The Aminothiol WR-1065 Protects T Lymphocytes from Ionizing Radiation-induced Deletions of the *HPRT* Gene[1]

L. Scott Clark,[2] Richard J. Albertini, and Janice A. Nicklas

Genetic Toxicology Laboratory, University of Vermont, Burlington, Vermont 05401

Abstract

Aminothiols, such as WR-2721 and its active free thiol, WR-1065, reduce mutations from ionizing radiation in exponentially growing cells. In this study, human noncycling G_0 T lymphocytes were exposed *in vitro* to γ-irradiation in the presence or absence of WR-1065. The five treatment groups were: (*a*) control; (*b*) treatment with 4 mM WR-1065; (*c*) treatment with 3 Gy of γ-radiation from a ^{137}Cs source; and (*d*) and (*e*) treatment with WR-1065 30 min prior to or 3 h after 3 Gy of γ-irradiation, respectively. A total of 224 cloned *HPRT* mutants representing 179 independent mutations were analyzed for genetic alterations using multiplex PCR. Ionizing radiation alone significantly increased the percentage of mutations with gross structural alterations compared to controls ($P = 0.02$). Although the frequency of such large structural mutations was not different from control cells treated with WR-1065 alone, this aminothiol significantly reduced their frequency among irradiated mutants ($P = 0.01$) when the radioprotector was present during the irradiation. Addition of WR-1065 3 h postirradiation also greatly reduced the percentage of gross structural alterations; however, due to small numbers, this was not statistically significant. This is the first demonstration that the antimutagenicity of WR-1065 in human cells specifically protects against these kinds of large-scale DNA alterations induced by ionizing radiation. WR-1065 and similar aminothiol compounds may afford protection against radiation-induced mutations through polyamine-like processes, *e.g.*, stabilization of chromatin structure, inhibition of cell proliferation, and influences on DNA repair systems.

Received 2/26/97; revised 6/25/97; accepted 6/27/97.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] This work was supported by Department of Energy Grant FG028760502, National Cancer Institute Grant CA30688, and American Cancer Society Grant CB-45.

[2] To whom requests for reprints should be addressed, at MD-68, Genetic and Cellular Toxicology Branch, Environmental Carcinogenesis Division, United States Environmental Protection Agency, Research Triangle Park, NC 27711. Phone: (919) 541-2248; Fax: (919) 541-0694; E-mail: clark.scott@epamail.epa.gov.

Introduction

Ionizing radiation, specifically γ-radiation, is an inducer of gross structural alterations in the *HPRT* (hypoxanthine-guanine phosphoribosyl transferase) gene and other mutation assay systems (1–6). Furthermore, ionizing radiations dramatically increase the percentage of human *in vivo HPRT* T-cell mutations with gross structural alterations of the gene, as demonstrated on Southern blots. Gross structural alterations are defined as total and partial deletions of the *HPRT* gene, as well as translocations and molecular weight changes indicating intronic deletions/insertions. Such changes represent 15% of the adult background, with the remaining 85% being base changes or small insertions and deletions that escape detection due to the limited sensitivity of Southern blot analysis (7–11). By contrast, Bradley *et al.* (9) found that 33% of the T-cell *HPRT* mutations in patients who had received diagnostic radionuclides showed Southern blot-defined gross structural alterations compared to 13% in pretreatment patients. Similarly, elevated percentages of T-cell mutations with the large-scale DNA changes were observed on Southern blots in radioimmunotherapy patients who had received ^{131}I and/or ^{90}Y-labeled antiferritin antibodies (38.3%) compared to preradioimmunotherapy patients (16.9%) or to normal controls (14.2%; Refs. 12–14). Also, using Southern blot techniques, O'Neill *et al.* (15) found similar results following *in vitro* γ-irradiation of human T lymphocytes, in which the percentage of gross structural alterations among the *HPRT* mutations increased to 75% after 3 Gy of irradiation. Therefore, the mechanisms by which ionizing radiations induce mutations result in large scale DNA changes.

N-(2-Mercaptoethyl)-1,3-diaminopropane (WR-1065) is the *in vitro* active species of the well-characterized radioprotector WR-2721, which requires *in vivo* metabolic activation by alkaline phosphatase. There is considerable interest in WR-2721 and WR-1065, because these agents appear to preferentially protect normal rather than neoplastic tissues against the deleterious effects of alkylating agents or ionizing radiations (16, 17). These and other thiol-containing compounds have been shown to exhibit antitransforming (18), anticlastogenic (19, 20), anticarcinogenic (21), and antimutagenic (22–24) properties in mammalian cells.

In murine studies, WR-2721 increases the life span and decreases the incidence of lymphocytic lymphomas in animals receiving neutron or γ-irradiations (25, 26). Decreases in *Hprt* mutant frequencies determined in splenic lymphocytes were also observed (27). Increases in cell survival *in vitro* in Chinese hamster cell lines have been observed only if the aminothiol, WR-1065, is administered prior to irradiation, but *Hprt* mutant frequencies decrease regardless of the time of administration (30 min prior to irradiation, 5 min postirradiation, or 3 h postirradiation) of the aminothiol (22, 28). Although the radioprotective effects of these agents have been shown to be antimutagenic and anticlastogenic, few data are available on the molecular nature of these protected mutations.

Because the mutagenicity of γ-irradiation is associated with increases in gross structural alterations, we examined the radioprotective effects of WR-1065 on T-cell mutations *in vitro*. Human G_0 T lymphocytes were incubated with WR-1065 30 min before or 3 h after 3 Gy of γ-irradiation from a ^{137}Cs source. We have reported elsewhere protection by WR-1065 against the *in vitro* T-cell *HPRT* mutagenicity of γ-irradiation, as evidenced by markedly reduced frequencies of radiation-induced mutation (29). The resulting mutants from these studies have now been analyzed at the molecular level using multiplex PCR to define the nature of the *HPRT* changes and to determine the kinds of mutations eliminated from the γ-irradiation mutation spectrum by this radioprotection.

Materials and Methods

Cellular Cloning of HPRT Mutants. *In vitro* mutagenicity experiments were performed as described previously (30, 31). *HPRT* mutants were isolated from selection plates inoculated with cells that had been allowed an 8-day expression interval following their irradiation with 3 Gy from a ^{137}Cs source, treatment with WR-1065, or treatment with WR-1065 30 min before or 3 h after 3 Gy of γ-irradiation. Mutant and wild-type *HPRT* isolates were determined only from experiments that demonstrated a minimum of a 6-fold or greater increase in mutant frequency with irradiation as compared to controls. Isolates were expanded to at least 10^6 cells using nonselection media [RPMI 1640, 20% HL-1 medium + 5% calf bovine serum (CBS), an interleukin 2 source (supernatant of mononuclear cells cultured with interleukin 2 during lymphokine-activated killer therapy), and irradiated *HPRT*$^-$ lymphoblastoid feeder cells (a gift of H. Liber and C. Denault, Harvard University, Cambridge, MA)], as described elsewhere (30). Fifty thousand cells [for multiplex PCR analysis of the *HPRT* gene or γ-*TCR* (T-cell receptor) gene analysis] or 5000 cells (for β-*TCR* gene analysis) were washed in PBS and microcentrifuged for 2 min at 6500 rpm. Supernatants were removed, and the microfuge tube containing the cell pellet was quick frozen in liquid nitrogen and stored at −70°C.

Multiplex PCR Analysis. Dry cell pellets were resuspended in 50 μl of lysis buffer containing 0.45% NP40, 0.45% Tween 20, 100 μg/ml proteinase K, and $T_{10}E_1$ [10 mM Tris-HCl (pH 8.0) and 1 mM EDTA (pH 8.0)]. Resuspended cell pellets were incubated at 50°C for 1 h, with a final incubation at 96°C for 10 min to inactivate the proteinase K. Cell lysates were then stored at −20°C until analysis by multiplex PCR. Five μl of cell lysate were added to a 45 μl multiplex *HPRT* PCR reaction, as described by Gibbs *et al.* (32). After 33 cycles, one-half of the reaction was analyzed on a 1.4% agarose gel stained with 0.5 μg/ml ethidium bromide. The presence of individual *HPRT* exons and changes in exon fragment size was visualized under a UV transilluminator. Individual isolates that represented total gene deletions were confirmed by an additional multiplex PCR using *HPRT* multiplex primers for exons 1, 6, and 9 and with the dystrophin primers for exon E as an internal control (33).

Determination of Independent Mutations. To quantify the number of independent *HPRT* mutations represented by the *HPRT* mutants isolated, the *TCR* gene rearrangement patterns of the *HPRT*-isolated mutants were analyzed by one of two methods. Cell pellets of 5000 cells were lysed, and first-strand DNA synthesis was completed using avian reverse transcriptase. The cDNA was then PCR amplified as described previously (34). This method used primers specific for the *TCR*-β gene. Alternatively, 5 μl of the cell lysate prepared for multiplex PCR of *HPRT* were PCR amplified using primers specific to the *TCR*-γ gene (35). In any given experiment, only the *TCR*-β or -γ gene was analyzed for clonality, unless confirmation required further analysis. After either amplification, the PCR-amplified products were digested with two different restriction enzymes (*Hin*FI and *Hae*III for *TCR*-β or *Rsa*I and *Bst*N1 for *TCR*-γ), and the digested products were electrophoresed on a NuSieve (FMC Bioproducts) agarose gel. Mutants demonstrating different restriction patterns by either of the methods were considered to have arisen in different *in vivo* clones and then considered to have arisen from independent mutations *in vivo*. However, mutants that showed identical patterns after digestion with both enzymes were further analyzed by digesting the PCR-amplified product with yet another restriction enzyme or by sequencing the cDNA PCR product. Mutants with the same *HPRT* alteration that also shared the same *TCR* sequence or shared the same *TCR* restriction pattern following digestion with a third enzyme were considered to have arisen from a single mutational event. The percentages of gross structural alterations observed in Table 1 reflect these corrections for independent mutations.

Statistical Analysis. Gross structural alterations of control, WR-1065 treatment alone, 3 Gy alone, or WR-1065 treatment 30 min prior to or 3 h after irradiation with 3 Gy (Table 1) were compared by Fisher's exact test. Specific *HPRT* alterations (Table 2) were analyzed by χ^2 analysis.

Results

A total of 224 *HPRT* mutant isolates and 42 wild-type isolates were obtained from 11 experiments (involving γ-irradiation and WR-1065 treatments) using blood samples from eight different individuals. Multiplex PCR was used to determine the percentage of mutants with gross structural alterations (defined as total deletions, partial deletions, or molecular weight changes of the *HPRT* gene, as shown by exon bandshifts).

Independence of the mutations was determined by PCR of *TCR* gene rearrangements. The percentages of gross structural alterations among the *HPRT* mutations for the different treatment groups are shown in Table 1. After correction for clonality by *TCR* analysis, it was found that 179 (80%) of the isolated mutants represented independent mutations. In the control mutants, 16.7% demonstrated gross structural alterations in the *HPRT* gene. The percentage of gross structural alterations among the *HPRT* mutations in the control group is therefore 14.6% after correction for clonality. The percentage of mutants with large-scale changes was similar for those exposed to WR-1065 alone, *e.g.*, 18.8% of mutants and 18.2% of mutations.

In the cell populations receiving 3 Gy of γ-irradiation and selected after an 8-day expression interval, 38.5% of the mutants and 35.3% of the mutations showed large-scale DNA changes. This elevation was statistically significant ($P = 0.02$) when compared to the two control populations (control and WR-1065 alone-treated cells). In contrast to this, the presence of WR-1065 during irradiation or added 3 h later dramatically reduced the percentages of radiation-associated gross *HPRT* alterations. Cells receiving 3 Gy of γ-radiation in the presence of WR-1065 showed 13.6% of the mutants and 11.9% of the mutations with gross structural alterations of *HPRT*. Both percentages were significantly reduced from what was observed when irradiated cells were not protected by WR-1065 ($P = 0.01$). Furthermore, the percentage of radiation-derived mutant and mutations with gross structural alterations when the WR-1065 was not added to the cell population until 3 h after their receiving the 3 Gy of γ-irradiation (8.7% and 12.5%, respec-

Table 1 *HPRT* multiplex PCR and clonality analyses for *HPRT* mutant clones from cells treated with and without WR-1065 and 3 Gy of γ radiation *in vitro*

Treatment	No. of wild-types isolated	No. of mutants isolated	No. of mutations isolated	*HPRT* multiplex PCR results[a]	*TCR* pattern results[b]	Frequency of mutants with structural alterations
None	14	60	48	50 no change	1–8-mer, 2–2-mer	
				4 shift in exon 6	1–4-mer	
				2 del. exons 2–9		
				1 del. exon 3		10 of 60 mutants (16.7%)
				3 total del.		7 of 48 mutations (14.6%)
WR-1065 alone	18	32	22	26 no change	1–3-mer, 1–7-mer	
				4 del. exon 3	1–3-mer	
				1 total del.		6 of 32 mutants (18.8%)
				1 shift in exon 2		4 of 22 mutations (18.2%)
3 Gy of radiation alone	3	65	51	40 no change	1–5-mer, 3–2-mer	
				3 del. exon 4	1–3-mer	
				6 total del.		
				2 del. exon 2	1–2-mer	
				7 del. exons 7–9	1–4-mer, 1–2-mer	
				1 del. exon 3		
				2 shifts exon 3		
				2 del. exon 9		
				1 del. exons 1–3		25 of 65 mutants (38.5%)
				1 del. exons 4–9		18 of 51 mutations (35.3%)
WR-1065 + 3 Gy[c]	5	44	42	38 no change	1–2-mer	
				2 del. exon 9	1–2-mer	
				2 del. exons 7–9		
				1 del. exons 2–3		6 of 44 mutants (13.6%)
				1 shift in exon 4		5 of 42 mutations (11.9%)
3 Gy + WR-1065[d]	2	23	16	21 no change	1–8-mer	
				1 del. exon 9		2 of 23 mutants (8.7%)
				1 del. exon 6		2 of 16 mutations (12.5%)
Total	42	224	179			

[a] del., deletion.
[b] mer, identical *TCR* isolates that have the same *HPRT* mutation.
[c] WR-1065 30 minutes prior to and during 3 Gy of gamma irradiation.
[d] WR-1065 3 hours after 3 Gy of gamma irradiation.

tively) was reduced. However, because of small numbers, this reduction did not achieve statistical significance. There were no statistically significant differences in the percentages of gross structural alterations when control cells, cells receiving WR-1065 alone, or cells receiving 3 Gy of γ-irradiation with WR-1065 added either before or after the irradiation were compared.

The percentages of total deletions, partial deletions, and point mutations were examined to determine to what extent each type of lesion was protected by the WR-1065 (summarized in Table 2). When the treatment of cells in the presence or absence of WR-1065 was examined, total deletions represented 9.09% of the mutations without WR-1065 treatment as compared to 1.25% of the mutations in WR-1065 treatments (P = 0.055). Subdividing treatments by WR-1065 and/or irradiation revealed that 11.8% of the mutations from irradiation treatments were total deletions. In contrast, no total deletions were detected in the combination aminothiol/irradiation treatment.

Discussion

We have reported previously that the γ-radiation-induced increase in *HPRT* mutations in human G_0 T lymphocytes (7–10-fold above control) is reduced to background levels when the aminothiol WR-1065 is present before or even 3 h after irradiation (29). The current study was conducted to determine what types of mutational events are protected by this amino-

Table 2 Analysis of *HPRT* alterations among *HPRT* mutations observed in cells from mutants treated or not treated with WR-1065

Treatment	Total deletions[a]	Partial deletions	Point mutations[a,b]
A			
No WR-1065/no irradiation	3 of 48 (6.25%)	4 of 48 (8.33%)	41 of 48 (85.4%)
WR-1065/no irradiation	1 of 22 (4.55%)	3 of 22 (13.6%)	18 of 22 (81.8%)
No WR-1065/irradiation	6 of 51 (11.8%)	12 of 51 (23.5%)	33 of 51 (64.7%)
WR-1065/irradiation	0 of 58 (0.00%)	7 of 58 (12.1%)	51 of 58 (87.9%)
B[c]			
No WR-1065	9 of 99 (9.09%)	16 of 99 (16.2%)	74 of 99 (74.7%)
WR-1065	1 of 80 (1.25%)	10 of 80 (12.5%)	69 of 80 (86.3%)

[a] Statistically significant (P = 0.055).
[b] Mutations that showed no gross structural alterations when analyzed by multiplex PCR were considered point mutations that include single-base changes and small insertions and deletions.
[c] No irradiation and irradiation-exposed groups from part A combined.

thiol. As has been shown in several studies, an increase in gross structural alterations (deletions, translocations, and inversions) is characteristic of γ-radiation-induced mutations. In the present study, cell populations exposed to 3 Gy of γ-radiation showed that 35.3% mutations had gross structural alterations, as determined by multiplex PCR. In general, multiplex PCR identifies deletions of one or more exons, but bandshifts representing small insertions or deletions can also be detected. Translocations or inversions are only rarely detected (as exon band loss) because of the requirement that the break occur in the small region amplified by an exon primer set (usually breaks occur in the much larger nonamplified intronic regions).

We believe that the signature for ionizing radiation is a high percentage of gross alterations (mutations other than small deletions and point mutations). Although the frequency of gross structural alterations reported here is lower than that reported by O'Neill *et al.* (Ref. 15; 75% gross structural alterations were detected after 3 Gy of γ-irradiation); we used multiplex PCR, whereas the O'Neill study used Southern blots. Southern blots detect deletions over 200–500 bases (but not small deletions), and most translocations and inversions (nearly all if restriction enzymes generating large fragments are used). In the O'Neill *et al.* (15) study, many structural alterations were detected through Southern blot analysis with both *Bam*HI and *Hin*dIII restriction digests, which examines nearly the entire gene. The high percentage of gross rearrangements detected in the O'Neill study indicates that many radiation-induced mutations involve translocations or inversions; this was supported by initial karyotype analysis.[3]

Multiplex PCR alterations, in particular total deletions, are reduced greatly from the postirradiation mutation spectrum when WR-1065 is added before or after irradiation. Therefore, a reduction in mutant frequency observed in our earlier study is due to protection from this class of DNA alteration. This reduction in mutant frequency occurred with no decrease in survival as compared to irradiation alone, indicating that WR-1065 is not eliminating cells containing premutagenic lesions. Our results in regard to total deletions are different from those of Denault and Liber (36), who studied lymphoblastoid cells irradiated in the presence of cysteamine, an endogenous aminothiol similar to WR-1065. They showed a preferential protection against lesions that lead to smaller deletions or rearrangements. Denault and Liber studied a cycling B lymphoblast cell line, whereas we studied noncycling human peripheral T lymphocytes, and it is known that certain stages of the cell cycle are more sensitive to γ-irradiation (37).

The protection against radiation- and chemical-induced mutagenesis afforded by aminothiol compounds has been attributed to free radical scavenging, direct chemical repair via hydrogen donation, and auto-oxidation (38). Although these mechanisms may account for some reduction of γ-radiation-induced *HPRT* alterations observed when WR-1065 is present during irradiation by reducing the initial damage, these mechanisms cannot account for the reduction of mutations in cells exposed to WR-1065 3 h after irradiation.

In regard to the effects of WR-1065 after irradiation, it is thought that aminothiols act similarly to polyamines. Polyamines can bind to and stabilize DNA, affect DNA replication, and modify DNA repair processes (39). WR-1065 and its disulfide form WR-33278, like polyamines, have been found to bind to DNA (40) and associate with nucleoproteins (41). WR-33278 has also been reported to effectively compete with polyamines for uptake via a well-characterized polyamine transport system into the lungs of rats (42).

In our previous studies, WR-1065 was shown to transiently delay G_0 lymphocytes after exposure to γ-radiation and mitogen stimulation in the G_1 phase of the cell cycle prior to the first S phase. This result is consistent with those of Murley *et al.* (43), who found that WR-1065 transiently delayed progression of the cell cycle in late S phase and G_2 phase. As noted, a delay in cell cycle progression could permit a longer time for more accurate repair of DNA damage.

Furthermore, WR-1065 has been shown to directly affect some enzymes involved in DNA function. The disulfide form of WR-1065 increases topoisomerase I activity (44), whereas WR-1065 decreased topoisomerase IIα activity (45). WR-1065 might also affect levels of DNA repair enzymes by a similar mechanism.

In summary, the aminothiol WR-1065 has demonstrated protection against γ-radiation-induced DNA alterations at the *HPRT* gene in human G_0 T lymphocytes. We have demonstrated that antimutagenicity is detected using this biomarker gene, and we suggest that humans can be monitored for antimutagenicity *in vivo* using the *HPRT* T-cell system. The hope is that such studies will demonstrate the efficacy of using aminothiols with traditional, cancer radiation therapies, so that the untoward late effects of treatment-induced secondary cancers can be eliminated or reduced.

Acknowledgments

We thank the Drug Synthesis and Chemistry Branch, Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, for providing WR-1065. We also thank D.J. Grdina for his expertise on radioprotectors.

References

1. Fuscoe, J., Zimmerman, L., Harrington-Brock, K., and Moore, M. Large deletions are tolerated at the *HPRT* locus of *in vivo* derived human T-lymphocytes. Mutat. Res., *282:* 255–262, 1992.

2. Fuscoe, J., Zimmerman, L., Fekete, A., Setzer, R., and Rossiter, B. Analysis of X-ray-induced *Hprt* mutations in CHO cells: insertions and deletions. Mutat. Res., *269:* 171–183, 1992.

3. Park, M., Hanks, T., Jaberaboansari, A., and Chen, D. Molecular analysis of γ-ray-induced mutations at the *HPRT* locus primary human skin fibroblasts by multiplex polymerase chain reaction. Radiat. Res., *141:* 11–18, 1995.

4. Albertini, R. J., Nicklas, J. A., O'Neill, J. P., and Robison, S. H. *In vivo* somatic mutations in humans: measurement and analysis. Annu. Rev. Genet., *24:* 305–326, 1990.

5. Straume, T., Langlois, R., Lucas, J., Jensen, R., and Bigbee, W. Novel biodosimetry methods applied to victims of the Goiania accident. Health Phys., *60:* 71–76, 1991.

6. Kyoizumi, S., Umeki, S., Akiyama, M., Hirai, Y., Kusunoki, Y., Nakamura, N., Endoh, K., Konishi, J., Sasaki, M., Mori, T., Fujita, S., and Cologne, J. Frequency of mutant T lymphocytes defective in the expression of the T-cell antigen receptor gene among radiation-exposed people. Mutat. Res., *265:* 173–180, 1992.

7. O'Neill, J., Nicklas, J., Hunter, T., Sullivan, L., and Albertini, R. Molecular analysis of mutations at the *HGPRT* locus in human T-lymphocytes: Banbury Report 28. *In:* Mammalian Cell Mutagens, pp. 249–260. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory, 1987.

8. Nicklas, J., O'Neill, J., and Albertini, R. Molecular analysis of *in vivo HPRT* mutations in human T-lymphocytes. I. Studies of low frequency "spontaneous" mutants by Southern blots. Mutagenesis, *2:* 341–347, 1987.

9. Bradley, W., Gareau, J., Seifert, A., and Messing, K. Molecular characterization of 15 rearrangements among 90 human *in vivo* somatic mutants show that deletions predominate. Mol. Cell Biol., *7:* 956–960, 1987.

10. Hakoda, M., Hirai, Y., Kyoizumi, S., and Akiyama, M. Molecular analysis of *in vivo HPRT* mutants from T cells from atomic bomb survivors. Environ. Mol. Mutagen., *13:* 25–33, 1989.

11. Lichtenauer-Kaligis, E., Thijssen, J., den Dulk, H., van de Putte, P., Tasseron-deJong, J., and Giphart-Gassler, M. Genome wide spontaneous mutation in

[3] B. Hirsch, personal communication.

human cells determined by the spectrum of mutation in *HPRT* cDNA genes. Mutagenesis, *8:* 207–220, 1993.

12. Nicklas, J. A., O'Neill, J. P., Hunter, T. C., Falta, M. T., Lippert, M. J., Jacobson-Kram, D., Williams, J. R., and Albertini, R. J. *In vivo* ionizing irradiations produce deletions in the *HPRT* gene of human T-lymphocytes. Mutat. Res., *250:* 383–396, 1991.

13. Albertini, R., Nicklas, J., Fuscoe, J., Skopek, T., Branda, R., and O'Neill, J. *In vivo* mutations in human blood cells: biomarkers for molecular epidemiology. Environ. Health Perspect., *99:* 135–141, 1993.

14. Nicklas, J., Falta, M., Hunter, T., O'Neill, J., Jacobson-Kram, D., Williams, J., and Albertini, R. Molecular analysis of *in vivo HPRT* mutations in human T-lymphocytes. V. Effects of total body irradiation secondary to radioimmunoglobin therapy (RIT). Mutagenesis, *5:* 461–468, 1990.

15. O'Neill, J. P., Hunter, T. C., Sullivan, L. M., Nicklas, J. A., and Albertini, R. J. Southern-blot analyses of human T-lymphocyte mutants induced *in vitro* by γ-irradiation. Mutat. Res., *240:* 143–149, 1990.

16. Phillips, T. L. Rationale for initial clinical trials and future development of radioprotectors. Cancer Clin. Trials, *3:* 165–173, 1980.

17. Yuhas, J. M., Spellman, J. M., and Culo, F. The role of WR2721 in radiotherapy and/or chemotherapy. Cancer Clin. Trials, *3:* 211–216, 1980.

18. Hill, C. K., Nagy, B., Peraino, C., and Grdina, D. J. 2-[(Aminopropyl)amino]ethanethiol (WR-1065) is anti-neoplastic and anti-mutagenic when given during ^{60}Co γ-ray irradiation. Carcinogenesis (Lond.), *7:* 665–668, 1986.

19. Gupta, R., and Devi, P. U. Protection of mouse chromosomes against whole-body γ irradiation by SH-compounds. Br. J. Radiol., *59:* 625–627, 1986.

20. Littlefield, L. G., Joiner, E. E., Colyer, S. P., Sayer, A. M., and Frome, E. L. Modulation of radiation induced chromosome aberrations by DMSO, an OH radical scavenger. I. Dose response studies in human lymphocytes exposed to 220kV X-rays. Int. J. Radiat. Biol., *53:* 875–890, 1988.

21. Milas, L., Hunter, N., Stephens, C. L., and Peters, L. J. Inhibition of radiation carcinogenesis by *S*-2-(3-aminopropylamino)ethylphosphorothioic acid. Cancer Res., *44:* 5567–5569, 1984.

22. Grdina, D. J., Nagy, B., Hill, C. K., Wells, R. L., and Peraino, C. The radioprotector WR1065 reduces radiation-induced mutations at the hypoxanthine-guanine phosphoribosyl transferase locus in V79 cells. Carcinogenesis (Lond.), *6:* 929–931, 1985.

23. Nagy, B., and Grdina, D. J. Protective effects of 2-[(aminopropyl)amino]ethanethiol against bleomycin and nitrogen mustard-induced mutagenicity in V79 cells. Int. J. Radiat. Oncol. Biol. Phys., *12:* 1475–1478, 1986.

24. Grdina, D. J., Peraino, C., and Hill, C. K. Protective effect of *S*-2-(3-aminopropylamino)ethylphosphorothioic acid against induction of altered hepatocyte foci in rats treated once with γ-radiation within one day after birth. Cancer Res., *45:* 5379–5381, 1985.

25. Carnes, B. A., and Grdina, D. J. *In vivo* protection by the aminothiol WR-2721 against neutron-induced carcinogenesis. Int. J. Radiat. Biol., *61:* 567–576, 1992.

26. Grdina, D. J., Carnes, B. A., Grahn, D., and Sigdestad, C. P. Protection against late effects of radiation by *S*-2-(3-aminopropylamino)-ethylphosphorothioic acid. Cancer Res., *51:* 4125–4130, 1991.

27. Grdina, D. J., Kataoka, Y., Basic, I., and Perrin, J. The radioprotector WR-2721 reduces neutron-induced mutations at the hypoxanthine-guanine phosphoribosyl transferase locus in mouse splenocytes when administered prior to or following irradiation. Carcinogenesis (Lond.), *13:* 811–814, 1992.

28. Grdina, D. J., Sigdestad, C. P., and Carnes, B. A. Protection by WR1065 and WR151326 against fission-neutron-induced mutations at the *Hgprt* locus in V79 cells. Radiat. Res., *117:* 500–510, 1989.

29. Clark, L., Albertini, R., and Nicklas, J. *HPRT* mutations in human T-lymphocytes reflect radioprotective effects of the aminothiol, WR-1065. Carcinogenesis (Lond.), *17:* 2647–2653, 1996.

30. O'Neill, J. P., Sullivan, L. M., and Albertini, R. J. *In vitro* induction, expression and selection of thioguanine-resistant mutants with human T-lymphocytes. Mutat. Res., *240:* 135–142, 1990.

31. O'Neill, J. P., McGinniss, M. J., Berman, J. K., Sullivan, L. M., Nicklas, J. A., and Albertini, R. J. Refinement of a T-lymphocyte cloning assay to quantify the *in vivo* thioguanine-resistant mutant frequency in humans. Mutagenesis, *2:* 87–94, 1987.

32. Gibbs, R., Nguyen, P., Edwards, A., Civitello, A., and Caskey, C. Multiplex DNA detection and exon sequencing of the hypoxanthine-guanine phosphoribosyltransferase gene in Lesch-Nyhan families. Genomics, *7:* 235–244, 1990.

33. Chamberlain, J., Gibbs, R., Ranier, J., Nguyen, P., and Caskey, C. Deletion screening of the Duchenne muscular dystrophy locus via multiplex DNA amplification. Nucleic Acids Res., *16:* 11141–11156, 1988.

34. Clark, L., and Nicklas, J. TCR β PCR from crude preparations for restriction digest with sequencing. Environ. Mol. Mutagen., *27:* 34–38, 1996.

35. de Boer, J., Curry, J., and Glickman, B. A fast and simple method to determine the clonal relationship among T-lymphocytes. Mutat. Res., *288:* 173–180, 1993.

36. Denault, C., and Liber, H. The effects of hypoxia and cysteamine on X-ray mutagenesis in human cells. Radiat. Res., *135:* 98–107, 1993.

37. Grdina, D. J., and Sigdestad, C. P. Chemical protection and cell-cycle effects on radiation-induced mutagenesis. Cell Prolif., *25:* 23–29, 1992.

38. Giambarresti, L., and Jacobs, A. Radioprotectant in Military Radiobiology. Orlando: Academic Press, 1987.

39. Brown, P. Mechanism of action of aminothiol radioprotectors. Nature (Lond.), *213:* 363–364, 1967.

40. Smoluk, G., Fahey, R., and Ward, J. Equilibrium dialysis studies of the binding of radioprotector compounds to DNA. Radiat. Res., *107:* 194–204, 1986.

41. Grdina, D. J., Guilford, W. H., Sigdestad, C. P., and Giometti, C. S. Effects of radioprotectors on DNA damage and repair, proteins, and cell-cycle progression. Pharmacol. Ther., *39:* 133–137, 1988.

42. Wyatt, I., Moore, R., and Smith, L. Competition for polyamine uptake into rat lung slices by WR2721 and analogues. Int. J. Radiat. Biol., *55:* 463–472, 1989.

43. Murley, J. S., Grdina, D. J., and Meechan, P. J. Accumulation of CHO cells in G_2 phase following exposure to WR-1065. Radiat. Res., *126:* 223–228, 1991.

44. Holwitt, E., Koda, E., and Swenberg, C. Enhancement of topoisomerase I-mediated unwinding of supercoiled DNA by the radioprotector WR-33278. Radiat. Res., *124:* 107–109, 1990.

45. Grdina, D. J., Constantinou, A., Shigemaztsu, N., and Murley, J. Inhibition of topoisomerase IIα activity in CHO K1 cells by 2-[(aminopropyl)amino]ethanethiol (WR-1065). Radiat. Res., *138:* 44–52, 1994.