Enhanced Chemosensitivity to CPT-11 with Proteasome Inhibitor PS-341: Implications for Systemic Nuclear Factor-κB Inhibition

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Abstract

Inducible activation of nuclear factor-κB (NF-κB) inhibits the apoptotic response to chemotherapy and irradiation. Activation of NF-κB via phosphorylation of an inhibitor protein IκB leads to degradation of IκB through the ubiquitin-proteasome pathway. We hypothesized that inactivation of proteasome function will inhibit inducible NF-κB activation, thereby increasing levels of apoptosis in response to chemotherapy and enhancing antitumor effects. To assess the effects of proteasome inhibition on chemotherapy response, human colorectal cancer cells were pretreated with the dipeptide boronic acid analogue PS-341 (1 μM) prior to exposure to SN-38, the active metabolite of the topoisomerase I inhibitor, CPT-11. Inducible activation of NF-κB and growth response were evaluated in vitro and in vivo. Effects on p53, p21, p27 and apoptosis were determined. Pretreatment with PS-341 inhibited activation of NF-κB induced by SN-38 and resulted in a significantly higher level of growth inhibition (64–75%) compared with treatment with PS-341 alone (20–30%) or SN-38 alone (24–47%; P < 0.002). Combination therapy resulted in a 94% decrease in tumor size compared with the control group and significantly improved tumoricidal response to treatment compared with all treatment groups (P = 0.02). The level of apoptosis was 80–90% in the treatment group that received combination treatment compared with treatment with single agent alone (10%). Proteasome inhibition blocks chemotherapy-induced NF-κB activation, leading to a dramatic augmentation of chemosensitivity and enhanced apoptosis. Combining proteasome inhibition with chemotherapy has significant potential to overcome the high incidence of chemotherapy resistance. Clinical studies are currently in development to evaluate the role of proteasome inhibition as an important adjuvant to systemic chemotherapy.

Introduction

The genotoxic effect of conventional anticancer therapy involving many chemotherapy agents and gamma irradiation results in the induction of apoptosis in cancer cells (1–3). The ability to inhibit apoptosis appears to be a principal mechanism by which resistant cancer cells are protected from chemotherapy and radiation (4–6). Cellular mechanisms that protect cancer cells against apoptosis include lack of a functional response mechanism to apoptotic stimuli (e.g., mutated or deleted p53 tumor suppressor gene), presence of an inhibitor to apoptosis (e.g., Bcl-2 and IAP3), and the expression of the multidrug resistance gene (MDR; Refs. 7–9). Recently, the inducible activation of the nuclear transcription factor NF-κB has been reported to inhibit the apoptotic response to chemotherapy and irradiation (10).

NF-κB, a key transcription factor involved with immune and inflammatory responses as well as cell growth, is regulated primarily through interactions with an inhibitor protein known as IκB (11). Stimuli that activate NF-κB typically induce the recently identified IκB kinase (IKK) complex to phosphorylate IκB on NH2-terminal serines, which leads to ubiquitination and subsequent degradation of the inhibitor by the proteasome. After IκB degradation, NF-κB translocates to the nucleus, where it regulates genes involved in a variety of processes, including the suppression of apoptosis (12, 13).

Recently, a gene therapy approach using recombinant adenovirus-mediated transfer of a modified form of IκBζ resulted in significant augmentation of chemosensitivity and enhanced induction of apoptosis in a xenograft tumor model in response to chemotherapy treatment (14). These findings suggested that NF-κB may represent an important molecular target for the purpose of enhancing the sensitivity of certain cancer cells to apoptotic stimuli. The use of gene therapy to deliver NF-κB inhibitors is relevant to certain cancers but is limited when considering widely disseminated metastases.

An alternative strategy for the inhibition of NF-κB activation is facilitated by inhibition of proteasome function [for a review of the proteasome function, see Elliott and Adams (15)]. The inhibition of proteolytic function effectively blocks degradation of cellular proteins that have undergone ubiquitination, such as IκB. In fact, proteasome inhibition is a well-established mechanism to block NF-κB in response to a variety of stimuli. A clear advantage to this therapeutic approach is the clinical availability of a systemically administered small molecule (PS-341, a potent, boronic acid dipeptide that is highly selective for proteasome inhibition; Ref. 16) that can potentially inhibit chemotherapy-induced activation of NF-κB and enhance the apoptotic response to chemotherapy. To evaluate this therapeutic approach, we hypothesized that inactivation of proteasome function will inhibit inducible NF-κB activation, resulting in increased levels of apoptosis in response to chemotherapy and enhanced antitumor effects in vivo. To test our hypothesis, we used PS-341 to evaluate the effects of proteasome inhibition on: (a) chemotherapy-induced NF-κB activation in colorectal cancer cells; (b) the levels of apoptosis after treatment with chemotherapy; and (c) tumor growth in a xenograft model treated with chemotherapy.

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3 The abbreviations used are: IAP, inhibitor of apoptosis protein; NF-κB, nuclear factor-κB; CPT-11, 7-ethyl-10-[4-[(1-piperidino)-1-piperidino]carbonyloxy]camptothecin; SN-38, 7-ethyl-10-hydroxy camptothecin; ATCC, American Type Culture Collection; FBS, fetal bovine serum; MTD, maximum tolerated dose; TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling; HRP, horseradish peroxidase.
Materials and Methods

Chemotherapy Agents. Camptothecin is a specific inhibitor of mammalian DNA topoisomerase I. The camptothecin analogue CPT-11 and its active metabolite SN-38 were generously provided by J. Malcyn (Pharmacia and Upjohn Co., Kalamaizoo, MI).

Cell Culture. The human colon cancer cell line LOVO was obtained from ATCC (Rockville, MD). The LOVO cells were grown in F-12 (Ham’s) with 20% FBS. The colon cancer cell lines KM12L4 (generous gifts of J. Fidler, U.T. M.D. Anderson Cancer Center, Houston, TX), and WiDr (obtained from ATCC) were grown in MEM with nonessential amino acids (Grand Island, NY) and 10% FBS. The colon cancer cell line CDD841 (ATCC) was grown in DMEM with 10% FBS. All media were obtained from Life Technologies, Inc. (Gaithersburg, MD) and supplemented with 100 μg/ml penicillin G and 100 μg/ml streptomycin. Cell cultures were maintained at 37°C.

Cell Growth Response. Human colorectal cells, including LOVO, KM12L4, WiDr, and CDD841, were seeded in six-well plates at 5 × 10⁴ cells/well. Cells were treated with PS-341 24 h after plating at the concentration of 1 μM for 1 h. The medium was refreshed, and 3 h later, the cells were treated with SN-38, the active metabolite of CPT-11, at a dose of 10 ng/ml for LOVO and KM12L4, a dose of 5 ng/ml for WiDr, and at a dose of 0.5 nM for CDD841. PBS, PS-341 alone, and SN-38 alone were used as control groups. Cells counts were performed daily.

NF-κB Activation Assay. Activation of NF-κB in response to treatment with chemotherapy and the proteasome inhibitor was determined by the electrophoretic mobility shift assay as described previously (17). Cells were cultured by seeding 1 × 10⁷ cells in 100-mm dishes. Once cultures reached 50–70% confluence, they were treated with PS-341 at the dose of 1 μM for 1 h. Three h later, cells were treated with SN-38 at the dose of 1 μM/ml. Cells were harvested at 0, 1, 2, and 6 h after treatment with SN-38, and nuclear extracts were prepared as described previously (17). In brief, cells were harvested and suspended in hypotonic buffer. The nuclei were pelleted and resuspended in a low-salt buffer to high-salt buffer. The soluble protein released by centrifugation was collected and stored at −70°C. The DNA probe used contains an NF-κB site (underlined) from the H-2κb gene (5'-CAGGGCTGGGGATTC-CCATCTCCAGTTTCACTCTC-3'; Ref. 14). Seven μg of nuclear extracts were preincubated with 1 μg of poly(deoxyinosinic-deoxyctydilic acid) in binding buffer (10 mM Tris, 50 mM NaCl, 20% glycerol, 0.5 mM EDTA, and 1 mM DTT) for 10 min at room temperature. Approximately 20,000,000 cpm of 32P-labeled DNA probe were then added and allowed to bind for 15 min. The complexes were then separated on a 5% polyacrylamide gel and autoradiographed.

Tumoricidal Response to Combination Therapy. The tumor growth response to combined treatment with the proteasome inhibitor and CPT-11 was assessed in a LOVO xenograft model. Tumors were established by injecting 5 × 10⁶ LOVO cells s.c. into the flank of nude mice (NCR nu/nu athymic nude mice, 5–6 weeks of age, female, and weight of 18–20 g; Taconic, Germantown, NY). Once tumors reached 1-cm diameter in size, the animals received CPT-11 at the dose of 1 μM for 1 h. Four μM-thick sections were cut and collected on charged and pre-cleaned microscope slides (Fisher Scientific, Pittsburgh, PA). The presence of apoptosis, determined by the TUNEL assay was performed using the in situ cell death detection kit (Boehringer Mannheim Corp., Indianapolis, IN) according to manufacturer’s instructions. The apoptotic indices of tumor sections within areas of viable tumor were estimated from the percentage of cells scored under a light microscope at ×100.

Western Blot Analysis. Total cell lysates were prepared by homogenizing the cell pellets in RIPA buffer (25 mM Tris (pH 7.5), 150 mM sodium chloride, 2 mM EDTA, 1 mM dimethyl sulfoxyl fluoride, 10 mM sodium orthovanadate, 50 mM β-glycerophosphate, 1% NP40, 5% deoxycholic acid, 1% SDS, and 20% glycerol) containing a mixture of protease inhibitors [25 μg/ml aprotinin, 10 μg/ml leupeptin, and 5 μg/ml pepstatin were combined with 50 μg of protein extract from cells treated with one of four treatment regimens including PS-341, SN-38, a combination of PS-341 and SN-38, or PBS (control)]. Four h after PS-341 treatment, the samples were electrophoresed on 10% SDS-PAGE gel overlayed with 4% acrylamide stacking gel and transferred to pure nitrocellulose membrane (0.45 μM pore size; Schleicher & Schuell, Hercules, CA), followed by blocking with 10% dry milk in 1× Tris-buffered saline containing 0.1% Tween 20 for 1 h at room temperature. The first antibody P53 (Anti-P53 mouse monoclonal IgG1; Santa Cruz Biotechnology, Santa Cruz, CA) was used to incubate the blot at room temperature for 1 h at a dilution of 1:2000. The second antibody was used to incubate, at room temperature, the dilution of 1:5000 for 30 min. Immunoreactive bands were visualized by ECL Western blotting analysis system (Amersham Life Sciences, Buckinghamshire, United Kingdom) and enhanced chemiluminescence. The ECL system was removed by incubating the blot in ECL strip buffer (100 mM 2-methylethanol, 2% SDS, and 62.5 mM Tris, pH 6.8) at 65°C for 30 min. P27 antibody (mouse polyclonal IgG1; Santa Cruz Biotechnology, Santa Cruz, CA) was used to reprobe the blot at the concentration of 1:1000 for 1 h. The secondary antibody (antimouse IgG-HRP; Promega, Madison, WI) was used at a concentration of 1:2000 for 30 min at room temperature. P21 antibody (goat polyclonal IgG1; Santa Cruz Biotechnology) at a concentration of 1:1000 was used to reprobe the blot after removing the ECL system. The secondary antibody used was antigoat IgG-HRP (Santa Cruz Biotechnology) at a concentration of 1:2000. Actin, a highly conserved protein expressed in all eukaryotic cells, was used to quantitate protein loading. The ECL system was removed by incubating the blot in ECL strip buffer (100 mM 2-methylethanol, 2% SDS, and 62.5 mM Tris, pH 6.8) at 65°C for 30 min. The first antibody, antigoat polyclonal IgG (Anti-P53 mouse monoclonal IgG1; Santa Cruz Biotechnology, Santa Cruz, CA), was used to reprobe the blot at the concentration of 1:500. The second antibody, antigoat IgG-HRP (Santa Cruz Biotechnology) was used at the concentration of 1:2500 to evaluate the actin expression.

Statistical Analysis. The ANOVA procedure was used to compare cell growth response and tumor growth response.

Results

Previously, it has been shown that the topoisomerase I inhibitor CPT-11, or its metabolite SN-38, induces NF-κB activation in cancer cells in vitro and in tumor models (14, 18). To determine the effect of proteasome inhibition on the level of NF-κB activation induced by exposure to chemotherapy in human colorectal cancer cells, cells were treated with proteasome inhibitor PS-341 prior to chemotherapy treatment (Fig. 1). Activation of NF-κB, assessed by electrophoretic mobility shift assay, was observed in all cell lines treated with SN-38 alone, with peak levels occurring at either 1 or 2 h after exposure to SN-38. Pretreatment with PS-341 prior to chemotherapy completely inhibited activation of NF-κB induced by exposure to SN-38 in all cell lines tested. Importantly, treatment with PS-341 alone did not result in activation of NF-κB.
positive control (PS-341). As was observed in Fig. 3 (A), the concentration of the proteasome inhibitor while controlling the dosage of the CPT-11 (Fig. 3B). As was observed in Fig. 3A, combined treatment of CPT-11 and the MTD (1.0 mg/kg) of PS-341 resulted in a sustained tumoricidal effect in which LOVO tumors continued to decrease in size throughout the course of therapy. Increasing the dosage of PS-341 that was administered in combination with CPT-11 resulted in a dose-dependent increase in tumor growth inhibition.

The significantly decreased tumor size that resulted from combined treatment of PS-341 and CPT-11 resulted in a 94% decrease in tumor size, compared with the treatment group receiving mock treatment. However, when PS-341 was combined with SN-38, cell counts were decreased by 64–75% compared with untreated controls, resulting in significantly improved growth inhibition when compared with treatment with PS-341 or SN-38 alone ($P < 0.002$; ANOVA). TUNEL staining for the presence of apoptosis indicates that the markedly increased growth inhibition observed in the treatment group receiving combined PS-341 and SN-38 is associated with a 4-fold increase in the level of apoptosis when compared with treatment with PS-341 or SN-38 alone (data not shown).

To determine whether pretreatment with PS-341 would enhance the anticancer effects of chemotherapy, human colorectal cancer cells were exposed to PS-341 prior to treatment with SN-38. Cells were harvested and assayed for nuclear translocation of NF-κB at 1, 2, and 6 h after chemotherapy treatment. Positive control (+) was KM12L4 cells treated for 2 h with 10 ng/ml tumor necrosis factor-α (a potent activator of NF-κB). The data shown are representative of one of two independent experiments.

PS-341 AUGMENTS CHEMOSENSITIVITY VIA NF-κB INHIBITION

To determine whether the chemosensitivity of tumors could be enhanced by combining treatment with the administration of the proteasome inhibitor, a LOVO xenograft model was used (Fig. 3A). In these experiments, systemic administration of the proteasome inhibitor or CPT-11 alone resulted in a 26 and 48% decrease in tumor size, respectively, when compared with controls receiving mock treatment. However, when PS-341 was combined with SN-38, cell counts were decreased by 64–75% compared with untreated controls, resulting in significantly improved growth inhibition when compared with treatment with PS-341 or SN-38 alone (Fig. 2). Combination treatment, using PS-341 and SN-38, resulted in a slight increase in the level of stabilization of p21, p27, and p53 when compared with single-agent treatment alone. Staining for actin demonstrated equal protein loading in all treatment groups sampled (data not shown). These data suggest that one potential mechanism of action of the observed anticancer effects of combination therapy may be through the stabilization of p53 and the cell cycle regulatory proteins p21 and p27. This also suggested that the increased apoptosis observed in combination therapy (data not shown) may be attributable to p53-dependent apoptosis. However, as shown in Fig. 2, an equivalent reduction in cell number is achieved in either p53 wild-type or mutant cell lines.
tumor model with the combination therapy, tumor sections were stained for the presence of TUNEL-positive cells after treatment (Fig. 5). The TUNEL-positive tumor cells at 24 h after treatment with CPT-11 or PS-341 accounted for ~10% of the total number of cells stained. This relatively low level of apoptosis is not significantly different from that observed in tumor sections obtained from untreated controls (data not shown). In comparison, the number of tumor cells that stained positively for apoptosis was 80–90% in the treatment group that received combined treatment with PS-341 and CPT-11. Thus, these findings suggest that the tumoricidal response to combined treatment with PS-341 and CPT-11 appears to result from markedly increased levels of apoptosis when compared with treatment with either agent alone.

**Discussion**

A growing body of evidence suggests that protection of cancer cells from cell death facilitates tumorigenesis by conveying a selection advantage to cancer cells, thereby disrupting the homeostatic balance of cell death and cell growth in favor of cells that use antiapoptotic survival mechanisms (19). In addition to its role in tumorigenesis, protection against apoptosis may also play a role in resistance to conventional treatments, such as chemotherapy and irradiation (19, 20). Survival mechanisms that function by the inhibition of the apoptotic response may be distinguished by their activity as either acquired or inducible. Acquired mechanisms of antiapoptosis are associated with genetic mutations involved in malignant transformation, such as mutation of the p53 tumor suppressor gene (21), overexpression of Bcl-2 (8), and overexpression of P-glycoprotein in multidrug resistance (7). In contrast, inducible mechanisms of antiapoptosis, are not directly involved in tumorigenesis and are only transiently expressed after exposure to genotoxic stimuli such as anticancer therapies (10, 14).

Recently, we and others have described the inducible antiapoptotic function of the transcription factor NF-κB as a principle mechanism by which cancer cells are protected from undergoing programmed cell death after exposure to tumor necrosis factor-α (10, 22, 23), gamma irradiation, and some chemotherapy agents (10). We have also reported that protection from apoptosis resulting from inducible NF-κB activation may be overcome by selective NF-κB inhibition using the super-repressor IκBα (10, 14, 18). In those studies, infection of tumor cells with a recombinant adenovirus that expresses a mutated form of the highly specific NF-κB inhibitor IκBα (super-repressor IκBα) significantly decreased inducible NF-κB activation in response to treatment with the topoisomerase I inhibitor CPT-11 in a wide variety of human colorectal cancer cell lines and the breast cancer cell line MCF-7 (18). The inhibition of inducible NF-κB resulted in enhanced chemosensitivity in vitro and in xenograft models of both colorectal cancer and sarcoma (14, 18). It was also demonstrated that the enhanced response to chemotherapy was associated with markedly increased levels of apoptosis after combination treatment (14, 18). The mechanisms responsible for the antiapoptotic effects of NF-κB activation after exposure to chemotherapy and gamma irradiation

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![Fig. 3. The effects of combination therapy using the proteasome inhibitor PS-341 and CPT-11 were assessed in a xenograft model. One-cm-diameter LOVO tumors were grown in the flanks of nude mice. The tumoricidal effects of systemic treatment was assessed (A) after pretreatment with PS-341 (1 mg/kg i.v. bolus injection) or vehicle alone, followed by i.v. bolus administration of CPT-11 (33 mg/kg) or vehicle. Treatment was administered biweekly (Monday and Thursday), and tumor diameter along two orthogonal axes was recorded every other day. Tumor volume was calculated by assuming a spherical shape of the tumor, using the formula: volume = 4/3 πr³, where r is ½ (mean diameter of the tumor), and recorded as the means for each treatment group (n = 8–10). Bars, SE. The dose response to increasing concentrations of PS-341 when used in combination with a fixed dosage of CPT-11 (33 mg/kg) was assessed in a LOVO xenograft model (B). The tumoricidal response to different concentrations of PS-341, including the MTD (1 mg/kg) or nucleic acid, was determined by pretreatment with an i.v. bolus administration of PS-341, followed by an i.v. bolus administration of CPT-11. Treatment was administered biweekly, and tumor diameter along two orthogonal axes was recorded every other day (n = 8–10). Tumor volume was calculated as described above.]

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![Fig. 4. Stabilization of cellular proteins mediated by combination therapy using proteasome inhibitor PS-341 and SN-38. A. Western blots of p53 in LOVO cells at 0, 6, 24, and 48 h after treatment with SN-38. The treatment group labeled SN-38 was pretreated with PBS alone prior to chemotherapy treatment; treatment group PS-341 was pretreated with PS-341 4 h prior to mock treatment; treatment group PS341/SN38 was pretreated with PS-341 4 h prior to treatment with SN-38. Immunoblot analysis was used to detect p21 (B) and p27 (C) from protein obtained from whole cell lysates using LOVO cells treated in a similar fashion to that described above.](cancerres.aacrjournals.org)
have not been fully elucidated. However, it is likely that downstream
gene targets that are NF-κB regulated, such as the IAP proteins
c-IAP1 and c-IAP2, are involved in this inducible survival mecha-

Several methods of inhibiting NF-κB activation are currently under
investigation in preclinical studies in our laboratory. One of the first
and most effective methods of NF-κB inhibition uses recombinant
adenovirus-mediated overexpression of the IκBα gene, described pre-
viously. However, the clinical feasibility of gene therapy approaches
for inhibiting NF-κB are currently limited by the requisite intratu-

cular delivery of the recombinant virus that expresses the NF-κB
inhibitor. Alternatively, a systemic means of inhibiting NF-κB may be
applicable in the treatment of widely disseminated metastatic disease.
In this setting, i.v. administered agents that block the antiapoptotic
response mediated by chemotherapy-induced NF-κB activation in
tumor cells may enhance the response of metastatic disease to con-
ventional drug treatment. To examine the potential role of systemic
NF-κB inhibition to enhance chemosensitivity of human cancer cells,
we have selected the proteasome inhibitor PS-341, which has been
shown to block NF-κB activation through stabilization of IκBα, to
combine with the chemotherapy agent SN-38/CPT-11.

The ubiquitin-proteasome pathway (specifically, the 26S protea-
some) is the principle mechanism by which cellular proteins, includ-
ing ubiquitinated IκBα, are degraded (24–26). Inhibition of the ubiq-
utin-proteasome pathway results in dysregulation of cellular proteins
involved in cell cycle control, promotion of tumor growth, and in-
duction of apoptosis (16). Recently, a novel class of anticancer agents
called proteasome inhibitors that yield effective anticancer responses
both in vitro and in vivo have been introduced into the treatment of
malignancy (16, 27). The dipeptide boronic acid analogue PS-341,
used in these experiments, inhibits the chymotryptic activity of the
proteasome in a potent, reversible, and selective manner (16). Protea-
some inhibition using this compound has demonstrated significant
cytotoxic activity against a range of human tumor cell lines that
comprise the National Cancer Institute in vitro screen and is well
tolerated in preclinical animal studies and in Phase I clinical trials
(16). The ability of proteasome inhibitors to regulate NF-κB activa-
tion has led many to speculate that these new compounds may have
important clinical implications in the management of both inflamma-
tory disease and malignancy (28).

This report addresses the issue of whether pretreatment of cancer
cells with PS-341 can effectively block the activation of NF-κB that
is induced by exposure to SN-38/CPT-11. As a second objective, we
have sought to determine whether NF-κB inhibition using PS-341
may augment the sensitivity of tumor cells to chemotherapy. Similar
to the findings we reported using the super-repressor IκBα (14, 18),
proteasome inhibition using PS-341 potently blocked the early acti-
vation of NF-κB at 2 h after chemotherapy treatment. In addition,
combining proteasome inhibition with chemotherapy treatment re-
sulted in a significant anticancer effect compared with treatment with
single agent alone. TUNEL assays, performed to determine whether
this increased anticancer effect from combination treatment was at-
tributeable in part to regulation of apoptosis, demonstrated markedly
increased levels of apoptosis compared with single agent treatment
alone. This observed response is comparable with the response ob-
served when NF-κB was selectively inhibited using the super-repres-
sor IκBα in our previous reports (14, 18).

Our findings suggest that the enhanced anticancer effects, observed
when PS-341 is combined with chemotherapy, are attributable to
inhibition of inducible NF-κB activation. However, potentially other
cell cycle regulatory processes and apoptotic response mechanisms
impacted by proteasome inhibition play a contributing role:

(a) Proteasome inhibition may result in prolonged binding of the
camptothecin to the topoisomerase-1 enzyme in a complex that under
physiological conditions would be degraded by the proteasome (29).
Enhanced sensitivity to SN-38/CPT-11 when a proteasome inhibitor is
coadministered may therefore result in part from the impaired metab-
olism and longer half-life of the topoisomerase 1-camptothecin com-
plex, resulting from impaired proteasome function. The addition of
the proteasome inhibitor to chemotherapy would thereby effectively
increase the duration of activity of the SN-38/CPT-11.

(b) The findings that p21, p27, and p53 are stabilized by chemo-
therapy or PS-341 treatment suggest that stabilization of these key cell
cycle regulatory proteins may be involved in the antitumor effects of
these molecules when used alone or in combination. The ability to
enhance chemosensitivity by proteasome inhibition, however, was
observed in cells expressing both wild-type p53 (CCD841 and
LOVO), mutant p53 (KM12L4 and WiDR), and Bcl-2 (KM12L4),
suggesting that inhibition of chemotherapy-induced NF-κB activation
using proteasome inhibition may occur independently of the func-
tional status of p53 in colorectal cancer cell lines.

Although a variety of mechanisms in addition to inhibition of
NF-κB may potentially contribute to the efficacy of combination
therapy using PS-341 and SN-38/CPT-11, additional studies will be
necessary to further elucidate this potentially complex response. Fur-
thermore, the ability of proteasome inhibition to enhance the sensi-

Fig. 5. Representative photomicrographs demon-
srating TUNEL staining of LOVO tumor sec-
tions after treatment with CPT-11 (left), PS-341 (center), and combination PS-341 and CPT-11 (right). Tumors were harvested 24 h after final treatment.
tivity of other genotoxic anticancer therapies without increasing toxicity in preclinical animal studies warrants further evaluation and is the focus of ongoing studies in our laboratory. Nevertheless, the dramatic augmentation of sensitivity to SN-38/CPT-11 observed both in vitro and in vivo reported here has significant potential to overcome the relatively high incidence of chemotherapy resistance seen in patients with metastatic disease. Clinical studies to evaluate the role of proteasome inhibition as an important adjuvant to systemic chemotherapy are currently in development.

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References

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