DNA REPAIR IN HUMAN FIBROBLASTS TREATED WITH A COMBINATION OF CHEMICALS

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ABSTRACT Excision repair of DNA damage was measured by the photolysis of bromodeoxyuridine incorporated during repair in normal human and xeroderma pigmentosum group C fibroblasts (XP C) treated with a combination of the carcinogens *N*-acetoxy-2-acetylaminofluorene (AAAF), and 4-nitroquinoline 1-oxide (4NQO). Repair was additive in normal and XP C cells treated with AAAF plus 4NQO, indicating that there are different rate limiting steps for removal of 4NQO and AAAF lesions.

INTRODUCTION

Previous studies on DNA excision repair in human cells treated with repair-saturating doses of ultraviolet (UV) and supposedly UV mimetic carcinogens showed that in repair-proficient cells the total repair was additive for UV and *N*-acetoxy-2-acetylaminofluorene (AAAF) (1), or for UV and doses of 4-nitroquinoline 1-oxide (4NQO) low enough not to inhibit unscheduled DNA synthesis (UDS) (2), implying that there are different rate-limiting steps in removal of the physical and the chemical damages. The results were more complicated for xeroderma pigmentosum (XP) cells in that UV plus AAAF showed appreciably less repair than after UV alone, whereas UV plus 4NQO showed an additive effect (1, 2). We concluded that for UV and AAAF repair-deficient XP cells have a different repair system, not just fewer repair enzymes than normal cells; and that the same long-patch repair system works on 4NQO damage in both normal and XP cells. In the present work we report the extent of repair after treatment with AAAF and 4NQO. We chose AAAF and 4NQO because they were supposed to be UV mimetics (3). Hence, we wished to see the pattern of repair after a combination of these agents in repair-proficient and deficient cell strains.

We used the technique of photolysis of bromodeoxyuridine (BrdUrd) (3) incorporated into parental DNA during repair to study excision repair after the various treatments. The technique is sensitive, gives repeatable results, and agrees well with other techniques used for measuring repair such as UDS and assays for UV-endonuclease sensitive sites (1, 4). The results show that in normal human and XP C cells repair is additive after the combined action of AAAF plus 4NQO.

MATERIALS AND METHODS

Cell Culture

Two strains of human fibroblasts: normal, Rid Mor (CRL 1220) and XP group C, Ge Ar (CRL 1161) from the American Type Culture Collection (Rockville, Md.) were used. Cells were grown in plastic

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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 (GIBCO, Grand Island Biological Co., Grand Island, N.Y.) and kept in a humidified 7.5% CO_2 atmosphere at 37°C. When cells reached confluency they were subcultured at a ratio of 1:3. Passages between 3 and 24 were used.

Cells were regularly checked for the absence of mycoplasma contamination by fluorescent staining with bisbenzimidazol compound 33258 (Hoechst Pharmaceutical Co., Kansas City, Mo.) as described by Chen (5).

Chemical Treatment

A stock solution of 5 mM AAAF (National Cancer Institute Standard Chemical Carcinogen Reference Repository) was dissolved in fresh $(CH_3)_2SO$ and stored in plastic vials under liquid nitrogen. AAAF was added to cells in culture medium containing serum to a final concentration of 10–20 μM. After 30 min at 37°C the medium was changed.

At stock solution of 10 mM 4NQO (ICN Pharmaceutical, Plainview, N.Y.) in Hank's balanced salt solution (GIBCO) was prepared and kept frozen at −10°C. Working solutions of 0.01–1 mM were prepared and added to cells in petri dishes to the required final concentration in culture medium for 30 min before medium change.

Bromodeoxyuridine Photolysis

A detailed description of this technique and methods of calculation have been described (1, 3). The usual technique involves repair in the presence of 2 mM hydroxyurea. [^{3}H]deoxyribosylthymine (dThd), 0.4 μCi/ml (6.7 Ci/mmol; New England Nuclear, Boston, Mass.) labeled cells were treated and allowed to repair in BrdUrd (Sigma Chemical Co., St Louis, Mo.) and treated [^{14}C]dThd (0.4 μCi/ml, 50 Ci/mol; New England Nuclear) labeled cells allowed to repair in dThd (Sigma Chemical Co.) for 24 h. The two groups of cells were harvested and mixed together and the regions repaired with BrdUrd selectively photolyzed by 313 nm radiation from a Johns Monochromator (6) (courtesy of J. C. Sutherland, Biology Department, Brookhaven National Laboratory). Sedimentation in alkaline sucrose gives a single-strand weight average molecular weight, M_w, of the DNA from cells incubated in BrdUrd or dThd and exposed to different 313-nm doses. The difference between their reciprocals, $\Delta(1/M_w) = (1/M_w)_{BrdUrd} - (1/M_w)_{dThd}$, is a measure of photolysis of BrdUrd incorporated into parental DNA during repair, hence of repair itself.

The doses of 313 nm are presented in terms of the number of breaks per BrdUrd residues that would

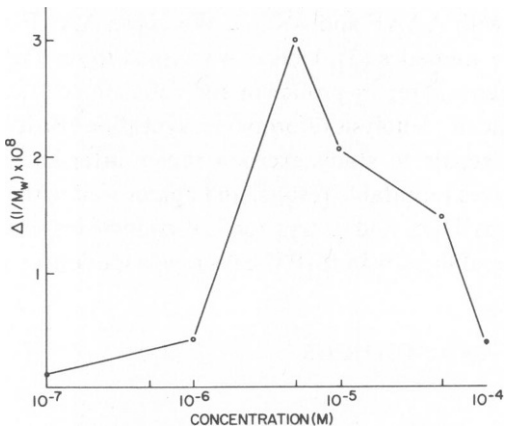

FIGURE 1 The relationship in normal human fibroblasts between $\Delta(1/M_w)$ as a result of photolysis by 313 nm and concentration of 4NQO. Cells were treated with the chemical as indicated and then incubated for 24 h in nonradioactive BrdUrd or dThd and exposed to a 313-nm photolytic doses of 7.2×10^{-2} breaks/BrdUrd.

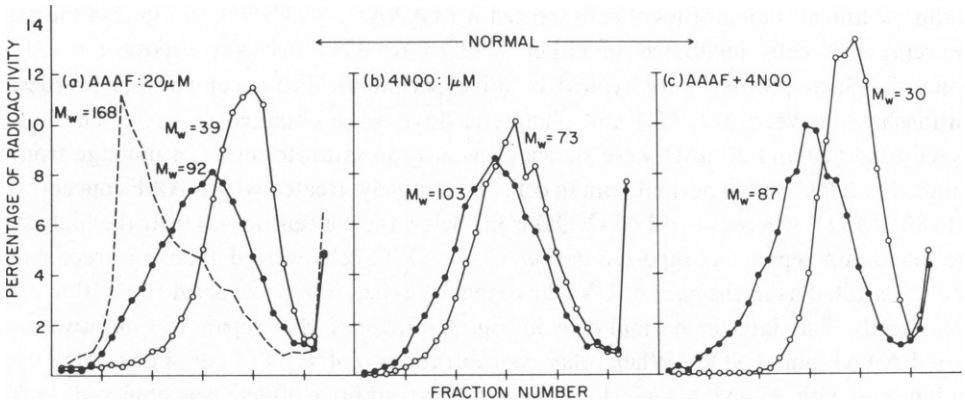

FIGURE 2 Alkaline sucrose gradient profiles of DNA from normal cells. Cells were treated as indicated and then incubated for 2 hr in nonradioactive BrdUrD (○) or dThd (●) and exposed to a 313 nm photolytic dose of 7.4×10^{-2} breaks/BrdUrd. M_w in millions are indicated on the profiles. The dashed line represents the profile of dThd DNA without 313 nm photolysis. Sedimentation to the left.

be made by exposure to the radiation source. In actual experiments the dose was measured with a calibrated photocell whose output multiplied by time could be expressed in breaks per BrdUrd. A typical dose of 4.5×10^4 J/m^2 corresponds to 3.7×10^{-2} breaks/BrdUrd.

RESULTS

Fig. 1 shows the relationship between excision repair expressed as $\Delta(1/M_w)$ and several concentrations of 4NQO after a photolytic dose of 7.2×10^{-2} breaks/BrdUrd. Repair reaches a maximum at ~5 μM followed by a rapid decline. When different photolytic doses were used, or when radioautography was tried (2), the same relation held. Therefore, in subsequent experiments the concentrations used did not exceed 5 μM. Fig. 2 shows some representative

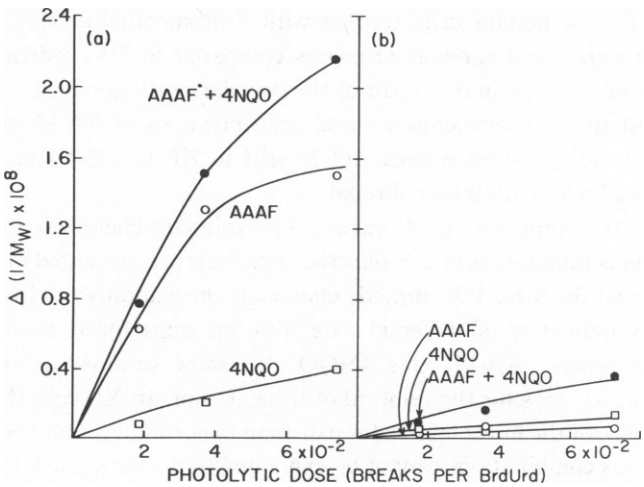

FIGURE 3 The relationship between $\Delta(1/M_w)$ and photolytic dose in normal human fibroblasts (*a*) and XP C (*b*). (*a*) Cells treated with 20 μM AAAF, 1 μM 4NQO, or the combination; (*b*) 10 μM AAAF, 1 μM 4NQO, or the combination.

alkaline sedimentation profiles of cells treated with AAAF and 4NQO. In Fig. 2 *A* the dashed line represents cells incubated in either BrdUrd or dThd without exposure to 313-nm photolysis. Such profiles were typical of all experiments. From comparable profiles, the relationships between $\Delta(1/M_w)$ and photolytic doses were obtained (Fig. 3). The doses of AAAF used (10 and 20 μM) were shown previously to saturate repair of damage from this chemical in XP C and in normal human cells, respectively, treated with AAAF concentrations up to 80 μM (1), whereas 1 μM of 4NQO is far below the concentrations near the highest that give maximum repair. As reported earlier (3, 7) XP C cells were defective in repair of the chemicals tested as in the case of UV; the extent of defect in repair varied from 10 to 50% of normal cells. The data for normal cells in Fig. 3 *A* indicate that repair was additive in cells treated AAAF plus 4NQO. When other concentrations of 4NQO (2 and 4 μM) were used in combination with 20 μM AAAF the same result (an additive effect) was observed. In XP C cells repair also was additive after treatment with AAAF plus 4NQO (Fig. 3 *B*).

DISCUSSION

Previous results showed that high concentrations of 4NQO resulted in a rapid decline of UDS in human (2, 7) and hamster (8) cells. In the present study we used an AAAF concentration that resulted in saturation of repair, and 4NQO concentrations that gave less than the highest repair level. Other reports previously showed an increase in UDS, though not a strict summation, in Syrian hamster cells treated with 20 J/m^2 UV and 1 μM 4NQO (8). Moreover, mouse cells were able to excise 4NQO-purine adducts as efficiently as normal human cells, but they showed a slower rate of removal of UV-dimers suggesting that the two types of damage may be removed by different repair pathways, or that in mouse cells the same excision repair system recognizes dimers more poorly than chemically induced lesions (9). It has been shown that different mammalian cell lines show striking differences in sensitivities to UV and 4NQO, indicating that the effects of UV on cells differ from 4NQO and that the repair process for UV-induced damage differ from that of 4NQO damage (10, 11). Studies by McCaw et al. (12) on human cells treated with 7-bromoethylbenz[a]anthracene showed different relative repair and survival responses compared to UV, indicating that different mechanisms may be involved in the repair of the two classes of damages.

In the design of these experiments we used concentrations of AAAF that saturate repair (20 μM AAAF in normal human cells and 10 μM in XP C cells) and a concentration of 4NQO that did not lead to inhibition of repair.

Our results on the combinations of various UV mimetic chemicals (1, 2) together with previous results on combinations of UV plus the chemicals are presented in Table I. In normal human cells UV and the three UV-mimetic chemicals show additivity of repair. We interpret this additivity as indicative of different rate limiting steps for removal of the UV- and chemical-induced lesions. AAAF plus 4NQO also show summation of repair indicating different rate limiting steps for the removal of these lesions. In XP cells the situation is more complicated because of the lower levels of repair than in normal cells and because inhibition of repair in the various combinations cannot be explained by a simple model. On the other hand the additivity of repair for UV plus 4NQO or AAAF plus 4NQO indicates different rate-limiting steps for removal of these lesions and, for the damages from these agents, XP cells act as if they have similar but lower levels of the repair system than normal cells. Brown

TABLE I
DNA REPAIR RESPONSES OF HUMAN CELLS TO COMBINATIONS OF UV AND ITS MIMETICS

Treatment	Cells	
	Normal human	Xeroderm pigmentosum C
UV + AAAF	additive*	inhibitory‡
+ ICR-170	additive	inhibitory
+ 4NQ0	additive	additive
AAAF + 4NQ0	additive	additive

UV data are from references 1 and 2.
*Approximately equal to the sum of the two separately.
‡Less than either.

et al. (13) reported that repair after UV plus 4NQO or UV plus AAAF was much less than the sum of each agent separately and that there was no additivity after treatment with 4NQO plus AAAF. They suggested that the differences between their results and ours might be due to: (*a*) different UV dosimetry, (*b*) different degrees of saturation of repair or, (*c*) treatments in different media. The first possibility does not seem reasonable because Brown et al. (13) observe 2.5×10^{-7} nuclease-sensitive sites per dalton after 10 J/m^2 of 254 nm and we observe 2.6×10^{-7} (1). The second possibility also seems unreasonable for UV, because the dose we usually use, 20 J/m^2, is within ~10% of the saturation value (4), but for 4NQO we (2) and Stich et al. (7, 8) do not observe saturation at high doses but Brown et al. (13) do. Most of our treatments were carried out in serum-containing medium in contrast to the serum-free medium used by Brown et al. (13). When we treated UV irradiated normal human cells with AAAF in serum free medium, however, the chemical did not inhibit the removal of nuclease sensitive sites (unpublished observations). Hence, we know of no good explanation for the differences between our observations and those of Brown et al.(13). Perhaps the differences are in the techniques used to measure repair. We used the photolysis technique whereas Brown et al. (13) used the repair replication technique. A direct comparison of these two techniques in the same system with the same chemicals would seem to be desirable.

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