Oral RDP58 Allows CPT-11 Dose Intensification for Enhanced Tumor Response by Decreasing Gastrointestinal Toxicity

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ABSTRACT
Cancer patients undergoing triple therapy (CPT-11, 5-fluorouracil, and leucovorin) often present with severe delayed diarrhea as a result of chemotherapy-induced gastrointestinal (GI) toxicity and inflammation. RDP58 is a novel, anti-inflammatory, d-amino acid decapeptide that inhibits the production of tumor necrosis factor α, IFN-γ, and interleukin 12, and has been shown to effectively inhibit clinical symptoms and intestinal inflammation in several rodent models of chemically induced colitis, nonhuman primates with spontaneous colitis, and humans with mild to moderate ulcerative colitis. We evaluated RDP58 as a potential protective agent in chemotherapy-induced GI inflammation. Oral administration of RDP58 significantly decreased the incidence of diarrhea and improved the survival rates of mice treated with toxic doses of CPT-11 or 5-fluorouracil. Histological analysis showed that RDP58 significantly reduced the destruction of the intestinal mucosa by inhibiting local overproduction of tumor necrosis factor α, IFN-γ, and interleukin 12 in vivo. Furthermore, RDP58 administration allowed the maximum tolerated dose of CPT-11 to be doubled in tumor-bearing mice resulting in significantly enhanced primary tumor responses and prolongation of time to relapse without a concomitant increase in GI toxicity. Our results suggest that RDP58 may have clinical utility in cancer therapy by preventing treatment-associated GI toxicity and potentially increasing the effectiveness of chemotherapy.

INTRODUCTION
The basis for the chemotherapeutic treatment of cancer is to ablate rapidly proliferating malignant cells. Most chemotherapeutic agents, however, also kill normal proliferating cells, such as those found in the mucosa of the gastrointestinal (GI) tract. Irinotecan (CPT-11) is a DNA topoisomerase I inhibitor used in the treatment of colorectal cancer and is known to generate GI toxicity in humans and animals (1, 2). Clinical symptoms of CPT-11 toxicity include mucositis and delayed diarrhea, and severe symptoms limit the completion of optimal dosing regimens in many patients (3, 4). Severe delayed diarrhea is a significant side-effect in cancer patients receiving triple therapy [CPT-11, 5-fluorouracil (5-FU), and leucovorin] (5, 6), and is the result of intestinal epithelial cell death, decreased crypt cell renewal, and destruction of the mucosal architecture (7). The tissue destruction induces an inflammatory response through the production of proinflammatory cytokines like tumor necrosis factor (TNF-α) by epithelial cells and infiltrating leukocytes (8). Moreover, TNF-α expression exacerbates the overall pathology initiated by CPT-11 cytotoxicity by increasing TNF-α-mediated apoptosis, and inducing tissue matrix degradation and vascular leakage that significantly add to the clinical toxicity of treatment.

RDP58 is a novel, d-isomer decapeptide with potent anti-inflammatory activity. RDP58 inhibits production of TNF-α, IFN-γ, and interleukin (IL)-12, and up-regulates heme oxygenase 1 activity in vivo (9–11). Previous studies show that oral administration of RDP58 reduced TNF-α production in colon biopsies taken from mice with dextran sodium sulfate-induced colitis (12, 13). Additionally, RDP58 reduced the overall clinical severity, delayed epithelial cell apoptosis, and preserved the intestinal mucosal morphology in these mice. RDP58 has also been shown to inhibit bloody diarrhea in rhesus and cynomolgus monkeys with spontaneous colitis (9), and appears to be effective in treating mild to moderate ulcerative colitis in a Phase II study in humans.1 Importantly, RDP58 has been shown not to be absorbed when taken orally and not systemically bioavailable. These studies suggest that RDP58 may also be therapeutic in chemotherapy-induced GI toxicity and inflammation. In the current study, we tested RDP58 in the mouse models of CPT-11 or 5-FU-induced diarrhea and intestinal inflammation in mice. We also tested whether RDP58 would allow an increase in CPT-11 dose without a concomitant increase in GI toxicity in two mouse tumor models. We found that RDP58 significantly reduced CPT-11-induced diarrhea, mucosal inflammation, and mortality in mice by suppressing the overproduction of proinflammatory cytokines TNF-α, IFN-γ, and IL-12 in vivo. Furthermore, the addition of oral RDP58 to the chemotherapy regimen allowed the doubling of the CPT-11 dose to generate an enhanced tumor response and prolongation of time to relapse without concomitant GI toxicity in mice. These data support the use of oral RDP58 in preventing severe GI toxicity associated with high-dose chemotherapy in humans.

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1 R. J. Tesi, personal communication.
MATERIALS AND METHODS

Animals. All of the in vivo studies were performed using 9–10-week-old BALB/c female mice (The Jackson Laboratory, Bar Harbor, ME). All of the mice were monitored daily and received food and water ad libitum. Animal protocols were approved by the SangStat Institutional Animal Care and Use Committee and were in accordance with the NIH guidelines for animal welfare.

Chemotherapeutic Agents. CPT-11 was purchased from Pharmacia and Upjohn (Kalamazoo, MI) as a ready-to-use aqueous solution. 5-FU was purchased from Sigma (St. Louis, MO). Cytarabine and CPT-11 was purchased from Chugai (Tokyo, Japan). RDP58 [NH₂-arg-norleucine (nle)-nle-arg-nle-nle-nle-gly-tyr-CONH₂] was synthesized using a solution-phase process, purified by high-performance liquid chromatography, and shown to be >90% homogenous by analytical reverse phase high-performance liquid chromatography (HPLC)

Table 1 Modulation of toxicity of CPT-11 and 5-FU by RDP58 in normal mice

<table>
<thead>
<tr>
<th>Therapy (mg/kg/day)</th>
<th>RDP58 (mg/kg/day)</th>
<th>Maximum weight loss</th>
<th>Diarrhea</th>
<th>Mortality</th>
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<td>CPT-11</td>
<td></td>
<td></td>
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<td>10</td>
<td>24.6 ± 1.9a</td>
<td>33</td>
<td>7</td>
</tr>
<tr>
<td>5-FU</td>
<td></td>
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<td></td>
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<td>10</td>
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<td>30</td>
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a P < 0.05 (ANOVA).

Cell Lines. CT-26 mouse colon carcinoma cells were generously provided by Dr. Lee Ellis (M. D. Anderson Cancer Center, Houston, TX). The cell line was grown as a monolayer in DMEM supplemented with 5% fetal bovine serum (Life Technologies, Inc., Rockville, MD), 1X nonessential amino acids, and 1 mM glutamine in a humidified, 37 °C incubator containing 5% CO₂ as described (14).

In Vitro Model of Chemotherapy-Induced GI Toxicty. Chemotherapy-induced GI diarrhea and inflammation was induced in mice by i.p. injection of either CPT-11 or 5-FU (15). Mice were randomized into treatment groups and given either three consecutive i.p. injections of CPT-11 (200 mg/kg/day, once daily [qd] x 3), two i.p. injections of 5-FU (100 mg/kg/day, qd x 2), or saline control. Mice were weighed and examined closely for signs of morbidity and diarrhea daily. Evidence of diarrhea was manifested by appearance of loose and watery stool on the mouse anus. In the initial studies, RDP58 was added to the drinking water at a concentration to deliver ~10 mg/kg/day based on the average daily water consumption per animal per cage. The amount of water consumed per animal per cage was recorded daily and the concentration of RDP58 adjusted accordingly to maintain 10 mg/kg/day dosing. Subsequent studies delivered RDP58 orally by 0.2-ml gavage. Body weights were recorded daily and maximum weight loss for each animal was calculated by subtracting minimal body weight recorded from the same animal upon entry to the study. RDP58-treated versus control (survival analysis).

Fig. 1 Effect of RDP58 on animal survival in normal mice treated with CPT-11 (A) or 5-fluorouracil (5-FU; B). A, mice were supplied with either plain water (control) or various doses of RDP58 (2.5, 5, and 10 mg/kg/day) in drinking water (n = 15 mice/treatment group) starting on day 3. Three consecutive i.p. injections of CPT-11 (200 mg/kg/day, once daily x 3) were administered on days 0, 1, and 2. Mouse survival was monitored continuously. B, mice received either plain water (control) or 10 mg/kg/day RDP58 p.o. from day −3 onward (n = 10/ treatment group, followed by administration of two i.p. doses of 5-FU on days 0 and 1 (100 mg/kg/day, once daily x 2). *, P < 0.05, RDP58-treated versus water control (survival analysis).
allowed to form. For studies using 4T1, animals were s.c. injected with $2.5 \times 10^5$ cells in 0.1 ml of HBSS in the abdominal mammary fat pad (16). Tumors were measured twice weekly in three dimensions using a caliper for the duration of the study, and tumor volumes (mm$^3$) were calculated using the formula $(\text{length} \times \text{width} \times \text{height})/2$. Tumor-bearing mice were randomized into treatment groups to obtain groups with similar average starting tumor volumes.

For studies in the CT-26 model, we used a modified protocol described previously by Boushey et al. (15). Briefly, starting on day 4, CPT-11 was injected i.p. daily for 3 days and followed by a 3-day recovery period. This 6-day regimen was repeated three times. RDP58 was delivered daily by oral gavage at the indicated doses starting on day 0 and continued for the duration of the study. Mice were euthanized at the end of the study, and the tumors were excised and weighed.

Mice in the 4T1 model were administered with CPT-11 daily for 3 consecutive days and followed by a 5-day recovery period. This regimen was repeated once. At the end of the study, lungs were harvested from mice and fixed in Bouin’s solution (Sigma). The total number of lung metastases was enumerated with the aid of a dissecting microscope (17).

**Quantification of Cytokine Production.** For cytokine analysis, 2-cm tissue biopsy segments were dissected from the mid-jejunum of animals and weighed. Tissue segments were then cultured for 12 h in 1 ml of RPMI 1640 containing 10% ultra low IgG fetal bovine serum (12). Cell culture supernatants were collected and clarified by centrifugation, and the levels of TNF-$\alpha$, IFN-$\gamma$, IL-12, IL-1$\beta$, and other cytokines were quantified using commercial ELISA systems (Biosource International, Camarillo, CA).

**Histological Analysis.** Segments of mid-jejunum, distal ileum, or descending colon were harvested at the indicated time points and fixed in 4% paraformaldehyde, processed, and paraffin-embedded (18). Prepared slides were stained with H&E, and morphology was examined and recorded using a Zeiss AxioShop 40 digital microscope equipped with AxioVision Image Analysis System. Both villus height and crypt depth were determined from 10–15 independent measurements of at least 5 different mice per assayed condition. All of the histological specimens were coded and analyzed by a technician blinded to the experimental conditions of each specimen.

The terminal deoxynucleotidyl transferase-mediated nick end labeling assay was performed to determine the level of apoptosis (TdT-FragEL; Oncogene Research Products, Boston, MA). Slides were counterstained with methyl green to aid the morphological evaluation and characterization of normal and apoptotic cells. Negative controls were generated by replacing TdT with water during the labeling step. The crypt epithelial apoptotic index was determined by determining the number of terminal deoxynucleotidyl transferase-mediated nick end labeling-positive cells compared with the total crypt cell count (19).

**Statistical Analysis of the Data.** All of the *in vivo* experiments were performed at least three times, and data points are expressed as mean ± SD. Statistical differences between treatment groups were determined by ANOVA. Survival analysis (log-rank test) was performed using the Fisher’s exact $t$ test. Dose response relationship was tested by Cochran-Armitage trend test. $P < 0.05$ were considered statistically significant.

**RESULTS**

**RDP58 Reduces CPT-11- or 5-FU-Induced Toxicity in Normal Mice.** CPT-11 is known to cause GI toxicity in mice resulting in body weight loss, diarrhea, and mortality (2), and we evaluated RDP58 in modulating GI toxicity in normal mice. Starting on day −3, RDP58 was given orally at 2.5, 5, or 10 mg/kg/day to mice injected with CPT-11 to induce diarrhea. The control treatment group received water only. Whereas only 26.7% of the control group survived CPT-11 treatment, addition
of RDP58 to the drinking water to deliver 2.5, 5, or 10 mg/kg/day significantly increased survival rates to 40.0%, 76.7%, and 93.3%, respectively (P < 0.05; Fig. 1A; Table 1A). The incidence of diarrhea was also reduced in an RDP58 dose-dependent manner. Only 33%, 40%, and 67% of animals receiving 2.5, 5, and 10 mg/kg/day RDP58, respectively, showed signs of diarrhea compared with 97% in the control group. Additionally, the survival rate was highly correlated to the decrease in the incidence of diarrhea (P < 0.05), suggesting that oral administration of RDP58 attenuates CPT-11-associated GI toxicity and mortality in a dose-dependent manner.

To determine whether the protective activity of RDP58 can be applied to other GI toxicity-causing cytotoxic agents, we tested RDP58 in mice treated with 5-FU. Mice were gavaged with 0.2 ml water or 10 mg/kg/day RDP58 daily starting on day −3 and then injected with 100 mg/kg 5-FU on days 0 and 1. RDP58 significantly improved animal survival from 10.0% in control mice to 90.0% in RDP58-treated mice (P < 0.05; Fig. 1B; Table 1B). RDP58 also reduced the incidence of diarrhea to 30% from 100% in 5-FU-treated mice (P < 0.05), suggesting that the protective effect of RDP58 against chemotherapy-induced GI side-effects is not restricted to a single antitumor drug.
RDP58 Inhibits Proinflammatory Cytokine Production in Vivo. The cytoablative effect of chemotherapy often results in increased intestinal permeability, mesenteric leukocyte infiltration, and the induction of proinflammatory cytokines in mice (15). To determine whether the reduction in clinical symptoms of CPT-11 toxicity are associated with RDP58-mediated inhibition of proinflammatory cytokine production in vivo, biopsies from the mid-jejunum region of the intestines from different treatment groups were cultured \textit{ex vivo}, and the levels of TNF-\(\alpha\), IFN-\(\gamma\), and IL-12 were quantified by ELISA. CPT-11 treatment resulted in significant overexpression of TNF-\(\alpha\), IFN-\(\gamma\), and IL-12 (\(P < 0.05\)); however, RDP58 administration significantly reduced the CPT-11-induced overproduction of these three cytokines (\(P < 0.05\); Fig. 2A-C). Consistent with its biological activity \textit{in vitro}, RDP58-mediated cytokine inhibition is specific because IL-1\(\beta\) is up-regulated by CPT-11 regardless of treatment (Fig. 2D). These data indicate that RDP58 reduces CPT-11-induced overexpression of TNF-\(\alpha\), IFN-\(\gamma\), and IL-12 \textit{in vivo}, and this activity is likely to contribute to the reduction of clinical symptoms of GI toxicity and mortality.

RDP58 Prevents CPT-11-Induced Morphological Damage and Apoptosis in Mouse Intestinal Tissue. Because oral RDP58 inhibits overproduction of TNF-\(\alpha\), IFN-\(\gamma\), and IL-12 \textit{in vivo}, we performed histological analysis to determine whether inhibition of proinflammatory cytokine production prevents damage of the intestinal morphology. Consistent with previous findings (20), CPT-11 treatment induced destruction of both mucosal villi and crypts in the jejunum, ileum, and colon compared with histology from control mice (Fig. 3A). In contrast, CPT-11-induced damage to intestinal mucosa was significantly minimized in all of the sections of the intestine examined in mice treated with RDP58 (Fig. 3A). These histological observations were confirmed by morphometric analysis. Whereas CPT-11-treated mice exhibited significant reduction in both villus height (Fig. 3B, panel a) and crypt depth (Fig. 3B, panel b) in the mid-jejunum and distal ileum regions (\(P < 0.05\)), this loss was minimized in mice receiving CPT-11 plus RDP58 (\(P < 0.05\)). These findings demonstrate that RDP58 helps to preserve the intestinal mucosa morphology by maintaining villus and crypt structure.

Mice with chemically induced colitis demonstrate local overexpression of TNF-\(\alpha\) that leads to significant cellular apoptosis in the crypt compartment of the intestine (12, 13). On the basis of our data, we performed terminal deoxynucleotidyl transferase-mediated nick end labeling staining of histological sections of mouse intestinal mucosa to determine whether the reduction of TNF-\(\alpha\) production by RDP58 results in a decrease in apoptotic cells. Whereas very few apoptotic cells were found in mucosal crypts in control animals (Fig. 4A, panel a), CPT-11 induced extensive apoptosis, particularly within the crypt compartment (Fig. 4A, panel b). However, epithelial cell apoptosis was markedly reduced in mice treated with CPT-11 plus RDP58 (Fig. 4A, panel c). Subsequent morphometric analysis revealed that the number of apoptotic crypt cells was reduced significantly in mice given RDP58 (\(P < 0.05\); Fig. 4B). The above results illustrate that RDP58 inhibits TNF-\(\alpha\)-mediated apoptosis in the crypt compartment, thereby protecting intestinal mucosa integrity in mice.

RDP58 Increases Both LD\(_{50}\) and Survival Time in CPT-11-Treated Mice. The data from the present study show that RDP58 inhibits diarrhea, inflammation, and apoptosis at a minimum CPT-11 dose sufficient to generate GI toxicity. We extended these studies to determine whether RDP58 still had a protective effect at higher doses of CPT-11. As shown in Fig. 5A, the LD\(_{50}\) of CPT-11 was \(\sim 160\) mg/kg/day (qd \(\times 3\)) in mice. In comparison, LD\(_{50}\) of CPT-11 was increased to \(\sim 240\) mg/kg/day (qd \(\times 3\)) when the mice were given oral 10 mg/kg/day RDP58 concurrently. Not only did RDP58 increase the LD\(_{50}\) of CPT-11 by \(\sim 50\%\), it also prolonged the survival time in mice receiving the higher doses compared with RDP58-untreated animals (\(P < 0.05\); Fig. 5B). These data show that RDP58 can protect mice from CPT-11-induced GI toxicity and mortality, and allow a higher maximum tolerated dose of CPT-11 up to 240 mg/kg/day (qd \(\times 3\)) without a concomitant increase in toxicity.
RDP58 Reduces CPT-11-Induced Mortality without Impairing Chemotherapy Efficacy in Tumor-Bearing Mice.

To evaluate the role of RDP58 in tumor-bearing mice, CT-26 murine colon carcinoma cells were s.c. injected into BALB/c mice on day –6, and solid tumors were allowed to form in vivo. Either RDP58 (10 mg/kg/day) or plain water (control) was provided to mice orally from day 0 until the end of the experiment. Starting on day 4, three courses of chemotherapy were administered to mice with injections of either CPT-11 or saline as control, as described in “Materials and Methods.” By the end of the experiment, a mortality rate of 40.0% was observed in mice treated with CPT-11 (Fig. 6A). However, mice receiving RDP58 tolerated the CPT-11 treatment well, and no mortality was observed \((P < 0.05)\), indicating that RDP58 increases animal survival in CPT-11-treated tumor-bearing mice.

To determine whether RDP58 treatment affects the efficacy of chemotherapy in tumor-bearing mice, tumors were surgically removed from mice upon termination of the experiment and weighed. As shown in Fig. 6B, CPT-11 reduced
We compared the maximum tolerated dose (MTD; CPT-11 beginning on day 4 as described in mg/kg RDP58 or water beginning on day 0 and three courses of toxicity. Tumor-bearing animals were given daily gavages of 10 enhanced tumor responses without the associated increase in GI toxicity. We hypothesized that inclusion of RDP58 into a higher-dose CPT-11 regimen for tumor efficacy study may generate enhancement of tumor volume reduction to ~85% of control with and without oral RDP58 added ($P < 0.05$; Table 2). Importantly, although the reduction in tumor volume was similar between these two groups, 100% of the animals in the 2× MTD CPT-11 plus RDP58 survived the treatment compared with only 50% survival in the 2× MTD CPT-11 alone group due to GI toxicity.

Additionally, 16.7% of mice recorded complete responses in the 2× MTD CPT-11 plus RDP58 groups, compared with none in the group receiving 1× MTD CPT-11. We also determined the time to relapse after completion of CPT-11 treatment in the surviving animals. In mice treated with 1× MTD CPT-11, it took an average of 7.2 days for tumors to regrow to a volume of 200 mm$^3$ (Table 2). In the 2× MTD CPT-11 only and 2× MTD CPT-11 plus RDP58 groups, time to relapse was significantly increased to 15.4 and 15.5 days, respectively ($P < 0.05$ versus 1× MTD CPT-11 group). These data demonstrate that RDP58, by preventing GI toxicity and mortality, can increase the effectiveness of chemotherapy to 2× MTD CPT-11, as measured by primary tumor response and time to relapse.

To determine whether the effect of RDP58 in allowing higher-dose chemotherapy is tumor model-specific, we performed a similar study in the 4T1 mammary adenocarcinoma tumor model. Mice were inoculated with 4T1 cells and were given two CPT-11 regimens as described in “Materials and Methods.” The MTD for CPT-11 in this model was 66.7 mg/kg/day (400 mg/kg total dose). At 1× MTD, there was a significant reduction in primary tumor volume (35.8%; $P < 0.05$) compared with control mice (Table 3). At 2× MTD CPT-11 only (133.3 mg/kg/day; 800 mg/kg total dose), a 56.7% reduction in primary tumor size was observed, and the mortality rate was 42% in this treatment group. In the 2× MTD CPT-11 plus RDP58 group, a similar reduction in the primary tumor size (59%; $P < 0.05$) was observed; however, in contrast to the 2× MTD only group, 100% of the animals survived CPT-11 treatment.

Unlike CT-26 carcinoma cells, 4T1 mammary adenocar-

![A graph showing the effect of RDP58 on animal survival and tumor size.](image)

**Fig. 6** Effect of RDP58 on animal survival (A) and tumor size (B) in tumor-bearing mice treated with CPT-11. Mice were inoculated with CT-26 murine colon carcinoma cells on day −6. Either water or RDP58 was provided p.o. throughout the experimental period (day 1 onward). Starting on day 4, mice were given three repeated 6-day chemo-treatment regimes consisting of 3 daily injections of either vehicle (saline) or CPT-11 followed by a 3-day recovery period ($n = 12$ mice/group). Mouse survival was recorded on a daily basis. Implanted tumors were extirpated upon the termination of the experiment and weighed. *, $P < 0.05$, CPT-11/RDP58 versus all other groups (survival analysis); #, $P < 0.05$, CPT-11 versus vehicle groups (ANOVA); bars, ±SD.

For more information, please refer to the full publication.

Table 2 | Modulation of chemotherapy effectiveness of CPT-11 by RDP58 in tumor-bearing mice

<table>
<thead>
<tr>
<th></th>
<th>CPT-11 (mg/kg/day)</th>
<th>RDP58 (mg/kg/day)</th>
<th>Mortality (%)</th>
<th>Tumor regression (%)</th>
<th>Time to relapse (day)</th>
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<td>66.7 (1X MTD*)</td>
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<td>85$^a$</td>
<td>15.5 ± 1.3$^a$</td>
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</tbody>
</table>

$^a$ MTD, maximum tolerated dose.

$^b$ P < 0.05 (ANOVA).

$^c$ P < 0.05 (survival analysis).
cinoma cells spontaneously metastasize to the lungs from the mammary fat pad (21). We also investigated the effect of 2× MTD CPT-11 with and without RDP58 compared with 1× MTD to reduce the rate of pulmonary metastasis and tumor burden in 4T1 tumor-bearing mice. In the 2× MTD CPT-11 plus RDP58 group, both the occurrence (58%) and the number of pulmonary metastases (2) were significantly lower compared with control mice (100% and 15, respectively; P < 0.05, with no mortality observed (Table 3). The 2× MTD CPT-11 alone group showed a similar response; however, treatment-related mortality was 42%. These findings suggest that the effect of RDP58 in allowing intensified CPT-11 treatment with better efficacy is not restricted to a single mouse tumor model, and can significantly reduce the incidence and overall tumor burden in a spontaneously metastatic model.

**DISCUSSION**

CPT-11 is a camptothecin derivative used to treat metastatic colorectal cancer (22), and severe, delayed diarrhea is one of the most common toxicities associated with this agent (3). Similarly, the incidence of diarrhea ranges from 26% to 56%, and the incidence of severe diarrhea is 5–23% in colorectal cancer patients receiving 5-FU and leucovorin as adjunct therapy (23). The physiological consequences of chemotherapy-induced severe diarrhea are potentially life-threatening and may involve hemodynamic collapse requiring immediate intensive care (6). In addition to the associated morbidity and mortality, interruptions or dose reductions during treatment may result in overall reduced effectiveness of therapy (24, 25). However, current treatment for chemotherapy-induced diarrhea using antidiarrheal agents such as loperamide is nonspecific and not effective (26).

Here, we used an established mouse model of CPT-11-induced GI toxicity and mortality to determine whether RDP58 can prevent proinflammatory cytokote overexpression, diarrhea, and mortality (14, 15, 26, 27). Additionally, we evaluated whether RDP58 would allow a higher tolerated dose of CPT-11 in tumor bearing mice to enhance tumor responses. Our data demonstrate that oral RDP58 therapy attenuates both CPT-11 and 5-FU-induced toxicity in mice by reducing body weight loss, occurrence of diarrhea, and mortality. We also determined that these protective effects do not interfere with the effectiveness of chemotherapy. In our data, we show that RDP58 allows the dose of CPT-11 to be doubled in mice without associated diarrhea and mortality. More importantly, the higher CPT-11 dose results in improved primary tumor regression, prolonged time to tumor recurrence, and reduced the rate of pulmonary metastasis. As a result, RDP58 not only reduces adverse effects of CPT-11, but allowed a higher tolerated dose to improve tumor response during chemotherapy.

We show that RDP58 is equally effective in two standard mouse tumor models, and that tumor responses in both models can be improved with the combination of RDP58 and higher dose therapy. We also demonstrate that RDP58 activity is not mouse strain-specific by assessing RDP58 in C57BL/6 mice. Female C57BL/6 mice were injected with either CPT-11 or 5-FU, and similar to studies performed in BALB/c mice, RDP58 decreased diarrhea and increased animal survival (data not shown). Thus, the protective effect of RPD58 on chemotherapy-induced toxicity is not strain- or tumor model-specific. In the present study, RDP58 was used prophylactically, and whereas diarrhea is prevalent during chemotherapy, not every patient develops GI toxicity. We investigated the efficacy of oral RDP58 therapy after the onset of CPT-11-induced diarrhea and observed protective activity, although to a lesser extent than prophylactic administration (data not shown).

The mechanism of action of RDP58 in attenuating chemotherapy-induced GI toxicity was also explored in this study. Chemotherapy with agents like CPT-11 is associated with increased intestinal permeability and leukocyte infiltration, resulting in overproduction of proinflammatory cytokines within the intestinal mucosa (15). Consistent with our data in the colitis models, RDP58 significantly reduced overproduction of proinflammatory cytokines such as TNF-α, IFN-γ, and IL-12 in CPT-11-induced inflamed bowel. The reduced production of inflammatory cytokatoes is associated with a conservation of mucosal morphology in CPT-11-treated mice receiving RDP58.

Other strategies to treat chemotherapy-induced diarrhea and mucositis have been evaluated in animal models. Other synthetic lipopeptides known to activate macrophages have been used to successfully prevent intestinal toxic effects in CPT-11-treated mice (14). Glucagon-like peptide 2 stimulates epithelial cell proliferation and inhibits crypt cell apoptosis (28), and reduces both chemotherapy-associated mucositis and indomethacin-induced enteritis (15, 18). Growth factors that stimulate proliferation and differentiation of cells of epithelial origin, including keratinocyte growth factor and fibroblast growth fac-

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**Table 3** Effect of RDP58 on 4T1 mammary fat pad tumor growth and metastasis in mice

<table>
<thead>
<tr>
<th>CPT-11 (mg/kg/day)</th>
<th>RDP58 (mg/kg/day)</th>
<th>Mortality (%)</th>
<th>Primary tumor size (mm³)</th>
<th>Incidence of pulmonary metastasis (%)</th>
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<td>6 (3–9) b</td>
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<td>58</td>
<td>2 (0–6) b</td>
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a MTD, maximum tolerated dose.
b P < 0.05 (ANOVA)
tor 2 have been shown to be protective (29, 30). In addition, cytokines such as IL-11 and IL-15 protect or accelerate recovery of chemotherapy-induced intestinal toxicity and damage in rodents (19, 20). Taken together, these other approaches can be used to prevent diarrhea and mucositis during chemotherapy. However, these agents are administered by s.c. or i.p. injection and may have systemic activities (15, 18–20, 29, 31). Thus, one advantage for RDP58 treatment is that it is an orally formulated, nonsystemically bioavailable drug, which will only suppress inflammatory cytokine production locally.

In summary, we demonstrate that RDP58 significantly reduces chemotherapy-induced GI toxicity and inflammation, and that this novel peptide holds promise as a new treatment for cancer patients experiencing GI toxicity and mucositis during chemotherapy. Moreover, the reduction of chemotherapy-induced complications may allow administration of higher dose chemotherapy regimens with improved tumor regression.

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