temporal Relationship and Antagonistic Effects of the Four Deoxyribonucleosides

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It is known that nucleosides may have antimutagenic and anticlastogenic effects. Here, we have investigated the influence of nucleosides on the induction of shattered chromosomes (fragmentation and/or pulverization of chromosomes of a mitotic cell) and of micronuclei by ultraviolet (UV) light and caffeine. Asynchronous cell cultures of a V79 subline of the Chinese hamster were irradiated at wavelength 254 nm using fluences up to 5.2 joules/m^2. Following irradiation, the cells were postincubated either with 1.0 mM or 2.0 mM caffeine alone or with caffeine plus the four deoxyribonucleosides (dXs) (concentration 0.1 mM each). After different incubation times (three to 24 hours), chromosome preparations were performed. In other experiments, synchronized cells were used. The percentage of metaphase spreads with shattered chromosomes and the percentage of cells with micronuclei were determined. Post-treatment with caffeine alone resulted in shattered chromosomes in a high percentage of cells at the first post-irradiation mitosis as described previously. Formation of cells with micronuclei was observed only after the appearance of mitotic cells with shattered chromosomes, the maximum percentage of cells with micronuclei being smaller than the maximum percentage of cells with shattered chromosomes. The strong potentiating effect of UV- light plus caffeine was significantly reduced, however, if the post-treatment was performed with caffeine plus nucleosides. A significant reduction was also observed in the percentage of micronuclei. An evaluation of the mitotic indices and of cell-cycle parameters indicates that the effect of nucleosides was not due to enhanced interphase death.

Key words: chromosome shattering, ultraviolet (UV) light, caffeine, nucleosides, antimutagens, micronuclei

INTRODUCTION

In a number of cell strains, especially in rodent cells, caffeine is known to potentiate the chromosome damaging effects of ultraviolet (UV) light and a number of chemical mutagens [Kihlman, 1974; Nilsson and Lehmann, 1975; Hartley-Asp, 1976; Kihlman, 1977;

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Parts of this investigation will be presented in the doctoral dissertation of M. Simickova to be submitted to the Faculty of Medicine, University of Freiburg i. Br.

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Roberts, 1978]. A striking phenomenon observed after UV irradiation ($\lambda = 254$ nm) and caffeine post-treatment is the frequent occurrence of cells with generalized chromosome shattering (GCS) (fragmentation and/or pulverization of all chromosomes of a mitotic cell) [Nilsson and Lehmann, 1975; T. Cremer et al, 1979].

In the present investigation, we have studied the influence of nucleosides on this phenomenon. These substances are known to have antimutagenic and anticlastogenic effects [Novick and Szilard, 1952; Kihlman, 1977; Gebhart, 1977]. Here, it is shown that the addition of deoxyribonucleosides to the postirradiation medium exerts a strong antagonistic effect on the induction of GCS. In addition, we examined the influence of nucleosides on the production of cells with micronuclei [Boller and Schmid, 1970; Countryman and Heddle, 1976]. The presence of nucleosides also reduced the percentage of micronuclei produced by UV light and caffeine.

MATERIAL AND METHODS

Cell Material and Culture Conditions

Cells of a subline of V79 Chinese hamster cells [Cremer et al, 1976] were used. This cell line has a modal chromosome number of 21 and a mean generation time of 13–14 hours. The cells were grown in Eagle's minimum essential medium (MEM) supplemented with 10% fetal calf serum (FCS), nonessential amino acids, and antibiotics (100 units/ml penicillin and 100 μg/ml streptomycin) in a humidified atmosphere with 5% CO_2. MEM (Flow Laboratories, Germany) was free from deoxyribonucleosides.

For experiments, cells were grown in 6-cm plastic petri dishes (Nunc/Denmark). Asynchronous cultures were inoculated after trypsination and grown two days before use. Synchronous populations were obtained by the mitotic shake-off procedure which resulted in at least 90% mitotic cells as revealed by direct chromosome preparations. The percentage of S-phase cells in these populations was small ($\leqslant 2\%$) as shown by pulse labeling with ^{3}H-thymidine prior to detachment.

UV Irradiation and Post-treatment

Asynchronous cells grown in petri dishes were labeled with ^{3}H-thymidine (0.1 μCi/ml, 5 μCi/mmol; Amersham Buchler) for 30 minutes prior to irradiation. Synchronized cells were used without prelabeling. Medium was removed before irradiation and the cells were washed twice with Hanks' solution (without phenol red). Thereafter, cells were irradiated from above, while covered with a 1 mm layer of Hanks' solution. A germicidal lamp (Sterisol 5143, Original Hanau) was used emitting predominantly at 254 nm wavelength. The fluence rate was determined to be 3.5 W/m^2 by means of a calibrated photodiode (United Detector Technology, Santa Monica, California). The duration of irradiation (0.75 seconds, 1.5 seconds) was controlled by openings in rotating masks above the cells which were moved by the wheel of a record player. This procedure allowed a precise adjustment of the irradiation time. Immediately after the irradiation, the cells were postincubated at 37°C in MEM with 10% FCS, either with caffeine (1–2 mM) alone or with caffeine plus the four deoxyribonucleosides (dXs) (deoxyadenosine, deoxycytidine, deoxyguanosine, and thymidine; concentration 0.1 mM each). Except for the addition of dXs, all treatments were identical and made in duplicate. After different incubation times (3–27 hours), in situ chromosome preparation was performed [Zorn et al, 1976]. Colchicine

(2 μg/ml) was added 3 hours before preparation. The dishes were air-dried and stained with aceto-orcein. The scoring of dishes was performed "blind," ie, in the absence of information about the treatment regimen. To examine chromosome damage and micronucleus formation , at least 50 metaphases and 500 interphase cells, respectively, were analyzed per dish; ie, at least 100 metaphase figures and 1,000 interphase cells were scored for each treatment and fixation time.

For statistical evaluation the binomial assumption was made, and the confidence limits $p_1 \leqslant p \leqslant p_2$ for proportions were determined [Beyer, 1974]. The observed frequency is p and p_1 and p_2 are the lower and upper limits of the 95% confidence interval, respectively. Throughout the text, the 95% confidence intervals are given as decimals. The actual data are expressed as a percent. The term "nonsignificant difference" between two observed frequencies p_I, p_{II} means that the 95% confidence intervals of p_I and p_{II} overlapped; if the term "signficant difference" is used, the 95% confidence intervals of p_I and p_{II} were clearly separated from each other.

Autoradiography

After microscopic evaluation as described above, pulse-labeled cells were covered with Ilford nuclear emulsion K2 and processed following standard procedures [Zorn, 1978]. Exposure time at 4°C was two weeks. In autoradiographs, the percentage of labeled metaphases was determined.

RESULTS

1. Classification of Metaphase Figures

The metaphase figures obtained following whole-cell irradiation (λ = 254 nm) of V79 cells and post-treatment with caffeine in the presence or absence of deoxyribonucleosides were classified in the following way [Zorn et al, 1977; Zorn, 1978; T. Cremer et al, 1980a]: class A: No recognizable alterations of chromosome morphology; class B: Metaphase plates with one, occasionally two alterations. In most cases, these alterations were achromatic lesions ("gaps") or chromatid breaks. Occasionally, chromatid exchange figures were found. Deviations from the modal chromosome number were not classified as aberrations; class C: All metaphase spreads containing more than two aberrations with the majority of chromosomes (11 and more) remaining intact, were classified as class C. In the whole-cell irradiation experiments presented here, class C figures were a very rare event (< 1%), in contrast to experiments in which only a small part of the cell nucleus was UV- microirradiated [Zorn et al, 1977; Zorn, 1978]; class D: This class contains metaphase spreads in which the majority of chromsomes (11 and more) showed an aberrant morphology, only one or several chromosomes remaining intact (Fig. 1a); class E: In metaphase spreads of class E, all chromosomes were affected (GCS), appearing fragmented and/or pulverized (Fig. 1b, c). In the majority, figures as shown in Figure 1c were observed. The term GCS is introduced as a descriptive one to avoid any prejudice concerning the continuity or discontinuity of the DNA strand. Following UV- irradiation alone (up to 7 joules/m^2) or caffeine post-treatment alone (1–2 mM), metaphase figures of classes C-E were rare events (< 1% each) [Zorn, 1978]. UV-irradiation plus caffeine post-treatment, however, induced GCS in a synergistic (potentiating) way up to almost 100% of all metaphase figures obtained.

Fig. 1. Chromosome shattering following UV irradiation (λ = 254 nm) of V79 cells and postincubation with caffeine. a) Aberrant morphology in the majority of chromosomes, only one or several chromosomes remaining intact: Class D; b, c) All chromosomes affected (generalized chromosome shattering, GCS): Class E; fragmentation of all chromosomes (b); pulverization of all chromosomes (c).

2. Percentage of Cells with GCS

To investigate the influence of the addition of the dXs on the amount of GCS, cells were treated under identical conditions except addition of dXs.

Figure 2 shows the result for asynchronous cells irradiated with 2.6 joules/m^2 or 5.2 joules/m^2 and post-treated with 1 mM caffeine with or without dXs. At 2.6 joules/m^2, the percentage of cells with GCS was low ($\leqslant$3%) in both cases (Fig. 2a, b), and no significant difference was observed.

Irradiation with 5.2 joules/m^2 and postincubation with 1 mM caffeine alone induced a maximum of 33% ($0.23 \leqslant p \leqslant 0.42$) of cells with GCS (Fig. 2c). This percentage was signficantly reduced, however, if dXs were added. In this case, a maximum of 12% ($0.06 \leqslant p \leqslant 0.20$) cells with GCS was obtained (Fig. 2d).

In Figure 3, the frequencies of metaphase figures classes A through E are shown for cells post-treated with 2 mM caffeine. At this concentration, irradiation with 2.6 joules/m^2 and post-treatment with caffeine alone had a considerable effect: Up to 40% ($0.30 \leqslant p \leqslant 0.50$) of cells with GCS were found (Fig. 3a). Again, the percentage of cells with GCS was significantly reduced if dXs were added (Fig. 3b), the maximum being 12% ($0.06 \leqslant p \leqslant 0.20$). The antagonistic effect of the addition of dXs was also observed following irradiation with 5.2 joules /m^2. While a maximum of 97% ($0.92 \leqslant p \leqslant 0.99$) of cells with GCS was observed without addition of dXs (Fig. 3c), the maximum was 78% ($0.69 \leqslant p \leqslant 0.86$) in the presence of dXs.

In Figure 4, the results of experiments with synchronized cells are shown. These cells were irradiated 3–5 hours after mitotic detachment and post-treated with 1 mM caffeine in the presence or absence of dXs. Again, a significant reduction of cells with GCS was obtained if nucleosides were added (Fig. 4a, b). After 15 hours incubation with 1 mM caffeine in the absence of nucleosides, the amount of cells with GCS was 15% ($0.08 \leqslant p \leqslant 0.24$) after irradiation with 2.6 joules/m^2 and 50% ($0.4 \leqslant p \leqslant 0.8$) after 5.2 joules/$m^2$. In the presence of dXs, the maximum percentages were 1.5% ($0 \leqslant p \leqslant 0.07$) and 3% ($0 \leqslant p \leqslant 0.08$) for the two doses, respectively. It is interesting to note that, in all cases with a significant reduction of the percentage of cells with GCS, the presence of nucleosides also significantly reduced the total aberration frequency (sum of percentages of cells with aberrations classes B through E, achromatic lesions excluded).

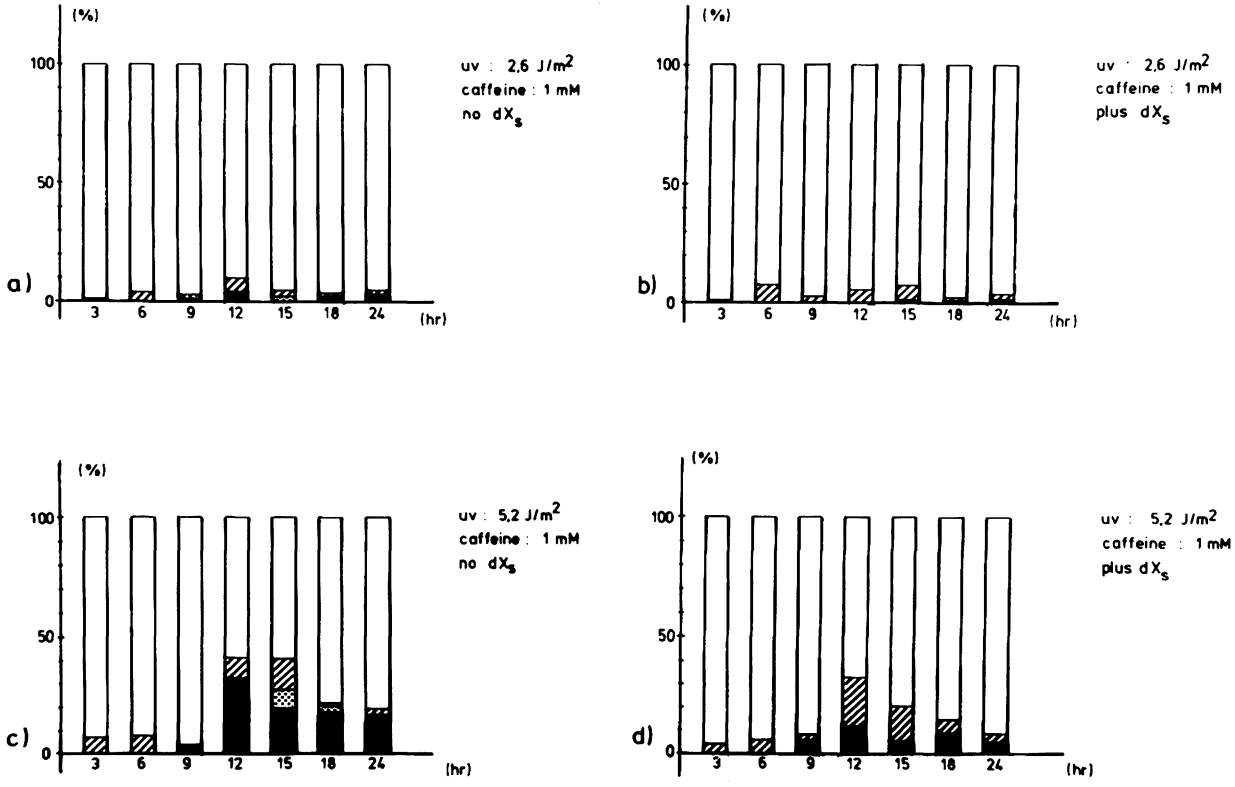

Fig. 2. UV irradiation (λ = 254 nm) of asynchronous V79 cells and caffeine post-treatment (1 mM) with or without the four deoxyribonucleosides (dXs; concentration 0.1 mM each); Induction of chromosome alterations. Abscissa: Incubation time following irradiation (hr); ordinate: Percentage of metaphase figures class A–E –□, no chromosomal alterations: Class A;▨, single defects (one, occasionally two aberrations): Class B;▩, aberrant morphology in the majority of chromosomes, only one or several chromosomes remaining intact: Class D;■, all chromosomes affected (fragmentation and/or pulverization): generalized chromosome shattering (GCS): Class E. (a) 2.6 joules/m^2, no dXs; (b) 2.6 joules/m^2, plus dXs. (c) 5.2 joules/m^2, no dXs, (d) 5.2 joules/m^2, plus dXs. For each value, at least 100 mitotic cells were scored.

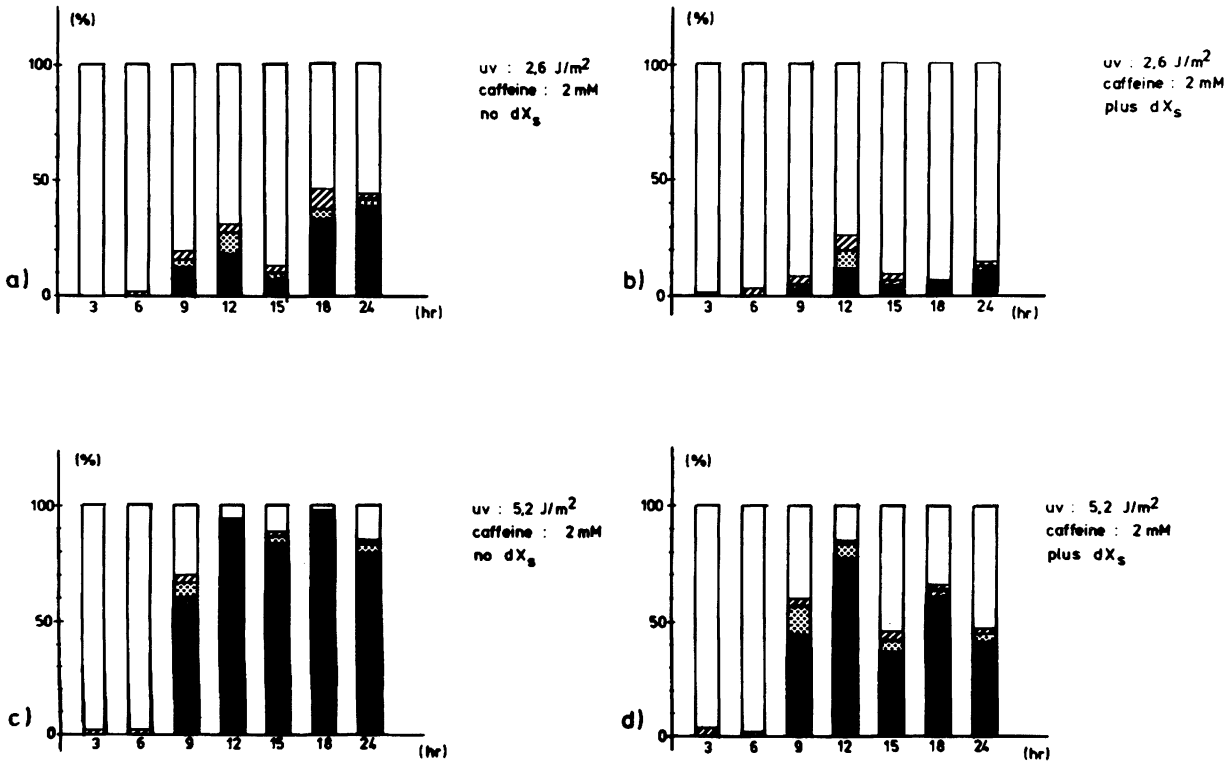

Fig. 3. UV-irradiation (λ = 254 nm) of asynchronous V79 cells and caffeine post-treatment (2 mM) with or without the four deoxyribonucleosides (dXs; concentration 0.1 mM each): Induction of chromosome alterations. Abscissa: Incubation time following irradiation (hr); Ordinate: Percentage of metaphase figures classes A–E (see legend to Fig. 2) –□, class A;▨, class B;▩, class D;■, class E. (a) 2.6 joules/m^2, no dXs; (b) 2.6 joules/m^2, plus dXs; (c) 5.2 joules/m^2, no dXs; (d) 5.2 joules/m^2, plus dXs. For each value, at least 100 mitotic cells were scored.

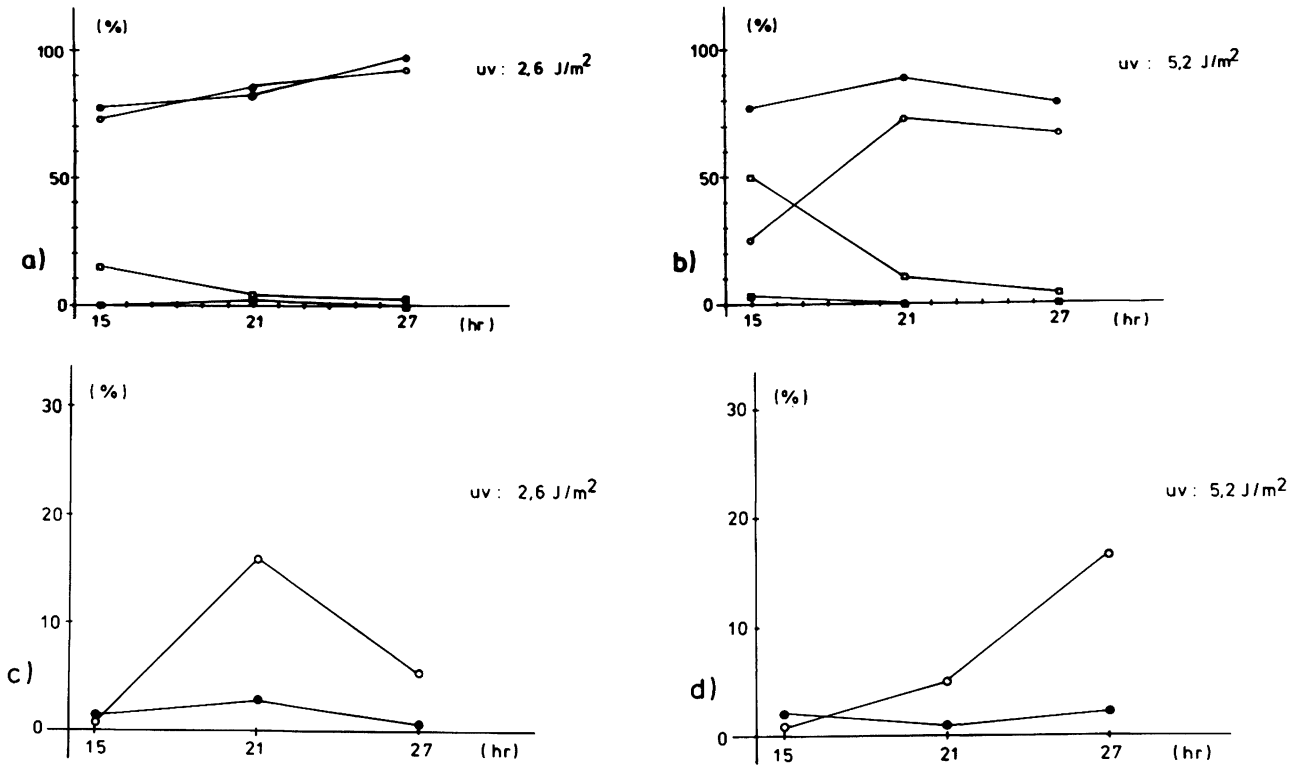

Fig. 4. UV-irradiation (λ = 254 nm) of synchronized V79 cells (GI and early S) and caffeine post-treatment (1 mM) with or without the four deoxyribonucleosides (dXs; concentration 0.1 mM each): Induction of generalized chromosome shattering and formation of micronucleated cells. a,b: Induction of generalized chromosome shattering (GCS). Abscissa: Incubation time following irradiation (hr); ordinate: Percentage of metaphase figures; classes A–E – ○, no chromosomal alterations (class A), caffeine post-treatment without dXs; ●, no chromosomal alterations (class A), caffeine post-treatment in the presence of dXs; □, all chromosomes affected (GCS), caffeine post-treatment without dXs; ■, all chromosomes affected (GCS), caffeine post-treatment in the presence of dXs. (a) 2.6 joules/m^2; (b) 5.2 joules/m^2. For each value, at least 100 mitoses were scored. (c, d) Induction of cells with micronuclei: Abscissa: Incubation time following irradiation (hr); Ordinate: Percentage of cells with micronuclei; ○, no dXs; ●, plus dXs. (c) 2.6 joules/m^2; (d) 5.2 joules/m^2. For each value, at least 1000 cells were scored.

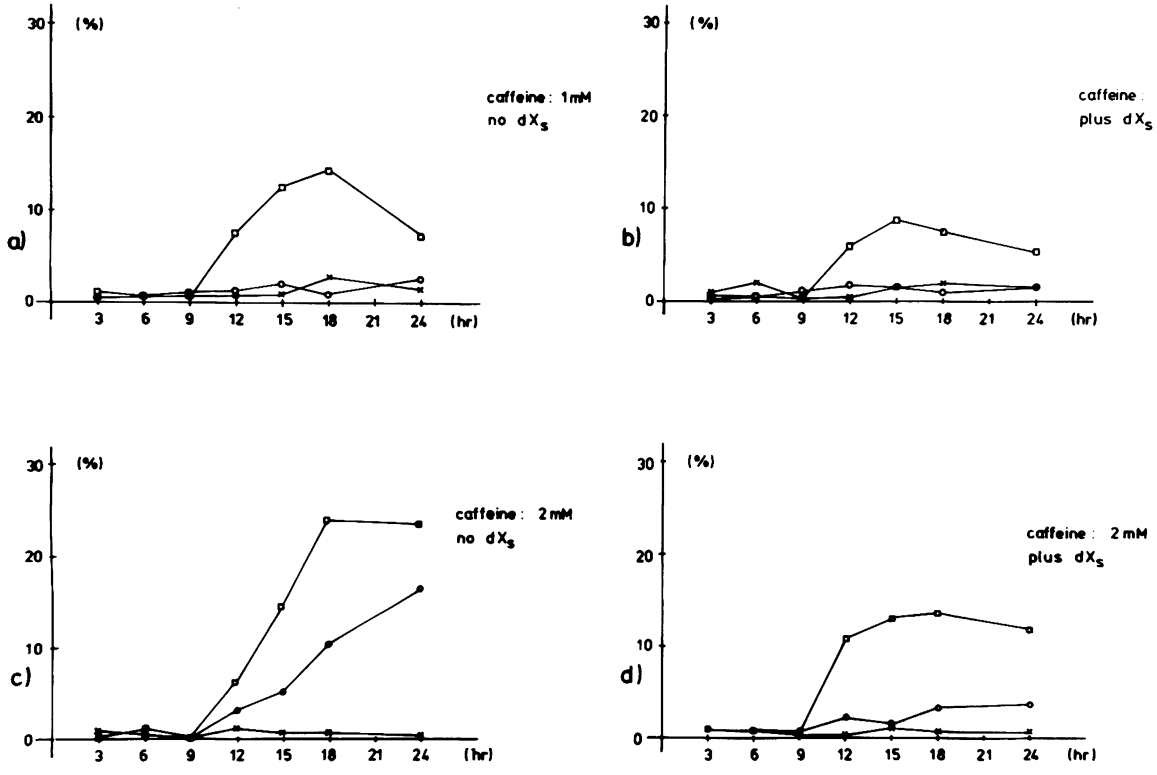

Fig. 5. UV-irradiation (λ = 254 nm) of asynchronous V79 cells and caffeine post-treatment with or without the four deoxyribonucleosides (dXs; concentration 0.1 mM each): Induction of cells with micronuclei. Abscissa: Incubation time following irradiation (hr); ordinate: Percentage of cells with micronuclei; x, O joules/m^2; 0, 2.6 joules/m^2; □, 5.2 joules/m^2. (a) 1 mM caffeine, no dXs; (b) 1 mM caffeine, plus dXs; (c) 2 mM caffeine, no dXs; (d) 2 mM caffeine, plus dXs. For each value, at least 1000 cells were scored. In the majority of cases (70%), micronucleated cells had one to three micronuclei.

3. Formation of Micronuclei

The percentage of cells with micronuclei obtained after UV-irradiation (2.6 joules/m^2 and 5.2 joules/m^2) without caffeine post-treatment or after caffeine treatment of unirradiated cultures was low ($\leqslant 2\%$, $0.01 \leqslant p \leqslant 0.03$), the differences being not significant. A significant increase (up to 14%, $0.11 \leqslant p \leqslant 0.16$), however, was observed following the combined treatment with 5.2 joules/m^2 and 1 mM caffeine (Fig. 5a). While 2.6 joules/m^2 and 1 mM caffeine post-treatment did not result in a significant increase of cells with micronuclei (Fig. 5a), 2 mM caffeine post-treatment produced a considerable effect (maximum 16%, $0.14 \leqslant p \leqslant 0.18$) even at this lower UV-dose (Fig. 5c). Addition of dXs significantly reduced the percentage of cells with micronuclei (Fig. 5b, d). The maxima of the percentage of micronucleated cells obtained in the presence of dXs were 8% ($0.06 \leqslant p \leqslant 0.10$) for 5.2 joules/$m^2$ plus 1 mM caffeine; 3.5% ($0.02 \leqslant p \leqslant 0.045$) for 2.6 joules/$m^2$ plus 2 mM caffeine; 13% ($0.10 \leqslant p \leqslant 0.15$) for 5.2 joules/$m^2$ plus 2 mM caffeine (without dXs – 24% ($0.21 \leqslant p \leqslant 0.26$)).

A comparison of the data presented for the induction of GCS (Figs. 2 and 3) and the results obtained for the production of micronuclei (Fig. 5) clearly shows that cells with micronuclei appeared only after a significant increase of cells with GCS was observed. For example, irradiation with 5.2 joules/m^2 and 2 mM caffeine post-treatment resulted in 60% mitotic cells with GCS after 9 hours incubation (Fig. 3c). At this time, the percentage of cells with micronuclei did not exceed the level of unirradiated controls.

A significant reduction of the percentage of cells with micronuclei by the addition of dXs was observed also in case of synchronized cultures (Fig. 4c, d). A comparison with the data presented in Fig. 4a, b shows that the formation of cells with GCS again preceded the appearance of cells with micronuclei.

4. Mitotic Index and Cell-Cycle Parameters

For all incubation times, the mitotic indices were determined. It was found that the addition of nucleosides did not significantly reduce the mitotic indices if compared with cells treated in the absence of dXs. In some cases, the mitotic index was even slightly higher when nucleosides were added. Furthermore, at 5.2 joules/m^2 plus caffeine post-treatment a reduction of the mitotic delay of approximately 3 hours was observed in the presence of dXs.

The duration of S-phase and of G_2 + prophase was estimated from the metaphase labeling index (MLI) curves according to the method of Evans and Scott [1964]. The results of these estimates are presented in Table I. Up to UV fluences of 2.6 joules/m^2, the differences in the duration of S-phase and G_2 + prophase were small to nonexistant whether nucleosides were added or not. At 5.2 joules/m^2 and postincubation with 1 mM and 2 mM caffeine, the duration of S-phase was observed to be reduced by approximately three hours in the presence of dXs. This fits well to the reduction of the mitotic delay derived from the mitotic index curves. No influence of dXs on the duration of G_2 + prophase was found at this UV fluence. It should be emphasized, however, that these measurements of cell-cycle parameters are only rough estimates since sampling times of 3 hours with colchicine were used to collect cells in metaphase. In any case, the mitotic indices and the cell-cycle estimates obtained from the MLI curves indicate that the reduction of the percentage of GCS and of miconucleated cells by the addition of the four deoxyribonucleosides cannot be explained by enhanced interphase death.

TABLE I. Duration of S-phase and G_2 + Prophase as Estimated from MLI Curves*

UV fluence	0 joule/m^2		2.6 joule/m^2		5.2 joule/m^2	
Post-treatment	S-phase (hr)	G_2 + prophase (hr)	S-phase (hr)	G_2 + prophase (hr)	S-phase (hr)	G_2 + prophase (hr)
0 mM Caffeine, 0 mM dXs	7.0	3.8	nd	nd	nd	nd
0 mM Caffeine, 0.1 mM dXs	6.0	3.8	nd	nd	nd	nd
1.0 mM Caffeine, 0 mM dXs	7.0	3.5	8.0	3.0	9.5	4.1
1.0 mM Caffeine, 0.1 mM dXs	6.5	4.2	6.5	4.2	6.8	4.3
2.0 mM Caffeine, 0 mM dXs	6.3	4.5	6.7	4.5	10.0	5.0
2.0 mM Caffeine, 0.1 mM dXs	7.0	4.5	6.1	4.7	7.0	5.0

*The cell-cycle parameters given were calculated from metaphase labeling index (MLI) curves according to the method of Evans and Scott [1964].

S-phase (hr): Estimated from the time interval between half of the maximum labeling index on the ascending limb to half the maximum labeling index on the descending limb of the first peak, minus the duration of the ^{3}H-thymidine treatment (30 minutes).

G_2 + prophase: Estimated from the time interval between the beginning of ^{3}H-thymidine treatment to the time when the labeling index reached half of the maximum value.

dXs: deoxyadenosine + deoxycytidine + deoxyguanosine + thymidine; concentration 0.1 mM each.

nd = not determined.

From growth curves of exponentially growing cells, a generation time of 13–14 hours was estimated.

DISCUSSION

1. GCS and Premature Chromosome Condensation

GCS (class E) was the most frequently observed class of altered chromosome morphology in the present experiments using whole-cell irradiation (λ = 254 nm) and post-treatment with caffeine. Two types of GCS were obtained: 1) Metaphase figures with chromatid breaks and/or gaps in all chromosomes (Fig. 1b); 2) metaphase figures with "pulverization" [Zorn et al, 1976; Vogel and Bauknecht, 1978] of the chromosomes (Fig. 1c). We have seen many examples of GCS where chromosomes with numerous chromatid breaks were still present besides pulverized chromosomes. This suggests that types 1 and 2 are closely related to each other. Type 2 was obtained in the large majority of metaphase figures with GCS. These figures resemble prematurely condensed chromosomes (PCC) during S-phase (S-PCC) [Johnson and Rao, 1970; Sperling and Rao, 1974] or G1-PCC following UV irradiation [Schor et al, 1975]. Therefore, we have considered the possibility that type 2 figures might be due to micronucleus-derived premature chromosome condensation [Obe and Beek, 1975]. The temporal relationship between the observation of shattered chromosomes and the production of micronuclei observed in our experiments, however, rules out such a possibility: Enhanced formation of micronuclei was observed after mitotic cells with shattered chromosomes appeared in the cultures (compare Figs. 2, 3, and 5).

Different mechanisms for the induction of GCS and PCC, however, do not exclude the possibility that pulverized chromosomes in metaphase plates with GCS indicate a failure of chromosome condensation. Whether the pulverized appearance of chromosomes in GCS figures is mainly due to a large number of DNA breaks or to a failure of chromosome condensation, remains to be investigated. A model developed by us previously to explain the induction of GCS by UV light and caffeine [Zorn et al, 1977; T. Cremer et al, 1980a, b] is compatible with both possibilities.

2. Antagonistic Effects of Nucleosides

The antimutagenic and anticlastogenic action of deoxyribonucleosides is well known [Novick and Szilard, 1952; Kihlman, 1977; Gebhart, 1977]. In the present investigation, it is shown that the synergistic effect of UV light plus caffeine on the induction of GCS and of micronucleated cells is significantly reduced by the addition of the four dXs to the post-irradiation medium. The evaluation of mitotic indices and cell-cycle parameters indicates that the effect is not due to enhanced interphase death.

A possible explanation of the antagonistic effects of nucleosides on the induction of chromosome shattering by UV light and caffeine may be obtained by findings of Collins and Johnson [1979] that the addition of nucleosides may enhance DNA repair synthesis in UV-irradiated Microtus agrestis cells. Unscheduled DNA synthesis following UV irradiation was observed also in the V79 line used in the present investigation [Zorn, 1978]. This suggests the following line of reasoning: First, it seems to be generally assumed that the synergistic action of UV light plus caffeine on the induction of chromosome aberrations is due to the inhibition of daughter strand repair (postreplication repair) of DNA photolesions [Nilsson and Lehmann, 1975; Kihlman, 1977; Roberts, 1978]. Here it is assumed that the inhibition of daughter strand repair by caffeine plays a decisive role also in the induction of generalized chromosome shattering: GCS is induced if the number of daughter strand repair sites exceeds a certain threshold which may vary from cell to cell [T.

Cremer et al, 1980a, b]. Second, addition of nucleosides to the postirradiation medium may enhance the excision of UV-induced pyrimidine dimers.

If so, the number of remaining DNA photolesions and, hence, the number of daughter-strand-repair sites becomes smaller in nucleoside-treated cells than in untreated ones. If this number falls below the threshold value characteristic for a given cell as first described, no GCS is induced. Although the model outlined above is far from proven, it offers a plausible mechanism for the antagonistic effect of nucleosides on GCS and fits well with the data available. It is also consistent with evidence obtained by Nakano and co-workers [1979] that the adverse effect of caffeine on the survival of UV-irradiated V79 cells is strongly diminished by an enhancement of the period of time available for excision repair. While the anticlastogenic effect of nucleosides may be due to their effect on excision-repair capacity, possible effects of an improved supply of cells with nucleosides on daughter-strand-repair capacity may also be considered.

Caffeine may have an inhibiting effect on the uptake and on the metabolism of DNA-precursors [Lehmann and Kirk-Bell, 1974] (C.A. Waldren, personal communication, 1980). The addition of nucleosides might overcome the adverse effect of a reduced supply of nucleosides. Furthermore, some interference of nucleosides with uptake and/or metabolism of caffeine, thus reducing the effective intracellular concentration of caffeine, should not be omitted from consideration.

CONCLUSIONS

Deoxyribonucleosides exert an antagonistic effect on the induction of GCS and micronucleus production by UV light and caffeine in V79 cells. It is suggested that by the addition of nucleosides, the excision repair capacity may be promoted, thus reducing the number of caffeine-sensitive sites of daughter strand repair (postreplication repair).

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