A Putative Role for Nicotinamide Adenine Dinucleotide-promoted Nuclear Protein Modification in the Antitumor Activity of N-Methyl-N-nitrosourea

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SUMMARY

Incubation of HeLa cells with the anticancer agent N-methyl-N-nitrosourea (MNU) results in: (a) depression of intracellular nicotinamide adenine dinucleotide levels; (b) stimulation of the chromatin-associated, chromosomal protein-modifying enzyme polyadenosine diphosphoribose [poly(ADP-ribose)] polymerase, which uses nicotinamide adenine dinucleotide as substrate; and (c) some fragmentation of cellular DNA. DNase treatment of HeLa nuclei in vitro also stimulates poly(ADP-ribose) polymerase activity, but not in nuclei derived from MNU-treated cells unless they have been subsequently incubated to allow for recovery from MNU damage. DNA polymerase activity is stimulated in vitro by poly(ADP-ribose) ribosylation of nuclear proteins. By using intact nuclei derived from MNU-treated HeLa cells, the repair via elongation of single-strand DNA breaks is demonstrated in vitro. This repair is dependent on DNA polymerase activity and is enhanced by adenosine diphosphate ribosylation of histones. Inhibition of poly(ADP-ribose) polymerase with nicotinamide results in extensive degradation of MNU-damaged DNA. Taken as a whole, these results suggest that poly(ADP-ribose) polymerase may play a role in the repair of alkylated damage to cellular DNA and that the inhibition of this enzyme in vivo might be exploited to potentiate the antitumor and carcinogenic activities of MNU.

INTRODUCTION

There is considerable evidence to indicate that monofunctional methylating agents such as MNU and streptozotocin are both carcinogenic and exhibit active cytotoxicity against tumor cells by virtue of their ability to alkylate cellular DNA. This alkylatation results in depurination (18) which, in turn, may lead to alkali-induced (2), chemical (17), or enzymatic (2) SSB in DNA. Presumably, such lesions represent the major biochemical basis for the usefulness of these drugs as antitumor, chemotherapeutic agents. However, eukaryotic cells contain the enzymatic potential for the rapid repair of such lesions (1, 5), and biological repair of DNA after the administration of these drugs may result in their reduced effectiveness with respect to both their chemotherapeutic and carcinogenic activities. The importance of such repair mechanisms in carcinogenesis is evident in the rare disease xeroderma pigmentosum, in which a defect in the repair of UV-induced thymine dimers is often accompanied by extensive metastatic epitheliomatosis. If the natural repair mechanisms for alkylation damage to DNA could be modified or inhibited, it is possible that the biological action of alkylating agents could be potentiated.

We were led to the studies outlined below by the following related observations. Schein et al. (11-14) had previously demonstrated in mice that the toxicity of the MNU derivative, streptozotocin, could be correlated with a depression of liver and pancreatic islet NAD concentrations, a phenomenon readily prevented by the concurrent administration of pharmacological doses of nicotinamide. A similar reduction in the NAD concentration of L1210 leukemia cells was observed after in vivo treatment with either streptozotocin or MNU (11, 13). This NAD reduction could also be prevented with nicotinamide, although nicotinamide did not reverse the antitumor activity of either agent; in fact, there was a suggestive increase in survival of tumor-bearing animals undergoing combined treatment. More recent data from our laboratories have shown that the activity of the nuclear enzyme, poly(ADP-ribose) polymerase, which utilizes NAD as a substrate for the poly(ADP) ribosylation of histones and other chromosomal proteins (15), is significantly stimulated when HeLa cells are pretreated with streptozotocin (16). Recent in vivo studies (7) have indicated that there is an extremely high rate of NAD turnover within eukaryotic nuclei, most of the degradation occurring via the generation of ADP-ribose, the active product of poly(ADP-ribose) polymerase. It seemed reasonable, therefore, that the previously noted depression of NAD levels in cells treated with methyl-nitrosourea agents might be a direct result of increased activity of this nuclear NAD-utilizing enzyme. Our past data had also indicated a possible physiological role for poly(ADP-ribose) polymerase in the repair of lesions in DNA. We had shown, for example, that this specific nuclear protein-modifying system causes the release of template-restrictive sites in both HeLa cell nuclei and chromatin for either exogenous or endogenous DNA polymerase (9, 10, 16), and
more recent data from our laboratory suggest that the latter effect is a result of the reduced affinity of ADP-ribosylated histones for DNA. Moreover, a greater release of template restriction was noted when poly(ADP-ribose) polymerase activity was stimulated by pretreatment with streptozotocin (16).

On the basis of these observations, then, it seemed appropriate to test whether poly(ADP-ribose) polymerase participates in the complex system of chromatin-associated reactions required for the repair of DNA damage in cells caused by the methylnitrosourea compounds.

**MATERIALS AND METHODS**

**Materials.** [3H]NAD (3.28 Ci/mmole), [3H]TTP (10 Ci/mmole), [3H]TdR (11 Ci/mmole), and [3H]tdR (54 mCi/mmole) were purchased from New England Nuclear, Boston, Mass. Avian myeloblastosis virus DNA polymerase was a kind gift from Dr. Jack Chirikjian (Georgetown University) and Dr. Takis Papas (National Cancer Institute, Bethesda, Md.). MNU and streptozotocin were from Southern Research Institute, Birmingham, Ala. SV40 DNA component I were obtained from Dr. Leonard Rosenthal (Georgetown University).

**Methods.** HeLa S2 cells were maintained at 37° in spinner flasks in Eagle’s S-MEM (Grand Island Biological Co., Grand Island, N. Y.) containing 10% fetal calf serum. Cells were harvested by centrifugation and washed with spinner salts, and detergent-washed nuclei were isolated by Dounce homogenization as previously described (9, 10).

HeLa cells (2 × 10⁶/ml) were incubated for 3 days in the presence of either [3H]TdR (0.5 μCi/ml) or [3H]tdR (0.05 μCi/ml), concentrated to 2 × 10⁶/ml in fresh S-MEM, and then incubated an additional 2 hr at 37°. MNU (7.5 mm final concentration) dissolved in 0.9% NaCl-10 mM sodium citrate, pH 4 (0.9% NaCl-citrate buffer) was then added to the 3H- or 14C-labeled cultures, respectively, and incubation was continued for 2 hr. The cells were then washed, and nuclei were isolated as described above and stored frozen at the concentration of 4 × 10⁷/ml.

DNA was isolated from MNU-treated and control cells by the method of Walker and Ewart (18).

Poly(ADP-ribose) polymerase activity in isolated nuclei was estimated as described previously (9, 16), and partially purified HeLa cell poly(ADP-ribose) polymerase was assayed under conditions described extensively before (8).

The effect of ADP ribosylation of nuclear proteins on the repair of MNU-induced SSB in DNA (Chart 4) was determined as follows. [3H]TdR-labeled nuclei from MNU-treated cultures and [3H]tdR-labeled nuclei from control cells were isolated as described above. MNU-treated nuclei (4 × 10⁶) were then incubated for 30 min at 37° in a reaction mixture (final volume 100 μl) which contained 4 μmoles Tris-HCl, pH 8; 0.05 μ mole β-mercaptoethanol; 0.6 μ mole MgCl₂; 5 μ moles NaCl; 0.1 μ mole ATP; 0.02 μ mole each of dATP, dGTP, dCTP, and TTP; and either 0.4 μ mole NAD or 0.4 μ mole NAD and 10 μ moles nicotinamide. The reactions were terminated by chilling at 0° for 5 min, followed by centrifugation at 0° for 5 min at 1000 × g. Cell pellets were then resuspended in lysis solution (0.25 n NaOH-0.15 M NaCl-15 m EDTA) before alkaline sucrose gradient centrifugation or were used to isolate DNA as described above.

For the alkaline sucrose gradient sedimentation studies, [3H]TdR-labeled, MNU-treated nuclei and [3H]tdR-labeled control nuclei in a common volume (300 μl) of lysis solution were layered onto 4.7 ml 5 to 20% linear sucrose gradients containing 0.1 n NaOH, 0.7 M NaCl, and 10 mM EDTA, and a 300-μl 50% sucrose cushion. The gradients were allowed to stand at room temperature for 30 min before centrifugation at 45,000 rpm for 45 min (20°) in a Beckman SW 50.1 rotor. The gradients were fractionated with a Densi-Flow IIc (G. D. Searle and Co., Columbus, Ohio), and 6 drop fractions were collected onto small squares of Whatman No. 1 filter paper before the estimation of acid-precipitable radioactivity.

DNA isolated from [14C]tdR-labeled, MNU-treated cells and control cells were subjected to sedimentation in 99% DMSO-sucrose gradients as follows. DNA, in a 150-μl volume containing 50 μl DMSO and 100 μl dimethylformamide (Eastman Organic Chemicals, Rochester, N. Y.), was layered onto 4.7 ml 5 to 20% linear sucrose gradients containing 99% DMSO, 10 mM Tris-HCl, pH 7.4; 10 mM LiCl, 1 mM EDTA; and a 300-μl 50% sucrose cushion. The gradients were centrifuged for 4.5 hr at 45,000 rpm (25°) in a Beckman SW 50.1 rotor and fractionated as described above. Acid-precipitable radioactivity was also determined as indicated in the preceding paragraph. In the DMSO-sucrose gradient sedimentation studies, SV40 DNA component I was used as a molecular weight marker.

**RESULTS**

In order to assess the effects of MNU and streptozotocin on DNA synthesis, HeLa cells were incubated for 2 hr with each of the drugs (7.5 mm final concentration), and measurements of [3H]tdR incorporation into DNA were performed with an aliquot of each cell suspension after 1 hr of incubation. Cell viability in the presence of either drug was essentially 100% at this time as tested by the trypan blue dye exclusion method. The data in Table 1 show that both MNU and streptozotocin resulted in a suppression of DNA synthesis in HeLa cells, but that at equal molar concentrations, MNU was the most effective inhibitor in this regard. This

<table>
<thead>
<tr>
<th>Addition of culture medium</th>
<th>[3H]tdR incorporation (cpm/15 min × 10⁻⁴)</th>
<th>% control</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>1.43</td>
<td>(100)</td>
</tr>
<tr>
<td>Streptozotocin (7.5 mm)</td>
<td>1.05</td>
<td>73</td>
</tr>
<tr>
<td>MNU (7.5 mm)</td>
<td>0.32</td>
<td>23</td>
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* C. Giri and M. Smulson, manuscript in preparation.
agent, therefore, was used in all subsequent experiments. After incubation, the cells were washed and nuclei were isolated before measurements of the activity of poly(ADP-ribose) polymerase, as shown in Chart 1. In confirmation of our earlier studies (16), as well as the recent work of others (19), incubation of cells with streptozotocin resulted in only a modest increase in the activity of this enzyme. The effect of MNU on the activity of poly(ADP-ribose) polymerase, however, was significant, with a nearly 2-fold increase in the extent of NAD incorporation evident under the experimental conditions utilized. Since preliminary unpublished observations from one of our laboratories (P. Schein) have indicated that free nicotinamide is methylated (in vitro) to only a very small extent by MNU, perhaps 5%, we tentatively conclude that the observed NAD depletion in intact cells or animals treated with MNU or its derivatives is a result, at least in part, of enhanced activity of poly(ADP-ribose) polymerase. Although it is difficult to correlate an enhanced activity measured in vitro with in vivo levels of NAD, it is of interest that there is significant NAD turnover in eukaryotic nuclei (7).

**Fragmented DNA and Poly(ADP-Ribose) Polymerase.** The possibility existed that fragmented DNA, produced as a result of MNU alkylation, might be causing the observed stimulation of poly(ADP-ribose) polymerase, since such fragmentation was occurring in vivo during our incubations (see Charts 4C and 5). Purified poly(ADP-ribose) polymerase requires both DNA and a nuclear protein acceptor for activity (15).

In order to test the effect of fragmented DNA on the reaction, a partially purified poly(ADP-ribose) polymerase activity recently described in HeLa cells (8) was assayed under conditions of optimal histone concentration with a variety of native and altered DNA's (Chart 2). In the absence of DNA, negligible ADP ribosylation occurred. The data indicated that DNA equally (25%) fragmented by either DNase I or II or by micrococcal nuclease activated the system at much lower DNA concentrations than did native DNA. Apurinic DNA was a poor substrate for the reaction, and DNA sheared by sonic disruption was approximately equal to native DNA. One interpretation of the in vitro data is that the stimulation of poly(ADP-ribose) polymerase by MNU in vivo might be mediated by SSB in DNA or other perturbations to DNA impaired in vivo by MNU. This concept is reasonable, as well, since we have previously shown in HeLa nuclei that there is a direct correlation between the extent of ADP ribosylation of chromosomal proteins and the reactivity of exogenously added bacterial DNA polymerase for DNA synthesis with this chromatin (10, 16). In addition, the data in Table 2 show the effect of poly(ADP-ribose) polymerase on the activity of 2 eukaryotic DNA polymerases toward HeLa chromatin. Endogenous HeLa nuclear DNA polymerase activity was stimulated almost 2-fold by the prior generation of poly(ADP-ribose). This enhancement was not due merely to NAD per se, since the effect was abolished by nicotinamide, a potent inhibitor of poly(ADP-ribose) polymerase.

In addition, the data in Table 2 show that exogenous avian myeloblastosis virus DNA polymerase can utilize HeLa chromatin for the polymerization of deoxynucleotides and that prior ADP ribosylation of nuclear proteins greatly increases this activity. These results, then, support a role for this
modification of histones in exposure of fragmented regions of HeLa chromatin DNA, subsequent to their utilization as primers for various DNA polymerases.

Fujiwara (1) has shown that MNU-induced fragmentation of HeLa cell DNA is repaired to maximum strand length (in vivo) after 4 hr of incubation, after the removal of the alkylating agent. In the experiments summarized in Chart 3, HeLa cells were incubated with MNU (7.5 mM final concentration) for 1 hr. As before, the activity of nuclear poly(ADP-ribose) polymerase was stimulated by such treatment (1546 dpm/µg protein versus a control value of 748). However, after 4.5 hr of posttreatment incubation in the absence of MNU, a time sufficient for the partial repair of SSB in DNA (1), the specific activity of the enzyme returned to a level comparable to that in cells not exposed to alkylating insult.

That fragmentation of DNA or, alternatively, some other effect of MNU on DNA might be signaling a cellular need for increased poly(ADP) ribosylation of nuclear proteins is also suggested by the differing effects of DNase I on the in vitro generation of poly(ADP-ribose) in nuclei, as shown in Chart 3. Miller (3) has previously demonstrated that the exposure of HeLa cell nuclei to small quantities of exogenous DNase markedly enhances the activity of poly(ADP-ribose) polymerase. Such an effect is also shown in Chart 3, in which poly(ADP-ribose) polymerase activity is seen to be stimulated by 64

### Table 2

<table>
<thead>
<tr>
<th>Process</th>
<th>HeLa DNA polymerase and exogenous AMV* DNA polymerase activity by poly(ADP) ribosylation of chromatin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nuclei</td>
<td>HeLa nuclei (4 x 10⁶) were preincubated for 30 min at 37°C under standard poly(ADP-ribose) polymerase conditions (9, 16) with additions as noted. Reaction was terminated by addition of 10 µl of buffer (0.32 M sucrose-2 mM KPO₄, pH 6.8-2 mM MgCl₂-3 mM CaCl₂) and centrifugation. Either endogenous DNA polymerase activity or exogenous AMV DNA polymerase (1 µg) activity was measured, with nuclei as source of DNA under standard conditions (10, 16). NAD had no effect on AMV DNA polymerase activity with activated calf thymus DNA as a source of template primer.</td>
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<table>
<thead>
<tr>
<th>Experiment</th>
<th>Addition to preincubation</th>
<th>Addition to DNA polymerase assay</th>
<th>Time of DNA polymerase assay (min)</th>
<th>TMP incorporation (dpm x 10⁻³)</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td></td>
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<td></td>
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<td>2</td>
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a-AMV, avian myeloblastosis virus.
b-see note.
and 72% after treatment of HeLa cell control nuclei with DNase I. The activity of this enzyme in nuclei derived from MNU-treated cells (already high compared to control values), however, was essentially unaffected by DNase I, presumably because SSB are already present in such nuclei (see Charts 4C and 5). Only after these breaks are allowed to be partially repaired in vivo can an effect by DNase I again be noted (24% stimulation).

If SSB do act as a signal for the stimulation of poly(ADP) ribosylation, one might have expected that the combined treatment with MNU and DNase I would have stimulated the enzyme to an even greater extent than was observed. It is possible, of course, that fragmented DNA can stimulate the enzyme only up to a fixed limit and that this limit was achieved by the MNU treatment alone. For example, it should be noted (Chart 2) that certain fragmented DNA preparations displayed quite narrow concentration-dependent stimulation of the enzyme compared to native DNA. Unfortunately, these types of DNA could not be tested directly in the experiment of Chart 3, since, here, nuclear chromatin instead of purified enzyme is used, and exogenously added DNA would tend to inhibit the enzyme assay. Alternatively, MNU may be affecting the nuclei in some other way (e.g., carbamoylation of proteins), so as to prevent a potentiation of the stimulatory effect by DNase I.

It is also interesting to note that, after MNU-induced damage has been allowed to be repaired during a 4.5-hr recovery period, DNase I no longer stimulates poly(ADP-ribose) polymerase to the same extent as it does in control nuclei (Chart 3). Again, some of the stimulatory damage of MNU may be irreparable in 4.5 hr, and any residual SSB may inhibit further enhancement of the enzyme by DNase I, even after the recovery period.

Similarly, other types of cellular damage effected by MNU such as carbamoylation of enzymes, including poly(ADP-ribose) polymerase, may require a much longer period of recovery before the nuclei are again responsive to the stimulatory effects of DNase I.

**MNU-induced SSB in the DNA of Intact Cells.** In an effort to determine whether MNU treatment of HeLa cells under the experimental conditions described above caused extensive SSB in nuclear DNA, cells were prelabeled with either [³²P]TdR or [³H]TdR as described in "Materials and Methods," washed, and then incubated with either MNU (7.5 mM final concentration) or, as a control, an equal volume of an alkaline detergent. As is evident from the data of Charts 4 and 5, the MNU treatment resulted in a significant fragmentation of the nuclear DNA. That most of the fragmentation of the DNA, as evidenced on the alkaline sucrose gradients, is in part the result of nicks introduced in vivo, rather than to alkali-labile, apurinic sites, is shown by the DMSO-sucrose gradient pattern (Chart 5). Such gradients are denaturing but should not be expected to hydrolyze phosphodiester bonds at apurinic sites.

**In Vitro Repair of MNU-induced SSB in HeLa Nuclei.** In order to determine whether NAD and poly(ADP-ribose) polymerase might serve a role in the repair of damaged DNA in vivo, an in vitro system was developed to measure the DNA polymerase- and polynucleotide ligase-promoted elongation and ligation of pieces of DNA in MNU-damaged nuclei.

**Figure 4.** Effect of ADP ribosylation of nuclear proteins on repair of MNU-induced SSB in DNA as determined by alkaline sucrose gradient centrifugation. [³²P]TdR-labeled nuclei from MNU-treated cultures and [³H]TdR-labeled nuclei from control cultures were isolated. MNU-treated nuclei were incubated in vitro under conditions optimal for DNA polymerase as described in "Materials and Methods," washed, and then incubated either with or without NAD. Sedimentation is from left to right. Shaded areas, differences in the population of DNA fragments. O, nuclei derived from MNU-treated cells; •, nuclei derived from control cells. The total amount of acid-insoluble counts recovered in A was 2% of counts recovered in B and C.

**Figure 5.** Effect of in vivo incubation of HeLa cells with MNU on the sedimentation behavior of isolated DNA in neutral DMSO-sucrose gradients. See "Materials and Methods" for details. Sedimentation is from left to right. O, DNA isolated from MNU-treated cells; •, DNA isolated from control cells. Arrow, peak position of SV40 component I marker DNA.
The details of this assay are described in "Materials and Methods" and in the legend to Chart 4. The in vitro system required (not shown) the presence of the 4 deoxynucleoside 5'-triphosphate substrates of DNA polymerase in order to demonstrate a size distribution change toward the heavy region of the gradient in [14C]TdR-labeled, MNU-treated nuclei.

**Possible Involvement of Poly(ADP-Ribose) in Eukaryotic DNA Repair.** The data in Chart 4 (B and C) demonstrate that poly(ADP-ribose) ribosylation of nuclear histones significantly enhances the in vitro repair of SSB in DNA induced in intact cells by MNU. Compared to control DNA, an approximate 2-fold increase (shaded areas) in the average size distribution of DNA in NAD-treated nuclei was noted. In contrast, nuclei incubated in the absence of NAD (Chart 4C) but with the substrates for DNA polymerase and polynucleotide ligase (ATP) showed little or no change in sedimentation toward the heavy end of the gradient. It should be noted that we could detect no sizable repair of DNA due to ADP ribosylation in non-MNU-treated nuclei, so that it is possible that considerable damage to DNA is a prerequisite for demonstration of this effect of NAD in HeLa nuclei. Alternatively, alkylated sites themselves may be required for involvement of poly(ADP-ribose) polymerase in repair.

It was important to demonstrate that the apparent enhanced repair of MNU damage to DNA shown above was not merely a result of NAD per se, but rather to the formation of poly(ADP-ribose) on nuclear proteins. Accordingly, nicotinamide, a potent inhibitor of poly(ADP-ribose) polymerase (10, 15), was incubated in vitro with NAD- and MNU-treated nuclei (Chart 4A). Contrary to expectations, the inhibition of poly(ADP-ribose) polymerase by nicotinamide did not merely suppress the enhancement of repair, but stimulated further extensive degradation of nuclear DNA in the nuclei (Chart 4A), as evidenced by an 80% loss of acid-insoluble counts recovered on the gradient. One interpretation of these data is that poly(ADP-ribose) polymerase is a necessary prerequisite for certain repair mechanisms in eukaryotic cells and that inhibition of the system leads to extensive exonucleolytic degradation of DNA, especially where extensive SSB or other changes in DNA due to MNU already exist. These observations with nicotinamide directly stimulated the studies described below with animals bearing L1210 tumor cells in an attempt to show potentiation of MNU anticancer activity with inhibitors of poly(ADP-ribose) polymerase.

**Potentiation of Antitumor Activity in the Intact Animal by Inhibition of Poly(ADP-Ribose) Polymerase.** One potential application of the studies described above would be the selective inhibition of ADP ribosylation of histones to enhance the antitumor activity of MNU. This would be accomplished by virtue of lowering the capacity of the tumor cell to repair SSB in DNA induced by the drug. Accordingly, we have tested this hypothesis directly, by performing in vivo antitumor studies in C57BL × DBA/2 F, mice bearing i.p.-injected L1210 leukemia cells (106) 2 days before treatment. The tumor-bearing animals were subjected to a regimen of either a single dose of MNU (35 to 75 mg/kg, i.p.) or the same dose schedule combined with 2 injections of nicotinamide (500 mg/kg), an inhibitor of poly(ADP-ribose) polymerase, at 12-hr intervals. The data (Table 3) show that the mean survival time of untreated animals (8.8 days) was extended by treatment with MNU and that for each dose of MNU there was an enhancement of survival time when treatment was combined with nicotinamide. No significance is attached to small differences in these data; however, the general trend of the results is totally consistent with a similar type of experiment (13) performed under somewhat similar conditions. These observations suggest that the in vivo inhibition of ADP ribosylation of nuclear proteins may have resulted in the enhanced toxicity of MNU for the leukemia cells.

**DISCUSSION**

Taken as a whole, the data presented above are highly suggestive of a pivotal role for NAD in the modification of nuclear proteins concerned with the maintenance of a balance between damage to DNA as occasioned by certain antitumor agents and the normal repair of such damage in vivo. The question arises, of course, as to why the generation of SSB in DNA or, alternatively, alkylated sites should act as a signal for increased levels of poly(ADP-ribose) polymerase activity as suggested by the above experiments and also those utilizing DNase I by Miller (3). It seems reasonable to assume, in the 1st place, that such insults to DNA as would be expected to result from the action of alkylating agents such as MNU would, in fact, trigger the mobilization and coordinate activity of a variety of enzymes concerned with the repair of such damage, and it is also unreasonable to expect that poly(ADP-ribose) polymerase might be 1 of these enzymes. We have recently found, for example, that in solution, ADP-ribosylated histones have a lesser affinity for DNA than unmodified histones. Accordingly, increased levels of histone ADP ribosylation in chromatin in response to (and presumably in the vicinity of) SSB might be expected to result in localized regions of relaxation of the chromatin architecture, thus facilitating the action of the repair enzymes themselves. Evidence that NAD may play a crucial role in the repair of MNU damage to DNA has been presented above (Chart 4).

We have also demonstrated above that the inhibition of the action of poly(ADP-ribose) polymerase after, or concomitant with, treatment with MNU results in: (a) in vitro, an increased degradation of the DNA isolated from the treated

**Table 3**

<table>
<thead>
<tr>
<th>Compounds tested</th>
<th>Mean survival time (days)</th>
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<tbody>
<tr>
<td>MNU (mg/kg)</td>
<td>Nicotinamide (500 mg/kg)</td>
</tr>
<tr>
<td>35</td>
<td>+</td>
</tr>
<tr>
<td>50</td>
<td>+</td>
</tr>
<tr>
<td>75</td>
<td>+</td>
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<tr>
<td>75</td>
<td>+</td>
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* Groups of 8 C57BL × DBA/2 F, mice were given i.p. injections of various compounds 2 days after L1210 tumor injection.
nuclei (Chart 4A), and (b) an increase in the survival time of animals bearing tumors. Because certain stages in the HeLa cell cycle have previously been shown to be especially sensitive to ADP ribosylation (16), these stages might, in the future, be exploited during nitrosourea chemotherapy in vivo.

Our past work has also shown that NAD concentrations are reduced in L1210 leukemia cells after exposure to MNU or streptozotocin and that nicotinamide, a potent inhibitor of poly(ADP-ribose) polymerase, does not decrease the antitumor activity of these agents, despite the fact that it does tend to restore normal intracellular levels of NAD. Previous studies with L1210 cells have, as well, demonstrated an increase in the survival time of host animals treated with both MNU and nicotinamide (13).

It should be mentioned that the mechanism of streptozotocin-induced islet cell toxicity (12) is mediated through a mechanism different from that of L1210 cell toxicity, and nicotinamide does not potentiate the action of this agent on pancreatic tumors. Instead, nicotinamide leads to the preservation of the biochemical stability and morphological integrity of the islet cell, presumably by maintaining pyridine nucleotide cofactors at optimum levels during the administration of the drug. Combined treatment with streptozotocin and nicotinamide results, in this case, in the formation of insulin-dependent islet tumors (6).

By demonstrating a potentially important involvement of NAD in the repair of methylation damage to DNA, it is hoped that the present studies also serve to further elucidate the many complexities of tumor biogenesis.

REFERENCES

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