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Comparative Intestinal and Testes Toxicity of Four Aminothiols in Irradiated and Nonirradiated Mice*†

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ABSTRACT

Intestinal and testicular toxicity in groups of nonirradiated and irradiated mice were investigated after intraperitoneal injection of aminothiol compounds or saline. Four aminothiols were studied. Three were prodrugs: WR-2721, WR-3689, and WR-151327 and one was the active form of WR-2721: WR-1065. Thirty minutes after injection, the mice were sham-irradiated or bilaterally exposed (whole body) to ^{60}Co γ-irradiation at a dose rate of 1 Gy per min to a total dose of 15 Gy. Four days after injection, mice were euthanised, and the intestines and testes were removed and histologically examined. The intestinal crypt cell number was increased in all the irradiated mice given WR-compounds compared to controls ($P < 0.05$). Interestingly, the crypt cell number in nonirradiated mice given WR-1065 was also greater than control or WR-2721 ($P < 0.05$) treated mice. Germinal cell numbers from testes of mice administered aminothiols prior to radiation decreased or did not change. Some swelling of the seminiferous tubules was also observed. The germinal cell numbers in sham-irradiated mice were also less than the controls. Thus, aminothiol addition can provide limited protection to intestinal crypt cells but not to germinal cells of the testes in response to γ-irradiation. There is also evidence that aminothiols are toxic to the germinal cell layer of the seminiferous tubules when given to sham-irradiated mice.

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Introduction

The search for chemical radioprotectors for use by physicians and workers in high radiation areas has been ongoing even since ionizing radiation was found to be damaging to cells. The National Cancer Institute Radioprotectant Screening Program found that aminothiols such as WR-151327, WR-3689 and WR-2721 are the most effective radioprotective agents.[2]

Hydroxyl radicals (·OH), superoxide ($O_2^{\cdot}$), hydrogen peroxide (H_2O_2), hydrated electrons (e_{aq}) and hydrogen atoms (H) are generally considered the active species following cell exposure to ionizing radiation. These species subsequently react with cell constituents generating various free radical pathways which can damage DNA and essential enzymes as well as cause the release of proteolytic enzymes. These mechanisms ultimately lead to cell death. Alternatively, free radicals can be beneficial by destroying invading microorganisms and cancer cells, by assisting in reproduction (sperm maturation, sperm transportation and fertilization), and initiating wound healing processes. Free radicals are also involved in neurotransmission, iodination of thyroid hormones and calcium mobilization of bone.[1]

The exact mechanism of action of radioprotectors is yet to be defined. It is possible that radioprotectors act as free radical scavengers or terminate free radical propagation by hydrogen transfer mechanisms. There is evidence that chemical protectors act as scavengers of hydroxyl radicals, reduce oxygen levels decreasing free radical formation, and chelate with histones on deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) shielding them from free radical attack.[3] Recent studies using Electron Spin Resonance (ESR) techniques have shown that the radioprotector WR-1065 can induce the production of the free radical nitric oxide, NO·.[14]

However, like all drugs, chemical radioprotectants are themselves toxic at sufficiently high concentrations.[10] For some radioprotective agents there is a narrow concentration range between protection and toxicity.[10] Just as certain cells in the body are more radiosensitive than others, it is probable that some cells may be more sensitive to chemical radioprotectants. Since crypt cells of the intestine and the germinal cells of the testis are known to be very radiosensitive, these cells were studied for sensitivity to chemical radioprotectants.

Aminothiols such as WR-151327, WR-3689, and WR-2721 are well established as intestinal radioprotectors[11–13] but may be chemical toxins of the testis.[15,16] For example, the literature reports radioprotection and cytotoxicity of the testis by the aminothiol WR-2721.[7,8] WR-151327, WR-2721, WR-1065, and WR-3689 were examined and their effects on intestinal and testes toxicity were compared. The crypt cells of the intestine and germinal cells of the testis were measured histometrically, four days post-injection in nonirradiated and irradiated mice.

Materials and Methods

The aminothiols tested, figure 1, were obtained from two sources.*†

Male mice CD2F1 eight to 10 weeks old were also obtained.‡ Mice were allowed food and water ad libitum. They were divided into two groups: nonirradiated and irradiated. Each group was divided into five subgroups. Each subgroup had eight mice. All drugs were dissolved in sterile saline and injected i.p. The radioprotectants were added at the

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‡ National Cancer Institute, Frederick, MD.

CHEMICAL STRUCTURES OF AMINOTHIOLS TESTED

WR-2721

$H_2N\text{-}(CH_2)_3\text{-}NH\text{-}(CH_2)_2\text{-}S\text{-}PO_3H_2$

WR-1065

$H_2N\text{-}(CH_2)_3\text{-}NH\text{-}(CH_2)_2SH$

WR-3689

$CH_3\text{-}HN\text{-}(CH_2)_3\text{-}NH\text{-}(CH_2)_2\text{-}S\text{-}PO_3H_2$

WR-151327

$CH_3\text{-}HN\text{-}(CH_2)_3\text{-}NH\text{-}(CH_2)_3\text{-}S\text{-}PO_3H_2$

FIGURE 1. WR-2721, WR-1065 and WR-3689 were obtained from the National Cancer Institute, Bethesda MD, and WR-151327 was obtained from US Biosciences, Cockenham, PA.

minimum dose required for mice survival 30 days post-irradiation. The aminothiol prodrugs WR-2721, WR-3689, and WR-15327 were administered at a concentration of 200 mg per kg body weight (BW) and the active drug WR-1065 at a concentration of 80 mg per kg BW. The control group was injected with saline.

The irradiation procedure was the same as previously described.[15] Mice were placed in plastic containers approximately 15 minutes before total-body irradiation. They were irradiated in a bilateral ^{60}Co γ-ray field at a dose rate of 1.0 Gy per min, receiving a total dose of 15 Gy. The nonirradiated mice were treated identically except for the actual irradiation. Four days later the mice were euthanised by lethal inhalation of methoxyflurane.§ Testes and jejunum (~1 cm long), taken 5 cm from the ligament of Trietz, were immediately removed and fixed in saline formalin fixative. The tissues were embedded in paraffin wax, cut at four microns and stained with hematoxylin and eosin. A Bioquant Hipad Digitiser attached to a light microscope was used to measure the circumference and the number of crypt cells in the intestine. The circumference and number of cells in the germinal cell layer of the testes were measured in a similar manner. The microscope slides were decoded into experimental groups and the raw data was statistically analysed using the Kruskal-Wallis Test. Post hoc evaluations were made using Dunn's Test at the five percent significance level.

Results

In figure 2A is shown the appearance of the intestine of a mouse four days after injection i.p. with saline. The villi are intact and there are cells visible in the crypts of Lieberkuhn. Four days after i.p. injection, the aminothiols caused no histological damage to the intestine. The appearance of the intestine of a mouse injected i.p. with the aminothiol, WR-3689 (200 mg per kg BW) is shown in figure 2B. The villi are intact and crypt cells are visible. Four days following 15 Gy whole-body irradiation, the intestine gave the typical pictorial image shown in figure 2C. The villi have become blunted and crypt cells cannot easily be detected. When mice were injected with an aminothiol 30 min prior to radiation, the intestine appears as shown for WR-3689 in figure 2D. The villi are intact, cells can be identified in the crypts of Lieberkuhn, and there is new cell growth identified by more densely populated and darker staining cells along the villi and

§ Trademark: Metofane, Pitman-Moore, USA.

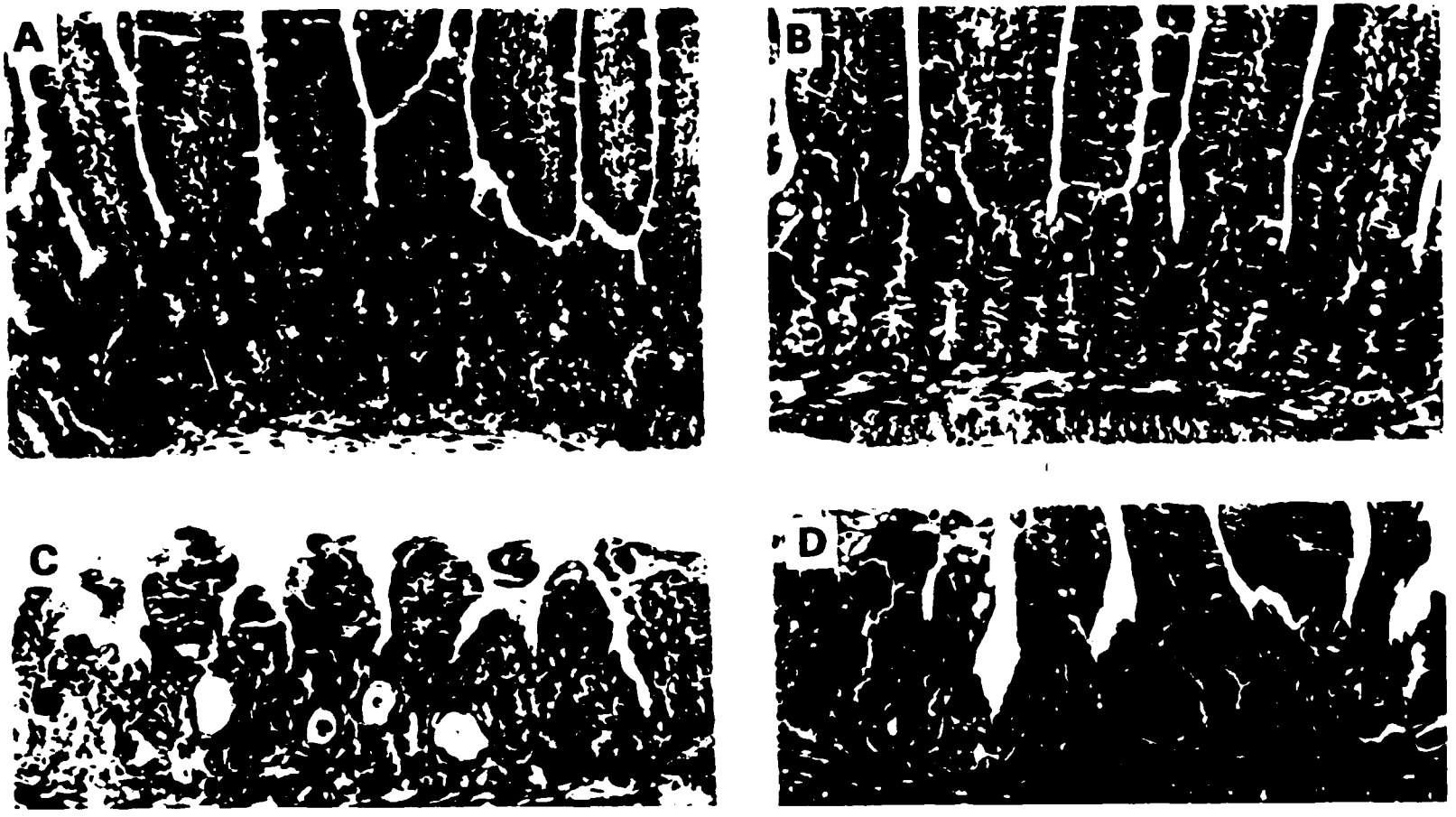


FIGURE 2 (A) Control intestine of a mouse injected with saline and euthanised four days later. The villi are intact and cells are present in the crypts of Lieberkuhn. (B) The intestine of a mouse given WR-3689 i.p. (200 mg per kg BW) and euthanised four days later. (C) The intestine of a mouse four days after 15 Gy whole-body irradiation from a ^{60}Co γ-ray source. (D) The intestine of a mouse given WR-3689 i.p. Thirty minutes after injection, the mouse was bilaterally exposed (whole-body) to ^{60}Co γ-irradiation at a dose of 1 Gy per min to a total dose of 15 Gy.

in the crypts. Similar results were obtained with WR-2721, WR-1065, and WR-151327.

Quantitative analysis of the numbers of crypts showed that i.p. injection of any of the aminothiols tested 30 minutes prior to 15 Gy whole-body irradiation resulted in a significant increase ($P < 0.05$) in the number of crypt cells when compared to the intestines of mice receiving irradiation alone. The mean cell number of crypt cells in irradiated intestines ± standard error of the mean (SEM) of eight mice was 1 ± 0.4. The results for the intestines of eight mice per group given aminothiols 30 minutes prior to irradiation are shown in figure 3B. The crypt cell numbers ± SEM were 24 ± 3 (WR-2721), 22 ± 6 (WR-1065), 30 ± 3 (WR-3689), and 15 ± 3 (WR-151327). Measurement of the circumference of these intestines showed no significant differences between groups ($P > 0.05$). When mice were given the aminothiols without irradiation the mean cell numbers from the intestines of mice given WR-2721, WR-3689, and WR-151327 showed no significant change compared to control ($P > 0.05$), figure 3A. However, there was a significant increase in crypt cell counts ($P < 0.05$) in mice given WR-1065, the active dephosphorylated form of WR-2721. WR-1065 is highly toxic, but the concentration of WR-1065 administered was equivalent to the concentration of WR-2721 required for radioprotection. There was no significant change in the circumferences of the intestines ($P > 0.05$).

An example of a transverse section of several seminiferous tubules of the testis from mice injected with saline i.p. and euthanised four days later is shown in figure 4A. Each seminiferous tubule is surrounded by an outer compact connective tissue and an inner basement membrane. Enclosed in the basement membrane are the specialized germinal epithelium and Sertoli's cells. The distinctive Sertoli's cell nucleus is ovoid or angular in shape, facilitating its discrimination from the spermatogenic cell that divides mitotically to produce several generations of cells. Intraperitoneal injection of 200 mg per kg BW WR-3689 caused degeneration of the germinal cell layer. In figure 4B is shown the transverse section of the seminiferous tubules from a mouse treated with WR-3689. Germinal cells are normally intermixed with Sertoli's cells along the basement membrane. In this case, there are occasional detachments of these cells. Also noted are small amounts of proteinaceous material within the lumen of the seminiferous tubules.

An example of a transverse section of the seminiferous tubules from mice administered saline i.p. and irradiated 30

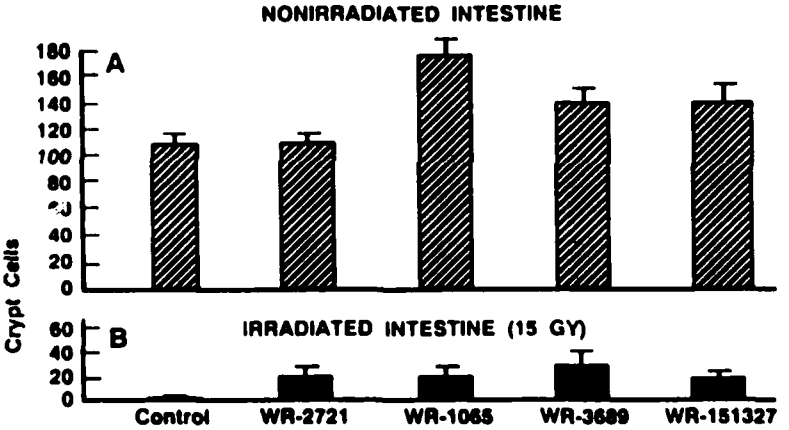
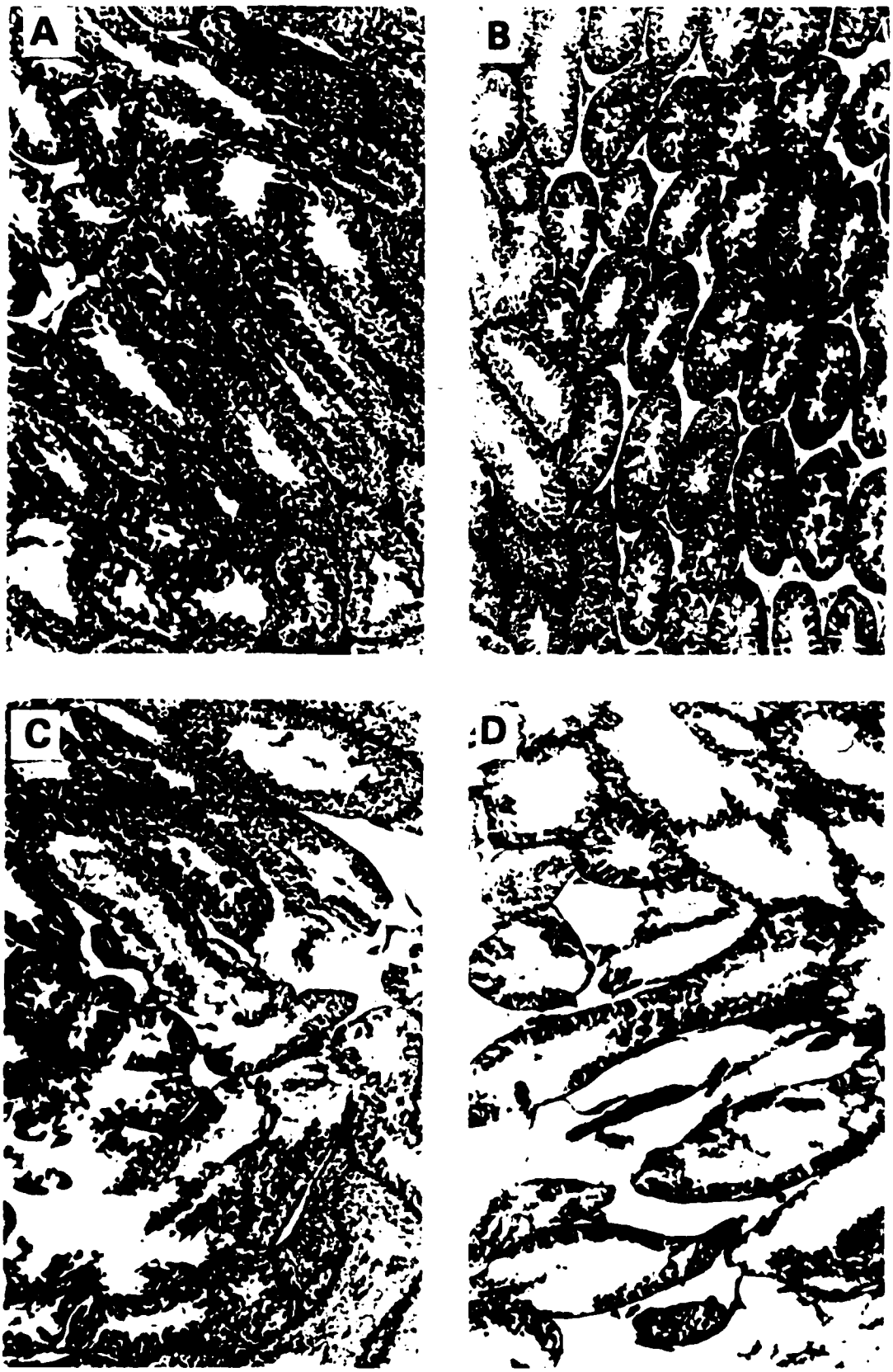


FIGURE 3. The numbers of crypt cells in intestines of (A) nonirradiated and (B) irradiated mice. Mice were injected i.p. with saline as control or with WR-2721, WR-1065, WR-3689, or WR-151327. Each group is the mean ± SEM of eight mice. All data was analysed by analysis of variance.


A
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min later with 15 Gy is shown in figure 4C. This photomicrograph indicated a reduction of cells in the germinal layer and absence of spermatozoa. The cells present had necrosis characterised by cytoplasmic swelling and vacuolization. Testes of mice injected with WR-3689 and 30 min later exposed to 15 Gy whole-body irradiation show visible depletion of the germinal cell layer four days later, figure 4D. The results for WR-2721, WR-1065, and WR-151327 were similar to WR-3689.

Four days after injection with aminothiols, nonirradiated mice had less cells present in the germinal cell layer (figure 5). The percentage decrease compared to the control group of eight mice was 34 percent with WR-2721, 38 percent with WR-1065, 24 percent with WR-3689, and 18 percent with WR-151327. The decrease in germinal cell number was statistically significant for WR-2721, WR-1065, and WR-3689 ($P < 0.05$). Statistically, there was no significant difference in the circumference of the seminiferous tubules of these mice ($P > 0.05$).

Following irradiation, there was a decrease in the number of cells present in the germinal cell layer compared to nonirradiated mice, ($P < 0.05$), figure 5. There was also a decrease in the germinal cell number from irradiated mice, pretreated with WR-2721, WR-1065, and WR-3689 when compared to the number of these cells in irradiated controls. This decrease in germinal cells was 19 percent for WR-2721, 18 percent for WR-1065, and 15 percent for WR-3689 (figure 5B). However, the results in figure 5B show that compared to irradiated controls, there is a seven percent increase in the number of germinal cells in irradiated mice pretreated with WR-151327. Only the decrease of 19 percent was significant for WR-2721 when compared to the control and WR-151327 ($P < 0.05$). The seminiferous tubules of the irradiated mice given WR-2721 and WR-3689 were swollen compare to the control mice and those given WR-1065 and WR-151327 ($P < 0.05$).

Discussion

These experiments showed that all the aminothiols tested were protective to the mitotic crypt cells in the irradiated intestine. Four days after injection of WR-2721, WR-1065, WR-3689, and WR-151327, there were significantly more crypt cells in the intestines of mice treated with these aminothiols compared to the intestines of untreated irradiated control mice ($P < 0.05$). Experiments by other investigators have also shown protection of the intestine by these aminothiols.[11–13] The present study compared the four aminothiols simultaneously in the same groups of animals and under the same radiation and experimental conditions.

Review of radiation protectors indicates that more than one mechanism may be involved in protection in biological systems.[3] Of importance are the mechanisms of interaction of the aminothiol radioprotectors with free radicals, since radiation-induced free radical production is a major cause of damage following exposure of biological systems to ionizing radiation. Recent studies have shown that the free radical nitric oxide ($NO^{\cdot}$) readily interacts with and is stabilized by thiols.[3] In the intestine, it has been shown that WR-1065 induces the production of $NO^{\cdot}$.[14] WR-2721 has also been shown to induce $NO^{\cdot}$ in the intestine; however, the effect of this drug is time

FIGURE 4. (A) The testis from a mouse injected with saline i.p. and euthanized 4 days later. (B) The testis of a mouse injected i.p. with WR-3689 (200 mg per kg BW) and euthanised four days later. (C) The testis of a mouse four days after 15 Gy whole-body irradiation from a ^{60}Co γ-ray source. (D) The testis from a mouse four days after WR-3689 (200 mg per kg BW i.p.) 30 minutes prior to whole-body exposure to γ-radiation from a ^{60}Co source.

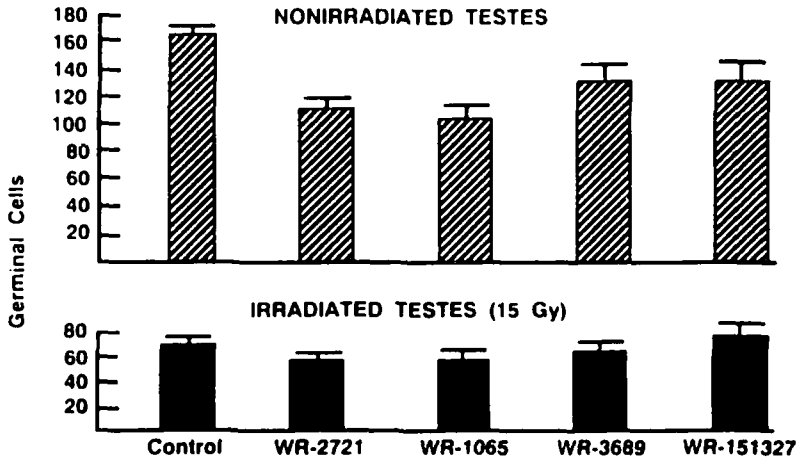

FIGURE 5. The number of germinal cells in the seminiferous tubules of the testes of (A) nonirradiated and (B) irradiated mice. Mice were injected i.p. with saline as control or with WR-2721, WR-1065, WR-3689 or WR-151327.

dependent, occuring as the WR-2721 is dephosphorylated to WR-1065. Furthermore, the involvement of NO in the conservation of gastric mucosal integrity has also been reported.[17]

In the present study, our experiments show WR-1065 increased the number of crypt cells ($P < 0.05$), but injection of the phosphorylated aminothiols had no significant change in crypt cell numbers in nonirradiated intestine. Therefore, it is possible that in the intestine the observed aminothiol/NO relationship contributes to the observed radioprotective effects of aminothiols in this organ.

Unlike the intestine, there was a decrease in the number of cells counted in the germinal cell layer of the nonirradiated testis, and this was significantly different for WR-2721, WR-1065, and WR-3689 ($P < 0.05$). The seminiferous tubules of the testes contain two kinds of cells, germ cells and Sertoli cells (figure 4).

The germ cell group represent the reproductive cells from the spermatogonial stem cells to spermatozoa. All but the spermatogonia are emeshed in the cytoplasm and are sustained structurally and nourished by the Sertoli cells,[6] figure 4. The specialized junctions which attach the Sertoli cells in the seminiferous tubules constitute a blood-testis barrier against exogenous substances.[6] Spermatogonia have free access to blood-borne nutrients and xenobiotics as they are located in the basal compartment (external to the barrier), and these are the cells which after several mitotic divisions move across the barrier to the luminal compartment to differentiate into preleptotene spermocytes.[6]

The chelating properties of WR-2721, WR-1065, WR-3689, and WR-151327 make it probable that they bind to metals and interfere with the synthesis of nucleoproteins by reducing the availability of sulfhydryl donors uniquely required by germ cells. The germ cells of the seminiferous tubules contain not only variants of histones found in somatic tissue but have at least five basic proteins rich in sulfhydryl containing amino acids unique to the germ cells.[18]

WR-151327 has been found to be less destructive to the germinal cell layer than WR-3689, which was less than WR-2721, with WR-1065 being the most destructive (figure 5). Cytotoxicity of the testes has been reported in nonirradiated mice given WR-2721[8] and WR-151327,[15,16] but our literature search revealed no studies carried out with WR-1065 or WR-3689.

At four days post-irradiation, none of the aminothiols injected protected the testes from radiation damage. There was no significant change ($P > 0.05$) in cell number when WR-151327, WR-3689, and WR-1065 were injected and a significant decrease ($P < 0.05$) in the cell numbers

from the irradiated control group when WR-2721 was injected 30 min prior to irradiation. Our results on WR-2721 are supported by Meistrich et al,[8] and our differences with other projects[1,7,9] can be explained by the experimental conditions, the concentration of aminothiol administered, and how "protection" of the testis is assessed. WR-151327 decreased the number of cells measured in the germinal cell layer in irradiated mice after 10 Gy irradiation and measured 10 days later;[15] however, our experiments showed no significant change ($P > 0.05$), suggesting that the damage to the testes increases with time.

In conclusion, our experiments show that aminothiol addition can provide limited protection to intestinal crypt cells but not to germinal cells of the testes in response to γ-irradiation. Furthermore, there is also evidence that aminothiols are toxic to the germinal cell layer of the seminiferous tubules when given to nonirradiated mice.

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References

1. BHARTIYA, J. H. C. and PAREEK, B. P.: Effects of S-2(3-aminopropylamino) ethylphosphorothioic acid (WR-2721) on the sensitivity of mouse spermatogonia A to radiation. Acta Radiol. Oncol. 23:65–68, 1984.
2. BROWN, D. Q., GRAHAM, W. J., MACKENZIE, L. J., PITTOCK, J., et al: Can WR-2721 be improved upon? Pharmac. Ther. 39:157–168, 1988.
3. CLANCY, R. M., MIYAZAKI, Y., and CANNON, P. J.: Use of thionitrobenzoic acid to characterize the stability of nitric oxide in aqueous solutions and in porcine aortic endothelial cell suspensions. Anal. Biochem. *191*:138–143, 1990.
4. ELLIS, L. C.: Free radicals in tissue culture. Part 1. Art to Science 9:1–6, 1990.
5. GRDINA, D. J. and SIGDESTAD, C.P.: Radioprotectors: The unexpected benefits. Drug Metabolism Rev. *20*:13–42, 1989.
6. HATAKEYAMA, S.: The male reproductive system. In: *Functional Endocrine Pathology*, vol 2. Kovacs K. and Asa, S. L., eds. St. Louis, Blackwell Scientific Publications, 1991, chapter 21, pp. 585–607.
7. MEISTRICH, M. L., FINCH, M. V., HUNTER, N., and MILAS, L.: Protection of spermatogonial survival and testicular function by WR-2721 against high and low doses of radiation. Int. J. Rad. Oncol. Biol. Phys. *10*:2099–2107, 1984.
8. MEISTRICH, M. L., FINCH, M., HUNTER, N., and MILAS, L.: Cytotoxic effects of WR-2721 on mouse testicular cells. Int. J. Rad. Oncol. Biol. Phys. *10*:1551–1554, 1984.
9. MILAS, L., HUNTER, N., and REID, B. O.: Protective effects of WR-2721 against radiation-induced injury of murine gut, testis, lung and lung tumor nodules. Int. J. Oncol. biol. Phys. *8*:535–538, 1982.
10. MONIG, H., MESSERSCHMIDT, O., and STREFFER, C.: Chemical radioprotection in mammals and man. In: *Radiation Exposure and Occupational Risks*. Scherer E., Streffer C., and Trott, K-R., eds. New York, Springer-Verlag, 1990, chapter 5, pp. 97–143.
11. MURRAY, D., MILAS, L., and MEYN, R. E.: Radioprotection of mouse jejunum by WR-2721 and WR-1065: Effects on DNA strand-break induction and rejoining. Rad. Res. *114*:268–280, 1988.
12. MURRAY, M. L., ALTSCHULER, E. M., HUNTER, N., FINCH, M. V., and MILAS, L.: Protection by WR-3689 against γ-ray induced intestinal damage: Comparative effect on clonic cell survival, mouse survival and DNA damage. Rad. Res. *120*:339–351, 1989.
13. SIGDESTED, C. P., GRDINA, D. J., CONNOR, A. M., and HANSON, W. R.: A comparison of radioprotection from three neutron sources and ^{60}Co by WR-2721 and WR-151327. Rad. Res. *106*:224–233, 1986.
14. STEEL-GOODWIN, L., ARROYO, C. M., GRAY, B., and CARMICHAEL, A. J.: EPR detection of nitric oxide dependent spin adducts in mouse jejunum. In: *Biology of Nitric Oxide. Part II. Enzymology, Biochemistry and Immunology*. Moncada, S., Marletta, M. A., Hibbs, J. B., Jr., and Higgs, E. A., eds. London, Portland Press, 1992, pp. 80–84.
15. STEEL-GOODWIN, L., KENDRICK, J. M., EGAN, J. E., and ECKSTEIN, J. M.: Pathological evaluation of WR-151327 administered orally in irradiated and nonirradiated male mice. Ann. Clin. Lab. Sci. 22:182–188, 1992.
16. VAN BEEK, M. E. A. B., DOAK, R. L., SIGDESTAD, C. P., and GRDINA, D. J.: Pathological effects of the radioprotector WR-151327 in mice. Rad. Res. *124*:79–84, 1990.
17. WHITTLE, B. J. R., LOPEZ-BELMONTE, J., and MONCADA, S.: Regulation of gastric mucosal integrity by endogenous nitric oxide: interactions with prostanoids and sensory neuropeptides in the rat. Br. J. Pharmacol. 99:607–611, 1990.
18. ZENICK, H. and CLEGG, E. D.: Assessment of male reproductive toxicity. A risk assessment approach. In: *Principals & Methods of Toxicology*. Editor Hayes, A. W., ed. New York, Raven Press, 1989, pp. 275–309.