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EXCISION REPAIR IN MAMMALIAN CELLS

Farid E. Ahmed, and R. B. Setlow

Department of Biology, Brookhaven National Laboratory,
Upton, New York 11973

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ABSTRACT Excision repair after combined treatments of UV and N-acetoxy-2-acetylaminofluorene (AAAF) was studied by three different techniques in cells proficient in UV excision repair and in cells deficient in UV repair. Two patterns of repair were observed: A) in repair proficient cells total repair was additive, and B) in repair deficient cells total repair was much less than additive—usually less than observed for separate treatments—and AAAF inhibited dimer excision. We conclude that in the 1st class of cells pathways for repair of UV and AAAF lesions are not identical, and in the 2nd class the residual excision enzymes are different from those in repair proficient cells.

INTRODUCTION

UV induced pyrimidine dimers and AAAF lesions in DNA are substrates for excision repair in human cells, and AAAF damage mimics UV damage in the following ways: A) both are repaired by a long patch mechanism (~ 100 nucleotides) (1), B) xeroderma pigmentosum (XP) cells deficient in repairing UV damage are also deficient in repairing AAAF damage (1-3), and C) XP cells are more sensitive than normal cells to the cytotoxic and mutagenic activity of both UV and AAAF (4). The amount of excision repair of dimers and AAAF lesions saturates at high doses (5,6). Hence, if AAAF truly mimicked UV damage one would expect that the amount of repair from a combined treatment using saturation doses would be less than the sum of the treatments separately. We investigated cell lines proficient in UV excision repair (normal human, XP variant, ataxia telangiectasia, Fanconi's anemia, and Cockayne syndrome) and repair deficient cell lines (Chinese hamster V-79, and XP groups C, D & E). Three techniques were used to measure repair: unscheduled DNA synthesis measured radioautographically (7), the photolysis of BrdUrd incorporated

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into parental DNA during repair (1), and the loss of sites sensitive to UV endonuclease (8).

RESULTS

The three techniques gave equivalent results (9, 10, and unpublished data) (Fig. 1 and Table 1). We observed the following: 1) repair of UV and AAAF damage saturates, 2) AAAF repair mimics UV repair qualitatively, 3) in repair proficient cells repair is additive at saturating doses of UV and AAAF, and 4) in repair deficient cells, repair due to combined treatment with both agents is much less than additive and actually is inhibited at saturating doses.

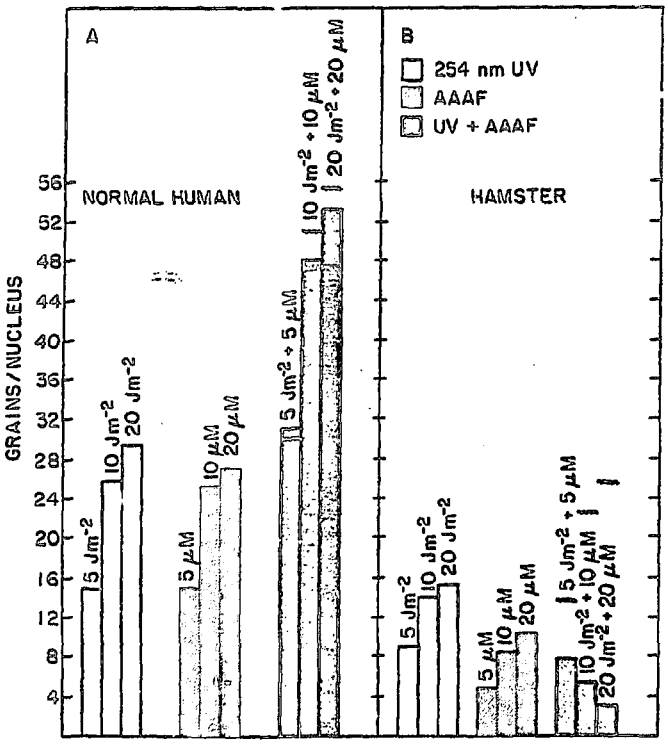

FIGURE 1. The average number of grains per nucleus for various UV doses, AAAF concentrations or combined treatments in normal human and V-79 cells. Wavy line indicates additivity (from references 9 and 10).

TABLE I

MEASURES OF EXCISION REPAIR IN HUMAN CELLS TREATED WITH UV, AAAF AND COMBINATIONS

Cell line	Unscheduled synthesis[a]			Endonuclease assay[b]		BrdUrd photolysis[c]		
	$20Jm^{-2}$	20μM	$20Jm^{-2}$+20μM	$20Jm^{-2}$	$20Jm^{-2}$+20μM	$20Jm^{-2}$	10μM	$20Jm^{-2}$+10μM
Normal human								
Fer Bel (CRL 1191)	18.6	16.1	33	27.5	27.4	3.5	1.4	4.9
Kid Mor (CRL 1220)	17.4	16.4	32.9	23.1	23.3	3.3	1.4	4.4
Ataxia telangiectasia								
NeNe (CRL 1347)	19.7	16.6	35	24.6	24.2	3.2	2.3	5.4
Se Fan (CRL 1343)	22.3	14	33.2	24.3	24.9	3.0	1.4	3.9
AT 4BI	19.6	14.4	35	26.3	26.4	2.2	1.4	3.4
Fanconi's anemia								
Ca Rol (CRL 1196)	15.4	13.9	28.2	27.5	27.4	3.4	0.9	4.4
Cockayne syndrome								
GM 1098	14.3	19.8	33.2	26.1	26.1	3.2	2.6	6.2
GM 1629				26.9	27.0	4.1	2.1	6.6
Xeroderma pigmentosum								
Variant; Wo Mec (CRL 1162)	23.0	17.1	37.2	24.7	24.0	2.8	1.1	4.3
C; Ge Ar (CRL 1161)	3.7	2.2	1.8	3.7	1.4	1.0	0.1	0.1
D; Be Wes (CRL 1160)	6.4	6.6	3.8	3.9	0.8	1.4	0.2	0.4
E; XP2R0 (CRL 1259)	10.8	6.4	2.8	19.3	9.6	4.8	1.0	3.8

[a] Grains/Nucleus incorporated in 3 hr (8 days exposure).
[b] Sites removed in 24 hr/10^6 Daltons.
[c] $\Delta(1/M_w)\times10^6$ at highest 313 nm dose (12 hr repair).

DISCUSSION

We have no unique explanation for the phenomena observed but suggest the following: since over the range of doses used the damage increases proportionately with dose, the measure of saturation of repair after UV and AAAF is due to saturation of repair systems and not of damage. The rate limiting step in the excision repair process seems to be the incision step (9,10). The following are possibilities to explain why incision is limiting at high doses: A) inaccessibility of lesions to endonucleases (this is ruled out by the fact that at very high UV doses ($\sim 100\ Jm^{-2}$) there is no decrease in the numbers of dimers removed (unpublished data)), B) limiting amount of enzymes or complexes of enzymes[1], C) limiting cofactors[2], and D) similar enzymes but the production of products that inhibit incision or accessibility. This also is ruled out by additivity in repair proficient cells.

[1]For example, incision activities might be part of a small number of repair enzyme complexes and if a later step in repair—say excision—was not completed a subsequent incision might not take place. In such a situation the rate limiting step would be excision but in most experiments would appear to be incision.

[2]For example, a DNA binding protein necessary for efficient endonuclease reactivity.

Some possibilities to explain the similarity between UV and AAAF repair are: A) same enzymes and cofactors or complexes of them working on both lesions, B) different enzymes or enzyme complexes but same cofactors, C) same enzymes but different cofactors, and D) similar or different excision systems but similar accessibility to endogenous enzymes[1]. We can rule out A because of the additivity observed in repair proficient cells and D does not explain the inhibitory effects observed in repair deficient cells.

Hence, we conclude: A) in repair proficient cells additivity implies that AAAF does not completely mimic UV as far as repar is concerned, and that there are different enzymes or complexes of enzymes and cofactors for incision of the two types of damage, B) inhibition of repair after a combined treatment in repair deficient cells seem due to less or different types of endonucleases and cofactors than in repair proficient cells for both UV and AAAF lesions.

We suggested models to explain these data (9,10). There are complexes of repair enzymes and cofactors specific for UV and AAAF in repair proficient cells, and major or minor products inhibit each other in repair deficient cells.

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[1]This possibility ascribes the defect in XP cells to the inaccessibility of their DNA.