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INHIBITION OF DNA-REPAIR AND DNA-SYNTHESIS BY HARMAN IN HUMAN ALVEOLAR TUMOR CELLS

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SUMMARY

The effect of the tryptophan pyrolysis product harman on colony forming ability, DNA-synthesis and DNA-repair was investigated in human alveolar tumor cells A549. Colony forming ability and overall DNA synthetic capacity decreased linearly with increasing harman concentration and reached values of 20 % and 29 % of untreated controls, respectively, at 200 μM. Harman also inhibited the repair of N-acetoxy-2-acetylaminofluorene induced DNA damage, as measured by the alkaline elution procedure. While incision of parental DNA occurred normally in the presence of harman the reconstitution of control molecular weights was strongly inhibited. These effects of harman may play a role in its co-mutagenic activity.

INTRODUCTION

Evidence has recently been obtained for the existence in the environment of chemicals which alone are non-mutagenic but which influence the mutagenicity of a second agent. In particular, indole derivatives such as the protein pyrolysis products harman and norharman have been shown to increase the mutagenicity of certain pro- and ultimate mutagens and to decrease that of others. This is of particular interest since these compounds have been detected in tobacco smoke and in charred meats (1, 2). Harman and norharman decreased

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the mutagenicity of benzo(a)pyrene (B(a)P) in Salmonella TA98 in the presence of S9 liver preparations (3, 4). It was shown that these compounds inhibited B(a)P metabolism by mouse hepatic microsomes as well as microsome mediated binding of B(a)P to DNA (4). Therefore, the observed effect of harman and norharman on B(a)P mutagenicity could be due to inhibition of metabolic activation. It should be noted that other reports described a stimulatory, i.e. co-mutagenic, effect of harman and norharman on B(a)P mutagenicity (5, 6). Harman and norharman were co-mutagenic for 2-acetylaminofluorene and its derivatives (3, 7). Co-mutagenicity was also demonstrated in combination with the ultimate derivative N-acetoxy-2-acetylaminofluorene (AAAF) which does not require metabolic activation for mutagenicity (7). In this case, the co-mutagens must act at the level of mutation initiation or -expression. We have attempted to obtain insight into the mechanism of co-mutagenicity in studies of the effect of harman on DNA-synthesis and on the repair of AAAF induced DNA damage in human alveolar tumor cells A549 (8). Harman was found to be a potent inhibitor of DNA synthesis and of the repair of parental DNA in AAAF treated A549 cells. These effects on DNA metabolism are expected to influence the mutagenic potency of AAAF.

RESULTS AND DISCUSSION

In a first series of experiments the effect of harman on the survival of the colony forming ability of human alveolar tumor cells A549 (8) was determined. A549 cells were treated for 48 hours with increasing concentrations of harman in dimethylsulfoxide (DMSO), cultured in fresh media for 11 to 14 days, and the colonies were fixed, stained and counted, according to standard procedures (9). The DMSO concentration was 0.4 % and had no effect on the colony forming ability. The data is given in Table 1. Not only was the colony forming ability decreased with increasing concentrations of harman but the size of the colonies was greatly reduced at 200 μM indicating a signifi-

Table 1 : Effect of Harman on the Survival of the Colony Forming Ability and DNA Synthetic Capacity of A549 Cells

Harman concentration μM	Survival colony * forming ability %	DNA synthetic capacity %
0	100	100
25	86	83
50	80	76
100	42	61
200	20	29

* Cells were plated at low density for 16-18 hours, exposed to the indicated concentrations of harman in DMSO (0.4 %) for 48 hours, the medium changed and colonies fixed and stained after 11-14 days (9). The values are derived from means of five replica plates.

cant increase in generation time. Harman was also found to be a potent inhibitor of overall DNA synthesis. This result was obtained in experiments in which A549 cells were prelabeled in their DNA with ^{14}C-thymidine, treated for 10 min with harman in DMSO and pulsed for 15 min with ^{3}H-thymidine in the presence of harman. The ratios of ^{3}H-over ^{14}C-radioactivity in trichloroacetic acid precipitable material was determined. The data was normalized to controls which were treated with DMSO only and is given in Table 1. It is evident that harman inhibited DNA synthesis in A549. Since only 10 min. preincubation with harman was required for inhibition of DNA synthesis it is likely that harman exerted its effect by direct interaction with chromatin rather than via inhibition of RNA- or protein-synthesis. The concentration dependence of the inhibition of DNA synthesis parallels the effect of the drug on colony formation.

The effect of harman on DNA repair was studied following exposure of A549 cells to the ultimate carcinogen AAAF. Monolayer cultures were prelabeled in their DNA with ^{14}C-thymidine, treated with 25 μM AAAF for 15 min. and then incubated for different lengths of time in fresh media in the presence and

absence of 200 μM harman in DMSO. The initial level of covalent carcinogen-DNA adducts in A549 cells at 25 μM AAAF is estimated at 160 μmoles adduct per mole DNA-phosphate (J. Remsen and P. Cerutti, unpublished observation). Following incubation, the cells were applied on a polyvinylchloride filter and the integrity of the labeled pre-existing "parental" DNA was analysed by the alkaline elution procedure according to a protocol described previously (10, 11). By this method single stranded DNA fragments of molecular weights below approximately 10^9 daltons are eluted rapidly from the filter with a tetrapropylammonium buffer pH 11.9 while DNA of higher molecular weights is eluted only very slowly. Elution curves were measured after increasing duration of post-treatment incubation by plotting the fraction of ^{14}C-DNA retained on the filter as a function of the amount of elution buffer which had passed through (not shown). The DMSO concentration in all samples was 0.4 % and had no effect on the elution curves. The time course of the fragmentation and subsequent reconstitution of parental DNA are shown in Figure 1 and was derived from the elution curves by plotting the fraction of ^{14}C-DNA eluted by 31.5 ml buffer (11). It is evident that parental DNA is rapidly fragmented following AAAF treatment. Immediate fragmentation was comparable regardless of whether harman was present or absent during incubation. In the absence of harman parental DNA was slowly reconstituted to the molecular weights of untreated controls over a time period of 20 to 30 hours. In contrast, in the presence of harman DNA fragmentation further increased during the first 4 hours of incubation and slow elongation only occurred after 16 hours. Therefore, harman did not affect immediate incision of damaged DNA but strongly inhibited a subsequent repair step. When the harman concentration was decreased to 100 μM the inhibitory effect on repair was much less pronounced but could still be recognized.

It should be appreciated that net fragmentation of parental DNA is measured in our experiments which is the result of several simultaneous

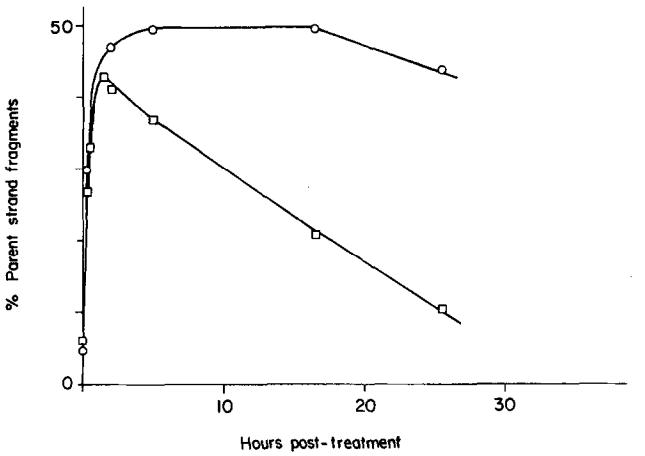

Figure 1 : Kinetics of parent strand fragmentation and elongation in AAAF-treated A549 cells in the presence and absence of harman. A549 cells were labeled for 3 days with low levels of ^{14}C-thymidine, chased for one day with media containing 10^{-6}M thymidine and replated at 3-4 x 10^5 cells per T25 Falcon tissue culture flask. Treatment was begun 24 hours after replating as described in the text. □, - harman o, + harman

enzymatic processes. It cannot be decided on the basis of our data whether harman inhibits lesion removal following incision, slows gap-filling or inhibits the ligation process. An effect of harman on gap-filling appears likely in view of its strong inhibitory effect on semi-conservative DNA synthesis. Harman may exert these effects because of its capacity to intercalate into DNA (12). Harman is a tryptophan derivative which is produced by pyrolysis. It is interesting to note that tryptophan photolysis products inhibited the media dependent resealing of X-ray induced single strand breaks in E. coli (13).

Our results suggest that the co-mutagenic effect of harman in Salmonella T98 with AAAF may be due to its effect on DNA-repair and/or DNA-synthesis. However, in contrast to the present work harman and AAAF were added simultaneously to the cultures in the mutagenesis studies (3, 7). Therefore, a direct effect of harman on the binding of AAAF to DNA cannot be excluded. Indeed, preliminary results show that harman strongly inhibited the total binding of

AAAF to bacteriophage T7 DNA *in vitro* (V. Wang and P. Cerutti, unpublished results). It is evident that harman and norharman affect several processes which are expected to modify the mutagenic potency of pro- and ultimate-mutagens. Even for ultimate mutagens it is not possible to relate their action to a single step in mutagenesis, therefore.

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