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Role of Poly(adenosine diphosphate ribose) in Deoxyribonucleic Acid Repair in Human Fibroblasts†

Michael R. James and Alan R. Lehmann*

ABSTRACT: We have investigated the role of poly(adenosine diphosphate ribose) in DNA repair in human fibroblasts by observing the effects of 3-aminobenzamide (3AB), a specific inhibitor of poly(ADP-ribose) synthesis, on various aspects of DNA repair. After treatment of human fibroblasts with dimethyl sulfate (DMS), 3AB retarded the joining of strand breaks; unscheduled DNA synthesis was unaffected after low doses of DMS but was stimulated after high doses. 3AB also enhanced the cytotoxicity of DMS. After γ irradiation there was a slight inhibition by 3AB of the rejoining of single-strand breaks but no effect on the rejoining of double-strand breaks, unscheduled DNA synthesis, DNA replicative synthesis, or cytotoxicity. There were no effects of 3AB on the repair of UV damage. On the basis of the different kinetics of the various steps of excision repair processes after different treatments of fibroblasts, our results are interpreted as evidence that the synthesis of poly(ADP-ribose) is involved in the ligation step of excision repair.

ADP-ribosylation is the covalent addition to protein of the ADP-ribose portion of an NAD molecule. ADP-ribosyltransferase (ADPRT; EC 2.4.99)[1] is a DNA-dependent nuclear enzyme which catalyzes both the ADP-ribosylation of chromosomal proteins and the further addition of ADP-ribose moieties to produce a homopolymer of up to 70 residues (Hayaishi & Ueda, 1977; Butt & Smulson, 1980; Purnell et

† From the Medical Research Council, Cell Mutation Unit, University of Sussex, Falmer, Brighton, BN1 9QG, United Kingdom. *Received December 29, 1981; revised manuscript received May 4, 1982.* This work was supported in part by EC Contract 166-76-1-BIO-UK.

[1] Abbreviations: 3AB, 3-aminobenzamide; ADPRT, adenosine diphosphoribosyltransferase; DMS, dimethyl sulfate; DSBs, double-strand breaks; MNU, *N*-methyl-*N*-nitrosourea; NAD, nicotinamide adenine dinucleotide; PBS, phosphate-buffered saline; SSBs, single-strand breaks; UDS, unscheduled DNA synthesis; UV, shortwave ultraviolet light; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; EDTA, ethylenediaminetetraacetic acid.

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al., 1980; Smulson & Sugimura, 1980).

Considerable evidence has shown that this enzyme is activated in response to treatment of cells with DNA-damaging agents (Berger et al., 1979, 1980; Durkacz et al., 1980; Cohen & Berger, 1981; Shall et al., 1981). Although many different nuclear proteins are apparently ADP-ribosylated, including a 110 000-dalton protein which is probably ADPRT itself (Ogata et al., 1980; Hayaishi et al., 1981), DNA repair is the only process in which poly(ADP-ribose) has been firmly shown to play a role, albeit an unknown one (Durkacz et al., 1980; Purnell et al., 1980; Shall et al., 1981). Treatment of cells with chemicals which alkylate DNA or with ionizing radiation causes a decrease in cellular NAD content, and this has been correlated with the observed stimulation of ADPRT (Juarez-Salinas et al., 1979; Skidmore et al., 1979; Jump et al., 1980; Rankin et al., 1980; Durkacz et al., 1980; Shall et al., 1981). Recently a number of inhibitors of the enzyme have been used in an attempt to ascribe a precise biological function to ADPRT. Very effective inhibition has been obtained with derivatives of benzamide (Purnell & Whish, 1980). These act by competitive inhibition and appear to be highly specific. One of these inhibitors, 3-aminobenzamide (3AB), has been found to prevent the decrease in NAD content in mouse L1210 cells after treatment with dimethyl sulfate (DMS) (Durkacz et al., 1980) and various other DNA-damaging agents (Shall et al., 1981). It also inhibited the repair of single-strand breaks induced in DNA by DMS and increased the cytotoxicity of DMS and *N*-methyl-*N*-nitrosourea (MNU) (Durkacz et al., 1980; Shall et al., 1981). These results agree with earlier experiments using other inhibitors (Skidmore et al., 1979; Nduka et al., 1980; Yamamoto & Okamoto, 1980; Shall et al., 1981).

In this report we extend these studies using 3AB to human fibroblasts, we have investigated the role of ADPRT in the repair of damage produced by shortwave ultraviolet light (UV), γ radiation, and DMS, and we have attempted to pinpoint the step in DNA repair in which ADPRT is required.

Materials and Methods

Materials. Cell culture media and sera were purchased from Gibco-Biocult, UK. Radiochemicals were from Amersham International, and other reagents and enzymes were from Sigma Chemical Co. 3-Aminobenzamide, kindly provided by Professor S. Shall, was synthesized as described elsewhere (Durkacz et al., 1981b).

Cell Culture. Experiments were performed on normal human fibroblast cell strains. Cells were maintained in Eagle's ME medium with 15% fetal bovine serum. For experiments, unless otherwise indicated, cells were distributed at 1×10^5 cells per 5-cm dish 2 days before use.

For UV irradiation, medium was aspirated from the Petri dishes which were wetted with phosphate-buffered saline (PBS) prior to irradiation by a shortwave UV source calibrated by a Latarjet meter. DMS solutions were made fresh each time by dilution in ethanol and then in serum-free medium immediately before use. Cells were treated for 30 min at 37 °C. X-ray and cobalt-60 γ irradiations were done in air at room temperature except where otherwise stated. Bleomycin was dissolved in Hepes-buffered medium, and dishes were kept in a 5% CO_2 atmosphere incubator during treatment. In all cases 3AB in warm medium was added to dishes at the end of the treatment.

Determination of NAD Content. The NAD content of cells was measured by an enzymatic cycling assay (Bernofsky & Swan, 1973). NAD was extracted from cells by adding 0.4 mL of 50% ethanol to PBS-rinsed monolayers and collected by scraping with a rubber policeman.

Sucrose Gradients. Repair of DNA single-strand breaks in cells treated with DMS or γ radiation was followed by sedimentation through alkaline sucrose gradients. Cells were prelabeled with [^{3}H]thymidine (1 μCi/mL; 1 Ci/mmol) or [^{14}C]thymidine (0.05 μCi/mL; 56 mCi/mmol) and at specified times after treatment were harvested by scraping in ice-cold saline–EDTA. Sedimentation in the SW50.1 rotor and analysis of results were as described previously (Lehmann & Kirk-Bell, 1972).

Alkaline and Neutral Elution of DNA. Elution of DNA from nitrocellulose filters followed the procedure of Fornace et al. (1976) for alkaline elution and Bradley & Kohn (1979) for neutral elution. Human fibroblasts were labeled for 72 h with [^{14}C]thymidine (0.008 μCi/mL). Chinese Hamster Ovary (CHO) cells were labeled with [^{3}H]thymidine (0.07 μCi/mL). Cells were chased for at least 3 h in nonradioactive medium. After irradiation of the human cells at 0 °C in air, fresh warm medium was added and incubation continued for the indicated times after which the medium was replaced with ice-cold PBS. Unirradiated CHO cells were havested by scraping, and aliquots were distributed to each dish of the human cells. These were then scraped off the dishes and transferred as a mixture with the CHO cells to the filters in the elution apparatus. After a 60-min lysis in 0.2% sarkosyl and 0.02 M EDTA, pH 10 (plus 0.5 mg/mL proteinase K for neutral elution), 45 mL of neutral or alkaline elution buffer was applied, and about 11 fractions were eluted and collected as described (Fornace et al., 1976; Bradley & Kohn, 1979). Radioactivity in each fraction and that remaining on the filters were assessed. For alkaline elution the relative rate of elution of ^{14}C dpm to the ^{3}H control was determined from the best-fit straight lines drawn through the datum points of the first five fractions on a semilogarithm plot. In the case of neutral elution where nonlinearity is often observed, the relative elution of ^{14}C to ^{3}H was calculated for the first nine fractions.

Repair Synthesis. The procedure used has been described in earlier work (Lehmann & Stevens, 1980). Normal human fibroblasts were grown for 4 days in arginine-free medium plus 5% fetal bovine serum. Before irradiation or during chemical treatment the cells were incubated in 10 mM hydroxyurea to inhibit residual semiconservative DNA synthesis. After treatment they were incubated for a further 4 h with 10 μCi/mL [^{3}H]thymidine (20–50 Ci/mmol), and 10 mM hydroxyurea with or without 3AB and then lysed in 0.2 M NaOH, 1% sarkosyl, and Cl_3CCOOH precipitated onto Whatman glass fiber circles for scintillation counting. Cell numbers were assessed on a few dishes. Results are expressed as ^{3}H cpm per 10^5 cells.

Replicative DNA Synthesis. Fibroblasts, prelabeled with [^{14}C]thymidine (0.015 μCi/mL; 56 mCi/mmol), were pulse labeled with 10–20 μCi/mL [^{3}H]thymidine (25–50 Ci/mmol) for 15–30 min at various times after irradiation or chemical treatment. Dishes were rinsed twice with PBS and lysed with 0.3 mL of 0.2 M NaOH and 1% sarkosyl, and duplicate samples were spotted onto glass-fiber filters which were washed in Cl_3CCOOH and ethanol for scintillation counting. Results are expressed relative to the untreated control value.

Survival. Reproductive survival was determined by colony-forming ability using the feeder layer method as has been fully described elsewhere (Arlett & Harcourt, 1980).

Results

NAD Content. A nontoxic concentration of 5 mM 3AB was used in all experiments. The effect of the inhibitor on cellular NAD content after treatment with DMS, γ radiation, and UV

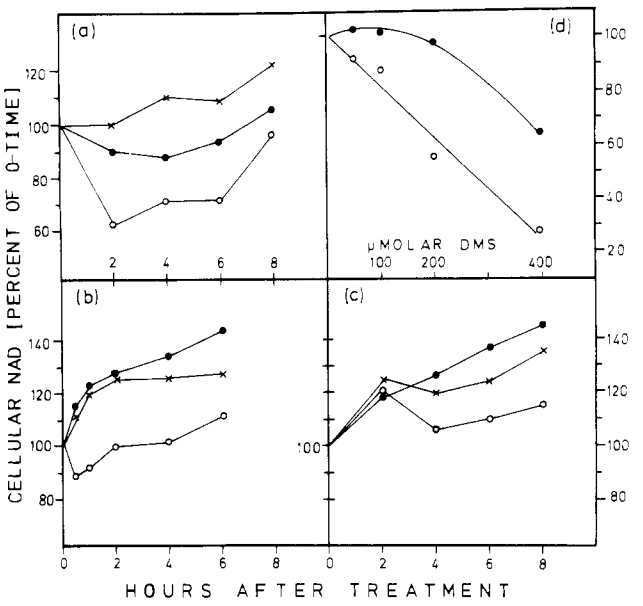

FIGURE 1: Effect of 3AB on NAD content in cells after various treatments. Results are expressed as a percentage of an untreated control at zero time; no correction was made for an increase in cell number during the experiments. Time course after (a) 100 μM DMS, (b) 10 krd of γ radiation, and (c) 50 J m^{-2} UV; (d) NAD content at 2 h after various DMS concentrations. (×) Untreated control; (○) treated sample; (●) treated sample plus 5 mM 3AB. The 100% values were (a) 308, (b and c) 438, and (d) 275 pmol of NAD/10^5 cells. Representative experiments shown.

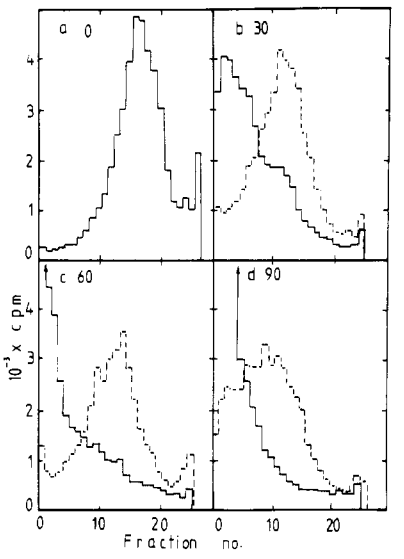

FIGURE 2: Rejoining of single-strand breaks produced by DMS. Cells labeled with [^{3}H]thymidine were exposed to 100 μM DMS. At the end of the treatment period, medium with (---) or without (—) 5 mM 3-aminobenzamide was added and incubation continued for 0 (a), 30 (b), 60 (c), or 90 min (d). The size of DNA was measured by sedimentation for 60 min at 25000 rpm in alkaline sucrose gradients as described under Materials and Methods. The number of breaks per 10^9 daltons calculated from these gradients are (a) 3.8, (b) 0.69 (−3AB) and 1.61 (+3AB), (c) <0.5 (−3AB) and 1.9 (+3AB), and (d) <0.5 (−3AB) and 1.1 (+3AB). Sedimentation is from right to left.

light is shown in Figure 1. The increase in NAD in control cultures seen during the experiment cannot be accounted for by cell growth and division and probably results from changing the medium after sham treatment (Rankin et al., 1980). At short times after treatment with 100 μM DMS (Figure 1a) or 10 krd of γ radiation (Figure 1b), there was a decrease in NAD content relative to that in the sham-treated controls. A decrease relative to controls was seen after UV but only at later times (Figure 1c). In all cases incubation with the inhibitor substantially prevented this decrease. The inhibition was less at DMS concentrations greater than 200 μM (Figure 1d), although the NAD loss may have occurred during the 20-min DMS treatment time. 3AB was not present during treatment to avoid possible interaction between DMS and 3AB. 3AB had only small effects on the NAD level in undamaged cells. The results in Figure 1 are similar to those obtained with various other cell types (Skidmore et al., 1979; Rankin et al., 1980; Durkacz et al., 1980; McCurry & Jacobson, 1981; Shall et al., 1981).

Strand Breaks. Sucrose gradient analysis demonstrated the considerable retardation by 3AB of the joining of single-strand breaks after DMS treatment (Figure 2). Thirty minutes after treatment some repair was evident, but this was much less than that in the control culture (Figure 2b). Even after a 90-min incubation (Figure 2d), strand breaks were still present in the culture containing 3AB.

After γ irradiation similar analysis often revealed a small effect, but this was not very reproducible (results not shown). We therefore used the more sensitive method of DNA elution from nitrocellulose filters to study repair of single-strand breaks after ionizing radiation and also to follow the kinetics of production and rejoining of single-strand incision breaks following UV irradiation. Parts a–c of Figure 3 show the elution profiles of DNA from cells exposed to different treatments and then incubated with or without 3AB, and parts d–f of Figure 3 show these data normalized and plotted as the relative elution as described under Materials and Methods. Figure 3a shows that at any fixed time after irradiation of the cells with 400 rd, the DNA from cultures treated with 3AB eluted in alkali more rapidly than that from cultures not treated with 3AB; i.e., more SSBs remained in the presence of 3AB. This is also seen in the normalized plot (Figure 3d). No such effect was seen during incubation after UV irradiation with 5 J m^{-2} (Figure 3b,e). When neutral elution was used to measure the joining of DSBs induced by 5 krd of γ radiation, again no difference was seen between cultures treated with and without 3AB; i.e., 3AB did not interfere with the rejoining of DSBs (Figure 3c,f).

Repair Synthesis. The polymerization step of DNA repair was assessed by the incorporation of [^{3}H]thymidine in quiescent fibroblasts (UDS; Figure 4). There was no effect of 3AB on UDS induced by UV (Figure 4b), by γ radiation (Figure 4c), or with the radiomimetic compound bleomycin (Figure 4d). After high doses of DMS (Figure 4a), there was more incorporation in the presence of 3AB than in its absence, by up to a factor of 2 at 400 μM DMS. The inhibitor had no effect on residual incorporation in untreated cells.

Replicative Synthesis. 3AB alone did not perturb semiconservative DNA synthesis [(×) in Figure 5d]. Its effect on the perturbation of DNA synthesis caused by mutagen treatment is shown in Figure 5. In dose–response experiments, in which DNA synthesis was measured 30 min after treatment, the only effects observed were possible small increases in the inhibition of DNA synthesis after low doses of γ radiation and DMS (Figure 5a,c). The kinetics of the recovery of DNA synthesis was unaffected by 3AB after UV and γ irradiation (Figure 5b,f), but after 125 μM DMS, DNA synthesis recovered in the presence of the inhibitor but more slowly than in cultures without the inhibitor, taking 4 h longer to reach control levels (Figure 5d).

Cell Survival. Colony-forming ability was measured after the various treatments; 5 mM 3AB was present continuously throughout the colony-forming period. The inhibitor alone was nontoxic up to about 9 mM (results not shown). The

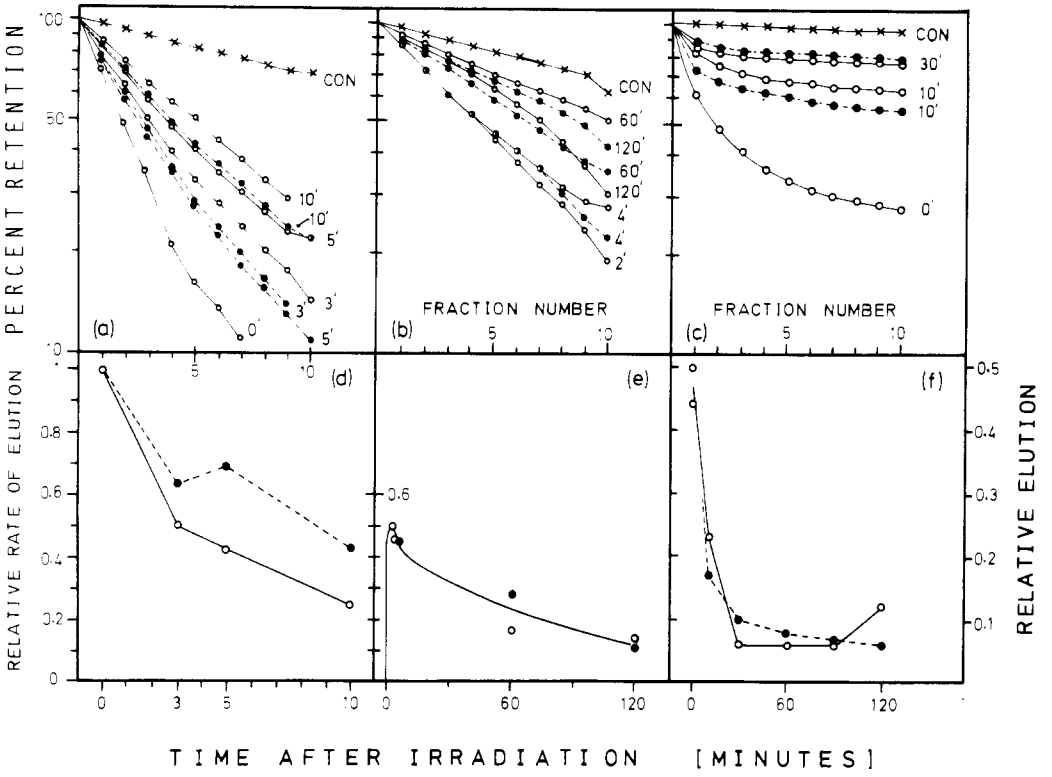

FIGURE 3: Elution of DNA from nitrocellulose filters. (a, d) and (b, e) show alkaline elution and (c, f) neutral elution of [^{14}C]DNA (○, ●) from cells irradiated with (a) 400 rd of X-rays (which produces about 1 single-strand break/10^9 daltons), (b) 5 J m^{-2} UV (which leads to the production of about 0.2 break/10^9 daltons), and (c) 5 krd of γ rays (which produces about 0.4 double strand break/10^9 daltons) and allowed different times to repair (time in minutes is shown for each profile). Untreated [^{3}H]DNA (×) was included on each filter as an internal control to which values were normalized. Relative rates of elution (d, e) or relative elution (f) were calculated from the elution profiles as described under Materials and Methods. Postirradiation incubation without (○) or with (●) 3AB.

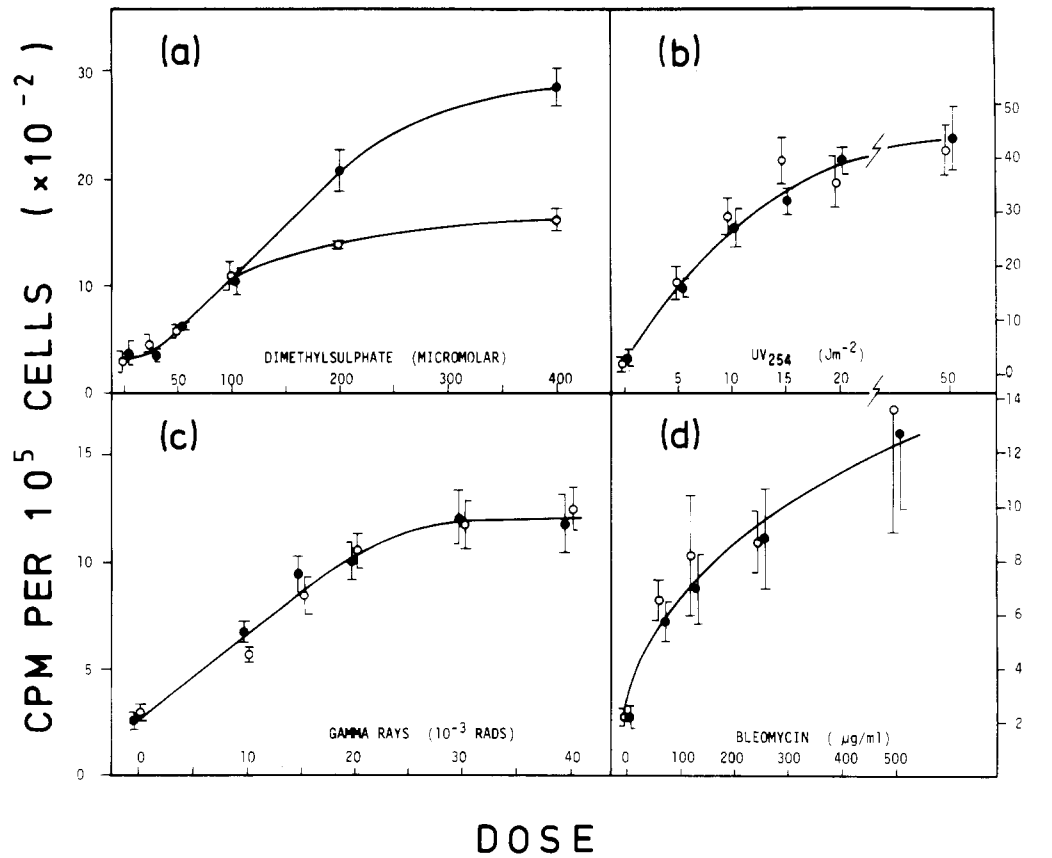

FIGURE 4: Effect of 3AB on repair synthesis induced by various agents. Experimental details were as given under Materials and Methods. The dose–response relationship between induced UDS and DMS (a), UV (b), γ radiation (c), and bleomycin (d) is shown for cells incubated without (○) or with (●) 3AB. The bars show the SE.

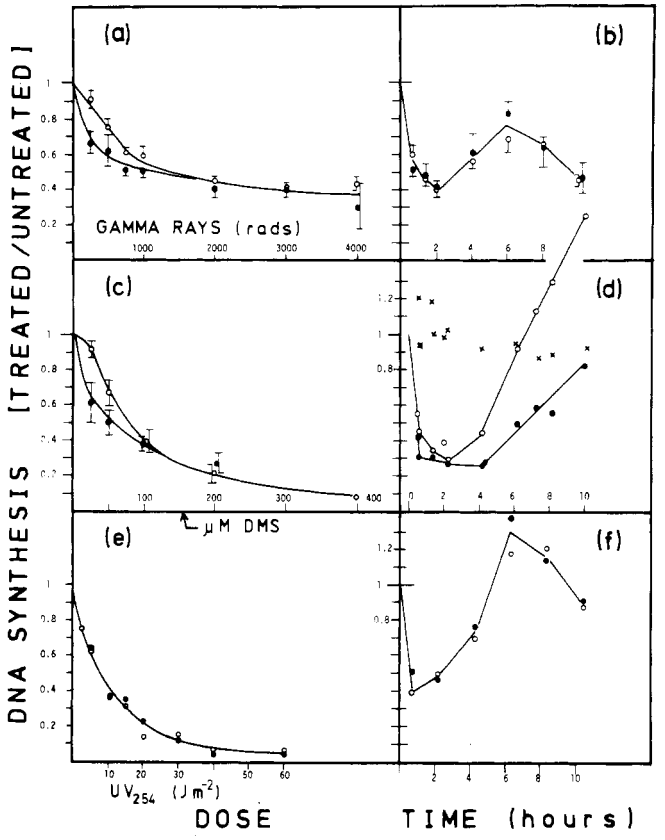

FIGURE 5: Replicative DNA synthesis after γ irradiation (a, b), DMS (c, d), and UV irradiation (e, f) without (O) or with (●) 3AB. In dose–response experiments cells were pulse labeled with [^{3}H]thymidine for 15 min beginning 30 min after the end of the treatment. In time–course studies the pulse time was 30 (UV and γ) or 75 min (DMS) at the times shown after 1 krd of γ radiation (b), 125 μM DMS (d), and 5 J m^{-2} UV radiation (f). The bars show SE; (d) two experiments; others show representative experiments. In (d) (×) represents untreated control with 3AB.

survival after treatment with the three agents is shown in Figure 6. Once again the only clear effect was with DMS where a substantial enhancement of toxicity by 3AB was observed.

Discussion

A large body of evidence has shown that the activity of

Table I: Summary of Effects of 5 mM 3AB on DNA Repair and Recovery in Human Fibroblasts after Treatment with DMS, UV Radiation, and γ Radiation

DNA damaging agent	rejoining of strand breaks	UDS	inhibition of DNA synthesis	recovery of DNA synthesis	toxicity
DMS	inhibition	stimulated after high doses	little effect	delayed	enhanced toxicity
γ rays	slight inhibition	no effect	little effect	no effect	no effect
UV	no effect	no effect	no effect	no effect	no effect

ADPRT is stimulated by fragmentation of DNA, both in purified systems (Benjamin & Gill, 1980b; Cohen & Berger, 1981) and in permeabilized cells (Berger et al., 1979; Juarez-Salinas et al., 1979; Skidmore et al., 1979; Jump et al., 1980; Durkacz et al., 1980; Benjamin & Gill, 1980a). The elegant experiments of Benjamin & Gill (1980a,b) showed that any type of DNA break would stimulate ADPRT, but the most efficient activator was DNA containing double-strand breaks with flush ends. Thus exposure of cells to any treatment which fragments DNA will activate ADPRT, and the associated decrease in cellular NAD content will be observed. This has been shown with ionizing radiation which produces breaks directly (Benjamin & Gill, 1980a,b) and after treatment with alkylating agents which rapidly give rise to enzymatic breaks during repair of the induced damage (Juarez-Salinas et al., 1979; Berger et al., 1979; Skidmore et al., 1979; Jump et al., 1980; Durkacz et al., 1980; Rankin et al., 1980; Shall et al., 1981). After UV damage, breaks are produced slowly during enzymatic repair. The reduction in NAD content is correspondingly slow and does not occur in cells from individuals with *Xeroderma pigmentosum*, in which excision repair is deficient (Berger et al., 1980; McCurry & Jacobson, 1981). In human fibroblasts we have observed changes in NAD content after treatment with these three types of agents (Figure 1), and the kinetics of these changes are similar to those found in other systems.

The finding of an increase in ADPRT activity as a result of DNA damage does not, however, imply that this activity is necessarily involved in repair of, or recovery from, this damage. Indeed our results on the effect of 3AB on DNA

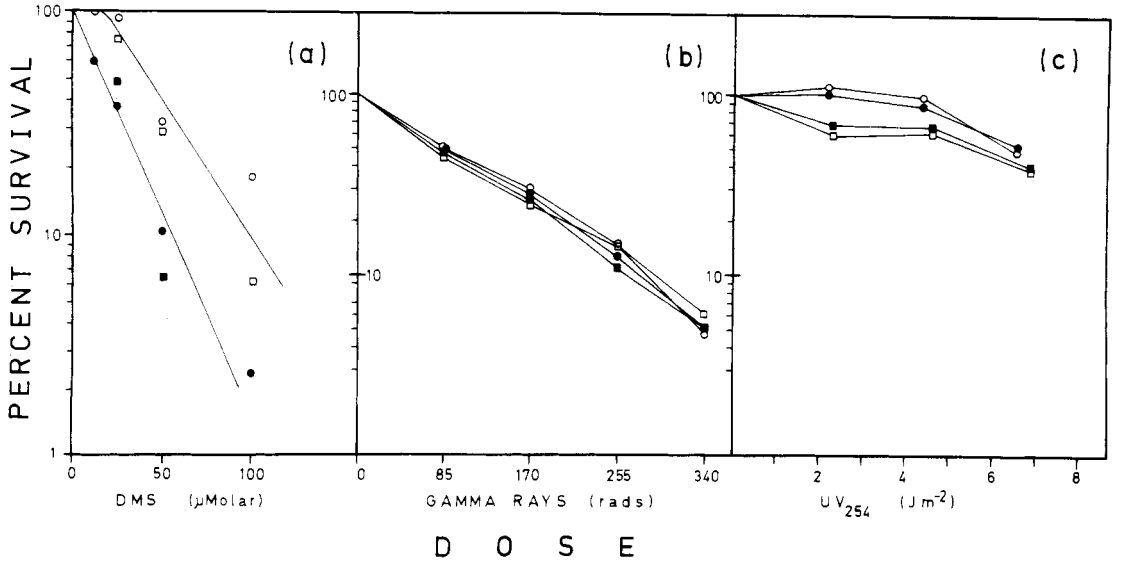

FIGURE 6: Survival of human fibroblasts after treatment with DMS (a), γ radiation (b), and UV light (c), without (open symbols) or with (closed symbols) 5 mM 3AB. Different symbols represent individual experiments. Plating efficiencies in these experiments were 50–80%.

repair in human fibroblasts, summarized in Table I, suggest that ADPRT is involved in repair of damage induced by DMS (as also found in L1210 cells), but in contrast it is unimportant for the recovery from damage produced by UV or γ irradiation. Possible reasons for this will be discussed below.

Involvement of ADPRT in the Repair of Damage Induced by DMS. Our experiments have attempted to locate the site of action of ADPRT in relation to DNA repair. Figure 2 showed that 3AB retarded the joining of strand breaks produced after treatment of human fibroblasts with DMS [although this inhibition was somewhat less marked than in similar experiments with mouse L1210 cells; see Durkacz et al. (1980)]. The assay of repair synthesis (Figure 4) showed that the polymerization step of excision repair did not require synthesis of poly(ADP-ribose). There was no effect of 3AB after low doses of DMS and a stimulation of up to 2-fold after high doses. This has also been confirmed with 3AB in mouse L1210 cells by using isopycnic gradients to measure repair synthesis (Durkacz et al., 1981a), and similar observations have also been made in human lymphocytes (Miwa et al., 1980; Berger & Sikorski, 1980) and cultured hepatocytes (Althaus et al., 1980). Such an increase in repair synthesis could result either from the repair of more DNA lesions with the same sized patches or from the repair of the same number of lesions but with larger patch size. We favor the latter explanation for the following reasons. We suggest that synthesis of poly(ADP-ribose) is involved in the ligation step of excision repair. Thus interference of this step (by 3AB) will produce long-lived strand breaks (as seen in Figure 2) which could allow nick translation by the repair polymerization system, resulting in larger repair patches under certain conditions. Similar conclusions have been reached by Durkacz et al. (1981a).

We further suggest that the delayed recovery of DNA synthesis (Figure 5) and the enhanced cytotoxicity of DMS (Figure 6) in the presence of 3AB result from the retarded rate of rejoining of strand breaks.

ADPRT and Radiation Damage: A Speculative Model. Our biochemical (Figures 2–5) and biological (Figure 6) experiments showed that synthesis of poly(ADP-ribose) was not involved in any biologically significant way in the recovery of damage produced by either UV or γ radiation. Only using the very sensitive alkaline elution assay (Figure 3) could any effect of 3AB be observed after ionizing radiation, and no effects at all were observed with UV. If, as suggested above, synthesis of poly(ADP-ribose) is involved in ligation, why does this involvement appear to be specific for repair of damage induced by alkylating agents? In order to explain this puzzling observation, it is necessary to consider the source of the breaks and the kinetics of their formation and repair after the three treatments. We will also take account of the very recent observations of Creissen & Shall (1982) that synthesis of poly(ADP-ribose) after DNA damage causes a rapid stimulation of DNA ligase activity (in extracts of L1210 cells) and that this stimulation is prevented by 3AB.

UV irradiation does not break DNA. Breaks are produced only during excision repair of UV damage, but it is well-known that such repair is slow, the incision step is rate limiting, and therefore only a very small number of breaks are present at any given time. For example after 10 J m^{-2} (10–20% survival) there are only about 0.4 break/10^9 daltons of DNA (Fornace et al., 1976). Basal levels of ligase are probably sufficient to seal these breaks. The ADPRT-stimulated increase in ligase activity is not necessary, and indeed the low steady-state level of breaks present after these fluences of UV may be insufficient to stimulate the synthesis of poly(ADP-ribose).

Ionizing radiation produces many breaks in DNA at doses commonly used in biochemical experiments (5–20 krd) but fewer at biological doses, e.g., 0.8 break/10^9 daltons after 300 rd (10–20% survival) (unpublished observations). These breaks are produced directly, and not during subsequent repair. Thus, although many breaks must be sealed by ligase, the steady-state level of breaks drops rapidly during repair, and once the initial breaks have been repaired, no or very few further breaks are formed, so again increased ligase activity may not be required.

After treatment with alkylating agents, as with UV, breaks are produced only during enzymatic repair, in this case by base loss, either from the action of DNA glycosylases or from spontaneous depurination, followed by the action of AP endonucleases. Unlike the situation with UV, however, these enzymes are not rate limiting, and a large number of breaks are seen after DMS treatment (Figure 2a; 4 breaks/10^9 daltons after 10–20% survival dose of 100 μM DMS). This number of breaks represents an equilibrium between the rate of production of these enzymatic breaks and the rate of their repair by polymerase and ligase. Since breaks will be produced continuously by enzymatic action for several hours, DNA containing many breaks would persist for some time, a situation which might be detrimental for the cell. However, the increase in ligase activity brought about by ADP-ribosylation will shift the above equilibrium such that the number of breaks present is greatly reduced (Figure 2b,c). Thus we interpret the results of Figure 2 as demonstrating, not the repair of breaks and its inhibition by 3AB, but a shift to a steady state of fewer persisting breaks brought about by ADPR-mediated increase in ligase activity, and a reduction of this shift by 3AB. The magnitude of the effects of inhibition of ADPRT by 3AB will on the above model be critically dependent on the precise kinetics of the various steps of the excision repair process. It is to be anticipated therefore that quantitative differences will be found between different cell types and different damaging agents.

ADPRT and DNA Replication. Edwards & Taylor (1980) recently proposed that poly(ADP-ribose) was involved in the inhibition of replicative DNA synthesis after ionizing radiation. They found that (1) the stimulation of ADPRT activity and (2) the inhibition of DNA synthesis by γ irradiation were lower in lymphoblastoid cells from individuals with the radiation-sensitive syndrome ataxia-telangiectasia than in normal cells [see also Houldsworth & Lavin (1980), Painter & Young (1980), de Wit et al. (1981), and Lehmann et al. (1982)]. They suggested that inhibition of DNA synthesis was dependent on poly(ADP-ribose) synthesis. Our results in Figure 5c do not support this contention. 3AB, which inhibited ADPRT activity, had little effect on the inhibition of DNA synthesis by γ irradiation.

In summary we find a clear involvement of poly(ADP-ribose) in repair of DMS damage in human fibroblasts and this appears to be at the level of ligase action. The use of the enzyme inhibitor suggests no biologically relevant role for poly(ADP-ribose) in the recovery of human fibroblasts from damage inflicted by ionizing radiation or UV light.

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