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Similarity in the Effect of Caffeine on DNA Synthesis after UV Irradiation between Xeroderma Pigmentosum Variant Cells and Mouse Cells

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The effect of UV irradiation on the rate of DNA synthesis was compared among normal human, xeroderma pigmentosum (XP, group A and variant) and mouse cells with and without caffeine in the culture medium after UV irradiation. At the same levels of survival, approximately 37%, all cells showed reduction in the rate of synthesis 0–3 h after UV irradiation followed by a recovery to normal or near-normal level 12 h later. In the presence of caffeine, no change in the recovery patterns was observed in normal human and XP A cells. XP variant cells and mouse cells showed little or no recovery in the presence of caffeine even after 12 h, when full recovery was obtained without caffeine. XP variant and mouse cells appear to have a common response in that post-irradiation treatment with caffeine inhibits reinitiation of UV-reduced DNA replication. Enhancement by caffeine of UV-killing in XP variant and mouse cells may be due to the retarded resumption of DNA replication.

Key words: Caffeine — UV — Xeroderma pigmentosum — DNA synthesis — Replicon initiation

The effect of UV-irradiation on the rate of semi-conservative DNA synthesis has been measured by many investigators by pulse-labeling cells with [^{3}H]thymidine as a function of time after UV-irradiation. It has been reported that the rate of the DNA synthesis was reduced to approximately half the initial level several hours after UV-irradiation, followed by a recovery to the normal level in mouse[1,2] and normal human fibroblast cells.[3–5] Recovery from the UV-induced reduction of the DNA synthesis has been shown to be related to the susceptibility of the cells to UV in the case of human fibroblast cells.[3] The recovery in fibroblast cells from xeroderma pigmentosum (XP)[2] patients, which are UV sensitive, is greatly reduced.[3,5–7] Also no recovery is observed in the cells from Cockayne's syndrome patients,[5,7,8] or in UV-sensitive mutant mouse cells[9] after UV-irradiation with a dose permitting complete recovery in normal cells. In XP variant cells which have slightly elevated sensitivity to UV, the rate of the recovery is retarded.[3]

In XP variant cells as well as rodent cells including mouse cells, UV-induced cell-killing has been enhanced by post-UV treatment with caffeine.[10–12] However, in normal human and XP fibroblast cells, excluding variant, the killing was not enhanced by caffeine. A block in the conversion of newly synthesized low-molecular-weight DNA to high-molecular-weight DNA has been suggested to be one of the causes of the inhibition by caffeine in UV-irradiated rodent and XP variant cells.[13–16] In the present study, similarity in the effect of caffeine on the rate of DNA synthesis after UV-irradiation was found between XP variant and mouse cells.

MATERIALS AND METHODS

The following human fibroblast cell strains: N1TO, normal human[17]; XP3OS, XP complementation group A[18]; XP5KA, XP variant; and mouse cell line, BALB3T3 were used in this study. All cells were grown in alpha-modified minimum essential medium (Flow Lab., USA) supplemented with 15% calf serum (Flow Lab.).

An appropriate number of cells (5×10^2–10^4) were inoculated into dishes (Lux Parmanox, USA, 6 cm) and incubated for 18 h. After the medium had been removed, the cells were exposed to UV light (254 nm) through the bottom of the dishes, which had 50% transmission of UV light (254 nm). Immediately after UV-irradiation, medium containing 2 or 5 m*M* caffeine was added to dishes followed by incubation for 24 h. Then the cells were grown in fresh medium without caffeine for 12–14 days until colonies formed. The colonies which consisted of more than 50 cells were scored after staining with 4% Giemsa (Merck Japan, Tokyo).

For measurement of rate of DNA synthesis, 10^5 cells were inoculated into a dish (3.5 cm) in which a glass fiber filter (Whatman, UK, GF/C 2.5 cm) had been placed. After a 24-h incubation, the cells on the filters were washed twice with phosphate-buffered saline (PBS). Then both sides of filters were exposed to UV. Immediately after UV-irradiation, the cells were incubated with fresh medium for various times followed by pulse-labeling with the medium containing [methyl-^{3}H]thymidine

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[2] Abbreviations: XP, xeroderma pigmentosum; UV, ultraviolet light; PBS, phosphate-buffered saline; UDS, unscheduled DNA synthesis.

(10 μCi/ml, 25 Ci/mmol, Amersham Japan, Tokyo) for 0.5 h. To investigate the effect of caffeine on the rate of the DNA synthesis, medium containing 2–5 m*M* caffeine was used from 30 min before UV-irradiation to the end of the incubation with the [^{3}H]thymidine. After labeling, the filters were successively washed with PBS, methanol, 5% ice-cold trichloroacetic acid and methanol. After the filters were dried, their radioactivity was measured with a liquid scintillation counter. The counts per minute of [^{3}H]thymidine incorporated in 0.5 h in UV-irradiated cells were regarded as proportional to the rate of the DNA synthesis, and were represented as percentage of the cpm in non-irradiated cells. The effect of unscheduled DNA synthesis (UDS) on the incorporation of [^{3}H]thymidine was negligible, because incorporation of [^{3}H]thymidine by UDS was far less than that by semiconservative DNA synthesis even in normal cells.

The UV light source was two 10-W germicidal lamps (Toshiba GL-10, Tokyo) whose dose was calibrated with a photometer (Topcon UV-254, Tokyo). The dose rate of UV was reduced by using a slit. Irradiation time did not exceed 2 min. Caffeine (0.5 *M*, Nacalai Tesque, Kyoto) was dissolved in PBS at 55°C and stored in a freezer. Caffeine solution was added to the medium just before use.

RESULTS

UV-survival curves of all strains in the absence and presence of caffeine are shown in Fig. 1. XP3OS cells, which belong to complementation group A, were 7 times more sensitive than normal human cells N1TO as judged by the D_0 value. XP5KA (XP variant) cells and mouse BALB3T3 cells showed intermediate sensitivities to UV, having D_0 values 1.2 and 1.4 times higher than that of N1TO cells, respectively. Treatment by 2–5 m*M* caffeine after UV-irradiation did not result in any sensitizing effect on the UV-survival of either N1TO or XP3OS cells (Fig. 1A). In both XP5KA and BALB3T3 cells, 2 m*M* caffeine enhanced UV-induced cell-killing as reported previously on XP variant cells and mouse cells.[10, 11, 13] The caffeine treatment enhanced UV-sensitivity 1.5 and 1.4 times in XP5KA and BALB3T3 cells, respectively (Fig. 1B).

Changes in the rate of DNA synthesis after UV-irradiation are shown in Fig. 2. The rate of the synthesis was reduced to about 50% at 3 h after 4 J/m^2 UV irradiation in all human cell strains. During subsequent incubation, the DNA synthesis gradually recovered to a near-normal level in N1TO cells, while the recovery of the DNA synthesis was much slower in XP5KA cells, and no recovery was observed in XP3OS cells in 12 h after UV-irradiation (Fig. 2A). When the UV dose yielding 37% survival (D_{37}) was given to each cell strain, all strains showed a reduction in the rate of the DNA synthesis followed by approximately the same rate of recovery to normal or near-normal levels 12 h later (Fig. 2B).

The effect of caffeine in the medium during post-UV incubation on DNA synthesis after equitoxic doses (D_{37})

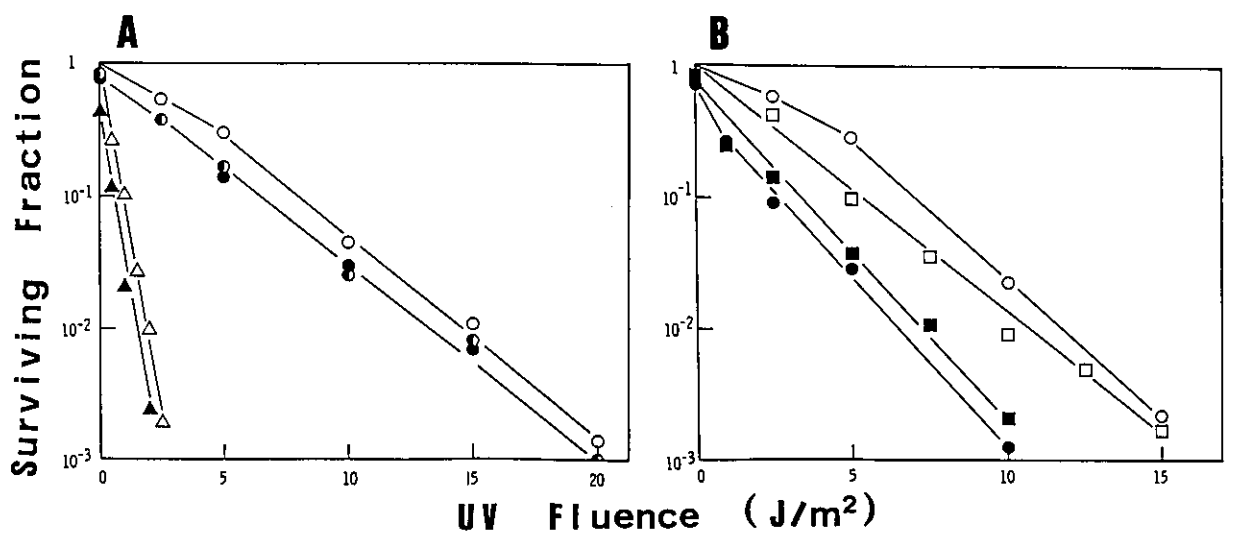

Fig. 1. Effect of caffeine on UV-survival. Cells were treated with the medium containing caffeine for 24 h beginning immediately after UV irradiation. A: ○, normal human, N1TO, no caffeine; ◐, N1TO 2 m*M* caffeine; ●, N1TO 5 m*M* caffeine; △, XP A, XP3OS, no caffeine; ▲, XP3OS, 2 m*M* caffeine. B: ○, mouse, BALB3T3, no caffeine; ●, BALB3T3, 2 m*M* caffeine; □, XP variant, XP5KA, no caffeine; ■, XP5KA, 2 m*M* caffeine.

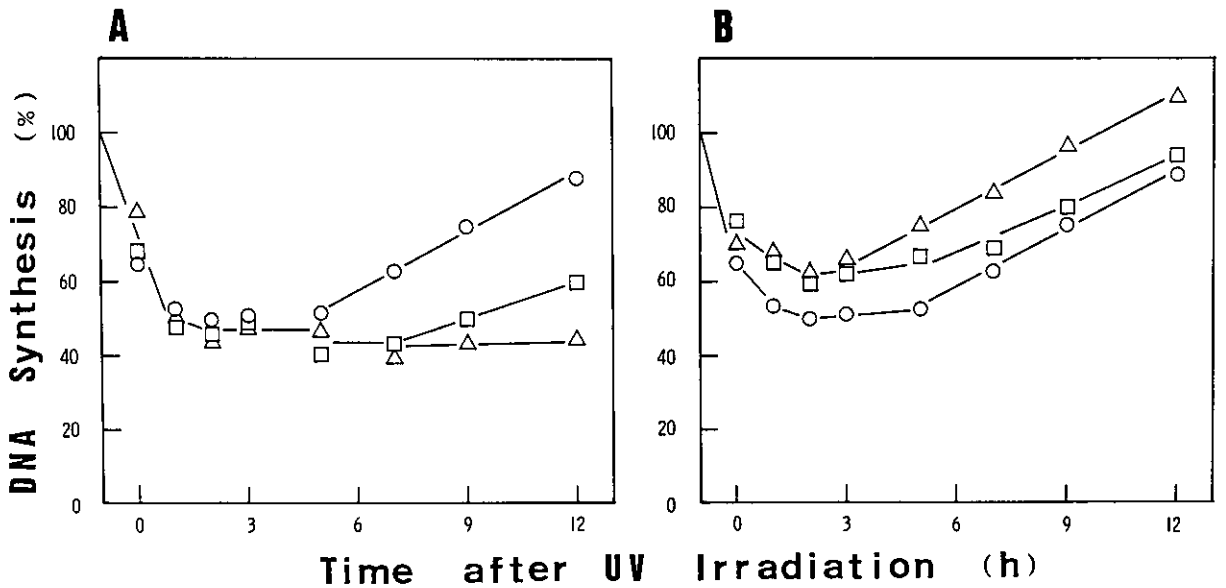

Fig. 2. Effect of UV irradiation on the rate of semiconservative DNA synthesis as a function of time after UV irradiation. ○, N1TO; □, XP5KA; △, XP3OS. A: all cell strains were irradiated with 4 J/m^2 UV. B: all cell strains were irradiated with UV at the dose giving a 37%-survival level; N1TO, 4 J/m^2; XP5KA, 3 J/m^2; XP3OS, 0.5 J/m^2.

of UV-irradiation is represented in Fig. 3. In N1TO and XP3OS cells, the DNA synthesis recovered to near-normal levels 12 h after UV-irradiation in N1TO and XP3OS cells even in the presence of up to 5 m*M* caffeine. In XP5KA cells, 2 m*M* caffeine caused a delay in the recovery of the DNA synthesis, and only slight recovery was shown 12 h after UV-irradiation. In mouse BALB-3T3 cells, 2 m*M* caffeine inhibited the recovery of the DNA synthesis completely 12 h after UV-irradiation. Caffeine alone reduced the amount of the DNA synthesis slightly in all cells (Fig. 3).

DISCUSSION

The reduction in the rate of semiconservative DNA synthesis by UV may be attributed to two principal causes, blocking of DNA chain elongation and interference with replicon initiation. Edenberg[19] showed that the UV-induced inhibition of DNA synthesis was caused by the halting of replicating forks at pyrimidine dimers. Cleaver *et al.*[20] suggested that the reduction in the rate of semiconservative DNA synthesis in UV-damaged cells could be due to a depression in the number of active replicons. They noted that the UV-produced change in the rate of the DNA synthesis was different from the change of molecular weight of nascent DNA as a function of time of incubation after UV-irradiation.

The recovery from the UV-induced suppression of the DNA synthesis may involve many cellular regulatory mechanisms including DNA repair. As shown in Fig. 2A, the rate of recovery appeared to be related to the excision repair capacity of the cells. XP3OS cells, which have about 0–3% of normal repair capacity,[18] were most reduced. Figures 1 and 2 also suggested that the rate of the recovery depended on the excision repair capacity of the cells and on the UV dose. The rates of recovery were, however, similar in all cells when the cells were irradiated with equitoxic doses (Fig. 2B). This may mean that the amount of damage remaining in DNA at a certain time after UV irradiation would play a critical role in the reinitiation of DNA synthesis.

Inhibition by caffeine of the recovery of DNA synthesis correlated well with caffeine-enhanced UV-killing in both XP variant and mouse cells (Figs. 1 and 3), and it appears to be caused by blockage of the conversion of nascent DNA molecules to high molecular weight, as reported by many investigators.[13–16] Another possible mechanism is interference with certain step(s) of excision repair by caffeine in XP variant and rodent cells, and this was supported by the following facts. 1) Host-cell reactivation of UV-irradiated adenoviruses was inhibited by caffeine in XP variant cells[21] and 2) liquid-holding recovery in UV-irradiated XP variant[22] and mouse BALB3T3 cells (Yagi, unpublished data) was inhibited by caffeine. Because UV-induced UDS[23] and excision of pyrimidine dimers[16] were not affected by caffeine, caffeine can be envisaged as blocking late step(s) in excision repair such as ligation of DNA[24,25] or restora-

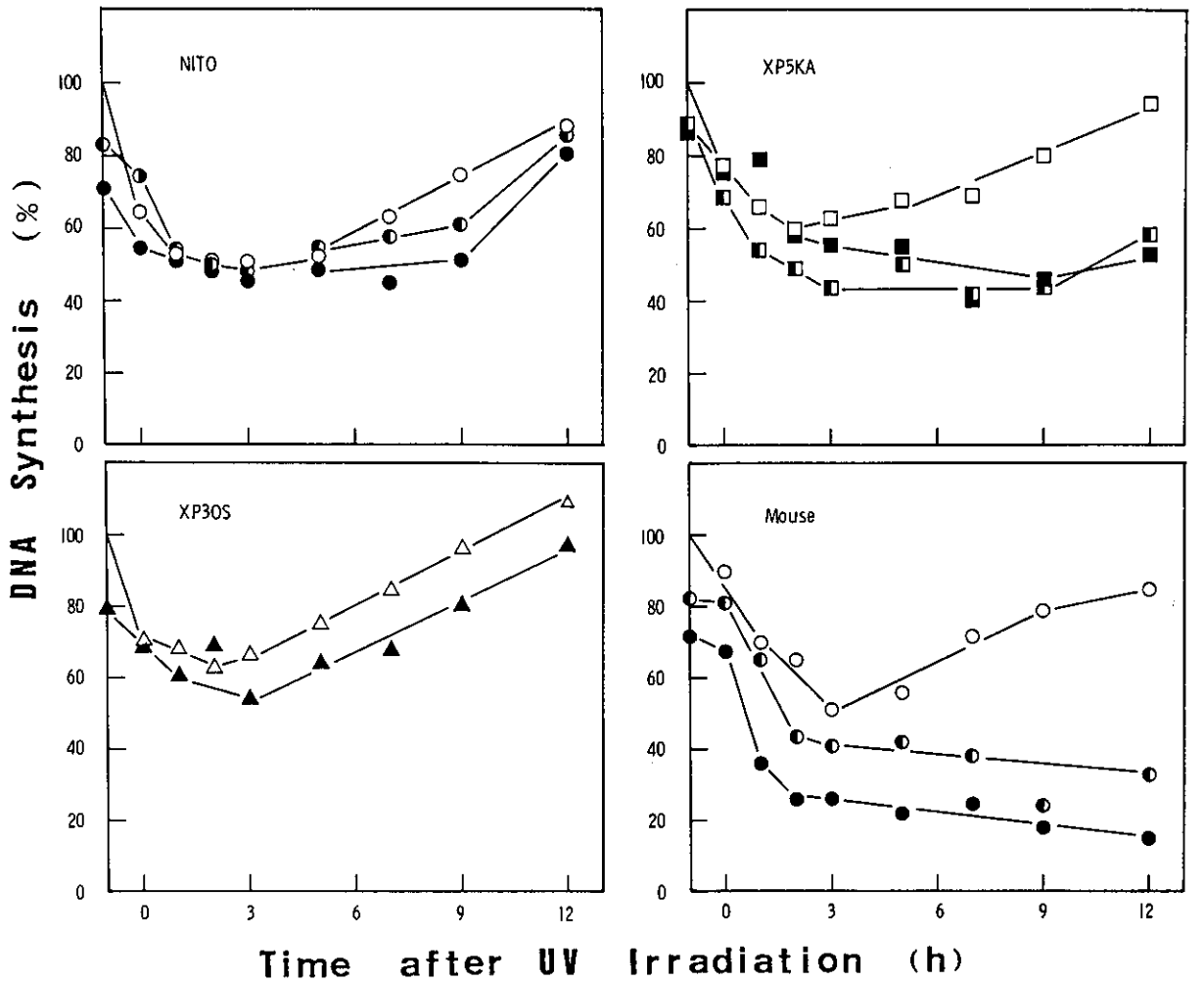

Fig. 3. Effect of caffeine on the rate of semiconservative DNA synthesis after UV irradiation at the dose giving a 37%-survival level; N1TO, 4 J/m^2; XP5KA, 3 J/m^2; XP3OS, 0.5 J/m^2; BALB3T3, 3.5 J/m^2. Open symbols, no caffeine; half-closed symbols, 2 m*M* caffeine; closed symbols, 5 m*M* caffeine.

tion of chromatin structure, particularly in XP variant and rodent cells.

We therefore conclude that XP variant and mouse cells have the common property that caffeine inhibits restoration of normal rates of semiconservative DNA replication in UV-irradiated cells. The enhancement by caffeine of UV-killing in XP variant and mouse cells may be caused by the retarded resumption of DNA replication.

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