ANALYSES OF DIFFERENTIAL SENSITIVITIES OF SYNCHRONIZED HELA S3 CELLS TO RADIATIONS AND CHEMICAL CARCINOGEN DURING THE CELL CYCLE: (II) ULTRAVIOLET LIGHT

Masami Watanabe and Masakatsu Horikawa

Division of Radiation Biology, Faculty of Pharmaceutical Sciences, Kanazawa University, Kanazawa 920 Japan

Received March 8, 1974

SUMMARY

UV-induction of thymine dimers in cellular DNA and their excision during different phases of the cell cycle of HeLa S3 cells were studied. Induction of thymine dimers was higher in the mitotic phase and the middle of the S phase than in the G_1 phase and from the late S phase to the early G_2 phase which are rather insensitive to UV. However, there is no significant difference in excision rate of UV-induced thymine dimers from the irradiated cells through the cell cycle. These findings indicate that the cyclic variation of UV-survivals during the cell cycle may be due to differences in the amount of thymine dimers in cellular DNA induced by UV-irradiation.

While the cyclic fluctuations in sensitivity to X- and UV-irradiation through the cell cycle of cultured mammalian cells has been demonstrated by many workers (1-7), the real nature of these fluctuations still remains to be solved. In a previous study (8) we established the optimum conditions for a combination of colcemid and harvesting techniques in order to obtain a large highly purified synchronized population from HeLa S3 cells and have compared the sensitivity of their colony-forming ability to X- and UV-irradiation and to the chemical carcinogen 4-NQO (4-nitroquinoline 1-oxide) during their cell cycle. In the present study we examined the induction and excision of thymine dimers in cellular DNA by UV-irradiation during different phases of the cell cycle, using the synchronized HeLa S3 population

obtained by this combined method (8), for elucidating the molecular mechanism of the fluctuation in sensitivity to UV-irradiation during the cell cycle.

MATERIALS AND METHODS

HeLa S3 cells grown in a medium containing 90% Eagle's MEM medium and 10% bovine serum were used (8). Exponentially growing HeLa S3 cells in Roux culture bottles (about 1 - 1.5 x 10^7 cells/bottle) were incubated for 24 hours in the medium containing 1 μCi of [^{3}H]-thymidine/ml (5 Ci/m mole; Daiichi Pure Chemical Co., Ltd, Tokyo). They were then rinsed, grown again for 6 hours in the medium containing 0.025 μg/ml of colcemid (Demecolcine, Nakarai Chemicals, Ltd. Kyoto) for cell synchronization. Synchronized mitotic cells were obtained by the combined method of colcemid and harvesting techniques described previously (8). Mitotic cells were collected and distributed into 90-mm glass petri dishes containing 10 ml of the normal culture medium and incubated at 37°C in a humidified 5% CO_2 incubator. After various periods of incubation, 2 x 10^6 cells were suspended in 1 ml of Eagle's MEM medium free of phenol red, spread over the surface of a 90-mm glass petri dish, and then irradiated with 200 ergs/mm^2 of UV (9). Either immediately or at various times after UV-irradiation, cells were collected from the dishes. Thymine dimers were analyzed chemically in a manner similar to that described by Trosko et al. (10).

RESULTS AND DISCUSSION

The cyclic fluctuation curve of sensitivity to UV-irradiation with 100 ergs/mm^2 through the cell cycle of HeLa S3 cells determined previously (8) is shown in Fig. 1. Sensitivity, measured as the loss of colony-forming ability after UV-irradia-

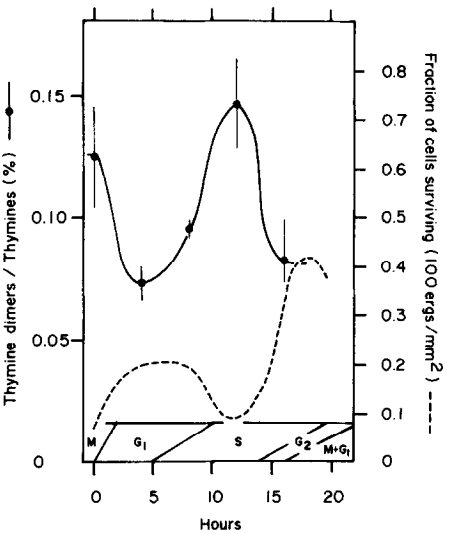

Fig. 1 The cyclic fluctuation in sensitivity to UV-irradiation with 100 ergs/mm^2 through the cell cycle of HeLa S3 cells, and the proportion of thymine dimers to thymines in PCA-insoluble fractions from the synchronous HeLa S3 cells immediately after irradiation with 200 ergs/mm^2 of UV, during the cell cycle. The bars indicate standard errors of the mean for five independent determinations for each point.

---

tion, increases late in the G_1 phase, reaches a maximum in the middle of the S phase, and decreases through the remainder of the S and G_2 phases. In addition, it can be concluded that the mitotic phase is as sensitive to UV as the middle of the S phase. These results agree partially with those found in synchronous HeLa S3 populations by Djordjevic and Tolmach (11).

The proportion of thymine dimers to thymines, calculated from their radioactivity in perchloric acid (PCA)-insoluble fractions from the synchronous HeLa S3 cells immediately after irradiation with 200 ergs/mm^2 of UV, during different phases of the cell cycle, is also shown in Fig. 1. As seen in this figure, the UV-induction of thymine dimers in cellular DNA during the cell cycle seems to be closely related to the cyclic variation of UV-survivals. That is, thymine dimers were highly induced in

the mitotic phase and the middle of the S phase, which are more sensitive to UV, than in the G_1 phase and from the late S phase to the early G_2 phase, which are rather insensitive to UV. Trosko et al. (12), who studied UV-induction of pyrimidine dimers during different phases of the cell cycle of Chinese hamster cells, showed that approximately 20% more pyrimidine dimers were induced in the early S phase than in the G_2 phase. On the other hand, Steward and Humphrey (13) showed that an average of 34% more thymine dimers were induced in the S phase than in the G_1 phase of Chinese hamster B-14 cells for doses up to 10^4 ergs/mm^2 of UV. In the present study, we did not determine the UV-induction of thymine dimers in the G_2 phase, to avoid contamination with mitotic phase cells which occurs with the decay of synchronization with time. However, we found that about 50% more thymine dimers were induced in the middle of the S phase than in the G_1 phase and from the late S phase to the early G_2 phase.

The results of experiments performed to determine the amount of excision of UV-induced thymine dimers from PCA-insoluble fractions of HeLa S3 cells at various times after irradiation with 200 ergs/mm^2, during the cell cycle, are shown in Fig. 2. Fig. 2a shows the percent of thymine dimers to thymines remaining in PCA-insoluble fractions at various times after irradiation in various phases of the cell cycle. Fig. 2b also shows percent excision of thymine dimers from PCA-insoluble fractions of HeLa S3 cells at various times after irradiation with 200 ergs/mm^2, during the cell cycle. As is seen in Figs. 2a and 2b, there is no significant difference in excision rate of UV-induced thymine dimers from HeLa S3 cells through the cell cycle. About 50% of thymine dimers were removed from PCA-insoluble fractions of HeLa

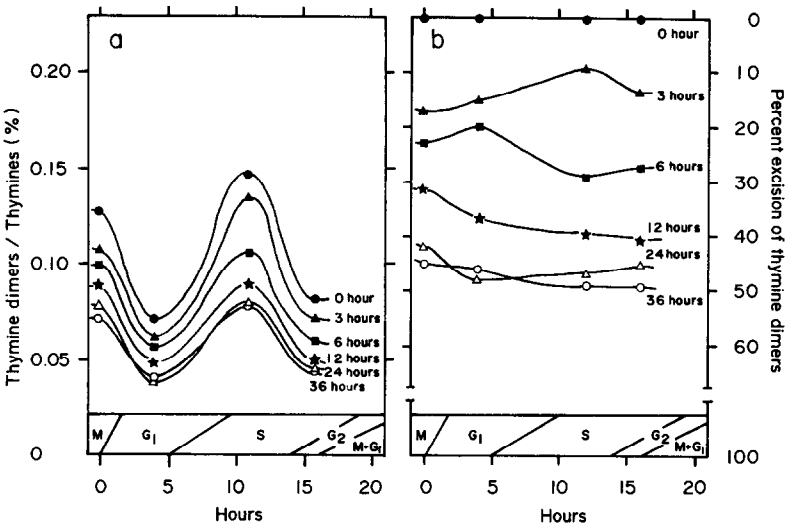

Fig. 2 a; The proportion of thymine dimers to thymines in PCA-insoluble fractions from HeLa S3 cells at various times after irradiation with 200 ergs/mm^2, during the cell cycle. b; Percent excision of UV-induced thymine dimers from PCA-insoluble fractions of HeLa S3 cells at various times after irradiation with 200 ergs/mm^2, during the cell cycle. Percent excision of UV-induced thymine dimers from HeLa S3 cells immediately after UV-irradiation at each phase of the cell cycle defined as zero. Each point shows the mean of five independent determinations.

S3 cells at various phases in the cell cycle during 24 hours after irradiation with 200 ergs/mm^2. This amount is comparable to the values previously reported for various human cell lines including HeLa cells (9,14,15). After 24 hours there is no further significant decrease in the proportion of thymine dimers to thymines.

These findings suggest that the cyclic variation of UV-survivals during the cell cycle of HeLa S3 cells is closely correlated with the differences in the amount of thymine dimers induced in cellular DNA by UV-irradiation, because thymine dimers are excised at almost the same rate from the cells of various phases in the cell cycle following UV-irradiation. Thus, more thymine dimers may remain in cellular DNA of the mito-

tic phase and the middle of the S phase than in the G_1 phase and from the late S phase to the early G_2 phase after UV-irradiation (Fig. 2a). These results are in contrast to the higher sensitivity to UV light of Xeroderma pigmentosum (XP) cells as compared with normal human skin fibroblasts due to failure of excision repair of UV-induced thymine dimers in XP cells (15,16).

In that case, we do not yet have any definite solution to the problem of why thymine dimers are induced highly in cellular DNA in the mitotic phase and the middle of the S phase. However, the following possibilities may be considered: (1) conformational changes of DNA- or chromatin-structure during the cell cycle; (2) changes in the location of DNA within the cells associated with DNA synthesis or cell division in the cell cycle; (3) changes in the amount of substances (including protein) to protect cellular DNA, as a filter, from UV light during the cell cycle; (4) the cells of a certain phase in the cell cycle have specific unknown enzyme(s) to repair or to enhance UV-damage induced in cellular DNA very rapidly immediately after UV-irradiation. Further studies to solve such a problem are now in progress.

ACKNOWLEDGMENTS

We are grateful to Dr. T. Sugahara, Kyoto University, for his many helpful discussions and reading of the manuscript. This study was supported financially by grants for Cancer Research from the Japanese Ministry of Education (Nos. 801019 and 801017).

REFERENCES

1. Terasima, T., and Tolmach, L. J. (1961) Nature 190, 1210-1211.
2. Terasima, T., and Tolmach, L. J. (1963) Biophys. J. 3, 11-33.
3. Erikson, R. L., and Szybalski, W. (1963) Radiation Res. 18, 200-212.

4. Sinclair, W. K., and Morton, R. A. (1963) Nature 199, 1158-1160.
5. Sinclair, W. K., and Morton, R. A. (1965) Biophys. J. 5, 1-25.
6. Rauth, A. M., and Whitmore, G. F. (1966) Radiation Res. 28, 84-95.
7. Djordjevic, B., and Tolmach, L. J. (1967) Radiation Res. 32, 327-346.
8. Watanabe, M., and Horikawa, M. (1973) J. Radiation Res. 14, 258-270.
9. Isomura, K., Nikaido, O., Horikawa, M., and Sugahara, T. (1973) Radiation Res. 53, 143-152.
10. Trosko, J. E., Chu, E. H. Y., and Carrier, W. L. (1965) Radiation Res. 24, 667-672.
11. Djordjevic, B., and Tolmach, L. J. (1967) Radiation Res. 32, 327-346.
12. Trosko, J. E., Kasschau, M., Covington, L. and Chu, E. H. Y. (1966) Radiation Res. 27, 535.
13. Steward, D. L., and Humphrey, R. M. (1966) Nature 212, 298-300.
14. Regan, J. D., Trosko, J. E., and Carrier, W. L. (1968) Biophys. J. 8, 319-325.
15. Setlow, R. B., Regan, J. D., German, J., and Carrier, W. L. (1969) Proc. Nat. Acad. Sci. USA 64, 1035-1041.
16. Cleaver, J. E. (1968) Nature 218, 652-656.