Protective Effects of S-2-(3-Aminopropylamino)ethylphosphorothioic Acid against Radiation Damage of Normal Tissues and a Fibrosarcoma in Mice

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ABSTRACT

S-2-(3-Aminopropylamino)ethylphosphorothioic acid (WR-2721) was investigated for its protective effect against radiation-produced damage of jejunum, testis, lung, hair follicles, and a fibrosarcoma of C3Hf/Kam mice. Most of these tissues were radioprotected, and the degree of radioprotection depended on the dose of WR-2721 and the time interval between administration of WR-2721 and radiation treatment. WR-2721 increased resistance of jejunal epithelial cells and spermatogenic cells to single doses of γ-rays by factors of 1.64 and 1.54, respectively. Protection against hair loss was less pronounced; the dose-modifying factor here was 1.24. The radiation-induced acute damage of the lung expressed by the increased formation of tumor nodules in the lung was not decreased by treatment of animals with WR-2721 before radiation. In contrast, WR-2721 augmented the radiation-induced enhancement of metastasis formation in the lung. WR-2721 protected fibrosarcoma micrometastases in the lung against therapeutic effect of radiation by a factor of 1.28. In contrast, this compound had no effect on the therapy of an 8-mm fibrosarcoma growing in the legs of mice.

INTRODUCTION

An approach for increasing the therapeutic ratio that has recently gained the interest of researchers is the selective radioprotection of normal tissues with WR-2721 (8, 27). The assumption is that this compound would, because of poor tumor blood supply, be less accessible to hypoxic tumor cells than to normal tissues and would thus afford less protection to tumor cells. A recent report by Yuhas (24) suggested that other mechanisms could also be responsible for this discriminatory radioprotection by WR-2721. In studies on radioprotection of the hematopoietic tissue, about 3 times higher doses of radiation were required for mice treated with WR-2721 to reduce the survival of hematopoietic colony-forming units to the same level. Also, WR-2721 increased the LD₅₀/₅₀ radiation dose of hematopoietic lethality by factors between 2.3 and 2.7 (28). The dose of radiation in WR-2721-treated mice had to be increased by factors of 2.4 (28) or 1.5 (15) to produce skin ulcerations and late skin damage. Radioprotection has also been reported to occur against damage of jejunum (9, 11), lung (9, 23), and esophagus (9), but not against brain and spinal cord damage (27).

The radioprotective effects of WR-2721 for tumors have been reported to be less than those for normal tissues (8, 27). In their recent review article, Yuhas et al. (27) reported the failure of WR-2721 to protect 12 solid tumors of experimental animals against radiation injury. Philips et al. (9) observed that WR-2721 did not protect murine EMT-6 solid tumors but protected murine P388 leukemia to the same degree as bone marrow. Yuhas and Culo (26) and Wasserman et al. (17) showed that WR-2721 fails to protect experimental solid tumors against chemotherapeutic agents, while greatly reducing the damage of normal tissues by these agents. More recently, however, Twentyman (14) reported that WR-2721 reduced the tumor-destructive effects of cyclophosphamide.

Most studies on WR-2721 radioprotection of solid tumors dealt with doses of radiation that were too low to produce tumor cures, and the effect of WR-2721 was determined by its effect on the radiation-caused delay in the tumor growth (17, 25, 29). Since the number of clonogenic tumor cells that remained after radiation might be quite high, any effect of WR-2721 on the radiosensitivity of tumor cells would be masked. Therefore, for investigations of the effect of WR-2721 on tumor-curable doses of radiation, assays other than the tumor growth delay, such as the TCD₅₀ assay, would be more sensitive. The sensitivity of the TCD₅₀ assay to the effects of WR-2721 can be understood by the consideration that tumors not controlled with the TCD₅₀ radiation dose can regrow from an average of approximately 0.7 cell (21) and that the average numbers of tumor cells that survive TCD₅₀ and TCD₅₀ doses are 2.3 and 0.1, respectively (based on the assumption of random killing). It can be anticipated from this that, if WR-2721 causes only one or 2 more cells to survive the TCD₅₀ level of radiation, it will greatly affect tumor control probability and the estimate of TCD₅₀.

Our studies were designed to investigate whether WR-2721 is capable of discriminating between tumors and normal tissues in its protective effects. The effects of this compound were studied for the following radiation injuries: loss of jejunal epithelial cells; loss of spermatogenic cells; lung tissue damage; hair loss; and limitation in leg extension. The tumor-radioproductive effect of WR-2721 was investigated using the lung colony assay, in which 4-day-old FSA micrometastases were
irradiated, and the TCD50 assay for 8-mm FSA growing in the legs of mice. At the time of irradiation, lung micrometastases were assumed to contain no hypoxic tumor cells, while 8-mm FSA were assumed to contain a large percentage of such cells (12). Single doses of γ-rays were used in all experiments.

MATERIALS AND METHODS

Mice

Inbred C3H/Kam mice of both sexes bred and maintained in our own specific-pathogen-free mouse colony were used. They were 9 to 14 weeks old at the beginning of the experiments. Within each experiment, mice of the same sex were housed 4 to 7/cage.

WR-2721

WR-2721 (Batch AJ-68.2), obtained from the Drug Synthesis and Chemistry Branch, National Cancer Institute, Bethesda, Md., was kindly supplied by Dr. David A. Pistenma. The drug was stored at 4° and, immediately before the experiment, was dissolved in 0.9% sodium chloride solution (pH 6.0) and injected i.p. in a volume equal to 0.01 ml/g body weight. The doses of WR-2721 ranged between 50 and 700 mg/kg body weight and were given 5 min to 5 hr before radiation. However, in most experiments, a 400-mg/kg dose was used 30 min prior to radiation. The LD50/30 of WR-2721 for most inbred strains of mice is above 600 mg/kg (22, 29).

Assays for Normal Tissue Radiation Damage

Gut. The microcolony assay introduced by Withers and Elkind (18) was used to assay the survival of crypt epithelial cells in the jejunum of mice exposed to ionizing radiation. Mice were exposed to WBI with single doses of γ-rays ranging from 1300 to 2600 rads delivered by a small-animal irradiator with a single 137Cs source. The radiation dose rate was 233 rads/min, and the distance between the source of radiation and mid-mouse was 28 cm. At Days 3.5 after irradiation, mice were killed, and the jejunum prepared for histological examination. The number of regenerating crypts in the jejunal cross-section was counted.

In order to construct radiation survival curves, the number of regenerating crypts was converted to the number of surviving cells by applying a Poisson correction for crypts regenerating from more than one stem cell (18). Lines were fitted to data points by least-squares regression analysis.

Testis. The assay used for determining stem cell survival of testis seminiferous tubules is described in detail by Withers et al. (19). Local irradiation of testes was performed with a small-animal irradiator with 2 parallel-opposed 137Cs sources at a dose rate of 917 rads/min. Each mouse (10 weeks old) was contained without anesthesia in a Lucite box placed in such a way that the testes were within the 3-cm-diameter irradiation field. Thirty-five days after irradiation, mice were killed, and the number of tubules sectioned were counted. The average number of colony-forming stem cells surviving per tubule cross-section was determined for each animal, and its geometric mean was computed for each group of 5 mice at a given dose point. Survival curves were obtained in the same manner as described for the gut assay.

Lung. It has been established by others (1, 16) and in our laboratory (7, 20) that exposure of mice to LTI leads to enhancement of formation of tumor nodules in the lung if mice were given tumor cells i.v. within 1 to 2 weeks after LTI. The cause of the increased yield of metastases is considered to be damage of the lung tissue produced by radiation (1, 7, 16, 20). Therefore, this assay can be used for studies of radiation-induced lung damage. Unanesthetized mice were exposed to 1000 rads LTI, delivered with a double-headed 137Cs irradiator at 917 rads/min. The irradiation portal was a 3-cm-diameter circle, with the interior margin at the level of the xiphisternum. One day after irradiation, mice were given i.v. injections of 2 × 106 viable FSA cells (more information about the FSA is given under “Assays for Tumor Response to Radiation”). Fourteen days after inoculation of FSA cells, the mice were killed, their lungs were removed, and the lung lobes were separated and fixed in Bouin’s solution. Colonies of tumor cells (metastases) appeared as white, round nodules on the surface of the yellowish lung and were counted with the naked eye. Groups consisted of 6 to 7 mice each.

Hair Loss and Reduction in Leg Extension. Hair loss (epilation) was examined on irradiated legs of mice in the TCD50 experiment (see below, TCD50 assay), 36 days after irradiation. Only mice having no recurrent tumors were used for the determination of radiation-induced hair loss. At each irradiation dose point, the number of mice having 100% epilation was scored. The ED50 was then determined by the logit method of analysis (2). Radiation-induced leg contraction (reduction in leg extension) was also determined on mice in the TCD50 assay that had no recurrent tumors present. It was measured at 9- to 12-day intervals, starting at Day 36 and ending at Day 100 after local leg irradiation using the method introduced by Dr. H. Stone.4 For measurements, mice in a Lucite jig with tails between the vertical posts of the jig had both the nonirradiated and irradiated legs extended over a scale measuring millimeters. Readings were made at the ankle. The leg extension reduction values were obtained by subtracting the length of the irradiated leg from that of the nonirradiated leg.

Assays for Tumor Response to Radiation

FSA is a methylcholanthrene-induced tumor (13) that has been used often in our laboratory for studies on different aspects of tumor radiobiology. Single-cell suspensions from this tumor were prepared by trypan digestion of necrotic tumor tissue (5). Viability of the cells was more than 95% as assessed by trypan blue exclusion and phase-contrast microscopy.

FSA Micrometastases in the Lung. The assay for testing radiation response of tumor micrometastases in the lung, previously developed in our laboratory (4) was used in the present study. Micrometastases were generated by i.v. injection of 106 viable FSA cells mixed with 106 heavily irradiated FSA cells into mice exposed to 600 rads WBI 1 day earlier. The purpose of WBI and the addition of heavily irradiated tumor cells was to increase the yield of tumor micrometastases in the lung (5, 6). Four days after injection of tumor cells, mice were exposed to single doses of LTI that ranged from 600 to 1650 rads, as described above. Sixteen days after LTI, mice were killed, and the number of lung nodules was determined. The number of lung nodules in mice not exposed to LTI was determined 14 days after tumor cell injection. The number of surviving clonogenic tumor cells was plotted for each irradiation dose, and survival curves were fitted to the data by least-squares regression analysis.

TCD50 Assay. Mice were given injections into the right hind thighs of 5 × 105 viable FSA cells. The resulting tumors were exposed to single doses of 3300 to 6200 rads γ-radiation when they grew to 8 mm in diameter, which occurred 8 to 11 days after tumor cell transplantation. Irradiation to the tumor was delivered from a dual-source 137Cs irradiator, as described for testes irradiation, the difference being that the mice were not contained in a Lucite box but rather were immobilized in a jig. During irradiation, the tumor was centered in the circular radiation field 3 cm in diameter. The dose rate was 917 rads/min. Mice were checked for the presence of tumor at the irradiated site at 9- to 12-day intervals for up to 100 days. TCD50 values were computed by the logit method of analysis (2).

Data Analysis

DMFs were computed using the method of Pike and Alper (10) for the gut, testis, and lung colony assays. The results with and without

* H. Stone, personal communication.

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WR-2721 were fit by linear least-squares regression; WR-2721 is dose modifying if the intercepts are not significantly different. In this case, the ratio of slopes is the estimate of the DMFs. The DMF for hair loss was determined by dividing ED_{50} of mice exposed to WR-2721 and irradiation with ED_{50} of mice irradiated only. The statistical evaluation of the difference in the number of lung metastases among different experimental groups (Tables 1 and 3) was performed by using the nonparametric Mann-Whitney U test. Differences between groups were considered significant if the p value of comparison was 0.05 or smaller.

RESULTS

Protection of Normal Tissues

Jejunum. Chart 1 shows the protective effect of different doses of WR-2721 ranging between 50 and 700 mg/kg i.p. given to mice 30 min before their exposure to a 1600-rad single dose of WBI. The protection was dose dependent, being more effective as the dose of WR-2721 increased up to about 400 mg/kg. However, doses larger than 400 mg/kg afforded no additional protection. Fig. 1 shows the histology of normal jejunum, jejunum exposed to 1600 rads, and jejunum of mice treated with both WR-2721 and 1600 rads.

Further experiments with jejunum were performed using a single 400-mg/kg dose of WR-2721. To determine whether the time interval between application of WR-2721 and gut irradiation is important in achieving radioprotection, WR-2721 was given to mice 5 min to 5 hr before irradiation with 1600 rads. The protection of jejunal crypts was achieved rapidly, within 5 min (Chart 2A). As soon as 10 min after WR-2721 administration, the protection reached a plateau and remained at that level for about 1 hr. Thereafter, the protective effect of WR-2721 began to decline but still remained at a significant level at 5 hr after the administration of the drug. The radioprotection was not achieved when WR-2721 was given after radiation. The number of jejunal crypts was 6.1 ± 1.2 (S.E.) in mice exposed to 1600 rads and 3.8 ± 0.6 in mice that received WR-2721 (400 mg/kg) immediately after radiation.

The following experiment was performed to determine the protective effect of WR-2721 against damaging effects of different doses of radiation. Mice were exposed to single doses of -rays ranging from 1300 to 2600 rads, and the survival of jejunal crypt epithelial cells was determined (Chart 3A). The protection was profound against the effect of all tested doses of radiation. The DMF was 1.64, with 95% confidence limits (1.61 to 1.67).

Testis. The degree of protection of testes was more dependent on the dose of WR-2721 than that of intestine (Chart 1B). With testis, the protection steadily increased with increase in the dose of WR-2721 and did not reach a plateau at doses up to 500 mg/kg (the highest we tested). WR-2721 was given i.p. 30 min prior to testes irradiation with 1400 rads. The histological appearance of testes from normal mice and mice exposed to radiation or radiation plus WR-2721 is shown in Fig. 2.

The protection of testes by WR-2721 was similar to the protection of jejunum in the rapidity of its onset. The dose of WR-2721 used in this experiment was 400 mg/kg. The best protection was achieved when the drug was given 10 to 15
associated confidence limits, were 1.64 (1.61 to 1.67) for gut, 1.54 (1.49 to 1.62) for testis, and 1.28 (1.15 to 1.41) for FSA lung metastases. WR-2721 was profound; the DMF was 1.54 (1.49 to 1.62).

In mice exposed to 5700 rads, the reduction in leg extension was 1.6 ± 0.4 mm at Day 36 after irradiation, and it steadily increased, reaching the value of 6.1 ± 0.7 mm at 100 days after radiation. In WR-2721-pretreated mice, these values were 0.2 ± 0.1 and 1.4 ± 0.2 mm at 36 and 100 days after irradiation, respectively.

In mice exposed to 5700 rads, the reduction in leg extension was 5.3 ± 0.8 mm at 36 days and 9.4 ± 0.8 mm at 100 days after radiation. WR-2721 reduced these values to 2.6 ± 1 mm at 36 days and 8.2 ± 1.1 at 100 days after radiation.

Hair Loss and Reduction in Leg Extension. Determinations of the protective effects of WR-2721 against hair loss and reduction in the leg extension caused by irradiation were performed on the same mice that were used in studies on the effect of WR-2721 on the TCD_{50} value of 8-mm FSA. The muscles of the right thighs of mice were treated by injection of 5 × 10^6 FSA cells. When tumors grew to 8 mm in diameter, they were irradiated with single doses of 3300 to 6200 rads γ-radiation. Approximately one-half of the mice received WR-2721 (400 mg/kg) 30 min prior to LTI. During 100 days after irradiation, mice were checked for tumor regression and regrowth (see "Tumor in the Leg: TCD_{50} Value"). At 36 days after tumor irradiation, mice that were without macroscopic tumor were checked for hair loss on the portion of the thigh exposed to radiation. The results presented in Table 2 show the proportion of mice with hair loss at each radiation dose level and also the ED_{50} value. WR-2721 protected against hair loss at all but the highest doses. The ED_{50} value for mice exposed to radiation only was 4073 (95% confidence limits, 3648 to 4548) rads, and for mice treated with WR-2721 and radiation it was 5043 (4639–5482) rads. The DMF was therefore, 1.24.

The length of the hind legs in their extended position, both irradiated and nonirradiated, was measured every 9 to 12 days from Day 36 until Day 100 after irradiation. Radiation limited the extension of the leg so that the distance between the femoroiliac and ankle joints was shorter than on the nonirradiated leg. The reduction in the leg extension was observed after all doses of radiation, and it was more prominent as the dose of radiation and the time from irradiation increased. These changes in the leg extension were less evident in mice that were given WR-2721 prior to irradiation. Chart 4 shows the extent of reduction in the leg extension after leg irradiation with 4200 or 5700 rads and the extent of protection against this effect of radiation by pretreatment of mice with WR-2721. The reduction of the leg extension was 1.6 ± 0.4 mm at Day 36 after irradiation with 4200 rads, and it steadily increased, reaching the value of 6.1 ± 0.7 mm at 100 days after radiation. In mice treated with WR-2721, these values were 0.2 ± 0.1 and 1.4 ± 0.2 mm at 36 and 100 days after irradiation, respectively.

Protection of FSA

Micrometastases in the Lung. Mice were exposed to 600 rads WBI and 1 day later were given i.v. injections of 10^5 viable FSA tumor cells mixed with 10^6 heavily irradiated FSA cells (10,000 rads). Four days after tumor cell injection, when lungs contained microscopic tumor foci, mice were exposed to 600 or 1000 rads LTI. Approximately one-half of the mice to be given LTI were given injections of WR-2721 (400 mg/kg) 30 min prior to LTI. One half of these mice were killed 16 days following LTI to determine the number of lung colonies, and the other half were not killed so that survival could be deter-
Table 1
Effect of WR-2721 on the LTI-induced enhancement of formation of artificial pulmonary metastasis of FSA in C3Hf/Kam mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment of tumor cell recipients</th>
<th>WR-2721 administration, time before LTI (min)</th>
<th>No. of lung metastases&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Experiment 1</th>
<th>Experiment 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>None</td>
<td></td>
<td>Mean ± S.E.</td>
<td>p&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Mean ± S.E.</td>
</tr>
<tr>
<td>2</td>
<td>1000 rads LTI</td>
<td></td>
<td>2.7 ± 0.9</td>
<td>&lt;0.001 (1)</td>
<td>4.3 ± 1.4</td>
</tr>
<tr>
<td>3</td>
<td>WR-2721 (200 mg/kg)</td>
<td></td>
<td>13.0 ± 1.6</td>
<td>NS&lt;sup&gt;d&lt;/sup&gt; (1)</td>
<td>22.6 ± 3.5</td>
</tr>
<tr>
<td>4</td>
<td>WR-2721 (400 mg/kg)</td>
<td></td>
<td>3.1 ± 1.4</td>
<td>NS (1)</td>
<td>4.1 ± 1.1</td>
</tr>
<tr>
<td>5</td>
<td>WR-2721 (200 mg/kg) + LTI</td>
<td></td>
<td>3.8 ± 0.5</td>
<td>NS (2)</td>
<td>11.1 ± 2.7</td>
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<tr>
<td>6</td>
<td>WR-2721 (200 mg/kg) + LTI</td>
<td></td>
<td>17.8 ± 2.7</td>
<td>NS (2)</td>
<td>23.9 ± 2.6</td>
</tr>
<tr>
<td>7</td>
<td>WR-2721 (400 mg/kg) + LTI</td>
<td></td>
<td>33.7 ± 4.6</td>
<td>&lt;0.001 (2)</td>
<td>55.3 ± 6.0</td>
</tr>
<tr>
<td>8</td>
<td>WR-2721 (400 mg/kg) + LTI</td>
<td></td>
<td>30</td>
<td></td>
<td>56.7 ± 6.4</td>
</tr>
</tbody>
</table>

<sup>a</sup> LTI and i.p. injection of WR-2721 were given 1 day before i.v. injection of 2 × 10<sup>4</sup> FSA cells.
<sup>b</sup> Fourteen days after i.v. injection of FSA cells, mice were killed for determination of the number of FSA nodules in the lung.
<sup>c</sup> Mann-Whitney U test relative to group indicated in parentheses.
<sup>d</sup> NS, not significant.

Table 2
Effect of WR-2721 on the radiation-induced loss of hair in C3Hf/Kam mice

<table>
<thead>
<tr>
<th>Irradiation dose (rads)</th>
<th>No. of mice with hair loss/total no. of mice&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Irradiation only</th>
<th>WR-2721 + irradiation</th>
</tr>
</thead>
<tbody>
<tr>
<td>3800</td>
<td>1/2</td>
<td>0/2</td>
<td></td>
</tr>
<tr>
<td>4200</td>
<td>2/5</td>
<td>1/7</td>
<td></td>
</tr>
<tr>
<td>4500</td>
<td>4/4</td>
<td>1/6</td>
<td></td>
</tr>
<tr>
<td>4800</td>
<td>6/6</td>
<td>1/5</td>
<td></td>
</tr>
<tr>
<td>5200</td>
<td>5/5</td>
<td>2/5</td>
<td></td>
</tr>
<tr>
<td>5700</td>
<td>5/5</td>
<td>4/5</td>
<td></td>
</tr>
<tr>
<td>6200</td>
<td>5/5</td>
<td>5/5</td>
<td></td>
</tr>
</tbody>
</table>

ED50<sup>a</sup>
95% confidence limit
3646-4558
4639-5482

<sup>a</sup> Mice with 8-mm FSA in the right hind thighs were exposed to single doses of radiation over that thigh (see Table 4), and 36 days later the presence of hair in the irradiated area was determined in mice that had no macroscopic tumor. WR-2721 (400 mg/kg i.p.) was given 30 min before irradiation.

Discussion:

- Two additional groups of mice were included: one group received no further treatment after being given tumor cells i.v.; and the other group received WR-2721, which was not followed by LTI. One half of the mice in each group were killed 14 days after tumor cell injection to determine the number of lung nodules, and the other half was spared to determine the survival of mice. The results are presented in Table 3. WR-2721 alone had no effect on either the number of lung nodules or the survival time. LTI reduced the number of tumor nodules in both WR-2721-untreated and -treated mice, but this reduction was less pronounced if the mice had been treated with WR-2721.

- WR-2721 also reduced the prolongation of survival of mice by radiation. The mean survival of mice exposed to 1000 rads LTI was 41.5 ± 3.5 days, and that for mice treated with both WR-2721 and LTI (p < 0.005) was 31.0 ± 0.9 days.

- Chart 2C shows the dependence of radioprotection on the time interval between injection of WR-2721 and LTI. The protection was already evident at 5 min after administration of WR-2721 (400 mg/kg), and it showed a steady increase by 30 min, after which time it remained more or less at a constant level to the end of the observation period of 3 hr. The whole experiment was performed in a manner similar to that described for the preceding experiment.

- Chart 3C shows the radiation dose-response curves for 4-day-old FSA micrometastases in the lungs of WR-2721-treated and untreated mice. Doses of LTI ranged between 750 and 1650 rads. WR-2721 (400 mg/kg i.p.) given 30 min before LTI protected against all doses of radiation. The DMF was 1.28 (1.15 to 1.41).

- Tumor in the Leg: TCD50 Value. The TCD50 assay was performed with 8-mm FSA growing in the leg. These tumors were generated by 5 × 10<sup>6</sup> FSA cells injected into muscles of the right thighs. Tumors grew to 8 mm diameter in 8 to 11 days. WR-2721 (400 mg/kg i.p.) was injected 30 min before irradiation. Bars, S.E.
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The time lapsed between WR-2721 and radiation treatments, and it varied for normal tissues and FSA micrometastases. The dose of WR-2721 for gut was around 400 mg/kg, whereas the protective dose for testis did not reach its plateau even at 500 mg/kg. These observations suggest that different tissues might be differently protected against acute radiation injury. The protective dose for testis did not reach its plateau even at 500 mg/kg. These observations suggest that different tissues might be differently protected against acute radiation injury.

DISCUSSION

Data presented in this paper show that WR-2721 can effectively protect several normal tissues against acute radiation injury. Also, this compound was radioprotective for FSA micrometastases in the lung, but it did not reduce the effect of radiation on 8-mm FSA growing in the leg. The effectiveness of radioprotection depended on the dose of WR-2721 and the time interval between administration of this compound and the radiation treatment. The effect of WR-2721 was tested against radiation damage of jejunum and testes. Both tissues were equally protected if more drug was given to animals. The optimal dose of WR-2721 for gut was around 400 mg/kg, whereas the protective dose for testis did not reach its plateau even at 500 mg/kg. These observations suggest that different tissues might require different doses of WR-2721 for maximal radioprotection.

Maximal protection against radiation damage depended on the time lapsed between WR-2721 and radiation treatments, and it varied for normal tissues and FSA micrometastases.
FSA micrometastases but not the tumor growing in the leg. Failure of WR-2721 to protect tumors in the leg can be ascribed to its limited accessibility to tumor cells responsible for tumor regrowth in the TCD50 assay. We reported earlier that a FSA with a diameter of 8 mm growing in the leg contains approximately 27% hypoxic cells and that these cells were responsible for postradiation recurrences when tumors were exposed to the TCD50 level of γ-radiation (12). This implies that hypoxic cells were the cells that were not protected by WR-2721. On the other hand, micrometastases in the lung, which most probably do not contain hypoxic cells, were significantly protected. These observations are in accordance with the earlier observation by Phillips et al. (9), who reported that a well-vascularized P388 murine leukemia was well protected by WR-2721.

It was suggested earlier that WR-2721 is passively absorbed by cells (3), and according to this suggestion cells in oxygenated portions of tumors should absorb more WR-2721 simply because they have better access to this compound than do cells in hypoxic regions of the tumor that have a deficient drug delivery because of the compromised blood supply. More recently, however, Yuhas (24) reported that the passive absorption of WR-2721 occurs only in tumor cells, whereas normal tissues actively concentrate WR2721 against a concentration gradient. Yuhas (24) considered the difference in the absorption to be the basis for selective protection of normal tissues by WR-2721 against the damage produced by both radiation and chemotherapy. Therefore, it is quite possible that both the poor accessibility of WR-2721 to hypoxic areas within tumors and the inability of tumor cells actively to absorb this drug have been causes for lack of protection of leg tumors from radiation damage.

In contrast to leg tumors, FSA micrometastases in the lung were protected by a factor of 1.28, but this protection was still smaller than that afforded to jejunum and testis. The lower DMF for FSA could be explained, not on the basis of reduced accessibility of WR-2721 to FSA cells (since lung micrometastases most probably do not contain hypoxic areas), but rather on the basis of inability of tumor cells actively to absorb WR-2721. Also, our observation that maximal radioprotection of lung metastases was achieved less rapidly than that of testes and jejunum implies that there is a difference in the rapidity of WR-2721 uptake between these tissues.

The observation that micrometastases but not 8-mm tumors were protected implies that the size of the tumor at the time of treatment might be an important factor in determining the therapeutic benefit of WR-2721 radioprotection. Accordingly the benefit of WR-2721 treatment should be greater in radiotherapy of larger tumors. This, however, might be true only for situations in which large single doses of radiation are used, as was the case in our present study. In the case of fractionated irradiation, some protection of tumors would be expected because of reoxygenation of hypoxic cells, which might thus become more accessible to WR-2721.

As already discussed, the dynamics of radioprotection was quite different between the gut and testis on the one hand and the FSA micrometastases on the other. Maximum radioprotection of gut and testes was achieved at 10 min, and that of lung micrometastases was reached at 30 min after WR-2721 administration. Therefore, in order to maximize the therapeutic gain, the difference in the dynamics of radioprotection between normal tissues and tumor should be used in such a way as to deliver radiation to the tumor at the time of the largest difference in radioprotective effect between the critical normal tissues and the tumor.

The DMF for hair loss was similar to that of lung micrometastases and thus also smaller than DMFs for jejunum and testis. An earlier report showed that the DMF for hair loss in mice was above 1.5 (9, 15). Other radiation changes for the skin, such as ulcerations and late damage, were protected against by factors larger than 1.5 (9, 15). The reason the DMF in our study is smaller than those reported earlier is not known. However, it should be noted that in the present study the effect was determined on the skin that was overlying the 8-mm tumor. It might be possible that the tumor of this size compromised the blood circulation in tissues that surrounded the tumor, including the skin, which would have resulted in deficient delivery of WR-2721 to the skin. Although this DMF value for hair loss might be different if radiation takes place in nontumorous legs, it is relevant for tumor therapy since it indicates that WR-2721 caused a sparing of normal tissues surrounding the tumor itself. A similar reasoning could be applied for the protective effect of WR-2721 against radiation-induced limitations in extension of the tumor-bearing leg.

It can be concluded that WR-2721 is a potent radioprotective compound but that the degree of radioprotection that this compound exerts varies from tissue to tissue and that it depends on the type of radiation damage. Highly proliferative tissues, such as the jejunal epithelium and spermatogenic cells, were efficiently protected. On the other hand, WR-2721 was not at all effective in protecting against changes in the lung capillary walls that lead to the increase in formation of tumor metastases from cells brought to the lung via circulation. WR-2721 was also capable of protecting FSA micrometastases in the lung, but not an 8-mm FSA growing in the leg. While not being able to protect leg tumors, WR-2721 protected normal tissues surrounding the tumor, which resulted in less pronounced radiation-induced loss of hair and loss of leg extension. Therefore, WR-2721 was therapeutically beneficial by increasing the therapeutic gain.

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Fig. 1. Photomicrographs of jejunal cross-sections. A, normal jejunum (contains about 160 crypts); B, jejunum irradiated with 1600 rads (contains only few regenerating crypts); C, jejunum irradiated with 1600 rads 30 min after WR-2721 (400 mg/kg; contains more than 100 regenerating crypts). H & E, x 50.
Fig. 2. Photomicrograph of testes cross-section. A, normal testis (tubuli contain spermatogenic cells); B, testis irradiated with 1400 rads (all tubuli depleted of cells); C, testis irradiated with 1400 rads 15 min after WR-2721 (400 mg/kg; cell regeneration is seen in 3 tubuli). H & E, x 320.
Protective Effects of S-2-(3-Aminopropylamino)ethylphosphorothioic Acid against Radiation Damage of Normal Tissues and a Fibrosarcoma in Mice

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