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● *Original Contribution*

COMBINED MODALITY RADIOPROTECTION: THE USE OF GLUCAN AND SELENIUM WITH WR-2721

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Glucan, WR-2721, and selenium, three agents with distinct radioprotective mechanisms, were evaluated in C3H/HeN mice for survival-enhancing and hemopoietic-regenerating effects when administered alone or in combinations before exposure to ^{60}Co radiation. At LD50/30 radiation doses (radiation doses lethal for 50% of mice within 30 days postexposure), dose reduction factors of 1.21, 1.02, 1.37, 1.51, and 1.66 were obtained following glucan (75 mg/kg i.v., −20 hr), selenium (0.8 mg/kg, i.p., −20 hr), WR-2721 (200 mg/kg, i.p., −30 min), glucan + WR-2721, and glucan + selenium + WR-2721 treatments, respectively. All treatments increased numbers of hemopoietic stem cells as measured by the day 12 endogenous spleen colony-forming unit (E-CFU) assay; the most significant E-CFU effects, however, were observed following glucan + WR-2721 and glucan + selenium + WR-2721 treatments. Combined modality treatments were also more effective than single-agent treatments at accelerating bone marrow and splenic granulocyte-macrophage colony-forming cell (GM-CFC) regeneration. These results demonstrate the value of multiple-agent radioprotectants.

Radioprotection, Glucan, Selenium, WR-2721, Drug interactions, Hemopoiesis.

INTRODUCTION

Recent radiation accidents at Chernobyl (U.S.S.R.) and at Goiania (Brazil) have again focused attention on the potential value of agents that could mitigate the biological effects of radiation exposure. Such agents, commonly called radioprotectants, could be valuable not only for individuals who may be exposed to radiation during accident rescue and/or clean-up activities, but also for astronauts who may be subjected to predictable radiation exposures, and for individuals undergoing radiotherapy.

During the past several decades, studies of numerous radioprotective agents have led to the realization that not all radioprotectants mitigate damage through similar mechanisms (9, 13, 20, 37). Because various radioprotective agents differ in mechanisms of action and in optimal administration times with respect to radiation exposure, the use of multiple agents may in some instances provide significantly better protection than single agents. In the studies described here, combinations of such agents were administered to mice and evaluated for effects on survival enhancement and on hemopoietic recovery. By design, individual agents were used at doses that induce minimal-to-no toxic or performance-degrading side effects.

These particular studies describe the combined use of glucan, WR-2721, and selenium. Glucan is a β,1-3 polyglucose immunomodulator isolated from the inner cell wall of the yeast *Saccharomyces cerevisiae* (10). This agent has been demonstrated to increase survival by enhancing host resistance to life-threatening postirradiation opportunistic infections and by accelerating hemopoietic regeneration (21, 22, 25, 28, 30). WR-2721 is a synthetic sulfhydryl compound shown to radioprotect by free radical scavenging, hydrogen atom donation, and induction of hypoxia (9, 35, 38). Selenium, an essential trace element, is a component of endogenous antioxidant systems known to be involved in the reduction of radiation-induced reactive oxygen species (8, 15, 37). Our results demonstrate that glucan administered in combination with WR-2721 enhances survival better than either agent administered alone, and that this effect can be further enhanced by the addition of selenium to this treatment

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combination. In comparison to single-agent treatments, combination treatments also more effectively accelerate postirradiation hemopoietic recovery.

METHODS AND MATERIALS

Mice

C3H/HeN female mice (~20 g) were used.[‡] Mice were maintained in an AAALAC (American Association for Accreditation of Laboratory Animal Care) accredited facility in micro-isolator cages on hardwood-chip, contact bedding and were provided commercial rodent chow and acidified tap water (pH 2.5) *ad libitum*. Animal rooms were equipped with full-spectrum light from 6 a.m. to 6 p.m. and were maintained at 70 ± 2°F with 50 ± 10% relative humidity, and at least 10 air changes per hour of 100% conditioned fresh air. Upon arrival, all mice were tested for *Pseudomonas* and quarantined until test results were obtained. Only healthy mice were released for experimentation. Prior to performance, all animal experiments were approved by the Institutional Animal Care and Use Committee of the Armed Forces Radiobiology Research Institute (AFRRI).

Glucan, WR-2721, and selenium

Endotoxin-free particulate glucan (10) was used[§] and intravenously (i.v.) administered (75 mg/kg) to mice ~ 20 hr before the start of irradiation. Selenium, as sodium selenite,[**] was intraperitoneally (i.p.) administered to mice ~ 20 hr before the start of irradiation at a dose of 0.8 mg selenium/kg. WR-2721 was obtained from Walter Reed Army Institute of Research (Washington, DC) and administered i.p. (200 mg/kg) ~ 30 min before the start of irradiation. All drugs were diluted in pyrogen-free saline and administered in a 0.5-ml volume. Control mice received 0.5-ml injections of pyrogen-free saline. Because no significant differences were observed among control mice receiving i.p., i.v., or both i.p. and i.v. saline injections, data from all saline-treated mice were pooled.

Irradiation

The AFRRI ^{60}Co source was used to administer bilateral total-body gamma radiation. Mice were placed in ventilated Plexiglas containers and irradiated at a dose rate of 0.4 Gy/min. Dosimetry was determined by ionization chambers as previously described (31). Radiation doses ranged from 6–16 Gy.

Survival assays

Irradiated mice were returned to the animal facility and cared for routinely. Survival was checked and recorded daily for 30 days; on day 31, surviving mice were euthanized by cervical dislocation. Each treatment group within each experiment consisted of ~10 mice. Experiments were repeated to obtain an "n" of at least 40 animals for each treatment group at each radiation dose. The percentage of mice surviving each radiation dose at 30 days postexposure was used to construct probit-plot survival curves (11, 12). Dose reduction factors (DRF's) were calculated by dividing the treatment LD50/30 radiation doses by the saline LD50/30 radiation dose.

Endogenous spleen colony-forming unit assay

Pluripotent hemopoietic stem cell recovery was evaluated using the endogenous spleen colony-forming unit (E-CFU) assay (32). Mice were exposed to 6–13 Gy of total-body radiation to partially ablate endogenous hemopoietic stem cells. Twelve days after irradiation, mice were euthanized by cervival dislocation; their spleens were removed, fixed in Bouin's solution, and the number of grossly visible spleen colonies counted. Each treatment group within each experiment consisted of ~5 mice. Experiments were repeated to obtain an "n" of at least 20 animals for each treatment group at each radiation dose. Student's t-test was used to determine statistical differences in E-CFU data.

Granulocyte-macrophage colony-forming cell assay

Hemopoietic progenitor cells committed to granulocyte and/or macrophage development were assayed by a modification of the *in vitro* granulocyte-macrophage colony-forming cell (GM-CFC) assay (27). Colonies (>50 cells) were counted after 10 days incubation in a 37°C humidified environment containing 5% CO_2. The cell suspensions used for these assays represented tissues from 3–12 normal, irradiated, or treated and irradiated mice at each time point. Cells were flushed from femurs with 3 ml McCoy's 5A medium[††] containing 5% heat-inactivated fetal bovine serum. Spleens were pressed through stainless-steel mesh screen, and the cells were washed from the screen with 6 ml medium. The total number of nucleated cells in each suspension was determined by hemocytometer. Experiments were repeated three times; data were pooled and analyzed using Student's t-test.

RESULTS

Survival-enhancing effects of glucan, WR-2721, and selenium

Compared to saline-treated mice, survival was enhanced by treatment with glucan, WR-2721, or the combination of these agents (Fig. 1). LD50/30 values of 7.82 [7.74, 7.89] Gy, 9.45 [8.22, 10.40] Gy, 10.73 [10.57, 10.89] Gy, and 11.77 [11.23, 12.29] Gy were obtained in mice treated with saline, glucan, WR-2721, and glucan + WR-2721, respectively. These values resulted in DRF's of 1.21

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[**] Sigma, St. Louis, MO.
[††] Gibco, Grand Island, NY.

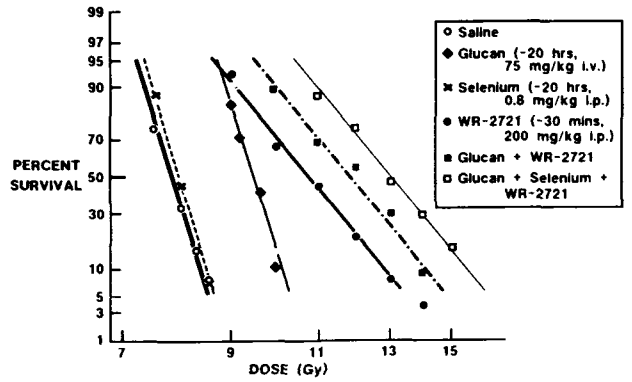

Fig. 1. Effects of combined glucan, selenium, and WR-2721 treatments on survival of irradiated C3H/HeN mice.

for glucan, 1.37 for WR-2721, and 1.51 for glucan + WR-2721 treatments. The slope of the survival curve of glucan-treated mice was identical (i.e., parallel) to that of saline-treated mice. The slopes of the survival curves of WR-2721-treated mice and glucan + WR-2721-treated mice were parallel to each other, however they differed significantly ($p < 0.001$) from those of saline-treated or glucan-treated mice. The administration of selenium in combination with glucan and WR-2721 further enhanced survival, resulting in an LD50/30 value of 12.97 [12.67, 13.27] Gy and a DRF of 1.66. The slope of this survival curve was parallel to those of WR-2721 and glucan + WR-2721. Selenium alone, or the administration of selenium in combination with glucan or WR-2721, did not enhance survival above that induced by saline, glucan alone, or WR-2721 alone. The LD50/30 values for selenium, selenium + glucan, and selenium + WR-2721 were 7.95 [7.68, 8.18] Gy, 9.58 [9.42, 9.75] Gy, and 10.88 [10.67, 11.03] Gy (data not graphed), respectively.

Effects of glucan, WR-2721, and selenium on hemopoietic regeneration

E-CFU studies. E-CFU numbers in saline-treated mice ranged from 7.2 ± 1.0 to 0.2 ± 0.1 following exposure to 6–8 Gy, respectively (Table 1). Glucan treatment significantly increased E-CFU numbers following all radiation exposures. Confluent colony formation (i.e., too many colonies to accurately count) was observed following 6 Gy, while 30.0 ± 2.5, 7.8 ± 1.0, and 3.6 ± 0.7 colonies were observed following 7, 8, and 9 Gy, respectively. WR-2721 treatment also significantly increased E-CFU numbers at all radiation doses, ranging from confluent colony formation at 6, 7, and 8 Gy to 10.4 ± 1.8, 6.7 ± 1.1, and 2.0 ± 0.7 E-CFU's, respectively, at 9, 10, and 11 Gy. Glucan + WR-2721 treatment increased E-CFU numbers more than either agent alone. For example, after a 9-Gy radiation exposure, 34.4 ± 3.9 E-CFU's were observed in mice treated with glucan + WR-2721 compared to 3.6 ± 0.7 E-CFU's in glucan-treated mice and 10.4 ± 1.8 E-CFU's in WR-2721-treated mice. Administration of glucan + selenium + WR-2721 even further increased E-CFU numbers; after an 11-Gy exposure, 8.4 ± 0.9 E-CFU's were observed in mice treated with all three agents compared to only 3.7 ± 0.5 E-CFU's in mice treated with glucan + WR-2721 ($p < 0.0001$).

Cellularity and GM-CFC studies. Recovery of bone marrow and splenic cellularity and GM-CFC's in 9-Gy-irradiated mice pretreated with glucan, WR-2721, selenium, or combinations of these agents was used to further evaluate the hemopoietic effects of these treatments (Tables 2, 3). Bone marrow cellularity in saline-treated mice was reduced to ~3% of normal at day 4 postirradiation and recovered to only ~14% of normal at day 13. Bone marrow cellularities in mice treated with WR-2721, glucan + WR-2721, or glucan + selenium + WR-2721 were significantly greater than that of saline control mice at all

Table 1. Effects of combined glucan, selenium, and WR-2721 on endogenous spleen colony formation in irradiated C3H/HeN mice

	Radiation Dose (Gy)							
	6	7	8	9	10	11	12	13
Saline	7.2 ± 1.0	1.6 ± 0.5	0.2 ± 0.1	0 ± 0	**	**	**	**
Glucan	confluent*	30.0 ± 2.5*	7.8 ± 1.0*	3.6 ± 0.7*	0 ± 0	**	**	**
Selenium	8.2 ± 1.1	4.5 ± 0.9*	0.9 ± 0.3*	0 ± 0	**	**	**	**
WR-2721	confluent*	confluent*	confluent*	10.4 ± 1.8*	6.7 ± 1.1	2.0 ± 0.7	0 ± 0	**
Glucan + WR-2721	confluent*	confluent*	confluent*	34.4 ± 3.9*†	25.0 ± 3.9†	3.7 ± 0.5†	0 ± 0	**
Glucan + Selenium + WR-2721	**	**	confluent*	confluent*@	confluent@	8.4 ± 0.9@	4.0 ± 0.8@	1.3 ± 0.5

Glucan (75 mg/kg i.v.) and selenium (0.8 mg/kg i.p.) were administered 20 hours prior to irradiation; WR-2721 (200 mg/kg i.p.) was administered 30 minutes prior to irradiation. Data represent mean ± standard error of spleen colony counts from at least 20 spleens.

* $p < 0.05$, with respect to saline values.
† $p < 0.05$, with respect to WR-2721 values.
● $p < 0.05$, with respect to Glucan + WR-2721 values.
** Not done.

Table 2. Effect of glucan, selenium, and WR-2721 on bone marrow and splenic cellularity in 9-Gy-irradiated C3H/HeN mice ($\times 10^6$)

	Day Postirradiation		
Bone Marrow	4	9	13
Normal 4.8 ± 0.53			
Saline	0.13 ± 0.02	0.54 ± 0.08	0.68 ± 0.11
Glucan	0.36 ± 0.05*	0.72 ± 0.09	0.94 ± 0.09
WR-2721	1.07 ± 0.11*	2.14 ± 0.32*	3.72 ± 0.58*
Glucan + WR-2721	1.37 ± 0.18*	3.42 ± 0.34*	3.94 ± 0.49*
Selenium	0.29 ± 0.02*	0.61 ± 0.07	0.74 ± 0.14
Glucan + Selenium + WR-2721	1.41 ± 0.12*	3.99 ± 0.56*	5.07 ± 0.73*
Spleen			
Normal 90.4 ± 10.8			
Saline	10.40 ± 0.83	8.30 ± 0.91	5.81 ± 1.11
Glucan	11.88 ± 0.79	12.12 ± 0.96*	12.71 ± 1.10*
WR-2721	13.37 ± 0.81*	16.29 ± 2.33*	26.42 ± 1.90*
Glucan + WR-2721	15.03 ± 0.83*†	33.10 ± 2.89*†	53.85 ± 3.62*†
Selenium	11.30 ± 0.94	8.80 ± 1.11	7.23 ± 0.79
Glucan + Selenium + WR-2721	17.77 ± 1.22*	38.16 ± 2.60*	79.00 ± 8.18*@

Glucan (75 mg/kg i.v.) and selenium (0.8 mg/kg i.p.) were administered 20 hours prior to irradiation; WR-2721 (200 mg/kg i.p.) was administered 30 minutes prior to irradiation. Data represent mean ± standard error of three experiments.
* $p < 0.05$, with respect to saline values.
† $p < 0.05$, with respect to WR-2721 values.
@ $p < 0.05$, with respect to Glucan + WR-2721 values.

time points evaluated; cellularities in these mice ranged from ~22–28% of normal at day 4 postexposure to ~77–105% of normal at day 13. Mice treated with only glucan or only selenium exhibited increased bone marrow cellularities at day 4 postexposure, but not at day 9 or 13. Splenic cellularity in saline-treated mice was reduced to ~12% of normal at day 4 postexposure and decreased to ~6% of normal at day 13. In contrast, splenic cellularities in all mice except those receiving selenium only, increased postirradiation; at day 13, cellularities in mice receiving glucan, WR-2721, glucan + WR-2721, and glucan + selenium + WR-2721, respectively, were ~14%, ~29%, ~60%, and ~87% of normal. In addition, splenic cellularity in mice treated with glucan + WR-2721 was significantly ($p < 0.005$) greater than that in WR-2721-treated mice, and splenic cellularity in mice treated with

Table 3. Effect of glucan, selenium, and WR-2721 on bone marrow and splenic GM-CFC recovery in 9-Gy-irradiated C3H/HeN mice

	Day Postirradiation		
Bone Marrow	4	9	13
Normal 5096 ± 612			
Saline	30 ± 4	2 ± 1	3 ± 1
Glucan	160 ± 21*	17 ± 1*	66 ± 5*
WR-2721	289 ± 33*	58 ± 4*	208 ± 13*
Glucan + WR-2721	626 ± 59*†	308 ± 41*†	465 ± 51*†
Selenium	97 ± 8*	17 ± 3*	26 ± 3*
Glucan + Selenium + WR-2721	776 ± 96*	698 ± 47*@	1014 ± 87*@
Spleen			
Normal 2531 ± 228			
Saline	0 ± 0	0 ± 0	0 ± 0
Glucan	0 ± 0	12 ± 2*	140 ± 9*
WR-2721	5 ± 1*	49 ± 3*	528 ± 56*
Glucan + WR-2721	8 ± 1*	166 ± 21*†	1345 ± 169*†
Selenium	0 ± 0	9 ± 1*	64 ± 4*
Glucan + Selenium + WR-2721	12 ± 1*@	294 ± 23*@	3555 ± 569*@

Glucan (75 mg/kg i.v.) and selenium (0.8 mg/kg i.p.) were administered 20 hours prior to irradiation; WR-2721 (200 mg/kg i.p.) was administered 30 minutes prior to irradiation. Data represent mean ± standard error of three experiments.
* $p < 0.05$, with respect to saline values.
† $p < 0.05$, with respect to WR-2721 values.
@ $p < 0.05$, with respect to Glucan + WR-2721 values.

glucan + selenium + WR-2721 was significantly ($p < 0.05$) greater than that in glucan + WR-2721-treated mice.

Femoral GM-CFC content in saline-treated mice was severely reduced following irradiation; at day 13 postexposure, the number of GM-CFC's per femur in these mice was ~0.06% of normal. Glucan, WR-2721, glucan + WR-2721, selenium, and glucan + selenium + WR-2721 treatments all significantly increased femoral GM-CFC numbers. At day 13 postexposure, the numbers of GM-CFC per femur in mice receiving these treatments were ~1%, ~4%, ~9%, ~0.5%, and ~20% of normal, respectively. In addition, glucan + WR-2721-treated mice exhibited significantly ($p < 0.01$) more GM-CFC's than WR-2721-treated mice, and glucan + selenium + WR-2721-treated mice exhibited significantly ($p < 0.005$) more GM-CFC's than glucan + WR-2721-treated mice. Although no splenic GM-CFC's could be detected at any time postirradiation in saline-treated mice (i.e., 0% of normal), all drug-treated groups did exhibit splenic GM-CFC recovery. At day 13, splenic GM-CFC numbers in mice treated with glucan, WR-2721, glucan + WR-2721, selenium, and glucan + selenium + WR-2721 were ~6%, ~21%, ~53%, ~3%, and ~140% of normal, respectively. As in the bone marrow, glucan + WR-2721 produced greater effects than WR-2721 treatment, and glucan + selenium + WR-2721 treatment produced greater effects than glucan + WR-2721 treatment.

DISCUSSION

The exposure of mammals to a single whole-body dose of ionizing radiation results in a complex set of symptoms whose onset, nature, and severity are a function of both total radiation dose and radiation quality (6). In general, radiation injury can be classified into three syndromes affecting the hemopoietic, the gastrointestinal, and the central nervous systems at progressively increasing radiation doses. The hemopoietic syndrome becomes evident at the lowest radiation doses (<10 Gy) and is manifested by hemopoietic stem cell depletion (5, 6) and ultimately by depletion of mature blood cells (2, 29), which, whether destroyed directly by irradiation or lost naturally through attrition, cannot be regenerated without hemopoietic stem cells. In turn, the loss of mature blood cells severely impairs antimicrobial immunity, and ultimately death ensues due to invasive opportunistic infections (3, 14).

A variety of agents can be radioprotective in the dose range of the hemopoietic syndrome. For example, if used at optimal doses, all three agents used in these studies (i.e., glucan, selenium, and WR-2721) can individually enhance survival to varying degrees following radiation doses that induce the hemopoietic syndrome (22, 26, 36, 38). In these particular studies, we have also demonstrated good radioprotective effects of suboptimal doses of these agents when used in combination. Radioprotection following administration of combinations of agents has previously been reported (1, 19). Maisin *et al.* evaluated primarily combinations of sulfhydryl agents, while Ainsworth *et al.* evaluated AET used in combination with endotoxin. Our studies differ in that we have evaluated the combined effects of three agents that radioprotect by distinct mechanisms. WR-2721 enhances survival by protecting cells from radiation-induced lethality through free-radical scavenging, hydrogen atom donation, induction of hypoxia, or combinations of these mechanisms (9, 35, 38). Hemopoietic stem and progenitor cells are among the cells best protected by WR-2721; however, WR-2721 also protects a variety of other cell types (9). Because WR-2721 is most effective when administered shortly before radiation exposure, either WR-2721 itself, or immediate metabolites, appear to mediate its radioprotective effects. Unlike WR-2721 which affects multiple cell types, glucan appears to act specifically on cells of hemopoietic origin (Dr. M. L. Patchen, unpublished data, September, 1988). If administered intravenously 15 min before radiation exposure some glucan-containing substances may also radioprotect by free radical scavenging or induction of hypoxia (18). However, evidence suggests that the glucan dose and injection schedule used in the studies presented here radioprotects by enhancing the proliferative capacity of surviving hemopoietic stem and progenitor cells (21, 22, 24, 25, 28). This effect, at least in part, appears to be mediated through the sustained induction of cytokines important in the stimulation and/or regulation of hemopoietic proliferation and function (23, 27). In contrast to either WR-2721 or glucan, induction of endogenous oxidative substances, such as glutathione peroxidase, is the probable mechanism through which selenium mediates its radioprotective effect (37).

The studies presented in this paper demonstrate that, compared to single glucan, selenium, or WR-2721 treatments, combinations of glucan + WR-2721 and glucan + selenium + WR-2721 produce additive-to synergistic survival-enhancing effects. The manner in which single versus combination treatments altered the slopes of their respective survival curves is interesting. For example, as has been shown previously (36), WR-2721 treatment protracts the survival curve, indicating that this agent enhances survival more effectively at high than at low radiation doses. In contrast, neither glucan (this paper) nor selenium (36) alone alter survival curve slopes, indicating equal protection with these agents at both low and high radiation doses. When used in combination with WR-2721, however, both glucan and selenium produce survival curves parallel to that of WR-2721, further increasing WR-2721's ability to selectively enhance survival better at high radiation doses.

Hemopoietic regeneration is also significantly accelerated in mice treated with glucan + WR-2721 and glucan + selenium + WR-2721 when compared to mice receiving single-agent treatments. Furthermore, the hemopoietic effects of these combinations as well as those of the single

agents paralleled their survival-enhancing effects, supporting the concept that accelerated hemopoietic regeneration reduces susceptibility to potentially lethal postirradiation infections, and consequently, enhances survival. Accelerated hemopoietic recovery has previously been demonstrated in mice receiving either WR-2721 or glucan before radiation (16, 21, 28, 33, 34). In the case of WR-2721, such recovery has been attributed to survival of a greater number of stem cells from which hemopoietic regeneration is initiated. In the case of glucan, such recovery has been attributed to enhanced proliferation of surviving stem cells. The further accelerated hemopoietic recovery observed here in mice treated with glucan + WR-2721 could be explained if WR-2721 preserved larger numbers of stem cells on which glucan could then exert its proliferative effects. Whether selenium further contributed to accelerated hemopoietic regeneration by reducing stem cell lethality (as suggested for WR-2721), enhancing stem cell proliferation (as suggested for glucan), or by yet undetermined mechanisms is not clear. We have previously demonstrated that pretreatment of mice with selenium (1.6 mg/kg) increased the radioprotective effect and decreased the lethal toxicity of WR-2721 (36). In the present study, a lower dose of selenium was used, which by itself did not have a radioprotective effect. Although there are a number of possible mechanisms by which selenium could act in conjunction with WR-2721 (37), the most probable mechanism, considering the dose and time of selenium administration, is induction of glutathione peroxidase. This selenium-containing enzyme may reduce stem cell lethality by reducing the level of deleterious hydroperoxides formed during radiation exposure, or perhaps even as byproducts in the metabolism of WR-2721.

The most effective radioprotective combination evaluated in these studies was glucan + selenium + WR-2721, which produced a DRF of 1.66. If used at sufficiently high doses, WR-2721 alone could produce greater DRF's. However, one of the goals of these studies was to evaluate whether relatively high DRF's could be obtained using doses of individual agents that lacked toxic or behavioral effects. High doses of WR-2721 are known to induce both toxicity and detrimental effects on performance (4, 7, 17). In fact, essentially all radioprotectants tested to date result in performance decrements at the best radioprotective dosages (Dr. Michael Landauer, oral communication, August, 1988). Although the side effects (e.g., nausea, vomiting, performance decrements) may not constitute major problems in clinical environments where patients are closely monitored and are not physically active, such effects would be particularly undesirable in radiation rescue and/or clean-up environments where performance and stamina could be critical to the success of the operation. Based on these considerations, doses of WR-2721 and selenium that induce only minimal toxicity (17, 36) were used in these studies. These agents administered in combination with glucan were not toxic; however, the behavioral effects of these combinations remain to be determined.

In conclusion, these studies suggest that a "cocktail" approach to radioprotection allows the exploitation of multiple protective mechanisms. In addition, such an approach allows doses of single agents to be reduced to nontoxic levels while maintaining respectable DRF's. The value of including a hemopoietic stimulant in such cocktails has also been illustrated. Future studies will determine whether agents operating through yet additional radioprotective mechanisms may further enhance the effectiveness of these cocktails.

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