Vol. 98, No. 3, 1981 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
February 12, 1981 Pages 823-828

HARMAN INHIBITS THE REMOVAL OF PYRIMIDINE DIMERS FROM THE DNA OF HUMAN CELLS

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Received December 29,1980

SUMMARY

Normal human fibroblasts were UV-irradiated and incubated for 6 hr with harman. The losses of sites, in the extracted DNA, sensitive to a UV specific endonuclease were determined as precision measures of the excision of UV-induced pyrimidine dimers. Harman inhibited excision, rising from ~ 30% inhibition at 200 μM to 75% inhibition at 600 μM.

INTRODUCTION

A number of agents inhibit one or more steps in excision repair e.g. acridines and caffeine in bacterial systems (1) and cytosine arabinoside and high concentrations of hydroxyurea in human systems (2-4). Harman, a pyrolysis product of tryptophan, inhibits the UV induction of mutations in Chinese hamster cells and, at the concentrations used (~ 40μM), inhibits slightly unscheduled DNA synthesis (5). It also has effects on the mutagenic activity of some aromatic hydrocarbons (see Reference 6 for a brief review), affects the repair of DNA of human cells exposed to N-acetoxy-acetylaminofluorene as indicated by the appearance and the failure of closure of DNA strand breaks estimated by the alkaline elusion technique. It was pointed out (6) that such data could not indicate whether harman inhibited the removal of lesions, slowed the filling of gaps, or inhibited ligation. Our data indicate that harman inhibits the removal of UV-induced pyrimidine dimers.

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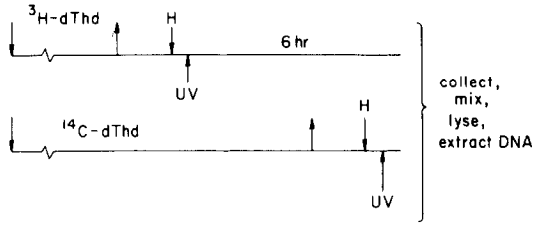

Figure 1. The experimental scheme used to detect the effects of harman (H) on the excision repair of pyrimidine dimers. After removal of the radioactive label, cells were incubated for two hours in complete medium. Harman was added and one-half hour later the cells were irradiated as indicated in Methods. At the completion of the experiment, the extracted DNA was treated with a UV specific endonuclease and sedimented in alkali so as to measure the number of pyrimidine dimers remaining. The difference between the number of nicks in the ^{14}C and ^{3}H labeled samples is the number of dimers removed in six hours.

MATERIALS AND METHODS

Cell culture. Normal human fibroblasts, Rid Mor (CRL 1220), from the American Type Culture Collection (Rockville, MD) were used. Cells were grown in plastic dishes in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum, 400 µg/ml L-glutamine, 140 U/ml penicillin and 140 µg/ml streptomycin (Grand Island Biological Co., Grand Island, NY) and kept in a humidified 7.5% CO_2 atmosphere at 37^{0}C. Cells were labeled by incubation for 36 hours in either [^{3}H]dThd, 0.3µCi/ml (6.6Ci/mmol) or [^{14}C]dThd, 0.06 µCi/mol(52Ci/mol).

Treatment. The general procedure is shown in Figure 1. Harman (Sigma) at 40 mM in $(CH_3)_2SO$ was diluted into the growth medium to give the desired concentration. Before UV-irradiation cells were washed two times with phosphate buffered saline. UV-irradiation, mostly 254 mm, was supplied by germicidal lamps giving a dose rate of 0.72W/m^{2}. After irradiation cells were incubated in medium containing harman.

Assay for endonuclease sensitive sites. A UV-endonuclease preparation from M. luteus was used to nick DNA at the sites of pyrimidine dimers (7, 8). Details have been described previously (8). The difference between the reciprocals of the number average molecular weight of the two DNA's in Figure 1 gives directly the numbers of sites removed in six hours. 20 J/m^{2} makes approximately 50 sites/10^{8} dalton. It is important that both DNA's be incubated with harman since, at high concentrations (400 - 600 µM), the incubation results in a decrease in the yield of pyrimidine dimers by approximately 10% (data not shown) presumably as a result of attenuation of radiation by harman. The precision of the double label method is high. In the absence of any incubation after UV, the two endonuclease-treated DNA's have molecular weights within 1% (Figure 2).

RESULTS AND DISCUSSION

Figure 3 shows typical alkaline gradients of endonuclease treated DNA from irradiated cells incubated with or without harman. There is a large increase in molecular weight during postirradiation incubation in the

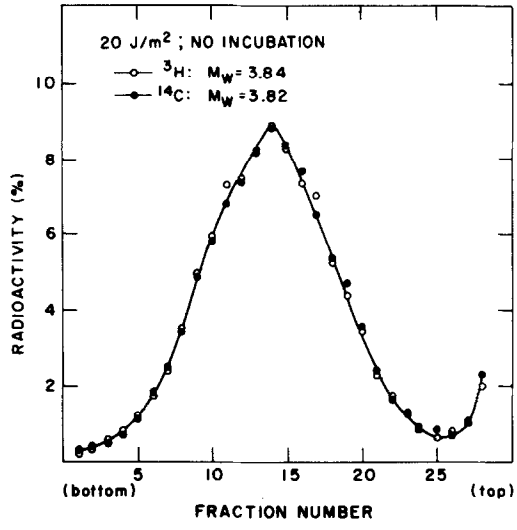

Figure 2. An alkaline sucrose gradient sedementation profile of endonuclease treated DNA from ^{3}H and ^{14}C labeled cells irradiated separately. After irradiation, the cells were mixed and the DNA extracted. Sedimentation in an SW-60 rotor at 50,000 rev/min for 150 min (^{3}H label approximately 3,000 cpm, ^{14}C label, 2,000 cpm).

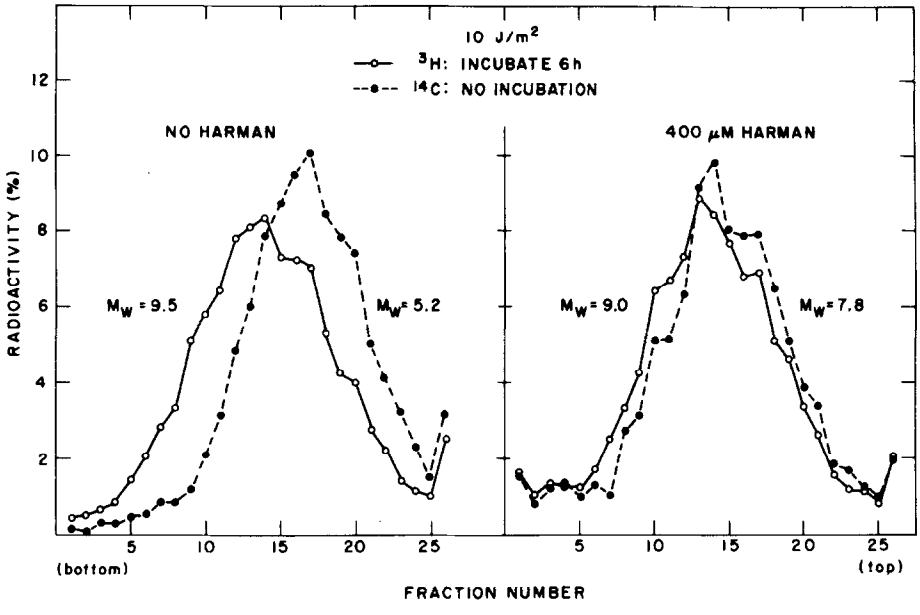

Figure 3. Alkaline sedimentation patterns of endonuclease treated DNA from irradiated cells, not treated or treated with 400 μM harman. ^{3}H-labeled cells incubated for 6 hr after irradiation and 14C-labeled cells not incubated. Sedimentation at 50,000 rev/min for 100 min. (^{3}H label, 4,000 cpm, ^{14}C label, 1,000 cpm.)

TABLE 1. The Inhibitory Effects of Harman on Removal of Endonuclease - Sensitive Sites in 6 hr

UV Dose (J/m^2)*	Harman (μM)	Sites Removed Per 10^8 daltons	Inhibition (%)
10	0	17.9	
	200	9.7	43
	400	8.6	50
	600	4.0	77
20	0	18.1	
	200	14.5	20
	400	7.0	61
	600	4.5	75

*Initial numbers of sites per dalton were 10 J/m^2:$25/10^8$; 20J/m^2:$50/10^8$.

absence of harman, but only a small one in its presence. (The differences in the molecular weights of the unincubated samples result, in part, from differences in the breakage during extraction and purification.) The data in Figure 3 indicate 17.1 sites removed/10^8 dalton in the absence of harman but only 8.6 in its presence -- a 50% inhibition. Data obtained from a number of similar experiments are shown in Table 1. Increasing concentrations of harman have increasing inhibitory effects on the removal of endonuclease sensitive sites. At the higher dose, 20 J/m^2, the rate of excision repair is saturated (9) and slight differences in dosimetry should not affect the numbers of sites removed.

We have not investigated in detail the persistence of the inhibitory effect of harman. However, if cells are treated with up to 600 μM harman for two hours, then incubated in its absence for one hour and irradiated and subsequently incubated in its absence, there is no inhibition of excision. The number of endogenously made single-strand nicks found at any instant in cells doing excision repair is small and difficult to detect by alkaline sedimentation. Incubation in harman makes little change in these numbers (less than $0.1/10^8$ daltons). Hence, the numbers of nicks observed in gradients, such as shown in Figure 2, represent nicks introduced by the endonuclease and not nicks accumulated during repair. Thus, the inhibitory

action of harman is on the initial endogenous incision step. However, since many of the steps in excision seem to be closely coordinated (3, 10), the action of harman is not necessarily on the incision step itself. Harman might inhibit a later step in repair and result in an overall inhibition of a repair complex that manifests itself as an inhibition of the early step in repair. The inhibition of excision observed in these experiments is somewhat less than the inhibition of DNA synthesis in unirradiated cells (6), but the latter is a replicative, not a repair polymerase function, and so the question remains open as to the mode of action by which harman affects excision repair.

Since harman affects both replication and repair and since the probability of a mutation arising depends upon replication on a damaged template, the two effects tend to counteract one another. Since harman seems to affect replication more than repair, one might expect that, if these were its only actions, it would decrease the mutation rate arising from UV-irradiation. One would expect a similar prediction would hold for N-acetoxy-2-acetylaminofluorene since this compound is a UV mimetic. However, harman seems to accentuate the mutagenicity of it (11). A full understanding of harman's mode of action does not yet seem to be in hand.

ACKNOWLEDGMENTS

This research was supported by the United States Department of Energy. A. C. was supported by the CNEN Italy.

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