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SELENIUM PRETREATMENT ENHANCES THE RADIOPROTECTIVE EFFECT AND REDUCES THE LETHAL TOXICITY OF WR-2721

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Although WR-2721, S-2-(3-aminopropylamino)ethylphosphorothioic acid, is an effective radioprotector, its use is limited by its toxicity. Combining WR-2721 with other agents might decrease its toxicity and/or increase its effectiveness. The effect of selenium (Se) pretreatment on the acute toxicity and radioprotective effect of WR-2721 was studied in male CD2F1 mice. Injection of 1.6 mg/kg Se 24 hr before WR-2721 (800–1200 mg/kg, IP) decreased the lethality of WR-2721 significantly. Lower doses of Se were also effective, but simultaneous administration was not effective. Se injection alone (1.6 mg/kg) 24 hr before cobalt-60 irradiation increased the survival (dose reduction factor, DRF = 1.1) significantly. A synergistic effect on post-irradiation survival was observed when Se was injected 24 hr before WR-2721 (200–600 mg/kg IP ½ before irradiation). For example, after exposure to 22 Gy (1 Gy/min), 30-day survival was 100% when mice were treated with both Se and 600 mg/kg WR-2721, and was 13% with WR-2721 alone. The DRF after 400 mg/kg WR-2721 was 2.6 with Se compared to 2.2 without Se pretreatment. Alkaline phosphatase activity in bone marrow cells and serum was significantly depressed after treatment with 1.6 mg/kg Se, suggesting that a retardation of conversion of WR-2721 to its active free sulfhydryl form through the action of alkaline phosphatase might be partly responsible for the effects of Se. Other possible mechanisms related to the antioxidant properties of Se are under investigation.

KEY WORDS: WR-2721; S-2-(3-aminopropylamino)ethylphosphorothioic acid; radioprotection; selenium; alkaline phosphatase; sulfhydryl compounds.

INTRODUCTION

The study of chemical radioprotectors *in vivo* and *in vitro* has led to a better understanding of the mechanisms of radiation damage, as well as providing potential practical applications. The first successful study of *in vivo* radioprotection is usually attributed to Patt *et al.*,[1] who observed protection of lethally-irradiated rats by cysteine injected before irradiation. During the past four decades, thousands of chemicals have been studied for their radioprotective effect. The radioprotector that provides the greatest protection against radiation-induced lethality in mice is S-2-(3-aminopropylamino)ethylphosphorothioic acid (WR-2721; ethiophos; amifostine).[2] The use of this drug for the protection of normal tissue in patients undergoing radiotherapy or chemotherapy or for accidental nuclear exposure is somewhat limited by the drug's toxicity, which includes nausea and vomiting in humans[3] and behavioral toxicity (performance decrement) in experimental animals.[4] Some research has been aimed at studying combinations of radioprotective agents, preferably those with different mechanisms of action.[5] This could allow the use of lower doses of the most

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effective agents, such as WR-2721. Studies from our laboratory have indicated that radiation sensitivity might be affected by agents that alter glutathione peroxidase activity,[6–8] including selenium.[9] For this reason, the combined radioprotective effect of selenium and WR-2721 was studied.

MATERIALS AND METHODS

Male CD2F1, (BALB/c × DBA/2)F1, mice were obtained from Charles River Laboratories, Wilmington, MA and quarantined and acclimated for at least 2 wks before experimentation. The mice were maintained in cages with filter lids and fed standard lab chow and acidified water, pH 2.4, *ad libitum*. Sodium selenite (Sigma Chemical Co., St. Louis, MO), WR-2721 and WR-1065, 2-(3-aminopropylamino)ethanethiol dihydrochloride, (Drug Synthesis and Chemistry Branch, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD) were injected IP in neutralized saline (pH 7–8) in a volume that was 1% of the mouse body weight. Mice in plexiglass boxes were irradiated bilaterally with cobalt-60 at 1 Gy/min.

In separate experiments, serum and bone marrow cells were obtained 24 hr after injection of selenium (Se) as sodium selenite for determination of alkaline phosphatase activity. Bone marrow was obtained from the femur by flushing with cold phosphate-bufferd saline, pH 7.0. Cells obtained on centrifugation were lysed by brief sonication. Alkaline phosphatase was determined by the method of Bowers and McComb,[10] and enzyme units were calculated from the change in absorbance over time.

RESULTS

Lethality from WR-2721 in CD2F1 mice is generally not observed at doses lower than 800 mg/kg. Administration of sodium selenite (1.6 mg/kg Se) 24 hr before lethal doses of WR-2721 (800–1200 mg/kg) resulted in a significant increase in the number of survivors (Table I). This dose of Se was approx. 1/4 the LD_{50} dose in this strain of mice. Simultaneous administration of Se did not reduce the toxicity of WR-2721.

The effect of Se pretreatment on postirradiation survival of animals treated approx. $\frac{1}{2}$ hr before irradiation with various doses of WR-2721 is shown in Table II. Se alone provided a mild radioprotective effect. All of the animals survived a radiation dose of

TABLE I
Effect of selenium pretreatment on acute toxicity of WR-2721. 7-day toxicity

SURVIVAL (%)

WR-2721 (800 mg/kg)	Se (1.6 mg/kg) + WR-2721 (800 mg/kg)	WR-2721 (1000 mg/kg)	Se (1.6 mg/kg) + WR-2721 (1000 mg/kg)	WR-2721 (1200 mg/kg)	Se (1.6 mg/kg) + WR-2721 (1200 mg/kg)
70**	100**	60*	90*	15**	60**

N = 20/group. Sodium selenite solution (1.6 mg/kg body wt) injected IP 24 hr before WR-2721 IP.
*$p < 0.05$; **$p < 0.01$

TABLE II
Effect of selenium pretreatment on radioprotection by WR-2721

30-DAY SURVIVAL (%)

Radiation dose (Gy)	Control (saline)	Selenium only	WR-2721 (200mg)	Se + WR(200)	WR-2721 (400mg)	Se + WR(400)	WR-2721 (600mg)	Se + WR(600)
8.5	50**	100**						
9.0	0***	100***						
10.0	0	0						
14.0			69*	100*				
16.0			0***	88***	100	100		
18.0					75[a]*	96[a]*		
20.0					17[a]***	83[a]***	38[b]**	100[b]**
22.0					6*	38*	13***	100***
24.0					0*	31*	8	25

Sodium selenite solution (1.6 mg Se/kg body wt) injected IP 24 hr before WR-2721 treatment IP. Bilateral cobalt-60 (1 Gy/min). N = 16/group, except [a], N = 24; [b], N = 8.
*p < 0.05; **p < 0.01; ***p < 0.001

9 Gy (900 rad), whereas those receiving saline all died within the 30-day period. The radioprotective effect of each dose of WR-2721 (200, 400, 600 mg/kg) was potentiated by Se pretreatment. Survival graphs of animals treated with WR-2721 (400 mg/kg) alone or the combination of WR-2721 and Se are shown in Figure 1. Probit analysis indicated that the DRF (dose reduction factor or ratio of the radiation dose with the drug to the radiation dose without the drug giving the same biological effect) was 1.1 for Se alone, but the DRF for WR-2721 of 2.2 was potentiated to 2.6 with Se pretreatment.

The effect of lower doses of Se and different time schedules of administration on the radioprotective effect of WR-2721 (400 mg/kg) at 20 Gy was determined. For WR-2721 alone, survival was 33% at 30 days. Pretreatment with 0.4 mg/kg Se at either −24 hr or −6 hr increased survival to 63% but pretreatment was not effective at −3 hr. Pretreatment with 0.8 mg/kg at −24 hr, −6 hr, and −3 hr provided protection of 88%, 75%, and 63%, respectively (N = 16/group). These values were not significantly different than those obtained with 1.6 mg/kg Se.

The radioprotective effect of WR-2721 compared to that of its free sulfhydryl derivative WR-1065 is shown in Table III. Se pretreatment at 0.4 or 0.8 mg/kg provided some potentiation of the radioprotective effect of WR-1065 when mice were irradiated at 13 Gy. Se pretreatment did not, however, protect against the lethality of higher doses of WR-1065.

Alkaline phosphatase activity in bone marrow cells taken 24 hrs. after Se injection (1.6 mg/kg) was significantly depressed with respect to controls: 1.63 ± 0.05 vs. 2.32 ± 0.11 units/mg protein; $N = 4$, $p < 0.05$. Serum alkaline phosphatase activity was also depressed after injection of 1.6 mg/kg Se: 63.8 ± 3.1 vs. 77.7 ± 3.6 units/liter; $N = 6$, $p < 0.05$. Serum alkaline phosphatase was not significantly altered after treatment with either 0.4 or 0.8 mg/kg Se.

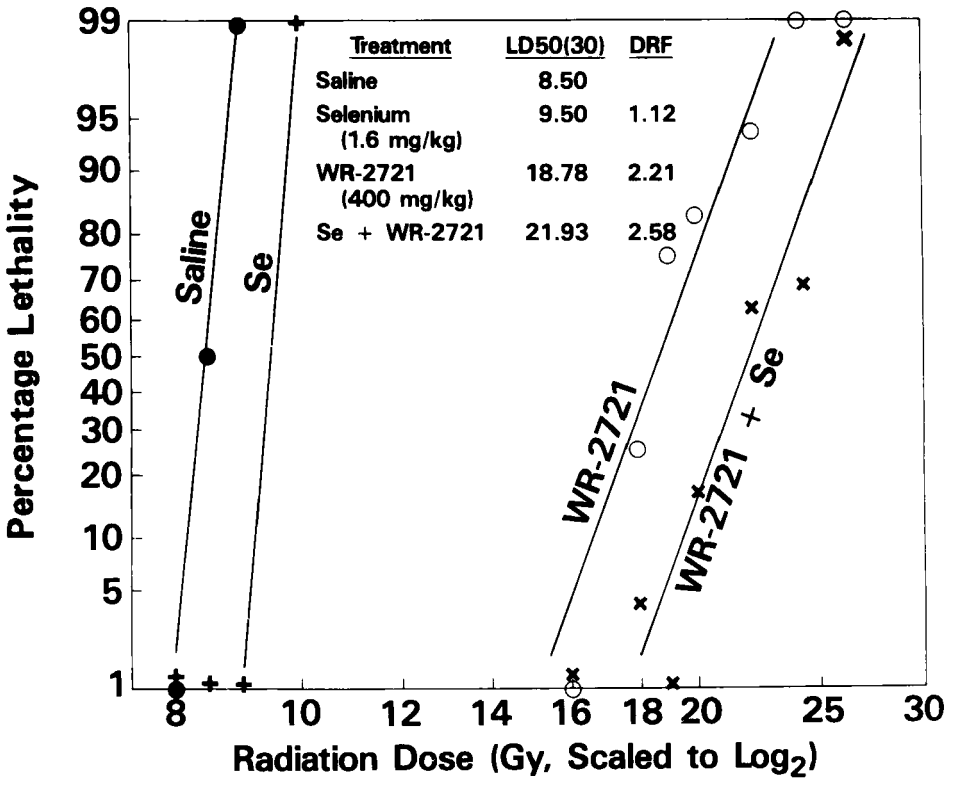

FIGURE 1 Probit analysis graph for percentage lethality of male CD2F1 mice at 30 days vs. radiation dose.

TABLE III
Comparison of radioprotective effects of WR-2721 and WR-1065 with selenium pretreatment. Irradiation with 13.0 Gy (1 Gy/min)

Pretreatment at −24 hr	30-day Survival (%)
WR-2721 (200 mg/kg)	
Saline	38
0.4 mg/kg Se	100
0.8 mg/kg Se	100
WR-1065 (200 mg/kg)	
Saline	13
0.4 mg/kg Se	38
0.8 mg/kg Se	88

N = 8/group

DISCUSSION

The results indicate that Se pretreatment enhances the therapeutic index of WR-2721, probably the most effective radioprotective agent, by decreasing its toxicity and enhancing its radioprotective effect. The DRF's (2.6 to 2.7) obtained due to Se pretreatment are among the highest reported in animals. One of the reasons for the effectiveness of WR-2721 is that it is administered as a phosphorylated derivative and is then dephosphorylated to its active form WR-1065 closer to the site of action.[2] The data indicate that some time is needed for the Se to be effective, suggesting biochemical induction. It is well established that Se has anticarcinogenic properties.[11] Pretreatment with Se, in the same range of pharmacologic doses used in the present experiment, can also reduce the toxicity of chemotherapeutic agents, such as *cis*-platinum.[12,13]

One possibility for the effects of Se observed in the present experiment is the inhibition of alkaline phosphatase, which would affect the conversion of WR-2721 to WR-1065 and thus alter either toxicity or efficacy or both. However, there was still a potentiation effect on the free sulfhydryl WR-1065, although the toxicity of this compound was not improved by Se pretreatment. This suggests there may be different mechanisms involved in reducing toxicity and promoting protection. There are many possibilities for other biochemical effects of Se in relation to the effects on WR-2721, but no data are reported here. The original reason for studying Se was as an inducer of glutathione peroxidase. Induction of this enzyme might result in a mild radioprotective effect.[9] Glutathione peroxidase could be involved in the toxicity reduction, since it is believed that hydrogen peroxide is a product of sulfhydryl oxidation.[14] Besides inducing glutathione peroxidase activity, Se injection may result in increases in other selenocysteine-containing proteins, quantitatively more important than the enzyme.[15] The role of these proteins in radioprotection is not known. On injection of sodium selenite, there is rapid formation of lower molecular weight organoselenium compounds, such as selenodicysteine and selenodiglutathione.[16] It is possible that some of these compounds formed have radioprotective potential, e.g., as demonstrated for selenomethionine *in vivo*[17,18] and *in vitro*.[19] Another possibility is increased synthesis of glutathione,[20] in response to the formation of oxidized glutathione, which is a byproduct of the formation of organoselenium compounds.[15] Some of the potential mechanisms by which Se improves the radioprotective effect of WR-2721 are under investigation.

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