WL-276, an Antagonist against Bcl-2 Proteins, Overcomes Drug Resistance and Suppresses Prostate Tumor Growth

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Abstract

Patients with hormone-refractory prostate cancer (HRPC) have an estimated median survival of only 10 months because of acquired drug resistance, urging the need to develop therapies against the drug-resistant HRPC phenotype. Accumulating evidence suggests that overexpressing antiapoptotic Bcl-2 family proteins is at least partially responsible for the development of drug resistance among HRPC patients. Antagonizing the antiapoptotic Bcl-2 family proteins, therefore, is one potential approach to circumventing drug resistance in HRPC. WL-276 was developed as a small-molecule antagonist against antiapoptotic Bcl-2 family proteins, with binding potency comparable to (−)-gossypol. Overexpressing Bcl-2 or Bcl-xL failed to confer resistance to WL-276. WL-276 also effectively induced apoptosis in PC-3 cells. In addition, three PC-3 cell lines with acquired drug resistance against standard cancer chemotherapies were more sensitive to WL-276 than the parent PC-3 cell line. The increased cytotoxicity toward drug-resistant PC-3 cells shows the clinical potential of WL-276 against HRPC that is resistant to conventional therapies. The anticancer activity of WL-276 was manifested in its suppression of PC-3–induced prostate tumor growth in vivo. The selective toxicity of WL-276 against drug-resistant PC-3 cells and its in vivo suppression of PC-3 prostate tumor growth suggest that WL-276 is a promising lead candidate for the development of Bcl-2 antagonists against drug-resistant HRPC. [Cancer Res 2008;68(11):4377–83]

Introduction

Prostate cancer is the most common malignancy and the second leading cause of cancer-related deaths among males in the United States with about 218,890 new cases and 27,050 deaths expected for 2007 (1). Whereas treatment for localized therapy can be highly effective, current treatments for metastatic prostate cancer are temporizing as the disease following initial remission in response to androgen ablation relapses to a hormone-refractory phenotype [hormone-refractory prostate cancer (HRPC)], a generally fatal form of prostate cancer (2). Currently, the best treatment against HRPC is docetaxel-based chemotherapy with an overall survival improvement by ~ 6 months. A major challenge to docetaxel-based chemotherapy for HRPC patients is the development of resistance, leading to the failure of treatment and patient death (3, 4). Therefore, there is an urgent need to search for approaches that control drug-resistant HRPC.

Drug-resistant HRPC is often genetically associated with the overexpression of antiapoptotic Bcl-2 family proteins, the key regulatory proteins in inhibiting apoptosis (4–9). Thus, antagonizing the antiapoptotic Bcl-2 family proteins is one promising strategy to treat drug-resistant HRPC. This is supported by the recent clinical trial results of oblimersen sodium (an antisense against Bcl-2 protein; refs. 10–12). Small-molecule antagonists against antiapoptotic Bcl-2 family proteins are also under intense investigation (13–19). (−)-Gossypol, for example, has been shown to enhance the response of PC-3 tumors to radiation therapy via inhibition of Bcl-XL (20). However, it remains to be determined whether any of these are effective against drug-resistant HRPC. Furthermore, none of these small molecules, as a single agent, have shown effective in vivo anticancer activity against HRPC.

WL-276 is a small-molecule antagonist against antiapoptotic Bcl-2 family proteins developed in our laboratory based on BH3I-1 (15). In this study, we showed that WL-276 had similar inhibitory activity against Bcl-2 protein and enhanced activity against Bcl-xL protein compared with (−)-gossypol (21). WL-276 effectively induced apoptosis in PC-3 cells at low micromolar concentrations. Overexpression of antiapoptotic Bcl-2 proteins failed to induce resistance to WL-276 in vitro. More excitingly, WL-276 showed enhanced toxicity against drug-resistant PC-3 cells. As a single agent, WL-276 effectively suppressed PC-3 cell–induced prostate cancer growth in vivo with no observable toxicity. WL-276 was metabolically stable as well. These studies, as detailed below, show the promise of developing WL-276–based Bcl-2 antagonists for the treatment of HRPC, especially the drug-resistant HRPC.

Materials and Methods

WL-276 syntheses. All commercial reagents and anhydrous solvents were purchased from vendors and used without further purification. Analytic TLC was done on EM Science silica gel 60 F254 (0.25 mm). Compounds were visualized by UV light or stained with p-anisaldehyde, potassium permanganate, or cerium molybdate solutions, followed by heating. Flash column chromatography was done on Fisher Scientific silica gel (230–400 mesh). Melting points were determined on a Thomas-Hoover Unimelt melting point apparatus 6406-K. 1H nuclear magnetic resonance (NMR) spectra were recorded on a Varian 300 MHz spectrometer and calibrated using an internal reference. Electrospray ionization mode mass spectra were recorded on a BrukerBioTOF II mass spectrometer. WL-276 was synthesized in our laboratory by the following procedures (Fig. 1). Briefly, NaOH (0.8 g, 200 mmol) was added to a suspension of 1-phenylalanine (1.65 g, 100 mmol) in water (150 mL). The mixture was stirred to complete dissolution. Carbon disulfide (0.6 mL) was then added and the mixture was stirred vigorously overnight. Aqueous solution of CICH2CO2Na (100 mL, 1 mol/L) was added and the mixture was stirred at room temperature for 8 h. Hydrochloric acid solution (5.5 mol/L) was then added to adjust the pH of the solution to pH 2–3, followed by refluxing the solution overnight. The reaction mixture was neutralized by saturated NaHCO3
Solution. Solvent was removed under reduced pressure and the residue was purified by silica gel chromatography eluting with 30% ethyl acetate in hexanes and 0.2% HOAc to give a cyclized product (1; 1.282 g, 45%). The solution of 1 (980 mg, 3.867 mmol) in dry toluene (70 mL) was mixed with 4-biphenylcarboxaldehyde (952 mg, 5.231 mmol) and NH4OAc (537 mg, 6.794 mmol) followed by refluxing for 16 h. Solvent was removed under reduced pressure. The residue was purified by silica gel chromatography eluting with 0% to 30% ethyl acetate in hexanes plus 0.2% HOAc to give a condensed product (2; 1.413 g, 90%). The solution of 2 (445 mg, 1 mmol) in dichloromethane (60 mL) was treated with p-toluenesulfonamide (188 mg, 1.1 mmol), N-ethyl-N’-(dimethylaminopropyl)carbodiimide (229 mg, 1.2 mmol), and 4-dimethylaminopyridine (61 mg, 0.5 mmol) and stirred at room temperature overnight. Solvent was removed under reduced pressure. The residue was purified by silica gel chromatography eluting with 0% to 15% ethyl acetate in hexanes plus 0.2% HOAc to give the desired product WL-276 as a yellow powder 430 mg (71%). TLC (ethyl acetate/hexanes, 1:3), acetate in hexanes plus 0.2% HOAc to give a cyclized product (980 mg, 3.487 mmol) in dry toluene (70 mL) was mixed with 4-(dimethylaminopropyl)carbodiimide (229 mg, 1.2 mmol), and 4-dimethylaminopyridine (61 mg, 0.5 mmol) and stirred at room temperature overnight. Solvent was removed under reduced pressure. The residue was then added into the mixture and further incubated overnight at 30°C. Ammonium acetate solution from Biovison (5 μL) was added to the sample and mixed well. Isopropanol (100 μL) was added and the solution was mixed well and kept at −20°C for 20 min. DNA pellet was obtained by centrifugation at 13,000 × g for 10 min. The pellet was washed twice with ice-cold 75% ethanol, dried, and resuspended in DNA Suspension Buffer (20 μL). Samples were loaded onto a 1.2% agarose gel containing 0.5 μg/mL ethidium bromide in both gel and running buffer. Electrophoresis was run at 30 V for 1 h. DNA was visualized with UV light and photographed.

**Cell viability analyses.** Jurkat cells (1 × 10^6 per well) were plated in a 96-well plate. PC-3 cancer cells (3,000 per well) were plated in a 96-well plate. The cells were treated with either a vehicle control or various concentrations of WL-276 for 24 h. At the end of each treatment, cell viability in each well was measured by using CellTiter Blue Cell Viability Assay kit (Promega) and normalized to the vehicle-treated control.

**DNA fragmentation.** DNA fragmentation was assessed with Apoptotic DNA Ladder Extraction Kit (Biovision). Briefly, PC-3 cells were treated with WL-276 for 6 h. Cells (2.0 × 10^6) were harvested and washed with PBS. The cells were suspended in DNA Ladder Extraction Buffer (50 μL). After incubation at 23°C for 10 s with gentle pipetting, the mixture was centrifuged for 5 min at 1,600 × g. The supernatant was transferred to a fresh tube and the cell pellet was extracted again with DNA Ladder Extraction Buffer (50 μL). The supernatants were combined and Enzyme A solution (5 μL) was added into the supernatant. The solution was mixed by gentle vortex and incubated at 37°C for 10 min. Enzyme B solution (5 μL) was then added into the mixture and further incubated overnight at 30°C. Ammonium acetate solution from Biovison (5 μL) was added to the sample and mixed well. Isopropanol (100 μL) was added and the solution was mixed well and kept at −20°C for 20 min. DNA pellet was obtained by centrifugation at 13,000 × g for 10 min. The pellet was washed twice with ice-cold 75% ethanol, dried, and resuspended in DNA Suspension Buffer (20 μL). Samples were loaded onto a 1.2% agarose gel containing 0.5 μg/mL ethidium bromide in both gel and running buffer. Electrophoresis was run at 30 V for 1 h. DNA was visualized with UV light and photographed.

**Caspase-3/-7 activity.** Apo-ONE Homogeneous Caspase-3/-7 Assay Kit (Promega) was used to measure caspase-3/-7 activity according to the manufacturer’s instructions. Briefly, after 24 h of WL-276 treatment, the cell culture medium was removed and fresh RPMI cell culture medium (50 μL) with Apo-One Caspase-3/-7 reagent (50 μL) was added to each well. The solution was mixed gently and incubated at 37°C for 45 min. The fluorescence intensity of each well was measured with excitation at 485 nm and emission at 530 nm. Caspase-3/-7 activity was normalized to the vehicle-treated control.

**Cytochrome c release.** Fractionation of PC-3 cells into mitochondrial and cytosolic fractions was done at 4°C following established procedures (23, 24). Briefly, 24 h after WL-276 treatment, PC-3 cells (20 × 10^6) were trypsinized and collected. The pellets were resuspended in fractionation buffer A (0.3 mL, 1 mmol/L HEPES-KOH, 0.1 mmol/L EDTA, 1 mmol/L EGTA, 250 mmol/L sucrose, pH 7.4) supplemented with protease inhibitor cocktail from Sigma. Cell disruption was done by passing the cells through a 23-gauge needle 20 times. The lysate was centrifuged at 800 × g for 10 min at 4°C to remove nuclei and unbroken cells. The supernatant was centrifuged at 10,000 × g for 10 min at 4°C to pellet the mitochondrial fraction and the supernatant was the cytosolic fraction. Cytosolic lysates

**Figure 1. Synthesis of WL-276.**
were vortexed for 10 s. The suspension was centrifuged at 14,000 × g for 10 min. The supernatant was recovered, 500 μL of which were mixed with a nitrocellulose membrane, blocked, and probed with cytochrome c primary antibody (Santa Cruz, Inc.). Bound antibodies were detected with appropriate peroxidase-coupled secondary antibody followed by detection using the SuperSignal chemiluminescence system (Pierce). Animals. Male athymic BALB/c nude mice obtained from Frederick Cancer Research Facility (National Cancer Institute, Frederick, MD) were maintained in a laminar airflow cabinet under pathogen-free conditions and used at 8 to 12 wk of age. All facilities were approved by the American Association for Accreditation of Laboratory Animal Care in accordance with the current regulations and standards of U.S. Department of Agriculture, U.S. Department of Health and Human Services, and NIH.

Xenograft tumor growth and WL-276 treatment. Cultured PC3-LN4 cells (60–70% confluent) were prepared for injection as previously described (25). Mice were anesthetized with isoflurane. Viable tumor cells (2 × 10⁶ in 0.2-mL PBS) were implanted s.c. into the flank. Formation of a bulla indicated a satisfactory injection. Beginning on day 3 after injection, groups of mice (16 mice per group) were then treated with lip. saline, 50 mg/kg WL-276, or 100 mg/kg WL-276 for 12 d. Tumor size, volume, and mouse body weight were assessed every 2 d. Mice were subjected to necropsy 24 h after the last WL-276 treatment. Tumors were removed and weighed. The tumors were quickly frozen in liquid nitrogen for Western blot analyses. At the time of sacrifice, the serum from each animal was collected for WL-276 quantification.

Induction of apoptosis in vivo. Frozen tumor tissues were pulverized using a mortar and pestle on dry ice. The pulverized tissue (weighing 1.2 g) was suspended in lysis buffer [100 μL, 15 mmol/L MgCl₂, 50 mmol/L HEPES (pH 7.4), 150 mmol/L NaCl, 8 mol/L urea, 0.1% Triton X-100] and a cocktail of protease inhibitors, sonicated for 1 min, and centrifuged at 15,000 rpm for 20 min. Recovered supernatants were frozen at −80 °C. Twenty micrograms of protein obtained from prostate tumor tissue homogenates were separated by SDS-PAGE, transferred onto a nitrocellulose membrane, blocked, and probed with poly(ADP-ribose) polymerase (PARP) primary antibody (Cell Signaling). Bound antibodies were detected with appropriate peroxidase-coupled secondary antibody followed by detection with the SuperSignal chemiluminescence system (Pierce).

Analyses of WL-276 in serum. On sacrifice, serum of each mouse was collected and stored at −80 °C until use. The serum level of WL-276 was determined by recovering WL-276 from the serum and quantifying the recovered WL-276 through high-performance liquid chromatography (HPLC) at 395 nm with 4-phenylphenol as the internal standard. Briefly, serum (200 μL) and a mixture of methanol and acetonitrile (1:1, v/v; 600 μL) were vortexed for 10 s. The suspension was centrifuged at 14,000 × g for 10 min. The supernatant was recovered, 500 μL of which were mixed with a solution of 4-phenylphenol (100 μmol/L, 500 μL, H₂O/methanol/acetonitrile 2:1:1). The solution was then analyzed by HPLC. HPLC analysis was done on a Beckman Coulter System Gold 126 solvent module with 168 detector. A Phenomenex Polar RP column (5 μm, 250 × 4.6 mm) was used for the analyses. The flow rate used was 0.6 mL/min. The mobile phase A was water with 0.1% acetic acid whereas B was acetonitrile with 0.1% acetic acid. The time program used for analyses was 40% A (0–5 min), 60% to 95% B (5–20 min), 95% B (20–25 min), and 5% to 40% A (25–27 min).

Statistical analysis. Most of the biological experiments, including the binding assays, cell viability assays, caspase-3/-7 assays, and HPLC quantification of serum concentrations of WL-276, were done at least twice with triplicates in each experiment. For DNA fragmentation, Western blot analyses, and in vivo xenograft tumor growth, at least two independent assays were done. Representative results are depicted in this report. Data were analyzed using Prism (GraphPad Software). Student’s t test was applied for comparison between groups using Prism. Differences were considered statistically significant at P ≤ 0.05.

Results WL-276 disrupts the binding interaction of Bak BH3 domain peptide with antiapoptotic Bcl-2 proteins. Based on our recent structure-activity relationship studies of BH3I-1 (21), we synthesized WL-276 as a putative antagonist against antiapoptotic Bcl-2 proteins (Fig. 1). We then examined its ability to disrupt the binding interaction of antiapoptotic Bcl-2 proteins with a Bak BH3 domain peptide (Fig. 2). (−)-Gossypol was used for comparison because it is the only small-molecule Bcl-2 antagonist with in vivo activity against HRPC (20) and one of the most potent antagonists reported to date. Compared with (−)-gossypol (Kᵢ = 10.1 μmol/L for Bcl-2 and Kᵢ = 24.7 μmol/L for Bcl-XL; ref. 21), WL-276 had similar potency (Kᵢ = 22.8 μmol/L for Bcl-2 and Kᵢ = 1.2 μmol/L for Bcl-XL). In fact, WL-276 had increased inhibitory activity against Bcl-XL protein than (−)-gossypol (20-fold improvement). Of note, the ex vitro inhibitory activities of (−)-gossypol against antiapoptotic Bcl-2 family proteins evaluated under our conditions are ~30 to 50 times lower than those reported before (14). The discrepancy of the ex vitro inhibitory activities of (−)-gossypol is likely due to the differences among the recombinant Bcl-2 proteins and the competitive peptides used in the assay systems (14, 21). Although the inhibitory activities of WL-276 are not in the nanomolar concentration range as the other reported small molecules (14, 18, 19, 26), it is comparable to those of (−)-gossypol.

WL-276 induces apoptosis in PC-3 cancer cells. As an antiapoptotic Bcl-2 family protein antagonist, we envision that WL-276 would be a potential candidate to treat HRPC. We therefore evaluated the in vitro cytotoxicity of WL-276 against PC-3 cell lines and explored its ability to induce apoptosis. The IC₅₀ of WL-276 was in the low micromolar concentration (13.6 μmol/L; Fig. 34), about two times less potent than the reported activity for (−)-gossypol (20). The relatively weak cytotoxicity of WL-276 compared with (−)-gossypol is likely due to the shorter drug treatment period used in our assay: our drug treatment was 2 days whereas that for (−)-gossypol was 5 to 7 days (20). The growth inhibition induced by WL-276 was attributable to apoptotic cell death, as evidenced by caspase-3/7 activation, DNA fragmentation.

Figure 2. WL-276 inhibits the binding of a Bak BH3 domain peptide to Bcl-2 or Bcl-XL protein. Analysis was carried out as described in Materials and Methods. In brief, Bcl-2 or Bcl-XL recombinant protein was first mixed with a fluorescein-labeled Bak BH3 domain peptide to form a protein-peptide complex, which resulted in fluorescence polarization (FP) increase. On the addition of WL-276, fluorescence polarization was measured, which reflects the amount of remaining protein-peptide complex.
WL-276 overcomes drug resistance in vitro. Because overexpressing antiapoptotic Bcl-2 family proteins is one mechanism for malignancies to acquire resistance to cancer therapies, we explored whether WL-276, as an antagonist against antiapoptotic Bcl-2 family proteins, may nullify such drug resistance. To test this hypothesis, two sets of Jurkat cells stably transfected with either Bcl-2 or Bcl-XL were acquired and characterized as detailed before (22). They showed extensive resistance to anticancer agents with

**Figure 3.** In vitro cytotoxicity and apoptotic induction by WL-276 in PC-3 cell line. A, dose-dependent effect of WL-276 on the cell viability of PC-3. The cells were exposed to WL-276 at the indicated concentrations for 48 h and cell viability was assessed with CellTiter Blue Cell Viability Assay kit. Points, mean (n = 3); bars, SD. B, levels of caspase-3/-7 induced by different doses of WL-276. Columns, mean, normalized to untreated cell sample (n = 3); bars, SD. *, P < 0.05; **, P < 0.01; ***, P < 0.001. PC-3 cells were treated with WL-276 at the indicated concentrations for 24 h and caspase-3/-7 activity was evaluated with Apo-ONE Caspase-3/-7 reagent. C, DNA fragmentation in PC-3 cells on WL-276 treatment. PC-3 cells were treated with WL-276 at the indicated concentrations for 6 h and DNA fragmentation was assessed by using Apoptotic DNA Ladder Extraction Kit. D, cytochrome c release in PC-3 cells on WL-276 treatment. PC-3 cells were treated with WL-276 at the indicated concentrations for 24 h and cells were fractionated into cytosolic fraction and mitochondrial fraction. The presence of cytochrome c in cytosol was assessed by Western blot analyses.

**Figure 4.** Sensitivity of various anticancer agents and WL-276 to Jurkat cells transfected with Bcl-2 or Bcl-XL. Jurkat cells were exposed to the drugs at the indicated concentrations for 24 h and cell viability was assessed with CellTiter Blue Cell Viability Assay kit. Columns, mean, normalized to untreated cell sample (n = 3); bars, SD. IC_{50} for Taxol and Fas ligand were not obtained because these two agents are cytostatic with the concentration range tested.
WL-276 inhibited tumor growth to ~30% of the tumor volume in the control group.

**WL-276 induces apoptosis in prostate tumors.** We next explored whether WL-276 suppressed prostate tumor growth in vivo through the induction of apoptosis by evaluating the cleavage of PARP, a well-known marker of apoptosis (27). WL-276 treatment at both dosages decreased the relative amount of full-length PARP (Fig. 6C), indicating the cleavage of PARP and the induction of apoptosis in tumor tissues treated with WL-276. The relative enhancement of apoptosis in the tumor tissues seemed to correlate with the reduction of tumor mass, supporting the notion that WL-276 suppresses prostate tumor development induced by PC-3 cells through the induction of apoptosis in cancer cells.

**WL-276 is stable in vivo and likely the active species to suppress tumor growth.** To determine whether WL-276 is the bioactive species in vivo for the observed anticancer activity or the activity may derive from WL-276 metabolites, we evaluated the stability of WL-276 in vivo and quantified the serum level of WL-276. We first examined the stability of WL-276 in serum. Briefly, authentic WL-276 was dissolved in serum collected from the control mice. The mixture was incubated at 37°C for varying periods of time followed by the recovery of WL-276 through extraction; its quantity and purity were analyzed by HPLC. As intermediates for WL-276 synthesis (Fig. 1), 1 and 2 were hypothesized as potential metabolites and therefore were analyzed. Based on HPLC profiles, no peaks other than WL-276 were detected, including 1 and 2 (Supplementary data). In addition, quantitative recovery of WL-276 was achieved, suggesting that WL-276 was stable in serum.

We then quantified the serum level of WL-276 from the mice treated with the two dosages of WL-276 by following the procedure above (Fig. 6D). WL-276 at 0.27 μmol/L was detected in the serum

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**Figure 5.** A, sensitivities of drug-resistant PC-3 cells against the corresponding anticancer agents used for the development of drug resistance. PC-3 cells were exposed to the drugs at the indicated concentrations for 24 h. Cell viability was assessed with CellTiter Blue Cell Viability Assay kit. Points, mean, normalized to untreated cell sample (n = 3); bars, SD. B, sensitivities of drug-resistant PC-3 cells against WL-276. PC-3 cells were exposed to the drugs at the indicated concentrations for 24 h. Cell viability was assessed with CellTiter Blue Cell Viability Assay kit. Columns, mean, normalized to untreated cell sample (n = 3); bars, SD. *, P < 0.05; **, P < 0.01; ***, P < 0.001.
the relative balance of proapoptotic Bcl-2 proteins and antiapoptotic Bcl-2 proteins between the drug-resistant PC-3 cell lines and the parent PC-3 cell line. Although the levels of antiapoptotic Bcl-2 proteins increased sensitivity to WL-276 compared with the control group. More importantly, drug-resistant PC-3 cells nullified drug resistance induced through the overexpression of antiapoptotic Bcl-2 protein (2-fold decrease in potency). Second, WL-276 (20-fold increase in potency) and moderately decreased potency against Bcl-XL protein (2-fold decrease in potency). Third, WL-276 effectively suppressed PC-3 tumor growth in vivo with near complete suppression at the dose of 100 mg/kg daily. Even at 50 mg/kg, WL-276 reduced tumor volume to ~30% of the control. Mechanistically, WL-276 suppressed tumor growth in vivo through the induction of apoptosis, consistent with its antagonism against antiapoptotic Bcl-2 family proteins. Lastly, WL-276 is metabolically stable in vivo, suggesting that the anti-cancer activity observed in vivo is likely due to WL-276 antagonism against antiapoptotic Bcl-2 proteins. Given its low micromolar activity against Bcl-2 and Bcl-XL proteins, it is possible that WL-276 may interact with cellular targets other than antiapoptotic Bcl-2 family proteins as well.

Considering the pivotal function of antiapoptotic Bcl-2 proteins in tumorigenesis, drug resistance, and metastasis of HRPC, it is possible that WL-276 represents a promising candidate for the development of selective therapies against drug-resistant HRPC. Further development of this candidate for the treatment of HRPC is currently under way.

Discussion

Current chemotherapeutic strategies for HRPC, a fatal form of prostate cancer, are not very effective because HRPC rapidly develops resistance to chemotherapy. Because overexpressing antiapoptotic Bcl-2 family proteins is one mechanism for HRPC to develop such resistance, small-molecule antagonist against antiapoptotic Bcl-2 proteins may be an effective treatment against drug-resistant HRPC.

Here, we identified WL-276, a small-molecule antagonist against antiapoptotic Bcl-2 family proteins, as a lead candidate to treat drug-resistant HRPC. First, compared with (−)-gossypol, a Bcl-2 antagonist with shown chemosensitizing activity against HRPC in vivo, WL-276 exhibited improved potency against Bcl-XL protein (20-fold increase in potency) and moderately decreased potency against Bcl-2 protein (2-fold decrease in potency). Second, WL-276 effectively suppressed PC-3 tumor cell growth through the induction of apoptosis in a dose-dependent manner. Third, WL-276 nullified drug resistance induced through the overexpression of antiapoptotic Bcl-2 proteins. More importantly, drug-resistant PC-3 cell lines showed increased sensitivity to WL-276 compared with the parent PC-3 cell line. Although the levels of antiapoptotic Bcl-2 proteins between the drug-resistant PC-3 cell lines and the parent PC-3 cell line showed no detectable differences (data not shown), the relative balance of proapoptotic Bcl-2 proteins and antiapoptotic Bcl-2 protein may vary among these cell lines, which may account for the selective cytotoxicity of WL-276 against the drug-resistant ones. The ability to eradicate Bcl-2 protein–induced drug resistance and to selectively eliminate drug-resistant PC-3 cancer cell lines strongly argue for the anticancer potential of WL-276 against drug-resistant HRPC. Fourth and most importantly, as a single agent, WL-276 effectively suppressed PC-3 tumor growth in vivo with near complete suppression at the dose of 100 mg/kg daily. Even at 50 mg/kg, WL-276 reduced tumor volume to ~30% of the control. Mechanistically, WL-276 suppressed tumor growth in vivo through the induction of apoptosis, consistent with its antagonism against antiapoptotic Bcl-2 family proteins. Lastly, WL-276 is metabolically stable in vivo, suggesting that the anti-cancer activity observed in vivo is likely due to WL-276 antagonism against antiapoptotic Bcl-2 proteins. Given its low micromolar activity against Bcl-2 and Bcl-XL proteins, it is possible that WL-276 may interact with cellular targets other than antiapoptotic Bcl-2 family proteins as well.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

Received 12/11/2007; revised 2/25/2008; accepted 3/14/2008.

Grant support: NIH grant CA114294 (C. Xing).

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