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DNA repair in human promyelocytic cell line, HL-60

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Abstract

The human promyelocytic cell line, HL-60, shows large changes in endogenous poly(ADP-ribose) and in nuclear ADP-ribosyl transferase activity (ADPRT) during its induced myelocytic differentiation. DNA strand-breaks are an essential activator for this enzyme; and transient DNA strand breaks occur during the myelocytic differentiation of HL-60 cells.

We have tested the hypothesis that these post-mitotic, terminally differentiating cells are less efficient in DNA repair, and specifically in DNA strand rejoining, than their proliferating precursor cells. We have found that this hypothesis is not tenable. We observe that there is no detectable reduction in the efficiency of DNA excision repair after exposure to either dimethyl sulphate or γ-irradiation in HL-60 cells induced to differentiate by dimethyl sulphoxide. Moreover, the efficient excision repair of either dimethyl sulphate or γ-irradiation induced lesions, both in the differentiated and undifferentiated HL-60 cells, is blocked by the inhibition of ADPRT activity.

Introduction

The human promyelocytic cell line, HL-60, can be induced to differentiate along either the monocytic or myelocytic lineages depending on the choice of the inducing agent (for review please see ref. 1). During dimethylsulphoxide (DMSO) induced myelocytic diiferentiation of HL-60 cells endogenous poly(ADP-ribose) increases substantially and there are large changes in the activity of the nuclear enzyme, ADP-ribosyl transferase, (ADPRT) (2). Breaks in DNA are an essential activator of nuclear ADP-ribosyl transferase (3) and we have demonstrated the transient appearence of DNA strand breaks in HL-60 cells differentiating along the myelocytic lineage (4). The transient appearance of these breaks raises the question whether these terminally differentiating cells are not proficient in DNA repair. Indeed, a number of studies have suggested that there may be a decrease in the DNA repair capacity of terminally differentiating cells including chick erythrocytes (5), chick neural retinal cells (6), murine neuroblastoma cells (7) and both avian and murine myotube nuclei

(8-10). Based on these and other similair observations it has been argued that in post-mitotic cells there is a less stringent requirement for efficient DNA excision repair, because there is no further need for the replication of the genome and that deficiencies in the integrity of the genome will not be passed to future generations. Indeed, deficiencies in DNA repair capacity, leading to the subsequent accumulation of mutations, has been proposed as a possible mechanism in cellular ageing and carcinogenesis (11-13). In contrast to these studies, other reports have demonstrated proficient DNA repair capacity in terminally differentiated mammalian neuronal cells (11,14), in guinea pig melanocytes (15) and in mouse epidermal cells (16). Comparison of repair capacity in rat and chick myotubes with their respective proliferating myoblasts has also failed to demonstrate a reduction in their repair capacity after the onset of terminal differentiation (17,18). Therefore, at the present time, there is some controversy as to the proficiency of DNA repair in post-mitotic, terminally differentiating cells. This issue is important not only for understanding the relationship, if any, between DNA repair and ageing but is of more direct importance in elucidating the mechanisms involved in alterations in DNA molecular weight which are detected in a number of examples of cytodifferentiation (18-23) and in the function of nuclear ADP-ribosyl transferase (ADPRT) in cell differentiation (24).

We have shown that during the onset of terminal differentiation in primary chick skeletal myoblasts, single-stranded DNA breaks are metabolically generated (18). Differentiation in normal human bone marrow CFU-gm cells (19) and in mouse erythroleukaemic cells is also associated with the formation of DNA strand-breaks (20,21). Resting, circulating human lymphocytes contain DNA strand breaks which disappear within four hours of mitogen stimulation (22,23).

Because of the widespread and important implications of these observations, it is necessary to ascertain whether these DNA strand-breaks are the passive product of alterations in DNA repair efficiency. In the present report, we have used a sensitive nucleoid sedimentation technique to examine the excision repair capacity of HL-60 cells following exposure to dimethyl sulphate (DMS) of γ-radiation, both before and after the induction of differentiation along the myelocytic lineage induced by DMSO.

Materials and Methods

Cells and culture conditions are described in the preceding manuscript (4). HL-60 cells were cultured in the presence of 1.25% (v/v)

DMSO for the indicated lengths of time. Cells were then collected by centrifugation at 500 x g, for 10 min. and resuspended in fresh medium at a density of 2 x 10^5 cells/ml. Cultures were then incubated for approximately 2 hours before exposure to the DNA damaging agent (either DMS or γ-radiation) unless otherwise specified.

An appropriate volume of a solution of DMS in absolute ethanol, at 1000 times the desired final concentration, was added to cultures. Because the half-life of DMS in culture medium at 37°C is very short (approximately 15 min.) the DMS was not washed away. A Cobalt-60 source, emitting approximately 1000 rads per min. was used for γ-irradiation. The exact rate of emission was calculated from a Cobalt-60 decay chart. After exposure to the DNA-damging agents, cells were incubated at 37°C for the indicated times prior to the estimation of DNA strand-breaks.

The effect of inhibition of adenosine diphophoribosyl transferase (ADPRT) activity on DNA repair was assayed after adding either 5 mM 3-amino benzamide or 2 mM 3-methoxybenzamide, which are competitive inhibitors of ADPRT activity (25), to the cultures immediately after the addition of DMS or following γ-irradiation. Alternatively, the acid analogues of these compounds, 3-aminobenzoic acid or 3-methoxybenzoic acid, which are poor inhibitors of ADPRT activity (25), were added to the cultures immediately after exposure to the DNA damaging agent, at the concentrations indicated above.

DNA repair was assayed by the estimation of DNA strand-breaks by the nucleoid sedimentation method previously described (4).

Results

DNA strand-breaks formed after exposure of cells to low doses of either γ-radiation or DMS can be easily quantitated, using the nucleoid sedimentation procedure. This method, described in the preceding paper (4), can accurately measure DNA strand-breaks formed by approximately 25 rads of γ-radiation or as little as 2.5 μM DMS (Fig.1). Ionising radiation produces 2 single-strand breaks per 10^{12} daltons of DNA per rad (26). HL-60 cell DNA has a molecular mass of 4 x 10^{12} daltons, calculated from a diploid DNA content of 10 pg per nucleus. Therefore, γ-irradiation of HL-60 cells produces 8 single-strand breaks per genome per rad. These calculations are used to establish the calibration scale in Fig. 1. This scale is used to estimate the approximate number of DNA strand breaks in subsequent experiments.

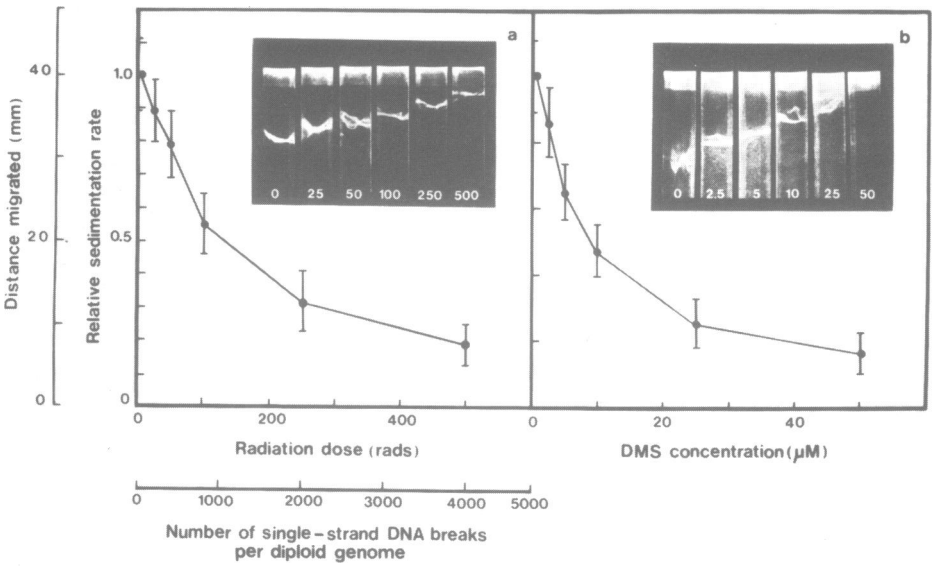

Fig.1. Estimation of DNA strand-breaks by the nucleoid sedimentation procedure. a) Sedimentation of nucleoids from HL-60 cells irradiated with the indicated dose of Υ-irradiation. **b)** Sedimentation of nucleoids from cells treated with the indicated concentration of DMS for 15 minutes. The error bars show the standard error of the mean of at least three separate measurements in each case and refer only to the actual distance migrated and not the relative migration rates. The standard errors for the relative sedimentation rates are smaller than the symbols in these figures. The insets are photographs of two nucleoid gradients against a vertical UV transilluminator. The figures in the insets indicate the dose of Υ-irradiation (a), or the concentration of DMS used (b).

Exposure of HL-60 cells to 100 rads or Υ-radiation produces 800 single-strand DNA breaks per dipoid genome in both undifferentiated, proliferating cells and in the post-mitotic cells induced to differentiate along the myelocytic lineage. These breaks are rapidly repaired in both cell types during a subsequent incubation of the cells and within 40 minutes the sedimentation rate of the nucleoids is restored to the control un-irradiated levels (Fig. 2). Increasing the Υ-irradiation dose to 400 rads decreases the relative sedimentation rate of the nucleoids to 0.35, corresponding to approximately 3 200 single-strand DNA breaks. This larger number of DNA strand-breaks takes longer to be repaired and even after 2 hours there is still some 200 single-strand breaks evident. However, either at 100 or at 400 rads of Υ-irradiation there is no detectable difference in the kinetics of repair of DNA strand-breaks between

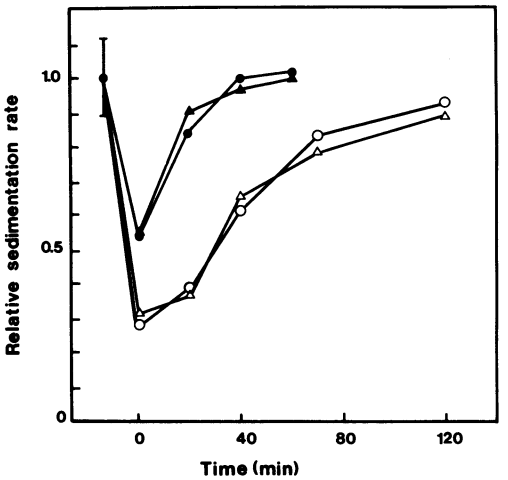

Fig.2. Repair of DNA strand-breaks induced by γ-radiation in HL-60 cells. Proliferating, undifferentiated HL-60 cells (● , ○) and post-mitotic, undifferentiated cells, which had been cultured in the presence of 1.25% (v/v) DMSO for 7 days (▲ , △) were irradiated with 100 (closed symbols) or 400 (open symbols) rads of γ-radiation. Cells were then incubated at 37°C for the indicated times prior to being applied to the nucleoid gradients.

proliferating, undifferentiated and the post-mitotic, differentiated cells (Fig. 2).

The exposure of HL-60 cells to 10 μM DMS for 15 minutes reduces the relative sedimentation rate of the nucleoids to 0.55, corresponding to approximately 800 single-strand breaks present at the time of assay, reflecting the balance between the rate of formation and the rate of removal of strand breaks. The total number of methylated products is much greater and repair will continue for many hours. There is no detectable difference in the kinetics of repair of DMS-induced DNA strand-breaks between undifferentiated cells, or in differentiating cells in cultures treated with DMSO for 2 days, or the post-mitotic, differentiated cells in cultures treated with DMSO for 7 days. Interestingly, as already demonstrated (4), the differentiating HL-60 cells (treated with DMSO for 2 days) contained physiologically formed DNA strand-breaks prior to DMS exposure (Fig. 3). The additional DNA strand-breaks produced by the exposure to DMS were repaired with apparently similair kinetics in these differentiating cells as in the control or in the differentiated cells. In all three cultures the steady-state level of detectable DNA strand-breaks has fallen to 5% the initial value by 160

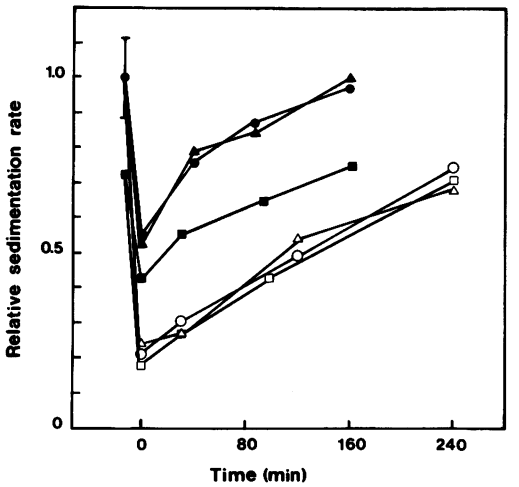

Fig.3. Repair of DNA strand-breaks induced by DMS in HL-60 cells. Proliferating, undifferentiated HL-60 cells (●, ○), differentiating cells from cultures treated with DMSO for two days (■, □), or post-mitotic differentiated cells from cultures treated with DMSO for 7 days (▲, △) were exposed to 10 μM (closed symbols) or 50 μM (open symbols) DMS. After 15 minutes (defined as time 0 of repair), cells were removed at the indicated times and their nucleoid sedimentation rate was determined.

minutes. However, in the differentiating cells approximately the same level of DNA strand breaks as was present prior to DMS treatment was maintained at 160 minutes (Fig. 3).

Increasing the DMS concentration to 50μM reduces the relative sedimentation rate of the nucleoids to 0.23, corresponding to approximately 4 000 single-strand breaks per dipoid genome. The repair of this much higher level of DNA strand-breaks takes a longer time and after 4 hours of incubation approximately 1 000 single-strand breaks are still observed (fig. 3); but again there is no detectable difference between differentiated, differentiating and undifferentiated cells.

The inhibitors of ADPRT activity, 3-aminobenzamide (5 mM) and 3-methoxybenzamide (2 mM), retard the repair of DNA strand-breaks formed by exposure to either 400 rads of γ-radiation (Fig. 4), or 10 μM DMS (Fig. 5). This inhibition of DNA repair is seen in both the the proliferating, undifferentiated and in the post-mitotic, differentiated cells. At corresponding concentrations the acid analogues of these compounds, 3-aminobenzoic acid and 3-methoxybenzoic acid, which do not inhibit ADPRT

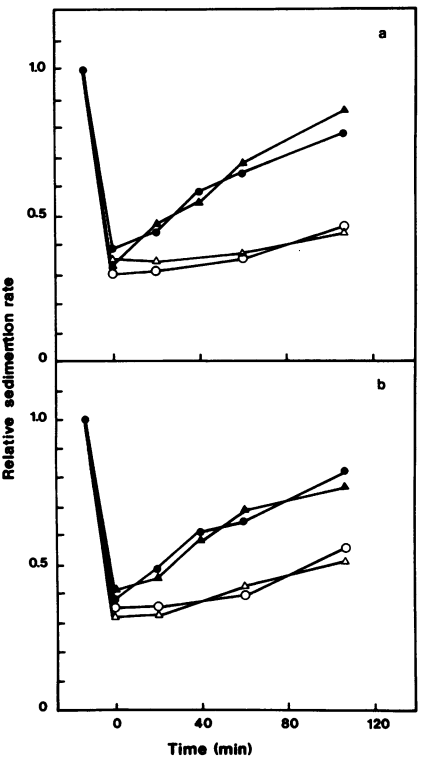

Fig.4. Effect of inhibition of ADPRT activity on the repair of DNA strand-breaks induced by Υ-radiation.
Proliferating undifferentiated (● , ○) and post-mitotic differentiated HL-60 cells (▲ , △) were irradiated with 400 rads of Υ-radiation. The progress of DNA strand ligation was then measured in the presence of ADPRT inhibitors and their non-inhibitory acid analogues. a) 5 mM 3-aminobenzamide (open symbols), or 5 mM 3-aminobenzoic acid (closed symbols). b) 2 mM 3-methoxybenzamide (open symbols), or 2 mM 3-methoxybenzoic acid (closed symbols).

activity, do not block the religation of DNA strand-breaks formed by exposure to either Υ-radiation or DMS (Figs. 4 & 5). At these concentrations neither the ADPRT inhibitors nor their acid analogues have a detectable effect on the rate of proliferation of control, undifferentiated, HL-60 cells even over three days of culture (Fig. 6). Up to 5mM concentrations of these two compounds there was no apparent effect on cell growth. At higher concentrations the rate of cell proliferation was significantly slowed down.

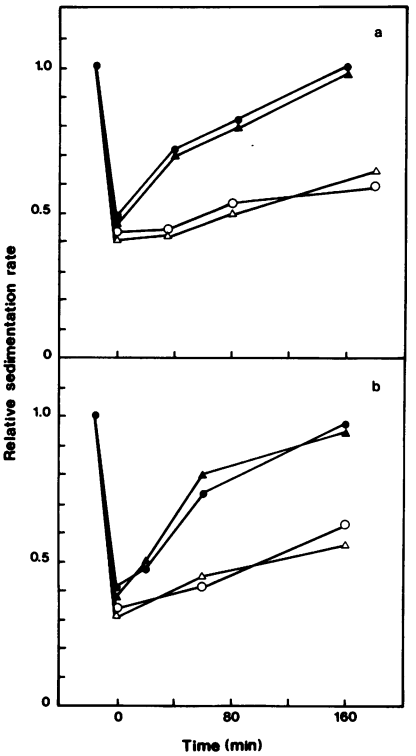

Fig.5. Effect of inhibition of ADPRT activity on the repair of DNA strand-breaks induced by DMS.
Proliferating undifferentiated (● , ○) and post-mitotic differentiated HL-60 cells (▲ , △) were exposed to 50 μ M DMS. The rate of DNA strand-ligation was then measured in the presence of ADPRT inhibitors and their non-inhibitory acid analogues. a) 5 mM 3-aminobenzamide (open symbols), or 5 mM 3-aminobenzoic acid (closed symbols). b) 2 mM 3-methoxybenzamide (open symbols), or 2mM 3-methoxybenzoic acid (closed symbols).

Discussion

The data presented here demonstrate that induction of differentiation by DMSO in the human promyelocytic leukaemic cells, HL-60, along the myelocytic lineage does not inhibit the ability of these cells to repair DNA lesions induced by exposure to either ionising radiation or treatment with the monofunctional methylating agent, DMS.

The differentiating HL-60 cells, after two days of treatment with DMSO, contain approximately 400 physiologically formed DNA strand-breaks per diploid genome. The additional DMS-induced DNA breaks are efficiently repaired, but approximately 400 DNA strand-breaks per diploid genome remain

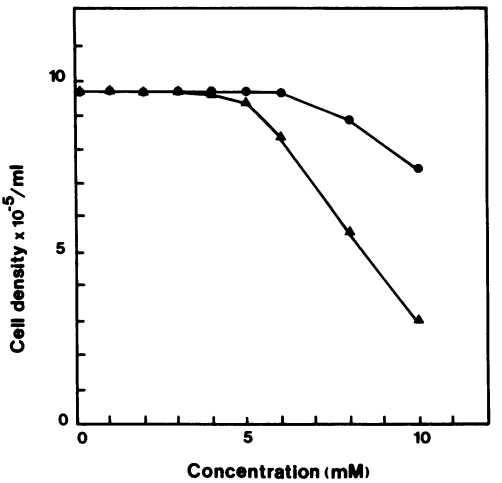

Fig.6. Effect of ADPRT inhibitors on HL-60 cell growth.
HL-60 cells from a logarithmically growing culture were sub-cultured into fresh medium at a cell density of 2 x 10^5/ml. 3-aminobenzamide (●), or 3-methoxybenzamide (▲) was then added at the indicated concentrations. The cell densities were determined three days later.

unligated. This suggests either that the physiologically-formed breaks are different from the DMS-induced breaks, or alternatively that the differentiating cells maintain a quantitatively similar number of breaks as was present prior to DMS treatment after the repair of the DMS induced breaks. The physiologically formed breaks are also eventually ligated during the next 24 hours in the course of differentiation (please see the preceding paper, ref. 4).

Comparison of the rate of removal of DNA strand-breaks induced by 100 and 400 rads of Y-radiation demonstrates that under both conditions approximately 20 single-strand DNA breaks per genome are religated per minute. This is an interesting observation suggesting that in both cases there was a 'saturating concentration' of DNA strand-breaks available to the DNA repair mechanism; or alternatively, that the rate of DNA strand ligation is independent of the 'concentration' of DNA strand-breaks present. A similar analysis of the kinetics of the rate of ligation of DNA strand-breaks formed by DMS cannot be made. This is because the detectable number of DNA strand breaks at any given time following exposure to DMS, is only a reflection of the net balance in the rate of formation and removal of DNA strand-breaks. The rate of repair of Y-induced DNA damage is

comparable to the rate observed in a number of other DNA repair-proficient cells. However, DMS-induced damage in proliferating, or in either differentiating or the post-mitotic differentiated cells appears to be repaired much more slowly in these cells compared to the mouse lymphoblastic cell line, L1210.

The present studies also demonstrate that in both the proliferating, undifferentiated cells and in the post-mitotic cells induced to differentiate along the myelocytic lineage, nuclear ADPRT activity is required for the efficient repair of DNA damage induced by either DMS or γ-irradiation. Nuclear ADPRT activity has also been shown to be required for the efficient excision repair of DNA damage induced by DMS in a number of other cells (for review see ref. 27 and 28).

The inhibition of ADPRT activity is specific to benzamide and its amide analogues, all of which, including the most commonly used compounds 3-aminobenzamide and 3-methoxybenzamide, block the activity of ADPRT; the acid analogues of these compounds do not inhibit ADPRT activity. However, the additional effects of high, lethal concentrations of benzamide derivatives on growth and nucleotide biosynthesis (29) are not specific to the amides but are randomly shared by the acid analogues as well. Therefore, the correct use of ADPRT inhibitors, that is, at non-toxic concentrations and in conjunction with their acid analogues, offers a perfectly valid means of testing the effect of specific inhibition of ADPRT activity in different biological processes, including DNA excision repair and cellular differentiation.

Acknowledgements

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