Preclinical Evaluation of WR-151327: An Orally Active Chemotherapy Protector

Dianna Green, Dennis Bensely, and Philip Schein

Vincent T. Lombardi Cancer Research Center and Department of Medicine, Georgetown University Medical Center, Washington, DC 20007 [D. G., D. B.], and U.S. Bioscience, West Conshohocken, Pennsylvania 19428 [P. S.]

ABSTRACT

Clinical trials are in progress to evaluate radio- and chemoprotection by the aminothiol 2-[(3-aminopropyl)amino]ethanethiol-dihydrogen phosphate ester (WR-2721; amifostine). Phase II and III clinical studies have demonstrated that i.v. administered WR-2721 protects against the toxicities of cis-diaminedichloroplatinum (II) and cyclophosphamide. In preclinical murine studies, we have now further characterized the chemoprotective properties of WR-2721, and have evaluated the protective ability of the related aminothiol S-3-(3-methylaminopropylamino)propylphosphorothionic acid (WR-151327) following p.o. administration. The P388 leukemia (i.p. tumor-i.p. cytotoxic drug on Day 1 after tumor) was used to determine antitumor efficacy. Single dose pretreatment with i.p. WR-2721 protects normal mouse tissues against the chemotoxicities of mitomycin C, cis-diaminedichloroplatinum (II), and doxorubicin. Bone marrow suppression and cytotoxic drug-induced lethality were reduced, without compromising P388 antitumor activity. Pretreatment with a single p.o. dose of WR-151327 was as effective as i.p. WR-2721 in protecting against the myelotoxicity and lethality of mitomycin C, cis-diaminedichloroplatinum (II), and cis-diammine(cyclobutanedicarboxylato)platinum (II), while P388 antitumor activity was maintained. These data support the clinical development of WR-151327 as a p.o. administered chemotherapy protector.

INTRODUCTION

WR-2721 (amifostine) is a unique chemotherapy and radiation therapy protective agent currently in Phase III clinical development. This drug arose from the efforts of a classified United States Army research project to develop a radioprotector that might be administered to troops exposed to nuclear warfare. Based on its efficacy and relative safety (1), WR-2721 was selected from approximately 4400 chemicals that were screened. In preclinical in vivo studies with mouse lung and rat mammary carcinomas, WR-2721 has demonstrated the ability to reduce the toxic effects of cisplatin and alkylating agent chemotherapy, without loss of antitumor activity (2–4). Protection is systemic, with reduced toxicity demonstrated for a wide range of organs, including bone marrow, kidney, and gastrointestinal tract. In vitro, WR-2721 reduced both the mutagenic and carcinogenic effects of drug and radiation therapy (5–7). This selective protection of normal tissues by WR-2721 can be correlated with preferential uptake of the active thiol metabolite, WR-1065, into non-carcinogenic tissues (8, 9). Calabro-Jones et al. (10) attribute this selectivity for normal tissues at least in part to their higher pH and alkaline phosphatase activity as compared to tumor. Phase II and early Phase III clinical studies with WR-2721 using the required i.v. route of administration demonstrated chemoprotection for cisplatin and cyclophosphamide (11–13).

Interest has recently been demonstrated for the derivative WR-151327, based on its ability to protect against lethal doses of radiation when administered p.o. (14, 15).

In the studies reported here, our laboratory has further characterized the profile of WR-2721 in mice, and has tested, for the first time, the ability of WR-151327 to provide chemoprotection using a p.o. route of administration.

Structures for WR-2721 and WR-151327 are presented in Fig. 1.

MATERIALS AND METHODS

Drugs. WR-2721 (NSC-296961) was obtained from U.S. Bioscience, and WR-151327 was kindly provided by Dr. James Piper, Southern Research Institute, Birmingham, AL. Immediately prior to use, both aminothiols were reconstituted at 4°C with lactated Ringer’s and 5% dextrose, adjusted to pH 7.3 with sodium bicarbonate. Cisplatin, carboplatin, nitrogen mustard (HN2), and doxorubicin were kindly provided by the Developmental Therapeutics Program, National Cancer Institute, Bethesda, MD, and mitomycin C was purchased from United States Biochemical, Cleveland, OH. Cisplatin, HN2, and doxorubicin were dissolved in sterile 0.85% sodium chloride (saline), carboplatin was dissolved in sterile water, and mitomycin C was dissolved in ethanol, and then diluted to 10:90 (ethanol:sterile water) concentration.

Experimental Animals. Female DBA/2F and male BAL/b × DBA/2F (CD2F1) mice, approximately 5 weeks of age, were obtained from Charles River Laboratories, Wilmington, MA. Mice were provided food and water, ad libitum.

Tumor Transplant. The murine P388 leukemia was obtained from the Tumor Repository of the National Cancer Institute, and the tumor was maintained i.p. in female DBA/2F mice by serial transplantation of 1 × 10⁶ P388 cells. Antitumor activity was determined in male CD2F1 mice, implanted i.p. with 1 × 10⁶ P388 cells on Day 0, with administration of drug(s) on Day 1. ILS was calculated as follows:

\[
\% \text{ILS} = \left( \frac{T - C}{C} \right) \times 100
\]

where T is the mean survival days of the drug-treated mice, and C is the mean survival days of the vehicle-treated mice.

There were 5 or 6 mice/treatment group, and 2 or 3 replicate experiments were performed for each study. Results were validated with the Student’s t test.

Toxicity for the Murine Hematopoietic System. Measurement of peripheral leukocyte (WBC) count was performed on normal male CD2F1 mice (5–8/group) using a 20-μl sample of retro-orbital sinus blood obtained on Day 4 following administration of drug(s). Blood samples obtained were diluted in 9.98 ml of the physiological diluent Isoton, and counted in a model ZBI Coulter Counter after lysis of red blood cells with Zapoglobin (Coulter Diagnostics, Hialeah, FL). WBC counts are expressed as a percentage of values obtained from control mice that received drug vehicle or no treatment.

For carboplatin, relative toxicity to pluripotent bone marrow stem cells was quantitated by assay of CFU-S. A modification of published methods was used (16, 17). DBA/2F female mice (5/treatment group) were administered carboplatin (i.p. or i.v.) with or without aminothiol pretreatment. Twenty h later, each experimental donor mouse was sacrificed and bone marrow was extracted from the femurs into McCoy’s 5A medium (GIBCO, Grand Island, NY) on ice. Nucleated marrow cells were quantitated, and 5 × 10⁶ cells were injected i.v. into isogenic recipient mice 2 h after they received 850 rads whole body radiation. Four irradiated recipient mice per experimental donor marrow were given injections. Nine days later, each recipient animal was sacrificed, and the spleen was removed and fixed in Bouin’s solution. Surface colonies were then counted with the aid of a dissecting microscope, and experimental (mean number of colonies ± SD) were compared with irradiated recipient mice that received 5 × 10⁶ nucleated marrow cells from drug vehicle-treated donor mice.
RESULTS

Murine P388 Antitumor Comparative Studies: Pretreatment with p.o. WR-151327 versus i.p. WR-2721. P388 antitumor activities were determined for LD₉₀ doses of mitomycin C and carboplatin, with and without aminothiol pretreatment. WR-151327 was administered p.o. and WR-2721, i.p. Male CD2F₁, mice, given i.p. implants of 1 × 10⁶ P388 leukemia cells, were administered cytotoxic drug as a single i.p. dose 1 day later. For mice pretreated with p.o. WR-151327, 900 mg/kg were administered by gavage (0.05 ml/10 g body weight) 30 min before cytotoxic drug; for i.p. WR-2721, 400 mg/kg were administered 25 min before cytotoxic drug. These aminothiol doses approximate one-half the published LD₁₀ (14, 15). As summarized in Table 1, there was no loss of P388 antitumor activity following single dose pretreatment with either aminothiol. Mitomycin C, 4.5 mg/kg, produced a 90% ILS, with or without i.p. WR-2721. Results with p.o. WR-151327 were comparable: 91% ILS for mitomycin C, 5 mg/kg, versus 90% with WR-151327 pretreatment. For carboplatin, 100 mg/kg, the P388 ILS was 76–78% (Table 1), and this antitumor activity was maintained with i.p. WR-2721 (87% ILS) and p.o. WR-151327 (78% ILS). Another clinically used alkylating agent, nitrogen mustard (HN2), was evaluated in combination with p.o. WR-151327. The P388 ILS for HN2, 3.1 mg/kg, was 58% versus 65% for HN2 and WR-151327 (Table 1).

Clinical trials have demonstrated the very short half-life of WR-2721 relative to the half-life of carboplatin (18). For this reason, the

Table 1 Murine P388 antitumor studies with i.p. WR-2721 or p.o. WR-151327
pretreatment

<table>
<thead>
<tr>
<th>Drug treatment</th>
<th>Average wt loss (−) or gain (+)</th>
<th>Deaths due to drug toxicity (%)</th>
<th>P388 (%) ILS (%)</th>
<th>35-day survivors (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cisplatin, 16 mg/kg i.p.</td>
<td>−2.5g (Day 7)</td>
<td>40</td>
<td>&gt;70</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>−2.0g (Day 9)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>−0.2g (Day 13)</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Cisplatin, 16 mg/kg i.p. + WR-151327, 600 mg/kg p.o.</td>
<td>0.2g (Day 7)</td>
<td>3.0g (Day 13)</td>
<td>None</td>
<td>&gt;111</td>
</tr>
<tr>
<td>Cisplatin, 16 mg/kg i.p. + WR-151327, 900 mg/kg p.o.</td>
<td>0.2g (Day 7)</td>
<td>1.2g (Day 9)</td>
<td>2.0g (Day 13)</td>
<td>None</td>
</tr>
</tbody>
</table>

* P388 leukemia cells (1 × 10⁶) were implanted i.p. into male CD2F₁ mice on Day 0.

P388 antitumor activity of carboplatin plus single dose WR-2721 pretreatment was compared with 2 doses of WR-2721. As summarized in Table 1, a 300-mg/kg i.p. dose of WR-2721 was administered 25 min before carboplatin (100 mg/kg), and a second 300-mg/kg WR-2721 dose was administered 4 h later. The resultant 86% ILS was comparable to 87% ILS with single dose WR-2721 pretreatment and 78% ILS for carboplatin alone.

P388 Studies with p.o. WR-151327 and High-Dose Chemotherapy. Combination aminothiol/high dose chemotherapy was also evaluated. A single p.o. dose of WR-151327, 600 or 900 mg/kg administered 30 min before high dose cisplatin, decreased cisplatin toxicity without compromising P388 antitumor activity. As summarized in Table 2, with 16 mg/kg cisplatin, the mean weight loss was 2.5 g on Day 7, 40% of deaths were attributed to cisplatin toxicity, and the P388 ILS was >70%, with 17% of the mice surviving on Day 35. WR-151327, 600 mg/kg p.o. administered 30 min before cisplatin, eliminated the platinum-induced toxicity deaths while the mice gained an average 0.2 g on Day 7, the P388 ILS was increased to >111%, and 33% of the mice were 35-day survivors. With 900-mg/kg WR-151327 pretreatment, deaths due to drug toxicity were eliminated, the average weight gain on Day 7 was 0.2 g, and the P388 ILS was >129%, with 17% survivors on Day 35.

Similar chemoprotection was achieved with high dose mitomycin C. A 7-mg/kg dose of mitomycin C was lethal to 20% of the mice, and the P388 ILS was 98% (Table 3). Pretreatment with WR-151327, 600 mg/kg, eliminated the deaths due to mitomycin toxicity and the P388 ILS was >70%, with 17% of the mice surviving on Day 35. WR-151327, 600 mg/kg p.o. administered 30 min before cisplatin, eliminated the platinum-induced toxicity deaths while the mice gained an average 0.2 g on Day 7, the P388 ILS was increased to >111%, and 33% of the mice were 35-day survivors. With 900-mg/kg WR-151327 pretreatment, deaths due to drug toxicity were eliminated, the average weight gain on Day 7 was 0.2 g, and the P388 ILS was >129%, with 17% survivors on Day 35.

Murine Studies of Peripheral Leukocyte (WBC) Depression. WR-2721 or WR-151327 pretreatment does not compromise P388 antitumor activity for platinum compounds, mitomycin C, nitrogen mustard, or doxorubicin.

Concurrent studies in normal mice evaluated the effects of aminothiol pretreatment on chemotoxicity. Following administration of a single i.p. LD₁₀ dose of each myelotoxic chemotherapeutic to normal mice, the nadir WBC count occurred on Day 4. WR-151327 administered p.o. was as effective as i.p. WR-2721 in decreasing the myelotoxicity of mitomycin C.

As summarized in Table 4, mitomycin C (4.5 mg/kg) produced a nadir WBC count of 57% of control; with 400 mg/kg WR-2721 pretreatment, the nadir WBC was 87% of control. WR-151327 (900 mg/kg) pretreatment p.o. increased the WBC nadir to 85% of control, versus 58% of control for mitomycin (5 mg/kg) alone.

For nitrogen mustard (3.1 mg/kg), pretreatment with 900 mg/kg p.o. WR-151327 eliminated the deaths due to HN2 toxicity (15% of the mice), with a nadir WBC of 86% of control, compared to 56% with nitrogen mustard alone.

Chemical Structure

WR-2721

\[ \text{H}_2\text{N(CH}_2\text{)}_2\text{NH(CH}_2\text{)}_2\text{SP}_2\text{H}_2 \]

WR-151327

\[ \text{CH}_2\text{NH(CH}_2\text{)}_2\text{NH(CH}_2\text{)}_2\text{SP}_2\text{H}_2 \]

\[ \text{H}_2\text{N(CH}_2\text{)}_2\text{NH(CH}_2\text{)}_2\text{SH}, \text{WR-1065; active metabolite of WR-2721.} \]

Fig. 1. Structures of WR-2721 and WR-151327.
While considerable emphasis has been appropriately placed on bone marrow toxicity, many other organs are also at risk, including the gastrointestinal tract, kidneys, lung, and the neurological system. Bone marrow colony-stimulating factors, which produce dose-dependent increases in peripheral granulocytes, are an important adjunct for preventing serious infection during higher or more frequent doses of chemotherapeutics (19), but with successive courses of chemotherapy, the progenitor cells in the bone marrow may be progressively destroyed (20). In addition, the colony-stimulating factors do not protect other organs from chemotoxicity. In the case of radiation therapy, clinical bone marrow toxicity does not occur frequently, whereas damage to normal tissues and organs encompassed within the radiation field is common and can result in long-term morbidity. As a result, there is a rationale for the development of a systemic protector, effective for both dose-intensive chemotherapy and radiation therapy, with the requirement that antitumor activity is not compromised.

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and 5). These murine studies are the first to demonstrate that p.o. administered WR-151327 reduces lethality for a range of anticancer agents, without compromising P388 antitumor activity.

In agreement with our studies, Treskes et al. (27) have recently reported that cisplatin and carboplatin are inactivated by WR-1065; coadministration of WR-2721 prior to either cytotoxic drug should result in minimal platinum drug inactivation in blood since WR-2721 disappears rapidly from blood into normal tissues. In nude mice, cisplatin efficacy for OVCAR-3 xenografts was not compromised by WR-2721 pretreatment, while cisplatin-induced nephrotoxicity and lethality were decreased (28).

In summary, the preclinical studies we report here demonstrate that i.p. WR-2721 protects normal mouse tissues against the myelotoxicity and lethality of mitomycin C, carboplatin, and doxorubicin without compromising P388 antitumor efficacy. Of relevance to our studies with doxorubicin, Ohnishi et al. (29) report that WR-1065, the dephosphorylated cellular uptake form of WR-2721, scavenged doxorubicin-derived superoxide anions in an in vitro heart mitochondria model. In BALB/c mice, Bhanumathi et al. (30) reduced doxorubicin cardiotoxicity with daily i.p. doses of WR-1065. Further preclinical studies of the potential of WR-2721 to reduce anthracycline cardiotoxicity are in progress in our laboratory. In addition, our murine studies demonstrate p.o. administered WR-151327 to be as effective as i.p. WR-2721 in chemoprotecting for mitomycin C, cisplatin, and carboplatin, and support the clinical development of WR-151327 as a p.o. administered chemoprotector.

REFERENCES


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