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Unscheduled Synthesis of DNA and Poly(ADP-Ribose) in Human Fibroblasts Following DNA Damage

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Unscheduled DNA synthesis has been measured in human fibroblasts under conditons of reduced rates of conversion of NAD to poly(ADP-ribose). Cells heterozygous for the xeroderma pigmentosum genotype showed normal rates of UV induced unscheduled DNA synthesis under conditions in which the rate of poly(ADP-ribose) synthesis was one-half the rate of normal cells. The addition of theophylline, a potent inhibitor of poly(ADP-ribose) polymerase, to the culture medium of normal cells blocked over 90% of the conversion of NAD to poly(ADP-ribose) following treatment with UV or N-methyl-N′-nitro-N-nitrosoguanidine but did not affect the rate of unscheduled DNA synthesis.

Key words: Xeroderma pigmentosum, poly(ADP-ribose), DNA repair, UV, N-methy-n′-nitro-N-nitrosoguanidine

Chromatin contains an enzyme that catalyzes the conversion of NAD to the unique biopolymer poly(ADP-ribose) [1, 2]. A wide variety of agents that cause damage to DNA are known to cause a rapid depletion of the cellular NAD pool [3–12]. The normal intracellular levels of poly(ADP-ribose) in 3T3 cells are very low but treatment with the potent DNA damaging chemical N-methyl-N′-nitro-N-nitrosoguanidine (MNNG) causes a rapid and transient increase in the levels of poly(ADP-ribose) which leads to depletion of the NAD pool [13]. Inhibitors of poly(ADP-ribose) polymerase potentiate the cytotoxicity of DNA damaging chemicals [14] which suggests that the synthesis of poly(ADP-ribose) in response to DNA damage is an adaptive response. Xeroderma pigmentosum (XP) cells are grossly deficient in unscheduled DNA synthesis (UDS) following UV treatment [15, 16] and our recent observation that these cells are also unable to convert NAD to poly(ADP-ribose) in vivo following UV treatment [17] has led us to examine the relationship between UDS and poly(ADP-ribose) synthesis in both normal cells and cells carrying the XP genotype.

METHODS

Normal human fibroblasts (IMR90), XP12BE (genetic complementation group A), and the parental heterozygote cell line of XP12BE were cultured and treated

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with UV and MNNG as described previously [17]. To determine the amount of UDS following UV or MNNG treatment, cells in 35-mm dishes were exposed to 10 μCi/ml of ^{3}H-thymidine (43 Ci/mmol) in the presence of 10 mM hydroxyurea. Following exposure, the medium was decanted and the cells were washed five times with ice cold phosphate buffered physiological saline and extracted with 0.5 ml of 0.1 M NaOH and 1 mM nicotinamide. The samples were placed on ice and adjusted to 20% (w/v) in tricholoroacetic acid. After standing on ice for at least 15 min, the precipitate was collected by centrifugation. The pellet was washed three times with ice cold 20% (w/v) trichloroacetic acid and dissolved in 88% formic acid and counted in an aqueous scintillation cocktail. Treatment of cell extracts in 0.1 M NaOH overnight at 37°C prior to precipitation with trichloroacetic acid did not affect the incorporation of radiolabel into acid insoluble material, indicating that no significant incorporation of radiolabel into RNA was occurring under these conditions.

RESULTS AND DISCUSSION

The studies of Yang et al, [18] have demonstrated that the repair of DNA lesions in human cells reduces both the cytotoxic and mutagenic effects of these lesions. Our previous studies have shown that poly(ADP-ribose) synthesis occurs in vivo as a rapid cellular response to DNA damage [13]. These observations along with those of Nduka et al [14] that inhibitors of poly(ADP-ribose) synthesis potentiate the cytotoxicity of DNA damaging chemicals suggest that the conversion of NAD to poly(ADP-ribose) is related in some way to DNA repair. The observations that cells homozygous for the XP genotype are grossly deficient in both UDS [15, 16] and conversion of NAD to poly(ADP-ribose) following UV treatment [17] are consistent with a requirement of poly(ADP-ribose) synthesis for UV induced UDS. Table I shows the amount of UDS in normal control cells and cells homozygous and heterozygous for the XP genotype (complementation group A) for a 4-hr period following UV or MNNG treatment. The rate of UDS is linear during the 4-hr period, thus the values shown represent rates of UDS under these conditions. Due to possible differences in the specific activity of the precursor pools, comparison of the absolute amounts of UDS between cell lines must be made with some caution; thus, Table I also shows the ratio of UV induced to MNNG induced UDS in each cell line. Cells carrying the XP genotype are repair proficient for MNNG [19]. The data shown confirm earlier studies that showed that the XP12BE cells are grossly deficient in UV induced UDS. In addition, the cells of the parental heterozygote show an amount of UDS similar to control cells despite the fact that the rate of conversion of NAD to poly(ADP-ribose) is approximately one-half that of the control cells under these conditions [17]. Other studies have also generally observed normal or near normal levels of UDS in cells heterozygous for the XP genotype [15, 16].

To examine further for a possible relationship between poly(ADP-ribose) synthesis and UDS, we have measured UDS following UV or MNNG treatment in normal cells in the presence of theophylline (Table II). Theophylline is a potent inhibitor of poly(ADP-ribose) polymerase [20] and addition of 2 mM theophylline to the culture medium blocks over 90% of the conversion of NAD to poly(ADP-ribose) [17]. The data show that the presence of theophylline has no appreciable effect on the UDS induced by either UV or MNNG under these conditions.

TABLE I. Unscheduled DNA Synthesis in Normal and Xeroderma pigmentosum Cells*

Cell line	Treatment (cmp/10^6 cells)		UV/MNNG ratio
	UV	MNNG	
IMR90	48,500	10,300	4.7
XP12BE	7,300	15,000	0.5
Parent of XP12BE	98,700	17,500	5.6

*Dishes of cells were treated with 60 J/m^2 of UV or MNNG (680 μM, 1 min) and incorporation of ^{3}H-thymidine (10 μCi/ml) was measured for a period of 4 hr in the presence of 10 mM hydroxyurea.

TABLE II. Effect of Inhibitors of Poly(ADP-Ribose) Synthesis on Unscheduled DNA Synthesis in Normal Human Fibroblasts*

Exposure	Unscheduled DNA Synthesis (cpm/10^6 cells)	
	Control	2 mM Theophylline
MNNG	9,646	9,440
UV	15,373	13,370

*Dishes of cells were treated with 60 J/m^2 of UV or 280 μM MNNG and incorporation of ^{3}H-thymidine (10 μCi/ml) was measured for a period of 2 hr in the presence of 10 mM hydroxyurea.

Studies with cells depleted of NAD have suggested that the conversion of NAD to poly(ADP-ribose) is required for UDS induced by MNNG [21]. The data presented here do not support a direct requirement of poly(ADP-ribose) synthesis for UDS induced by either UV or MNNG treatment. However, the data presented here do not rule out a direct requirement for poly(ADP-ribose) synthesis for the removal of a specific subclass of lesions or for steps in DNA repair subsequent to UDS. The studies of Durkacz et al [22] are consistent with a requirement for poly(ADP-ribose) synthesis for the rejoining of DNA strand breaks following excision repair of damage caused by dimethylsulfate, although alternative explanations are also possible. Our previous studies of the kinetics of poly(ADP-ribose) synthesis in vivo in response to DNA damage have shown that poly(ADP-ribose) formed is also rapidly degraded in vivo [13]. Thus it is also possible that the conversion of NAD to poly(ADP-ribose) may be needed in a regulatory role involved with the modulation of some aspect of DNA repair or the modulation of some other cellular response that must be coordinated with DNA repair.

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