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TOXICITY AND RADIATION PROTECTIVE EFFECT OF WR-77913 IN BALB/c MICE

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Although compounds providing protection against irradiation have been known since 1949 (PATT et coll.), it was not until many years later that some radiation protectors were found to be potentially applicable to human radiation therapy (YUHAS & STORER 1969 b). Screening of the S-phosphorylated thiols synthesized by ÅKERFELDT (1959) has demonstrated the most effective radiation protector to be S-2-(3-aminopropylamino)-ethyl phosphorothioic acid (WR-2721; PIPER et coll. 1969), since it not only is less toxic than cysteamine (YUHAS & STORER 1969 a), but it protects irradiated skin and bone marrow preferentially over tumor (YUHAS & STORER 1969 b) and provides selective normal tissue protection in several other normal tissue-tumor systems (HARRIS & PHILLIPS 1971, LOWY & BAKER 1972, YUHAS 1972, UTLEY et coll. 1976). More recently, other phosphorothioic acid compounds have been synthesized (DAVIDSON et coll. 1980) which may provide either decreased toxicity or increased protection as compared with WR-2721. This report presents data regarding the toxicity and radiation protective effect of sodium hydrogen-S-(3-amino-2-hydroxypropyl) phosphorothioate (WR-77913), a phosphorothioate of mercaptopropylamine.

Materials and Methods

Female BALB/c mice from Jackson Laboratories (Bar Harbor, Maine, USA) were used throughout these experiments. Twelve-week-old animals were used for the toxicity, bone marrow, central nervous system (CNS) and tumor protection experiments and 8-week-old mice for the gut protection experiments. The animals were housed 5 per cage in environmentally controlled rooms with 12 hour dark/light cycles, and fed standard mouse chow and water ad libitum.

Tumors. The EMT6 tumor of the BALB/c mice is maintained in this laboratory by serial in vivo/in vitro passages. A single tumor cell suspension was obtained by trypsinization of tumor freshly excised from source animals and 2×10^5 viable tumor cells in 5 μl of suspension were transplanted into the gastrocnemius of the right hind leg of the experimental animals. The tumors were irradiated 5 days after transplant, at a volume of about 150 mm^3. Tumors were measured with calipers at the time of irradiation and daily thereafter and volumes were calculated as the volume of an ellipsoid on the basis of 3 diameters.

Drugs. WR-2721 and WR-77913 were provided by the Drug Synthesis and Chemistry Branch (DTP, DCT, NCI, NIH). Immediately before their use, the drugs were dissolved in distilled water at a concentration such that the stated amount of drug in mg/kg of body weight was contained in 0.01 ml of solution. The drugs were injected intraperitoneally in a single dose, 30 min before irradiation in the radiation protection experiments.

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Table 1

Radiation protection in the gut of 8-week-old female BALB/c mice. Maximum tolerable dose defined as $LD_{10/30}$

Drug	Dose (mg/kg)	Per cent maximum tolerable dose	$LD_{50/7}$ (Gy) with 95% confidence limits	Dose modifying factor
None	–	–	7.91 (7.68–8.13)	–
WR-2721	300	51.9	13.09 (12.70–13.49)	1.65
WR-2721	150	26.0	12.35 (11.99–12.73)	1.56
WR-77913	1 500	47.9	15.44 (15.06–15.83)	1.95
WR-77913	750	23.9	14.21 (13.84–14.59)	1.80

Irradiation. A ^{60}Co unit with a source to target distance of 60 cm providing a dose rate of ≅2Gy/min was used for the bone marrow, gut and tumor protection experiments. Mice were confined for whole body irradiation to shallow, subdivided lucite containers thick enough to provide full build-up at the skin. Segmental irradiation of the tumor bearing limb was done by restraining the animals in lucite holders with the right hind leg brought into the radiation field by securely taping the foot and ankle. The exposed limbs were covered with tissue equivalent material to provide full build-up at the skin. A Gammacell (Atomic Energy of Canada Ltd.) experimental irradiator, providing a dose rate of ≅ 50 Gy/min, was used for the CNS protection experiments. Unanesthetized air breathing mice were used throughout these experiments.

Endpoints. The drug toxicity was evaluated as mortality at 30 days, although all deaths due to drug toxicity occurred invariably within 72 hours of the injection. No deaths attributed to drug toxicity were observed with the doses of WR-2721 or WR-77913 used in the protection experiments. CNS, gut and bone marow deaths were scored as mortality at 2, 7 and 30 days, respectively. LD_{50} (doses of drug or irradiation lethal to half of the treated animals at the stated time) were calculated by probit analysis (FINNEY 1963). Where protectors were used, dose modifying factors (DMF) were calculated as the ratio of the LD_{50} for the protected group divided by the LD_{50} for the radiation only group.

Results

As previously reported (MENDIONDO et coll. 1982) the $LD_{50/30}$ toxic for WR-77913 was 3574 mg/kg and that for WR-2721 was 678 mg/kg. The maximum tolerable doses (MTD), defined as the $LD_{10/30}$, were 3 132 mg/kg and 578 mg/kg for WR-77913 and WR-2721, respectively.

The protective effect of WR-77913 and WR-2721 against radiation induced bone marrow death is shown in Fig. 1. At doses nearing the MTD, WR-2721 appeared to be slightly superior but WR-77913 was more effective than WR-2721 at doses equivalent to one half or one quarter MTD. The protection against gut radiation death also appeared to be higher with WR-77913 than with WR-2721 at doses equivalent to one half and one quarter MTD (Table 1). For these experiments, 8-week-old mice were used, with an $LD_{50/7}$ of 7.91 Gy for the control non-protected group. Excellent protection was again achieved with 750 mg/kg of WR-77913, with a DMF=1.80.

Protection against acute CNS radiation death was not observed with either WR-2721 or WR-77913. The $LD_{50/2}$ was consistently lower for animals given the protectors than for the control group, and significantly lower for those pretreated with WR-77913 than for those given WR-2721 (Table 2).

Preliminary data on tumor protection are shown in Fig. 2. Mice bearing 150 mm^3 EMT6 tumor transplants were irradiated with or without prior administration of WR-77913 or WR-2721. Non-irradiated controls reach a volume of 1 000 mm^3 in 5 days while tumors treated with 20 Gy reach that size in 15 days. A slight protective effect occurred if the animals were given either WR-77913 or WR-2721, with the radiation induced growth delay decreased from 10 to 7.5 days. The protective effect appears to be similar for both protectors at the drug doses used.

Discussion

The efficacy of sulphydryl compounds as radiation protective agents was first demonstrated by

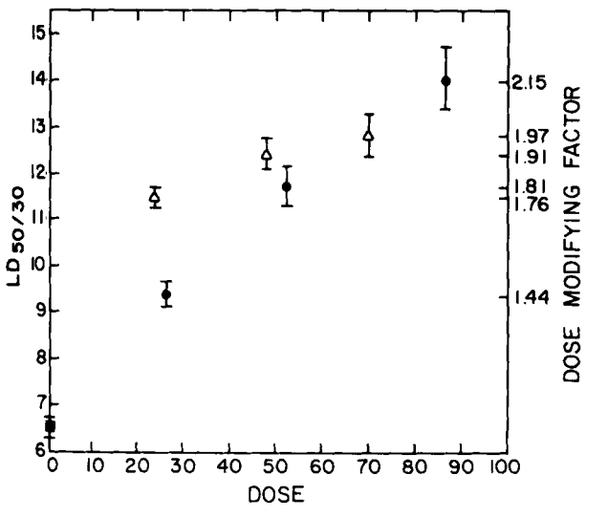

Fig. 1. Bone marrow radiation protection with WR-2721 (●) and WR-77913 (△). Control (■). Radiation $LD_{50/30}$ (in Gy) and dose modifying factors as a function of dose of protectors expressed as per cent maximum tolerable dose. The MTD for WR-2721 and WR-77913 are 578 mg/kg and 3 132 mg/kg, respectively.

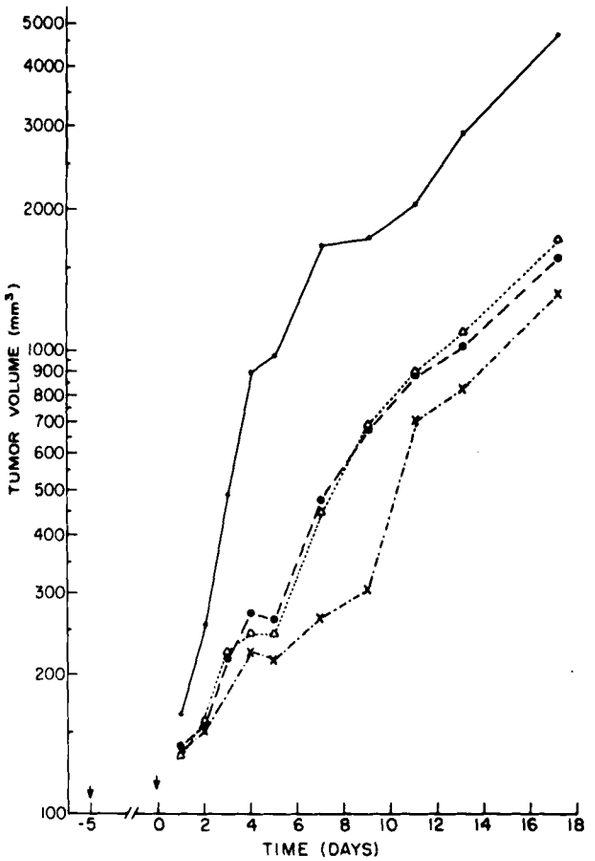

Fig. 2. Growth curves of EMT6 tumor transplants after treatment. Transplant on day −5, irradiation on day 0. Control (—). 20 Gy (–.–) WR-2721, 500 mg/kg, and 20 Gy (– – –). WR-77913, 2 200 mg/kg and 20 Gy (- - - -).

Table 2

Radiation protection in the central nervous system of 12-week-old BALB/c mice

Drug	Dose (mg/kg)	$LD_{50/2}$ (Gy) with 95% confidence limits	Dose modifying factor
None	–	453.77 (448.37–459.24)	–
WR-2721	500	389.92 (371.45–409.31)	0.86
WR-2721	300	411.08 (404.17–418.11)	0.91
WR-77913	2 200	320.22 (287.03–357.26)	0.71

PATT in 1949. Later, many compounds have been tested for their ability to protect against the effects of ionizing radiation. The decarboxylated cysteine derivative, cysteamine, was found to be the best protector in the sulphydryl class, giving DMF of approximately 1.3 for intestinal death (SIGDESTAD et coll. 1976b) and 1.8 for hematopoietic death (BACQ 1965). In 1959, ÅKERFELDT reported the synthesis of a thiophosphate derivative of cysteamine which was characterized by a phosphate group covering the sulphydryl. These phosphorothioic acids were shown to provide significant increases in radiation protection as compared with the compounds containing sylphydryl groups alone (HANSEN & SÖRBO 1961, ÅKERFELDT 1963).

Further synthesis and screening of phosphorothioic acids demonstrated the most effective radiation protector to be WR-2721 (PIPER et coll.). Its ability to protect normal tissues preferentially over tumors is of particular interest. Excellent protection has been demonstrated for normal tissues such as small intestine (YUHAS & STORER 1969 a, PHILLIPS et coll. 1973), bone marrow (PHILLIPS et coll.), skin (LOWY & BAKER), oral mucosa (UTLEY et coll. 1978, GRIGSBY & MARUYAMA 1981), salivary gland (UTLEY et coll. 1978) and esophagus (PHILLIPS et coll.). The possibility of improving the therapeutic ratio by preferential protection of normal tissues in an experimental normal tissue-tumor system was demonstrated by YUHAS. Other reports show preferential uptake of labeled WR-2721 in normal tissues over tumor (WASHBURN et coll. 1974, YUHAS et coll. 1980) and little or no protection in a number of experimental tumors (YUHAS et coll.).

Protection of some critical normal tissues such as kidney and lung is, however, modest (PHILLIPS 1980) and no protection has been obtained for the

brain (YUHAS & STORER 1969a). When used in a multiple dose regimen, the toxicity of WR-2721 increases and the drug dose per injection must be reduced. This results in slightly less protection of skin with a fractionated irradiation regimen, although the degree of protection observed for the intestine was essentially the same (as determined by the intestinal mucosa microcolony assay; UTLEY et coll. 1976). There is a need for chemicals that can efficiently protect tissues not adequately protected by WR-2721, and compounds which exhibit less toxicity may also be useful in a fractionated setting.

DAVIDSON et coll. recently reported on the toxicity and protective activity of several chemically synthesized compounds, among them WR-77913. This drug reportedly was exceptionally well tolerated in mice and dogs and had excellent protective activity against just supralethal irradiation. SIGDESTAD et coll. (1976a) showed good protection of the intestinal epithelium against roentgen or fission neutron irradiation with WR-77913. Recently, MENDIONDO et coll. reported on toxicity experiments with WR-77913. The drug $LD_{50/30}$ was 3574 mg/kg and interestingly, only a slight decrease in the $LD_{50/30}$ occurred if WR-77913 was given in combination with WR-2721, demonstrating that doses of both compounds nearing the MTD could be given without increasing the toxicity.

Adequate bone marrow protection was obtained with WR-2721 with doses as low as one quarter MTD (Fig. 1). In comparison with WR-2721, WR-77913 was slightly inferior at high doses but more effective at comparable lower doses. A similar effect was found for protection of the mouse intestine, measured by the radiation $LD_{50/7}$ (Table 1). These data suggest that the dose response curve for WR-77913 is shallow above drug doses equivalent to one quarter MTD, a potentially useful characteristic in a multifraction drug-radiation regimen.

No protection was observed for the brain as determined by the acute radiation $LD_{50/2}$ (Table 2) and the inability to protect CNS may be a limitation common to the phosphorothioic acids currently available. The fact that the $LD_{50/2}$ was consistently lower for WR-77913 treated animals than for the control mice may even suggest additive CNS toxicity. WR-77913 exerts a modest degree of protection on the EMT6 tumor, as shown by the growth curves in Fig. 2. The degree of protection is, however, at the doses used, no higher than that offered by WR-2721.

Some of the attractive characteristics of WR-77913 are its low toxicity and adequate protection of bone marrow and intestine at low drug doses, which might be used to an advantage in a multiple drug dose regimen. The lack of additive toxicity with WR-2721 suggests the possibility of significant increases in radiation protection with the combination of both compounds. Further investigation of the protective effect of WR-77913 in other normal tissue systems appears justified.

SUMMARY

The toxicity and radiation protective effect obtained by intraperitoneal injection of WR-77913 have been investigated in BALB/c mice. The toxic $LD_{50/30}$ was 3574 mg/kg. When WR-77913 was given 30 min before whole body irradiation adequate protection was observed against bone marrow and gut death. Dose modifying factors of 1.91 and 1.76 for bone marrow and 1.95 and 1.80 for gut death were obtained with drug doses roughly equivalent to one half and one quarter of the maximum tolerable doses (MTD). No protective effect was observed against central nervous system injury. Preliminary experiments with the EMT6 tumor show limited tumor protection with a dose of WR-77913 equivalent to 70 per cent of the MTD. Because of its low toxicity, adequate bone marrow and gut protection at doses equivalent to one quarter MTD and limited protection of the EMT6 tumor, WR-77913 deserves further investigation to determine its value in multiple drug dose regimens and its capability to protect other normal tissues.

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