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NICOTINAMIDE STIMULATES REPAIR OF DNA DAMAGE IN HUMAN LYMPHOCYTES

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SUMMARY: Nicotinamide stimulates the amount of DNA repair synthesis that occurs when freshly isolated, normal human lymphocytes are treated with UV irradiation, N-methyl-N'-nitro-N-nitroso guanidine, or dimethyl sulfate. Stimulation of DNA repair synthesis is concentration dependent and reaches a maximum between 2 to 5 mM nicotinamide. In contrast, DNA synthesis in cells that have not been subjected to DNA damage is not affected by nicotinamide at concentrations below 2 mM and is inhibited by concentrations between 2 to 5 mM. In the same concentration range, nicotinic acid has no effect on the rate of DNA synthesis in the presence or absence of DNA damage.

INTRODUCTION: Human cells conduct DNA repair synthesis in response to a variety of treatments that cause DNA damage (1-4). While a number of agents are capable of inhibiting DNA repair synthesis in eukaryotic cells (5,6), none have been described that can stimulate this process except for those agents that can initiate DNA repair by themselves. We now report that the amount of DNA synthesis that occurs in human lymphocytes treated with UV irradiation, MNNG[1], or DMS is markedly stimulated by simultaneous treatment of the cells with nicotinamide. In contrast, nicotinamide has essentially no effect on DNA synthesis in cells that have not previously been treated with DNA damaging agents. Thus, in resting human lymphocytes, nicotinamide appears to interact with DNA damaged cells to specifically stimulate the DNA repair process. To our knowledge, this is the first report of an agent that stimulates DNA repair synthesis in cells that have already sustained DNA damage but has no effect on DNA synthesis in undamaged cells.

METHODS: Normal human peripheral blood lymphocytes were isolated on Ficoll-Hypaque gradients, suspended at 2 x 10^6/ml in phosphate buffered saline and UV irradiated with a total dose of 20J/m^2 as previously described (4,6,7). Cells

[1]Abbreviations used: MNNG = N-methyl-N'-nitro-N-nitroso guanidine; DMS = dimethyl sulfate.

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were suspended in alpha modified Eagles medium buffered with 25 mM HEPES, pH 7.2 and supplemented with 10% fetal calf serum. For UV experiments, control cells were subjected to the same procedure except that they were not irradiated. For chemically induced DNA damage, MNNG or DMS was freshly dissolved in DMSO, diluted in saline and added to cells in culture medium to give final concentrations of 20 µg/ml MNNG or 0.1 mM DMS and 0.2% DMSO. Control cells for the chemical treatments were also adjusted to contain 0.2% DMSO. Immediately after the DNA damaging treatments, the medium in all cultures was adjusted to contain 10 mM hydroxyurea. Nicotinamide or nicotinic acid was then added to the indicated cultures. The cultures were incubated at 37°C for 30 min; [^{3}H]dThd (Specific Activity 56 Ci/mmole) was added at a final concentration of 200 µCi/ml and incubations were continued at 37°C. Incorporation of radioactivity into trichloroacetic acid precipitates was determined as previously described (4,6,7).

To bypass potential problems of pool size and precursor transport, cells were rendered permeable to exogenously supplied nucleotides as previously described (8-10) and DNA synthesis was measured in reaction systems containing 1 x 10^5 permeable cells, 33 mM HEPES, pH 7.8, 9 mM $MgCl_2$, 70 mM NaCl, 5 mM ATP, 0.1 mM dATP, 0.1 mM dCTP, 0.1 mM dGTP, and 0.29 µM [Me-^{3}H]dTTP (Specific Activity 140 x 10^6 dpm/nmole). Reaction tubes were incubated at 37°C for 30 min. Reactions were terminated by addition of an excess of cold 10% trichloroacetic acid, 2% sodium pyrophosphate. Cell pellets were prepared for scintillation counting as previously described (8-10). All results are presented as the means of triplicate assays which showed less than 10% variation.

RESULTS AND DISCUSSION: Table I shows the effect of nicotinamide on the level of DNA synthesis in control cells and in cells treated with UV irradiation, MNNG, or DMS. The untreated lymphocytes showed a low level of thymidine incorporation which was not affected by treatment with either nicotinamide or nicotinic acid. When lymphocytes were UV irradiated with 20J/m^2 there was a marked increase in thymidine incorporation into DNA. This abrupt increase in thymidine incorporation shows the characteristics of the repair mode of DNA synthesis. First, it occurs immediately after subjecting the cells to treatments which damage their DNA. Second, it occurs in the presence of hydroxyurea. Third, using alkaline cesium chloride gradients of lymphocyte DNA labeled with bromodeoxyuridine during the period after the DNA damage, we have shown that the DNA synthesized under these conditions has the same density as the parental DNA and is therefore the product of the repair mode of DNA synthesis (4).

Addition of 2 mM nicotinamide to the DNA damaged cells resulted in an increase in DNA repair synthesis that was even greater than that which occurred following DNA damage alone. In contrast, nicotinic acid had no effect on the levels of DNA synthesis in either the control or DNA damaged cells. The addi-

TABLE I: Effect of Nicotinamide on DNA Synthesis in Human Lymphocytes

Incubation Conditions	DNA Synthesis (Incorporation [^{3}H]dThd) dpm/10^6 cells/6 hrs		
DONOR 1	Untreated	UV Irradiated	
Control	2,003	29,788	
+ 2mM Nicotinamide	1,925	59,509	
+ 2mM Nicotinic Acid	1,917	29,732	
DONOR 2	Untreated	DMS	MNNG
Control	2,199	7,723	7,300
+ 2mM Nicotinamide	2,281	17,862	15,168
+ 2mM Nicotinic Acid	2,369	6,858	7,936

tion of nicotinamide to MNNG or DMS treated cells also resulted in greater stimulation of DNA repair synthesis then did treatment with the damaging agents alone. This ability of nicotinamide, but not nicotinic acid, to stimulate the level of DNA repair synthesis in DNA damaged cells has now been confirmed with lymphocytes prepared from more than 20 different normal donors.

To rule out the possibility that the stimulation of thymidine incorporation could be due to an effect of nicotinamide on thymidine uptake or pool size we conducted studies in which cells were UV irradiated, incubated with nicotinamide for 4½ hrs then made permeable to nucleotides and combined with a reaction mixture containing [^{3}H]dTTP, dGTP, dCTP, dATP, ATP, and Mg^{++} to assess the intrinsic rate of DNA synthesis. In this assay, the effective nucleotide pool sizes are those supplied in the reaction mix and DNA synthesis is independent of any variation in endogenous pools of these substrates (9,10). The results presented in Table II show that UV irradiation produced a marked increase in the rate of DNA repair synthesis in the permeable cells and that incubation of the UV irradiated cells with nicotinamide produced an additional 2-fold increase in the rate of DNA repair synthesis. Thus, when problems with transport and pool size are completely bypassed, nicotinamide continues to have a direct stimulatory effect on the rate of DNA repair synthesis.

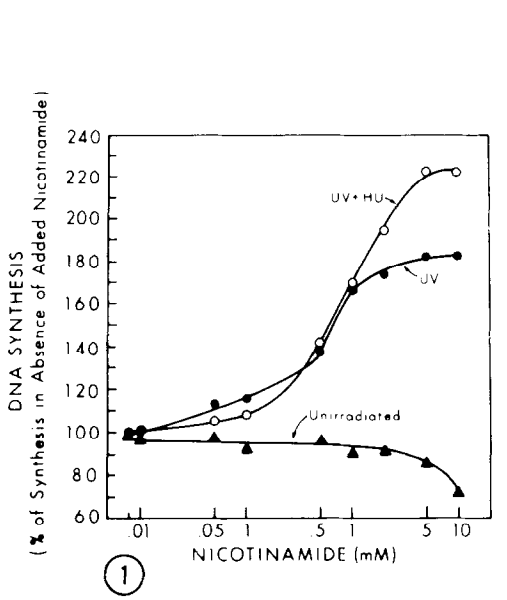
TABLE II: Effect of Nicotinamide on DNA Synthesis in Permeable Human Lymphocytes

Incubation Condition	Incorporation [^{3}H]dTMP dpm/10^6 cells/30 mins	
	Untreated	UV Irradiated
Control	1,480	5,570
2mM Nicotinamide	1,200	10,100

Fig 1 shows that DNA repair synthesis was maximally stimulated by nicotinamide in the range of 2 to 5 mM. It also shows that the nicotinamide stimulation of DNA repair was similar in both the presence and absence of hydroxyurea. In contrast, the level of DNA synthesis in undamaged cells was not significantly affected at nicotinamide concentrations up to 2 mM and it was partially inhibited in the range of 5 to 10 mM nicotinamide.

To evaluate the effect of nicotinamide on the time course of DNA repair synthesis, control and irradiated cells were incubated in the presence of [^{3}H]dThd and samples were removed at hourly intervals to measure the amount of DNA synthesis. The top part of Fig 2 shows that the UV irradiated cells conducted more DNA repair synthesis than the control, unirradiated cells and that the UV irradiated, nicotinamide treated cells conducted even more DNA repair synthesis than the cells that were treated with UV irradiation alone. Similar differences occurred when these experiments were carried out for as long as 24 hrs.

The lower half of Fig 2 shows the hourly rates at which cells incorporated [^{3}H]dThd into DNA. The UV irradiated cells incubated in the presence or absence of nicotinamide showed an identical increase in the rate of DNA repair synthesis during the first 2 hrs following irradiation. Thereafter, the rate of DNA repair in the UV irradiated cells began to decline while the rate in the UV irradiated, nicotinamide treated cells continued to increase. Four hrs after irradiation, the rate of DNA repair synthesis in the nicotinamide treated cells reached its maximum level and then began to decline. However, even after 8 hrs in culture, the irradiated cells incubated in the presence of nicotinamide maintained a higher rate of DNA repair synthesis than did the cells that were UV irradiated alone.



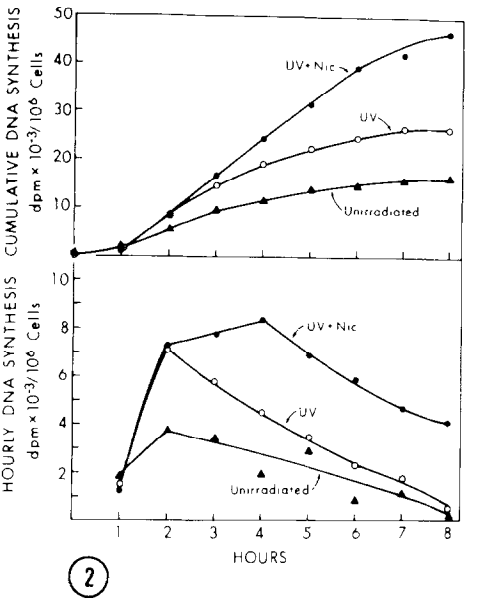


FIGURE 1: Concentration dependence of nicotinamide stimulation of DNA repair synthesis in normal human lymphocytes. Cells were subjected to 20J/m^2 of UV irradiation. Unirradiated cells were incubated in α MEM, 10% fetal calf serum (▲—▲). Irradiated cells were incubated in the same medium in the presence (O—O) or absence (●—●) of 10 mM hydroxyurea. Nicotinamide was present at the indicated concentrations. [^{3}H]dThd (Specific Activity 56 Ci/mmole) was added to all cultures at a final concentration of 200 μCi/ml. Cultures were incubated for 6 hrs at 37°C then incorporation of radioactivity into trichloroacetic acid precipitates was determined. All values are expressed as a percent of the values which were obtained at 8 μM nicotinamide which was the basal concentration of nicotinamide in α MEM. The basal values for DNA synthesis in the medium containing 8 μM nicotinamide were as follows: control unirradiated cells, 60,600 dpm/10^6 cells/6 hrs; UV irradiated cells, 36,200 dpm/10^6 cells/6 hrs; and UV irradiated hydroxyurea treated cells, 26,200 dpm/10^6 cells/6 hrs.

FIGURE 2: Time course of nicotinamide stimulation of DNA repair synthesis in human lymphocytes. Lymphocytes were subjected to 20J/m^2 of UV irradiation. Control and irradiated cells were suspended in α MEM, 10% fetal calf serum. 2 mM nicotinamide was added to the indicated cultures. [^{3}H]dThd (Specific Activity 56 Ci/mmole) was added to cultures at 200 μCi/ml and cultures were incubated at 37°C for 8 hrs. At 0 time and at hourly intervals thereafter samples were removed from each culture and TCA precipitates were prepared to measure the incorporation of [^{3}H]dThd. All points represent means of triplicate assays which showed less than 10% variation. The top part of the Figure displays the cumulative incorporation of radioactivity from time 0 until the time indicated on the abscissa. The lower panel shows the accumulation of radioactivity during the hour preceding each time point indicated on the abscissa. These latter data were obtained by subtracting the counts obtained during the previous hour from those obtained at the hour indicated on the abscissa. Unirradiated control cells (▲—▲); UV irradiated cells (O—O); UV irradiated, 2 mM nicotinamide treated cells (●—●).

The time course studies suggest the possibility that after 2 hrs in culture, the UV irradiated cells ran out of some component required for the continuation of DNA repair, whereas the nicotinamide treated cells were not depleted of this factor and were therefore able to carry out DNA repair to a greater extent and for a longer time. The studies outlined in Table II demonstrate that even in nucleotide permeable cells the rate of DNA repair was greater in the UV irradiated, nicotinamide treated cells than in the cells subjected to UV irradiation alone. Since these permeable cell preparations were supplied with an excess of dNTPs and ATP (8-10) it is clear that none of these nucleotides could represent the depleted components responsible for the lower rates of DNA repair in the cells that were not treated with nicotinamide.

While the mechanism by which nicotinamide stimulates DNA repair synthesis remains to be determined, the finding that nicotinamide specifically stimulates this process in cells that have already sustained DNA damage, suggests that it might be useful to prevent the development of mutations in cells that have been subjected to DNA damage by environmental mutagens or by the agents used in cancer therapy. In addition, nicotinamide might be useful in stimulating DNA repair in patients suffering from genetic disorders of these processes (11).

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REFERENCES:

1. Cleaver, J.E. (1968) Nature 218, 652-656.
2. Painter, R.B., and Cleaver, J.E. (1969) Rad. Res. 37, 451-466.
3. Regan, J.D., and Setlow, R.B. (1974) Cancer Res. 34, 3318-3325.
4. Berger, N.A., Sikorski, G.W., Petzold, S.J., and Kurohara, K.K. (1979) J. Clin. Invest. 63, 1164-1171.
5. Cleaver, J.E. (1969) Rad. Res. 37, 334-348.
6. Berger, N.A., Kurohara, K.K., Petzold, S.J., and Sikorski, G.W. (1979) Biochem. Biophys. Res. Commun. 89, 218-225.
7. Mendelsohn, J.S., Skinner, S.A., and Kornfeld, S. (1971) J. Clin. Invest. 50, 818-826.
8. Berger, N.A., Adams, J.W., Sikorski, G.W., Petzold, S.J., and Shearer, W.T. (1978) J. Clin. Invest. 62, 111-118.
9. Berger, N.A., and Johnson, E.S. (1976) Biochim. Biophys. Acta 425, 1-17.
10. Berger, N.A. (1978) Methods Cell Biol. 20, 325-339.
11. Setlow, R.B. (1978) Nature 271, 713-717.