DEFECTIVE REPAIR OF N-ACETOXY-2-ACETYLAMINOFLUORENE-INDUCED LESIONS IN THE DNA OF XERODERMA PIGMENTOSUM CELLS*

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Summary: Cells from normal individuals and from individuals with xeroderma pigmentosum were treated with the carcinogen N-acetoxy-2-acetylaminofluorene. Repair of the damage to DNA was estimated by incubating the cells in BrdUrd and then photolyzing the BrUra-containing repaired regions with 313-nm radiation. By this criterion normal cells repair the damage in a fashion similar to ultraviolet damage, but xeroderma pigmentosum cells (defective in ultraviolet repair) are defective in repair of the carcinogen-induced damage.

Introduction: Human cells are capable of removing a large fraction of ultraviolet (UV)-induced lesions in their DNA. Fifty percent of the cyclobutane pyrimidine dimers are excised in the first 12–24 hours after irradiation (1,2). Cleaver (3) was the first to show that cells from patients with the hereditary disease xeroderma pigmentosum (XP) are defective in the repair of UV damage, as judged by unscheduled synthesis and repair replication. Cells from such patients are incapable of excising dimers (2,4), presumably because they lack a functional UV-endonuclease (2,5), the enzyme that initiates the repair process by nicking the DNA next to a dimer.

2-Acetylaminofluorene (AAF) and its derivatives are carcinogenic in mice and hamsters. These species are capable of converting AAF to N-hydroxy-AAF, and the latter is thought to be esterified to N-acetoxy-AAF, which is believed to be the proximate carcinogen (for review see ref. 6). N-acetoxy-AAF is highly

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reactive and forms both protein and nucleic acid derivatives. In view of the reactivity of N-acetoxy-AAF with DNA, and since treatment with this chemical stimulates unscheduled DNA synthesis in human lymphocytes (7) and inactivates transforming DNA more when the DNA is assayed on repair-deficient bacteria than on wild-type cells (8), we examined repair events in the DNA of human cells after treatment with this agent. As an assay we employed a technique that utilizes the photolysis of 5-bromodeoxyuridine (BrdUrd) (9,10) to yield an estimate of the number of repaired regions in the cellular DNA and an indication of the average size of the repaired regions. Our results indicate that extensive repair occurs in the DNA of normal human cells exposed to N-acetoxy-AAF, whereas there is a much lower level of repair in XP cells.

Materials and Methods: The cells employed were diploid fibroblasts derived from skin biopsies of normal subjects and from patients with XP, and an aneuploid tumor cell line derived from an amelanotic melanoma from a patient with uncomplicated XP. A further description of this XP tumor cell line will appear elsewhere (11). Cell culture methods and details of the BrdUrd photolysis repair assay have been described previously in detail (9,10).† Briefly, cellular DNA is labeled by an overnight incubation in [^{3}H]dThd (2 μCi/ml) or $^{32}PO_4$ (10 μCi/ml). The labeling medium is removed and replaced with 5 ml regular growth medium for 2 hours. The medium is made 10^{-4} M in BrdUrd (^{3}H-labeled cells) or, as a control, 10^{-4} M in dThd (^{32}P-labeled cells), and N-acetoxy-AAF (in 50 μl of dimethyl sulfoxide) is added.‡ Hydroxyurea is added to a concentration of 2 mM, to inhibit semiconservative replication (12) and thus avoid any competition for the added nucleosides during

†Since the earlier reports (9,10), we have carefully calibrated our absolute dosimetry at 313 nm. The earlier exposures should be multiplied by 1.52 to obtain the correct values reported here.

‡Although N-acetoxy-AAF is not removed from the culture medium, the treatment is similar to a short-time one because the agent not only decays in solution (8) but also may react with the calf serum in the medium.

repair synthesis. After ~20 hours the cells are harvested with an EDTA solution (2) and irradiated with a high flux of 313-nm radiation. If damage to DNA is repaired and BrdUrd is inserted into repaired regions, the irradiation makes these regions alkali labile, and the decrease in sedimentation constant, as observed in an alkaline sucrose gradient, of the ^{3}H-labeled DNA gives a measure of the number of repaired regions. The DNA from treated ^{32}P-labeled cells incubated in dThd acts as a control for the estimation of nonspecific breakage by 313 nm.

Results and Discussion: Figure 1a shows the results of a typical N-acetoxy-AAF experiment with normal human skin cells. The decrease in molecular weight of the DNA as a result of 313-nm irradiation of treated cells incubated in BrdUrd, as compared to those incubated in dThd, is indicative of a large amount of repair. We show elsewhere (13) that the numbers and sizes of the repaired regions observed in these normal cells exposed to 7×10^{-6} M N-acetoxy-AAF are similar to those observed after about 50 ergs/mm^2 of 254-nm radiation (~1 lesion per 8×10^6 daltons and 20–30 BrUra residues per repaired region).

Figure 1b shows the results of a similar experiment using XP cells. The specific decrease in molecular weight induced by 313 nm in the DNA of BrdUrd-incubated normal cells is not observed in these XP cells. The same decreases in molecular weights are observed in cells incubated in BrdUrd and in dThd.[§] Thus there has been little repair synthesis in comparison to that in normal skin cells.

Figure 2 summarizes data from a number of experiments on normal and XP cells treated with N-acetoxy-AAF. The data are expressed as differences between the reciprocals of the weight-average molecular weights ($1/M_w$) of cells incubated in

[§]Shortly (10–30 min) after the beginning of treatment of normal and XP cells with 7×10^{-6} M N-acetoxy-AAF there is a 15-30% decrease in molecular weight. The decrease disappears with continued incubation of normal cells but does not disappear in XP cells. We have observed consistently that the DNA of treated XP cells incubated in dThd is more sensitive to 313 nm than the DNA of normal fibroblasts. We interpret these results as indicating that AAF remains bound to XP DNA and photosensitizes the DNA to 313 nm.

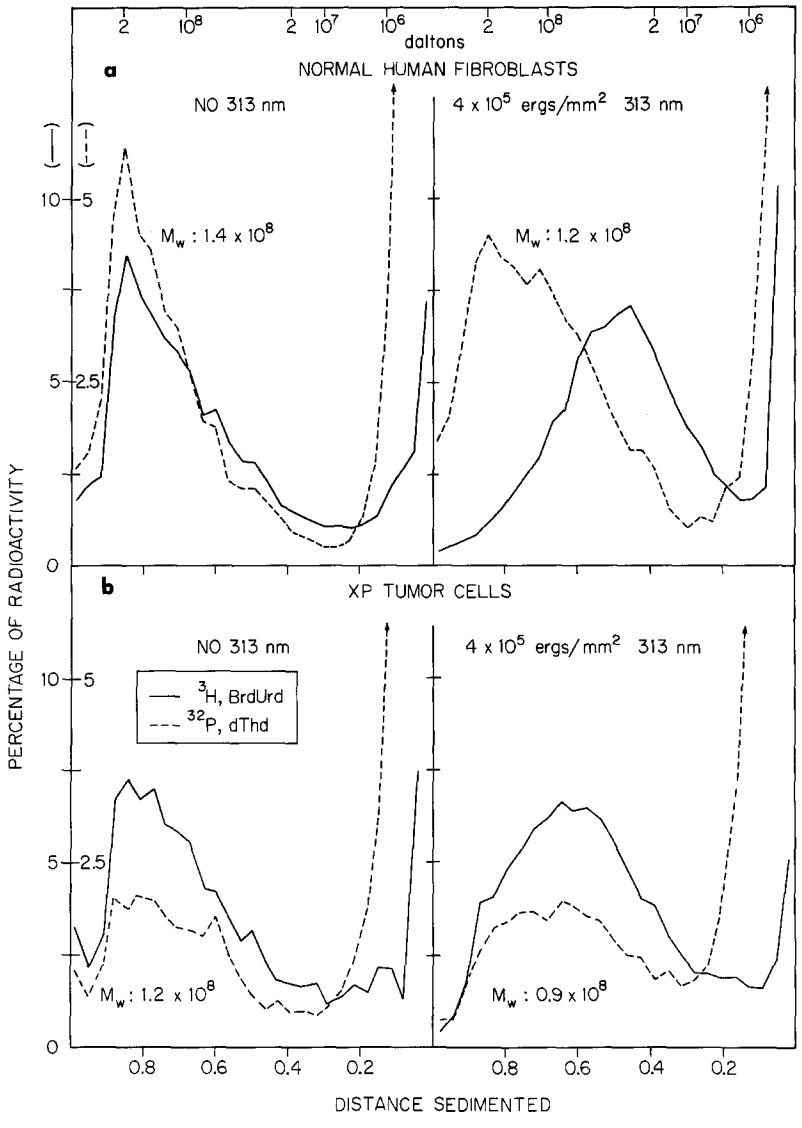

Fig. 1. Sedimentation patterns in alkaline sucrose of the radioactivity from human cells treated for 20 hours with 7 X 10^{-6} M N-acetoxy-AAF. Cells labeled with [^{3}H]dThd were incubated in BrdUrd and those labeled with $^{32}PO_4$ were incubated in dThd. Labeled cells were mixed together, exposed to 313-nm radiation, and sedimented in alkaline sucrose in a Beckman SW-56 rotor at 30,000 rev/min for 3 hours. The acid-insoluble, double-labeled samples were collected and analyzed as before (10). Weight-average molecular weights (M_w) were computed for the material that sedimented between 0.25 and 0.95 of the distance from the gradient. The ^{32}P-labeled material at the top of the gradients represents phospholipid, phosphoprotein, and unhydrolized RNA. (a) Normal human fibroblasts. (b) Tumor cells from an individual with xeroderma pigmentosum.

BrdUrd and cells incubated in dThd. In normal cells 313-nm irradiation results in large changes in $1/M_w$. In XP cells, however, the changes are minimal.

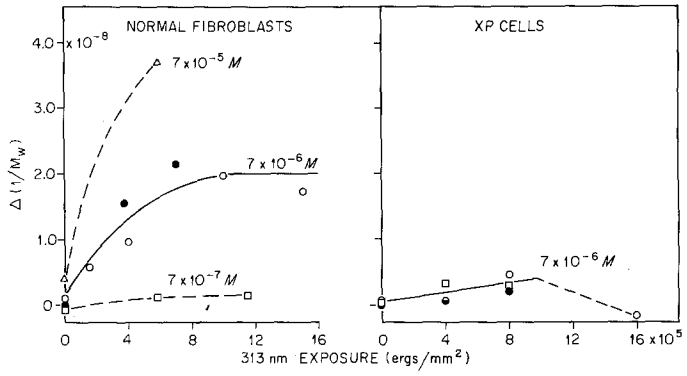

Fig. 2. The effect of 313-nm exposure on cells treated with the indicated concentrations of N-acetoxy-AAF for 20 hours. The difference $[(1/M_w)_{BrdUrd} - (1/M_w)_{dThd} \equiv \Delta(1/M_w)]$ is a measure of the numbers of breaks in repaired regions resulting from 313-nm exposure. The different symbols for normal cells represent separate experiments, those for XP cells represent different cell lines.

Although XP cells are clearly defective in repairing UV-induced lesions (2,3, 4,9) and show reduced levels of unscheduled DNA synthesis after treatment with 4-nitroquinoline 1-oxide (ref. 14), they show normal levels of unscheduled synthesis and strand rejoining after exposure to ionizing radiation (5, 15). In both XP and normal cells, however, there is little manifestation of repair synthesis after exposure to nitrogen mustard (16). Thus the data reported here suggest that the kind of damage caused by N-acetoxy-AAF treatment of human cells may be different from that induced by either ionizing radiation or nitrogen mustard. Since the primary defect in XP cells seems to be a defective UV-endonuclease (2,5), one could interpret the defective repair of AAF lesions in XP cells as indicating that repairable AAF-induced lesions and UV-induced lesions make similar topological distortions in DNA.

These data also indicate that fibroblasts and tumor cells from individuals with XP are defective not only in the repair of UV damage to DNA but in the repair of some chemically induced base damage as well. One would expect such cells to be very sensitive to a wide class of chemical carcinogens. The data reinforce the notion that there is a close — perhaps causal — connection among carcinogens, damage to DNA, and the repair of damaged DNA.

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References

1. Regan, J. D., J. E. Trosko, and W. L. Carrier, Biophys. J. 8, 319 (1968).
2. Setlow, R. B., J. D. Regan, J. German, and W. L. Carrier, Proc. Nat. Acad. Sci. U.S.A. 64, 1035 (1969).
3. Cleaver, J. E., Nature 218, 652 (1968).
4. Cleaver, J. E., and J. E. Trosko, Photochem. Photobiol. 11, 547 (1970).
5. Cleaver, J. E., Proc. Nat. Acad. Sci. U.S.A. 63, 428 (1969).
6. Miller, J. A., and E. C. Miller, Progr. Exp. Tumor Res. 11, 273 (1969).
7. Lieberman, M. W., R. N. Baney, R. E. Lee, S. Sell, and E. Farber, Cancer Res. 31, 1297 (1971).
8. V. M. Maher, E. C. Miller, J. A. Miller, and W. Szybalski, Mol. Pharmacol. 4, 411 (1968).
9. Regan, J. D., R. B. Setlow, and R. D. Ley, Proc. Nat. Acad. Sci. U.S.A. 68, 708 (1971).
10. Regan, J. D., R. B. Setlow, M. M. Kaback, R. R. Howell, E. Klein, and G. Burgess, Science 174, 147 (1971).
11. Regan, J. D., E. Klein, and R. B. Setlow, in preparation.
12. Pfeiffer, S. E., and L. J. Tolmach, Cancer Res. 27, 124 (1967).
13. Setlow, R. B., and J. D. Regan, in preparation.
14. Stick, H. F., and R. H. C. San, Mutat. Res. 13, 279 (1971).
15. Kleijer, W. J., P. H. M. Lohman, M. P. Mulder, and D. Bootsma, Mutat. Res. 9, 517 (1970).
16. Cleaver, J. E., Mutat. Res. 12, 453 (1971).