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Low Doses of Neutrons Induce Changes in Gene Expression

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ABSTRACT

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Studies were designed to identify genes induced following low-dose neutron but not following γ -ray exposure in fibroblasts. Our past work had shown similar modulation of transcripts for α -tubulin, β - and γ -actins, ornithine decarboxylase, and interleukin-1 following both neutron and γ -ray exposure. However, differences in the expression of β -protein kinase C and *c-fos* genes were observed, with both being induced following γ -ray, but not neutron exposure. Recently we have identified two genes that are induced following neutron, but not γ -ray, exposure: *Rp-8* (a gene induced by apoptosis) and the long terminal repeat (LTR) of the human immunodeficiency virus (HIV). *Rp-8* mRNA induction was demonstrated in Syrian hamster embryo (SHE) fibroblasts and was found to be induced in cells exposed to neutrons administered at low (0.5 cGy/min) and at high dose rate (12 cGy/min). No induction of other apoptosis-associated genes such as *Rp-2*, *bcl 2*, and *Tcl-30* was observed. The induction of transcription from the LTR of HIV was demonstrated in HeLa cells bearing a transfected construct of the chloramphenicol acetyl transferase (CAT) gene driven by the HIV-LTR promoter. Measures of CAT activity and CAT transcripts following irradiation demonstrated an unresponsiveness to γ rays over a broad range of doses (10–300 cGy). Twofold induction of the HIV-LTR was detected following neutron exposure (48 cGy) administered at low (0.5 cGy/min) but not high (12 cGy/min) dose rates. Ultraviolet-mediated HIV-LTR induction, however, was inhibited by low-dose-rate neutron exposure. These results are interesting in light of reports that *Rp-8* is induced during apoptosis and that HIV causes apoptosis.

INTRODUCTION

Many studies have shown the effects of DNA-damaging agents on endogenous gene expression (1-5), including genes such as those encoding HIV (3,6-9) and other viruses (10,11). Past work from our group has examined effects of low-dose γ -rays or neutrons on gene expression, demonstrating radiation-mediated transcriptional changes evident at doses as low as 1 cGy (4,5). In addition, work in our laboratory has focused on different patterns of gene induction following exposure to γ rays or neutrons (4,12,13). We have focused recent efforts on identifying genes induced following neutron, but not γ -ray, exposure. The experiments reported here document the induction of HIV-LTR and *Rp-8* (an apoptosis-associated transcript) following neutron, but not γ -ray, exposure. This is interesting in light of the fact that *Rp-8* is induced during apoptosis and HIV is reported to cause apoptosis.

MATERIALS AND METHODS

Cell Lines/Culture Conditions.

All experiments were performed using Syrian hamster embryo (SHE) fibroblasts used in previous experiments (4,5) or with HeLa cells stably transfected with a CAT construct driven by the HIV-LTR promotor. HeLa cells were generously provided by Dr. K. Valerie (6).

Radiation Exposures.

Doses and dose rates are as described in Results. For γ -irradiations, a ^{60}Co source was used. All neutron irradiations used the JANUS reactor of the Center for Mechanistic Biology

and Biotechnology at Argonne National Laboratory, which has a 0.3% γ -ray contamination of its neutron beam (14).

Exposures to 254-nm ultraviolet radiation were administered using a General Electric (GE30T8) 30-W germicidal lamp which was contained in a sterile hood. The irradiations were done 55.6 cm from the source.

CAT Assays.

The cells were trypsinized from the plates, washed, and counted. Equal numbers of cells were lysed by three freeze/thaw cycles. The lysate was then incubated in a reaction mixture containing 40 μ M 3 H-chloramphenicol (1343.1 GBq/mmol or 36.3 Ci/mmol, NEN Research Products) and *n*-butyryl coenzyme A. CAT transfers the *n*-butyryl group of the coenzyme A to the chloramphenicol, which changes its solubility in xylene. The activity, measured by liquid scintillation, directly relates to the amount of CAT in the reaction mixture.

Northern Blots.

RNA preparations, Northern blots, and hybridizations were performed as described (4,5). The pCAT probe was generously provided by Dr. Michael Getz (Mayo Clinic, Rochester, Minnesota), Rp-8 cDNA by Dr. G. P. Owens (University of Colorado, Denver), and Tc130 cDNA by Dr. S. Bourgeois (Salk Institute, LaJolla, California).

RESULTS

Effects of Ultraviolet Light on HIV-LTR-Driven CAT Expression.

Initial experiments were designed to document dose- and dose-rate effects of ultraviolet radiation on HIV-LTR-driven CAT expression 24 h following exposure to the

damaging agent (Fig. 1). The level of induction of CAT activity (driven by the HIV LTR) was correlated with the length of exposure (dose) of the ultraviolet light. Twelve- and 13-fold induction levels were detected following 20 s of exposure to the lamp. In additional experiments designed to compare the effects of ultraviolet dose rate on induction of CAT, we compared a specific dose of radiation (25 J/m^2) administered at different dose-rates ($0.279\text{--}0.427 \text{ J/m}^2\cdot\text{s}$). These results (Fig. 2) showed a larger induction of CAT activity following exposure to high dose rates than following exposure to low dose rates of ultraviolet radiation. Induction following TPA exposure (Fig. 2) was also observed. The effects of an exposure time of 20 s under a UV lamp is equivalent to 25 J/m^2 .

Effects of Ionizing Radiation on HIV-LTR-Driven CAT Expression.

Table 1 presents results of experiments demonstrating that JANUS neutrons, when administered alone at low dose rates, can cause induction of HIV-LTR-CAT. However, when given following UV induction at either low or high dose rates, repression of HIV-CAT expression was observed.

We also determined the effects of γ -rays at two doses (100 and 300 cGy) and two dose-rates (one, not all and 150 cGy/min) on HIV-LTR-CAT expression. Results of these experiments showed little effect of γ -rays at 1 h or 24 h following exposure to γ -rays administered at either low or high dose rates. We also examined the effects of γ -rays combined with UV on HIV-LTR-CAT. These results established UV-mediated induction of HIV-LTR-CAT, but no effect of γ -rays when administered on untreated or on UV-exposed cells.

Effects of Ionizing Radiation on Apoptosis-Associated Transcripts.

HIV expression has been found to cause apoptosis in many cell types (15). For that reason, we examined the expression of several apoptosis-associated transcripts (*Rp-8*, *Rp-2*,

Tcl 30; 16,17). Only mRNA for *Rp-8* was detectable in fibroblasts 1 h following neutron exposure (rather than high dose-rate) neutron exposure.

DISCUSSION

This work has shown effects of ultraviolet, γ -ray, and neutron irradiations on transcriptional activation of the HIV promoter as assayed in a CAT expression vector system. While ultraviolet light induced significant transcription from the promoter, neutrons caused moderate induction and γ -rays and sunlight had no effect. These results suggest different molecular pathways for transcriptional effects occurring intracellularly following exposure to ultraviolet, low-LET ionizing (γ -rays), or high-LET ionizing (neutrons) radiations. Previous work from our group has shown different molecular responses of high- and low-LET radiations (4,5,10).

The fact that some DNA-damaging agents (TPA, UV, and neutrons) induce HIV transcription while others (sunlight, γ -rays) do not suggests (a) that transcriptional activation of HIV is not dependent on DNA damage, (b) that sunlight/ γ -rays are capable of repressing the HIV-transcriptional response, or (c) that only specific types of DNA damage are able to elicit the response. Our results examining the effects of UV and γ -rays on HIV transcription demonstrate that the second case is not likely. Past work by Stein, *et al.* (7) has supported the idea that DNA damage is a required intermediate step for UV-induced HIV-1 expression. That work combined with our results reported here would suggest (a) that the DNA damage must be of a specific type for induction of HIV, (b) that this induction requires an additional cellular signal (such as membrane damage, protein phosphorylation, etc.) caused by some but

not all clastogenic agents, and/or (c) that induction following neutron exposure utilizes a different cellular pathway than induction following UV exposure.

Our results demonstrated only twofold induction of HIV and threefold induction of *Rp-8* following neutron exposure. This is not unusual since most responses to neutrons involve only two- to threefold changes in specific transcripts (4,5). What is interesting, however, is the finding that neutron exposure is able to repress UV-induced HIV transcription. This suggests potential use of neutrons and/or neutron-induced products in the control of HIV expression.

Induction of *Rp-8* mRNA following neutron exposure has suggested an association of apoptosis and HIV induction in the absence of full AIDS virus production. This suggests that apoptosis/HIV induction may be associated more with a common transcriptional pathway than with induction of apoptosis by HIV itself.

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FIGURE LEGENDS

Figure 1. Effects of ultraviolet lamp exposure on expression of CAT activity (number of seconds under UV light) with CAT transcription being driven by HIV-LTR. CAT control is a fixed amount (0.1 μ M) of CAT to allow for intra-experimental comparisons.

Figure 2. Effects of TPA and ultraviolet light on CAT activity with CAT transcription being driven by HIV-LTR. Control results are the total activity in cells not exposed to any damaging agents. Cells exposed to TPA were given a concentration of 25 ng/ml or 50 ng/ml with harvest time either 24 h or 48 h following addition of TPA. For UV-B lanes, cells were exposed to a total dose of 25 J/m² at three different dose rates (0.279, 0.378, or 0.427 J/m²·s). For cells exposed to the UV lamp, exposure time was varied (5 to 20 s).

Figure 3. Northern blot of RNA derived from untreated (0) and neutron-exposed SHE cells 1 h following completion of the exposure hybridized to ³²P-labeled *Rp-8* cDNA probe. Cells were treated with 6, 12, 24, or 36 cGy administered at a dose-rate of 0.5 cGy/min or 12, 24, 36, or 48 cGy administered at a dose-rate of 12 cGy/min.

Table I. Effects of Neutrons of HIV-LTR-CAT Expression

Treatment	n ₀ Dose rate (cGy/min)	Relative expression ^a
None	0	1.0 (0.4)*
UV ^b	0	2.2 (.13)
n ₀ ^c	0.5	2.0 (.23)
UV + n ₀ ^d	0.5	1.4 (.15)*
n ₀	12	1.2 (.18)*
UV + n ₀	12	1.6 (.26)*

^aExpression was measured at CAT-specific mRNA in stably transfected HeLa cells.

Relative expression in untreated cells was set at 1.0. All other values are expressed relative to that. Standard deviations are in parentheses.

^bUV = ultraviolet exposure at 254 nm of 10 J/m²·s dose rate. Cells were harvested 24 h later.

^cn₀ = Neutron dose of 48 cGy given at different dose rates. Cells were harvested 1 h later.

^dUV + n₀ = UV dose given 24 h prior to indicated neutron exposure.

*Indicates significantly different from ultraviolet treatment alone at $p < .05$.

Time under UV light

Figure 1

Figure 2

RP8

n_0

Figure 3

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