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METABOLIC CONSEQUENCES OF DNA DAMAGE: DNA DAMAGE INDUCES ALTERATIONS IN GLUCOSE METABOLISM BY ACTIVATION OF POLY(ADP-RIBOSE) POLYMERASE

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SUMMARY: In this communication we show that activation of poly(ADP-ribose) polymerase by DNA damage can produce drastic alterations in carbohydrate metabolism. We examined alterations in NAD^+, $NADP^+$, ATP and glucose-6-phosphate in L1210 murine leukemia cells, following exposure to different concentrations of N-methyl-N'-nitro-N-nitrosoguanidine. Treatment of cells with 20 µg/ml MNNG produced rapid depletion of NAD^+ and ATP. The G-6-P pool showed a biphasic change: first the pool size decreased, then increased to a level greater than that present in control cells. Nicotinamide treatment prevented the total depletion of NAD^+ and this in turn helped preserve the ATP pools and prevented the biphasic alteration in G-6-P pool sizes.

Poly(ADP-ribose) polymerase is a chromosomal enzyme that uses NAD^+ as substrate to synthesize poly(ADP-ribose) (1). The enzyme is activated by DNA strand breaks, and thus the synthesis of poly(ADP-ribose) usually increases during the cellular response to DNA damage (2,3). We have previously shown that extensive DNA damage can activate poly(ADP-ribose) polymerase to a sufficient degree to consume large quantities of NAD^+ and consequently deplete cellular pools of this nucleotide (4,5). In the present study we found that treating cells with N-methyl-N'-nitro-N-nitrosoguanidine produced rapid alterations in carbohydrate metabolism as shown by drastic changes in cellular glucose-6-phosphate levels. These alterations in carbohydrate metabolism are dependent on the activation of poly(ADP-ribose) polymerase by the DNA damage. Thus, in addition to the genetic effects produced by DNA damaging agents,

Abbreviations: ADP-ribose, Adenosine Diphospho-ribose; MNNG, N-methyl-N'-nitro-N-nitrosoguanidine; Me_2SO, Dimethyl Sulfoxide.

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there are also drastic metabolic consequences which are mediated by activation of poly(ADP-ribose) polymerase.

METHODS

L1210 cells were maintained in logarithmic growth between 0.3-1.0 x 10^6 cell/ml at 37°C in α modified Eagle's medium buffered with 25 mM Hepes, pH 7.2 and supplemented with 10% fetal calf serum, 50 units/ml penicillin and 50 µg/ml streptomycin (4). Cells were treated with MNNG that was freshly dissolved in Me_2SO and then diluted in saline. Controls performed with equivalent concentrations of Me_2SO showed that the solvent had no effect on nucleotide pools. Some cultures were adjusted to contain 2 mM nicotinamide, 15 minutes before treatment with MNNG. At the indicated time points, duplicate samples of cell suspension were removed, cell counts were performed, viability was determined with Trypan blue and cells were collected by centrifugation. Perchloric acid extracts were prepared and analyzed for NAD^+, $NADP^+$, ATP and G-6-P using enzymatic cycling techniques (6,7,8).

RESULTS AND DISCUSSION

Treatment of L1210 cells with 20 µg/ml MNNG results in the rapid production of DNA strand breaks, a consequent increase in poly(ADP-ribose) synthesis, and decreases in DNA, RNA and protein synthesis (4). As shown in Table 1, these cells undergo a characteristic depletion of NAD^+ pools due to maximal activation of poly(ADP-ribose) polymerase. The depletion of NAD^+ leads to a consequent depletion of ATP pools. It is interesting to note that while NAD^+ pools are totally depleted in response to 20 µg/ml MNNG, the much smaller $NADP^+$ pools are reduced but not depleted. The

TABLE 1: Metabolite Pools (pmol/10^6 Cells) in L1210 Cells Following Treatment with 20 µg/ml MNNG

Hours	NAD^+	ATP	G-6-P	$NADP^+$
0	430	2730	168	32
0.5	31	790	36	32
1	17	521	46	28
3	1.6	58	226	8
6	0	0	40	3

L1210 cells were treated with the indicated concentration of MNNG at time 0. At the indicated times cells were counted and duplicate samples were collected by centrifugation. Perchloric acid extracts were made, neutralized and metabolite assays were performed on each sample in duplicate using enzymatic cycling techniques.

relative maintenance of the $NADP^+$ pools probably reflects the inability of poly(ADP-ribose) polymerase to use $NADP^+$ as substrate as well as the lack of significant conversion of $NADP^+$ to NAD^+. The eventual decrease in $NADP^+$, that occurs between three and six hours after exposure to 20 μg/ml MNNG, probably reflects the depletion of NAD and ATP and consequent lack of de novo $NADP^+$ synthesis.

Following treatment of the L1210 cells with 20 μg/ml MNNG there are drastic alterations in carbohydrate metabolism as demonstrated by a biphasic shift in levels of glucose-6-phosphate. During the initial hour following MNNG treatment, the G-6-P content falls in association with the decrease in NAD^+ and ATP. However, at three hours following treatment with 20 μg/ml MNNG, there is a marked increase in the G-6-P content such that the cells contain more G-6-P than they did before treatment with the DNA damaging agent. The increase in G-6-P is transient and by six hours, the cellular level of this metabolite is considerably reduced.

Treatment of L1210 cells with MNNG at 5 or 10 μg/ml results in qualitatively similar decreases in NAD^+ and ATP pools and similar shifts in G-6-P. As shown in Figure 1, panel 4, G-6-P undergoes an initial fall, followed by an increase to a peak at 3 hours, followed by a distinct fall by 6 hours after damage. The involvement of poly(ADP-ribose) polymerase in these alterations of G-6-P levels is suggested by the effect of 2 mM nicotinamide. We have previously shown that nicotinamide can retard, but not completely inhibit the activity of poly(ADP-ribose) polymerase in DNA damaged cells (4). When the L1210 cells were treated with 5 μg/ml MNNG in the presence of 2 mM nicotinamide, the activation of poly(ADP-ribose) polymerase was retarded and the rate of NAD^+ depletion was slower. Under these conditions, the G-6-P level showed a gradual decrease to 30% of its initial value, however it never showed the biphasic response. Thus the drastic alterations in G-6-P pool sizes and its biphasic response following high levels of DNA damage are associated with drastic lowering of NAD^+ pools and can be partially prevented by inhibitors of poly(ADP-ribose) polymerase.

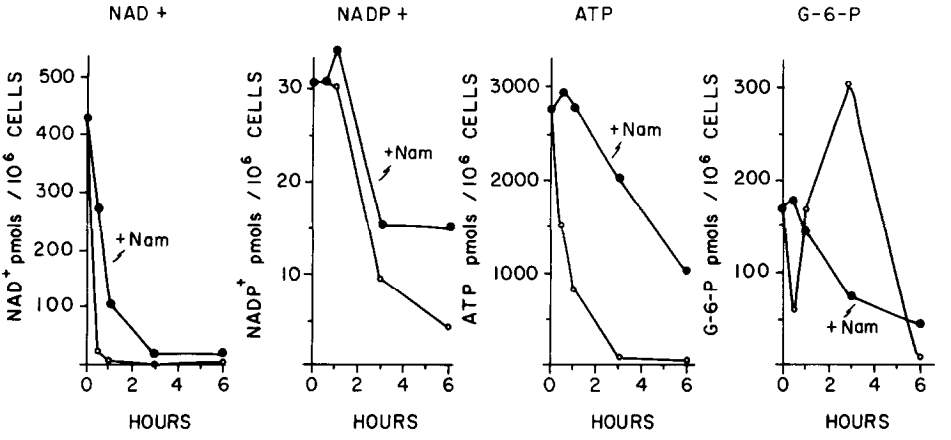

Fig. 1 L1210 cells in log phase growth were incubated in regular growth media or in media containing 2 mM nicotinamide. MNNG was added to a final concentration of 5 μg/ml. At the indicated times, cells were removed for counting and perchloric acid extracts were made, processed and metabolites analyzed utilizing enzymatic cyclic techniques described above. Cells for 0 time values were removed just before addition of MNNG. From left to right graphs are for NAD^+, $NADP^+$, ATP and G-6-P. (o) Cultures treated with MNNG; (●) Cultures pretreated with 2 mM nicotinamide before treatment with MNNG.

As indicated in Table 2, cells treated with 2 μg/ml MNNG showed only a small decrease in G-6-P. At six hours after damage, G-6-P remained at 63% of the control level. Table 2 also shows that the NAD^+ pool was reduced following treatment of cells with 2 μg/ml MNNG, however its rate of fall was not as fast as in the cells treated with 20 μg/ml. The lowest level of NAD^+ reached after damage with 2 μg/ml MNNG occurred at 3 hours and was approximately 5% of the level in control cells. By six hours after damage these cells began to recover their NAD^+ levels. Thus preservation of the

TABLE 2: Metabolite Pools (pmol/10^6 Cells) in L1210 Cells Following Treatment with 2 μg/ml MNNG

Hours	NAD^+	ATP	G-6-P	$NADP^+$
0	430	2730	168	32
0.5	186	3010	179	31
1	70	2870	137	33
3	21	2180	134	10
6	53	2030	105	11

Same as Table 1.

NAD^+ pool at 5% of the control level appears to be sufficient to preserve ATP and G-6-P levels at more than 60% of the control, predamage level.

These metabolic pool changes occurring in response to DNA damage can be explained by a process that is initiated when DNA damage activates poly(ADP-ribose) polymerase. As NAD^+ is consumed in the synthesis of poly(ADP-ribose), NAD pools are depleted, there is a consequent decrease in glycolysis and a failure to provide energy for ATP synthesis, leading eventually to ATP depletion. Since NAD^+ is depleted before ATP, there is a period when sufficient ATP remains to facilitate conversion of glucose to G-6-P. However, glycolysis can proceed no further than glyceraldehyde 3-phosphate since conversion of this metabolite to 1,3 diphosphoglycerate requires NAD^+. It is probable that the continued utilization of ATP to convert glucose to G-6-P and the inability to process this metabolite past glyceraldehyde 3-phosphate results in the pathway backing up and the accumulation of G-6-P. Since $NADP^+$ pools are relatively well preserved initially, the accumulated G-6-P can still be metabolized by the hexose monophosphate shunt. The shunt pathway can then remain active until ATP is depleted and no further G-6-P can be synthesized. This pathway provides an explanation for the previous observation that some cell types convert from glycolysis to the hexose monophosphate shunt pathway in response to DNA damage (9).

These studies show that activation of poly(ADP-ribose) polymerase in response to DNA damage mediates rapid and drastic alterations in metabolic pool sizes and pathways. These alterations may result in significant, indirect changes in cell viability before the genetic effects of DNA damage become manifest. These indirect metabolic consequences of DNA damage may account for increased susceptibility to DNA damage of certain cells or individuals expecially those with pre-existing alterations of metabolic pool sizes.

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