REMOVAL OF ACETYLAMINOFLUORENE FROM THE DNA OF CONTROL AND REPAIR-DEFICIENT HUMAN FIBROBLASTS

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SUMMARY

Removal of covalently-bound acetylaminofluorene (AAF) from DNA was studied in confluent (non-dividing) control and repair-deficient human fibroblasts. Control fibroblasts and fibroblasts from individuals with Fanconi's anemia and ataxia telangiectasia removed about 50% of the bound AAF in 48 hours. Fibroblasts from individuals with xeroderma pigmentosum (XP) (complementation groups A and C) removed little AAF while XP variants appeared to remove AAF as well as, or almost as well as, control cells.

INTRODUCTION

It is now clear that a variety of syndromes, including xeroderma pigmentosum, Fanconi's anemia, and ataxia telangiectasia, are associated with some deficiency in DNA repair (1-10). DNA repair synthesis in response to damage produced by the carcinogen N-acetoxy-2-acetylaminofluorene (NA-AAF) has been studied (3,4,11,12), but to date no one has looked at the removal of AAF from the genomes of normal and repair-deficient human cells. This problem is of interest because of recent observations on cultured mouse cells indicating that pyrimidine dimers and AAF

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molecules may be removed by different mechanisms (13, see also 14). For these reasons we evaluated the removal of covalently bound AAF in human fibroblasts known to be deficient in the ability to repair various DNA lesions.

MATERIALS AND METHODS

Fibroblasts were obtained from the American Type Culture Collection (ATCC) and grown to confluence in Dulbecco's modified Eagle's medium with 10% fetal calf serum and penicillin and streptomycin in 150 mm petri dishes (Falcon 3025) at 37°C and 10% CO_2. All experiments were done in a 37°C room equipped with a CO_2 incubator. Cells in dishes containing 20 ml of culture medium were treated with 9-^{14}C-NA-AAF (ICN; 36.9 mC/mmol) in 0.1 ml anhydrous dimethylsulfoxide (Pierce Chemical Co.) to yield a final NA-AAF concentration of 1 μM. Six to 10 dishes were pooled and used for each point. To achieve uniformity from experiment to experiment, one lot of NA-AAF was used; it was dissolved in ethyl acetate and aliquoted into vials. After the solvent was removed under N_2, NA-AAF was stored desiccated at -20°C. Dishes were treated with NA-AAF and 15 minutes later the radioactive medium was removed and replaced with conditioned medium. Forty-five minutes later (1 hour after NA-AAF addition), "100% time points" were taken (duplicates or triplicates). No removal was detected between 15 minutes and 1 hour, and, in order to handle a large number of petri dishes efficiently, 1 hour values were used (13). The cells were harvested, and DNA was prepared from nuclei by CsCl centrifugation; specific activities and μmol AAF/mol DNA-P were calculated from these gradients (11, 13, 15). Confluent cultures (G_1) were used to eliminate any effect of the cell cycle on removal and obviate the need to correct specific activities for DNA synthesis. There was some variation in binding levels from experiment to experiment (Table 1). The reason for this variation is unknown, but for any one experiment there was good reproducibility (Table 1, column 4).

RESULTS

Binding and removal of AAF were studied according to the protocol outlined in Materials and Methods. Initial binding levels ranged from 9 to 23 μmol AAF/mol DNA-P, or about 1 AAF molecule per 40,000 to 110,000 bases (Table 1). At these relatively low levels of binding, the <u>percentage</u> of AAF removed with time is constant and independent of the amount bound (13).

Our data indicate that control fibroblasts and fibroblasts from patients with Fanconi's anemia (HG-261, Ce Rel) and ataxia telangiectasia (Sa Gru) remove 40-48% of the AAF by the

Table 1. Binding of Acetylaminofluorene to DNA of Cultured Fibroblasts

Cell line	Source	ATCC Number	μMole AAF/Mole DNA-P at 1 hr
El San	Control	CRL-1222	23.45 (± 0.48)[b]
Jay Tim	XP-Group A	CRL-1223	9.12 (± 0.42)
Gor Do	XP-Group C	CRL-1204	13.50 (± 0.30)
Wo Mec	XP-Variant	CRL-1162	20.00 (± 0.73)
Pe Hay	XP-Variant	CRL-1258	15.50 (± 0.05)
Be Tim	XP-heterozygote[a]	CRL-1254	14.03 (± 0.67)
HG 261	Fanconi's anemia	CCL-122	10.12 (± 0.56)
Ce Rel	Fanconi's anemia	CRL-1196	18.42 (± 0.82)
Sa Gru	Ataxia telangiectasia	CRL-1312	15.76 (± 0.54)

[a]Mother of Jay Tim

[b]All values are ± 1/2 range, except for the Wo Mec value which is ± standard deviation.

end of 24 hours (Figure 1). Additional removal takes place between 24 and 48 hours, but at a slower rate. In contrast, XP cells from complementation groups A and C (Jay Tim and Gor Do) remove little damage (4% by 24 hours, 8% by 48 hours). Although only one set of experiments is shown here, similar experiments using El San and Jay Tim and different lots of NA-AAF gave similar results. In addition, our previous results indicated that fibroblasts from another XP patient (Cay Wen; Group D) removed roughly 13% of the AAF in 24 hours (13) and are in agreement with our present findings. Two XP variant fibroblast strains (Wo Mec and Pe Hay) remove AAF from DNA as well as, or almost as well as,

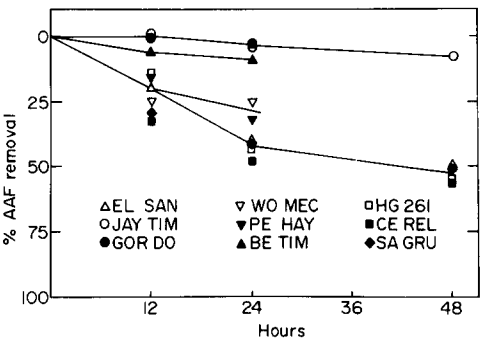

Fig. 1. Removal of AAF by control fibroblasts (El San) and by fibroblasts from patients (see Table 1).

control cells. Additional experiments would be needed to determine whether or not the small differences observed here are real. The relatively small extent of removal by the XP heterozygote strain Be Tim (7% at 12 hours and 9% at 24 hours; 37% and 22.5% of El San values, respectively) also needs further evaluation. Hydrolysis of DNA from cells treated with 9-^{14}C-NA-AAF and subsequent thin layer chromatography indicated that there appear to be two major products: N-(deoxyguanosine-8-yl)-2-acetylaminofluorene (∿80-85%) and 3-(deoxygluanosine-N^2-yl)-2-acetylaminofluorene (∿15-20%) (13, see also 16 and 17). Thus it is clear that most of the removal represents loss of N-(deoxyguanosine-8-yl)-2-acetylaminofluorene. Unfortunately, too little material was available for the chromatographic separations necessary to determine the extent of removal of the minor product.

DISCUSSION

These data have a number of important implications. (1) Because little or no AAF is lost from XP-A and XP-C cells, loss of AAF from DNA must be the result of an active cellular process requiring cellular enzymes. (2) Evidence from cultured mouse cells suggests that removal of pyrimidine dimers and AAF

from DNA may occur by different mechanisms (13, see also 14). It is also known that XP-A and XP-C cell types are poor at repairing not only NA-AAF damage but also ultraviolet radiation damage. Thus to the extent that these mechanisms are different, it appears that they are under similar genetic control in human fibroblasts. (3) The mechanism that accounts for AAF removal is evidently not the same as that which provides for removal of bases damaged by ionizing radiation: Our finding that fibroblasts from individuals with Fanconi's anemia and ataxia telangiectasia are competent to remove AAF contrasts with data that these cells are deficient in their ability to excise γ-ray-induced base damage (9,10). Lymphocytes derived from Fanconi's anemia patients are unable to repair damage by bifunctional (cross-linking) alkylating agents (7,8), but at present it is not clear how these relate to our findings.

Poon and collaborators (18) have suggested that Fanconi's anemia fibroblasts (HG-261) have a defect in repair of pyrimidine dimers and have attributed this finding to the inability of these cells to excise dimers. In contrast, we find that AAF is removed normally by these cells; however, our results and theirs are not strictly comparable since, in their studies, 200 ergs mm^{-2} resulted in about 80 times as much damage (dimers) as was produced by 1 μM NA-AAF (calculated on the basis of 0.7% of thymines as dimers at a fluence of 200 ergs mm^{-2}, ref. 8, Figure 3 and our highest level of damage Table 1, El San). Our results are similar to those of Regan, Setlow, Carrier and Lee (19) who found that Fanconi's anemia fibroblasts were capable of removing dimers.

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