Potentiation of Cisplatin Antitumor Activity Using a Vitamin D Analogue in a Murine Squamous Cell Carcinoma Model System

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ABSTRACT

In a murine squamous cell carcinoma (SCC) model, we have demonstrated that both 1,25-dihydroxycholecalciferol (1,25-D3) and the analogue 1,25-dihydroxy-16-ene-23-yne-cholecalciferol (Ro23-7553) have significant in vitro and in vivo antitumor activity. We have examined here the cell cycle effect of 1,25-D3 and Ro23-7553 on SCCVII/SF tumor cells by quantitating nuclear DNA using a detergent-trypsin method via flow cytometry analysis. Both 1,25-D3 and Ro23-7553 resulted in a significant increase of cells in G2-M, with an accompanying decrease of cells in G1 phase. The ability to arrest cells in G2-M has been exploited by combining Ro23-7553 with the cytotoxic agent cisplatin (cis-diamminodichloroplatinum; cDDP). Using the in vitro clonogenic assay, pretreatment with Ro23-7553 for 24–48 h significantly enhanced cDDP-mediated tumor cell kill as compared to concurrent treatment with Ro23-7553 and cDDP or cDDP alone. To examine the effect of Ro23-7553 and cDDP in vivo, C3H/HeJ mice with 9–14-day SCC tumors were treated either for 3 days with varying i.p. doses of Ro23-7553 or for 7 days continuously through the use of Alzet pumps, and on the last day of Ro23-7553 treatment, cDDP (1–6 mg/kg) was administered. Using the in vivo excision tumor cell clonogenic assay, in which tumors were removed from animals 24 h after cDDP treatment and plated in a clonogenic assay, pretreatment with Ro23-7553 markedly enhanced cDDP-mediated clonogenic tumor cell kill, even at low doses of cDDP as compared to cDDP treatment alone. Similarly, a significant decrease in fractional tumor volume and increase in tumor regrowth delay was observed when animals were pretreated before cDDP with Ro23-7553 as compared to either agent alone. These results demonstrate a significant enhanced antitumor effect with Ro23-7553 pretreatment before cDDP both in vitro and in vivo and suggest that Ro23-7553 may potentiate cDDP cytotoxicity through effects on cell cycle progression.

INTRODUCTION

Vitamin D and vitamin D analogues have been shown to have antiproliferative and differentiating effects in vitro and in vivo in a variety of normal and malignant cell types (1–12). Using a murine SCC model system, we have demonstrated that the active metabolite of vitamin D or 1,25-D3 and the analogue Ro23-7553 as single-agent therapy have significant in vitro and in vivo antitumor activity both in the treatment of established tumors as well as in the prevention of tumor growth or induction (12). In this model at high doses, 1,25-D3 has been shown to inhibit tumor growth but with severe hypercalcemia, whereas Ro23-7553 results in decreased tumor growth without significant hypercalcemia. In addition, we have demonstrated significant antitumor activity of both 1,25-D3 and the 1,25-D3 analogue Ro25-6760 in the metastatic syngeneic Dunning rat prostate adenocarcinoma model MLL with the capacity to induce primary and metastatic tumor growth inhibition.3 In vitro in the MLL model system, 1,25-D3 has been shown not only to be growth inhibitory but also to arrest cells in G2-M. The ability of 1,25-D3 to induce a G2-M-S phase block effect has been demonstrated in vitro in human lymphoid, leukemia, and solid tumor cell lines (13–15).3 In HL-60 cells, these effects have been shown to be mediated through the cyclin-dependent kinase inhibitor p27 (16).

cDDP is one of the most widely used anticancer agents and has been shown to kill tumor cells through the formation of covalent, bifunctional DNA adducts (17, 18). Inhibition of DNA synthesis has been observed in cells following platination (19, 20), and as a result, it has been reported that cells actively synthesizing DNA are most sensitive to cDDP (21, 22). Additionally, cells sensitive to cDDP appear to arrest in G2-M and go on to die by apoptosis at concentrations of cDDP that do not inhibit DNA synthesis (23). In contrast, however, cells have also been shown to demonstrate increased cDDP sensitivity when in the G1 phase of the cell cycle (24, 25). Donaldson et al. (25) have determined that tumor cells or fibroblasts in G0-G1, just prior to DNA replication, are most sensitive to cDDP cytotoxicity. The cells that are blocked in G0-G1-S have been shown to remain maximally sensitized upon release with progression through the cell cycle. The ability of 1,25-D3 to exert a G0-G1-S phase block in tumor cells and the potential of cDDP-mediated tumor cell kill in relation to the cell cycle suggest a possible interaction between these two agents. As a result, we have examined the effect of 1,25-D3 on cell cycle and the antitumor activity in vitro in vivo of 1,25-D3 in combination with cDDP in the SCC tumor model system. For these studies, we used the 1,25-D3 analogue, Ro23-7553, which has identical biological activity to the parent, 1,25-D3, without inducing hypercalcemia when administered at the same doses (12).

MATERIALS AND METHODS

Animals and Tumor Model Systems. Inbred female C3H/HeJ mice aged 6–10 weeks were obtained from The Jackson Laboratory (Bar Harbor, ME). The mice were virus antibody free, age and weight matched for experimental use, and fed a balanced rodent diet ad libitum. The animals and experiments used in these studies were approved by the institutional animal care committee according to USPHS guidelines. The SCCVII/SF murine squamous cell model was maintained in vivo in C3H/HeJ mice as described previously (12) by s.c. inoculation of 5 × 10⁶-log phase tissue culture cells in the right flank of the animal. The SCCVII/SF cell line, a murine, rapidly growing, nonmetastasizing squamous tumor line, was maintained in vitro in RPMI 1640 (Life Technologies, Inc., Grand Island, NY) supplemented with 12.5% inactivated FCS (HyClone, Logan, UT) and 1% penicillin-streptomycin sulfate (Life Technologies, Inc.).

Reagents. The active metabolite 1,25-D3, and the nonhypercalcemic vitamin D analogue Ro23-7553 were obtained through the kindest courtesy of Dr.
Milan Uskokovic (Hoffman-LaRoche, Inc., Nutley, NJ). The steroid hormones were stored initially in pure powder form in a sealed light-protective vessel at 4°C. For use, the drug was reconstituted in 100% ethanol and maintained as described previously (12). CDDP (Bristol-Myers Squibb Co., Princeton, NJ) was diluted in 0.9% saline and injected i.p. at various doses in a total volume of 0.2 ml.

**In Vitro Clonogenic Assay.** The *in vitro* cytotoxicity of drug on tumor cells was determined as described previously (12) with minor modifications as described below. Briefly, murine SCCVIIJSF cells were pretreated with either 2 or 4 × 10^5 Ro23-7553. Although neither 1.25-D_3_ nor Ro23-7553 is stable for long periods in tissue culture medium, antiproliferative effects are observed at 24-h, 48-h, and 7-day incubation times (12). After a 48-h incubation with Ro23-7553, cells were treated for 2 h with varying concentrations of CDDP, washed with RPMI 1640 plus FCS, and plated in various dilutions in six-well tissue culture plates (Corning Costar Corp., Cambridge, MA). Following a 7-day incubation at 37°C in 5% CO_2_, monolayers were washed with saline, fixed with 100% methanol, and stained with 10% Giemsa stain, and colonies were enumerated under light microscopy. The surviving fraction was calculated by dividing the cloning efficiency of treated cells by the cloning efficiency of untreated controls.

**In Vivo Excision Clonogenic Tumor Cell Survival Assay.** The effect of Ro23-7553 and/or cDDP on tumor cells *in vivo* was determined by a modification of the assay as described previously (26). Briefly, SCCVII/SF tumor-bearing animals at 14 days postimplantation were treated i.p. for 3 days with Ro23-7553 at either 0.5 mg/kg/day or at varying doses of 0.03125–0.5 mg/kg/day. On day 3, animals also received an i.p. dose of either 6 mg/kg or varying doses of 1–6 mg/kg of cDDP. After 24 h, aliquots of minced tumor were dissociated enzymatically for 60 min at room temperature with a mixture of type 1 collagenase (37.5 mg/ml; Sigma Chemical Co., St. Louis, MO), DNase (55 mg/ml; Sigma), and EDTA (1%; Life Technologies, Inc.). Viable tumor cells as determined by trypan blue were then plated at various dilutions. After 7 days of incubation, colonies were counted, and numbers of clonogenic cells per gram of tumor were enumerated. The mean ± SD cell yield, cloning efficiency, and number of clonogenic cells for control (no treatment) tumors (*n* = 40) averaged 139.4 ± 38.2 × 10^6 viable tumor cells/g tumor, 27.0 ± 0.56%, and 37.5 ± 13.3 × 10^6 clonogenic tumor cells/g tumor, respectively. The surviving fraction per gram of tumor is defined as the number of clonogenic tumor cells per gram of treated tumor divided by the number of clonogenic tumor cells per gram of control (untreated) tumor. We have shown previously that the *in vivo* excision of clonogenic assay is an accurate measure of control antitumor activity and that a surviving fraction less than 0.1 always correlates with an actual decrease in tumor volume and an increase in tumor regrowth delay (26–29).

**Cell Cycle Analysis.** Determination of the effect of 1.25-D_3_ or Ro23-7553 on the SCCVII/SF tumor cell cycle *in vitro* was achieved using a detergent-trypsin method to stain tumor cell nuclei for quantitative DNA analysis via flow cytometry, as described by Vindelov *et al.* (30). Briefly, tumor cells were seeded on six-well tissue culture plates (Corning Costar Corp.) at varying concentrations determined to produce 80–90% confluence upon harvest following the desired incubation interval. After a 24-h incubation, 1.25-D_3_ or Ro23-7553 was added to the cell culture. At various time points, the supernatant was removed, and 0.05% trypsin (Life Technologies, Inc.) in Ca^{2+}- and Mg^{2+}-free PBS was added to the monolayer. The resulting single-cell suspension was diluted in medium and centrifuged at 350 × g for 10 min, and the supernatant was removed. The pellet was isolated, and citrate buffer solution (40 mM trisodium citrate, 250 mM sucrose, and 5% DMSO) was added prior to storage at −75°C for up to 1 month. The cells were processed and stained as described by Vindelov *et al.* (30). The stained nuclei were filtered through 30 μm nylon mesh (Spectrum, Houston, TX) prior to flow cytometry analysis using a FACStar machine (Becton Dickinson, San Jose, CA). Cell cycle analysis was computed using the sum of broadened rectangles model with the DNA software from Becton Dickinson.

**Tumor Regrowth Delay.** SCCVII/SF tumor cells (5 × 10^6) were inoculated s.c. into the flank of the leg of C3H/HeJ mice. On day 9 postimplantation, as the tumors were palpable (approximately 5 × 5 mm), animals were randomized for treatment with i.p. Ro23-7553 (0.214 μg/kg/day) by Alzet micro-osmotic pump 1007D (Alza Corp. Palo Alto, CA) for continuous delivery of Ro23-7553 for 7 days. At the end of Ro23-7553 treatment (day 7), 6 mg/kg cDDP was injected i.p. Control animals received either treatment alone or no treatment. No-treatment animals were given injection of vehicle (PBS) alone, or sham pumps were implanted. Tumor growth was assessed by measuring the tumor diameter with calipers three times weekly. Tumor volumes were calculated by the formula: volume = length × (width squared)/2. Posttreatment volumes were expressed as a fraction of pretreatment volume at the time of initial treatment. Tumor regrowth delay was calculated as the mean ± SD of the difference in time in treated and control tumor volumes to reach four times the pretreatment volume.

**Statistical Analysis.** One-way ANOVA was used to assess the level of significance between treatment groups.

**RESULTS**

**In Vitro Effects of 1.25-D_3_ and Ro23-7553 on Cell Cycle.** Using the murine squamous cell carcinoma model, we have demonstrated previously that 1.25-D_3_ and Ro23-7553 have significant *in vitro* and *in vivo* antitumor activity both in the treatment of established tumors as well as in the prevention of tumor initiation or induction (12). To determine the potential of 1.25-D_3_ and Ro23-7553 to influence cell cycle progression, cultured tumor cells were treated initially with 1.25-D_3_, at half of the IC_{50} or 2 nm for 12–72 h and then subjected to DNA analysis via flow cytometry. As shown in Fig. 1, 1.25-D_3_ resulted in a progressive, time-dependent G_0-G_1–S phase block. The percentage of cells in G_0-G_1 (greater than 90% in 72 h) were increased significantly, whereas those in S phase were decreased as compared to no-treatment control cells. At 24 h after 1.25-D_3_ treatment, no significant difference was observed in the percentage of cells in G_2-M for 1.25-D_3_-treated (7.5 ± 1.3%) versus control (8.0 ± 0.8%). By 72 h, however, 3.0 ± 0.8% of 1.25-D_3_-treated cells were in G_2-M as compared to 12.7 ± 1.0% for control cells (*P* < 0.001; ANOVA). At 72 h, control plates had reached confluence, whereas treated cells appeared static and were not confluent. It also should be noted that direct cytotoxicity by trypan blue at this concentration of 1.25-D_3_ was minimal. Similarly, to examine the effect of Ro23-7553 on cell cycle, SCC cells were treated for 48 h with Ro23-7553 at 2 nm. As shown in Fig. 2, Ro23-7553 treatment at this dose resulted in a significant...
Significant enhancement of cDDP-mediated cytotoxicity was observed even at low doses of cDDP.

In Vivo Excision Clonogenic Tumor Cell Kill. To examine the effect of Ro23-7553 on in vivo cDDP-mediated antitumor activity, we used the excision clonogenic kill assay. This approach has been used extensively in our laboratory (26–29), and an increase with in vivo clonogenic cell kill correlates with a decrease in fractional tumor volume as well as an increase in tumor regrowth delay. SCCVII/SF tumor-bearing animals at 14 days postimplantation were treated i.p. for 3 days with 0.5 mg/kg/day of Ro23-7553. On the 3rd day, animals received varying doses of cDDP. After 24 h, tumors were harvested, dissociated, and plated in the clonogenic assay for a 7-day incubation. As shown in Fig. 4, pretreatment for 3 days with the Ro23-7553 before cDDP resulted in a significant enhancement of clonogenic cell kill when compared to animals treated with cDDP or Ro23-7553 alone. A significant increase in clonogenic tumor cell kill was observed at each cDDP dose tested as compared to cDDP alone. As shown previously (12), Ro23-7553 alone (zero point) resulted in a modest decrease in surviving fraction.

To determine the effect of varying the Ro23-7553 dose in this assay, SCC tumor-bearing mice were treated daily for 3 days with Ro23-7553. On day 3, cDDP was administered at 6 mg/kg. As shown in Fig. 5, Ro23-7553 was capable of significantly enhancing cDDP-mediated tumor cell kill even at the lowest doses tested as compared to cDDP or Ro23-7553 alone. It should be noted that animals in either experimental approach did not become hypercalcemic or experience weight loss at any of the doses tested of Ro23-7553 (data not shown).

Tumor Regrowth Delay. Studies were extended to determine whether decreases in surviving fraction translated into significant changes in fractional tumor volume and to examine the effect of
here, we have examined the effects of both 1,25-D$_3$ and Ro23-7553 on cell cycle in SCC tumor cells as well as the potential to exploit these cell cycle changes by combining Ro23-7553 with the conventional cytotoxic agent cDDP.

1,25-D$_3$ has also been shown to modulate cell cycle progression. In HL-60 cells, a human myelomonocytic leukemia cell line, 1,25-D$_3$, has been shown to arrest cells in G$_1$ (13, 14) and to be mediated through the cyclin-dependent kinase inhibitor p27 (16). 1,25-D$_3$-mediated arrest in G$_0$-G$_1$ has also been observed in human breast cancer lines (15). Studies in our laboratory have demonstrated in MLL cells that 1,25-D$_3$ treatment for 24 h at half the IC$_{50}$ results in a significant increase in G$_0$-G$_1$ and decrease in cells in S phase. We present evidence here that similar results have been obtained with SCC cells with a significant time-dependent increase observed in G$_0$-G$_1$ and a decrease in S phase. We are assuming that the in vitro cell cycle effects observed with 1,25-D$_3$ are contributing to the enhanced cDDP-mediated cell kill observed in vitro as well as in vivo. Indeed, whether cells arrested in G$_0$-G$_1$ are actually more sensitive to the effects of cDDP remains to be determined. We have preliminary evidence that cell cycle arrest can be observed in vivo in the tumors of mice treated with 1,25-D$_3$.

cDDP exerts its cytotoxic effect by interacting with cellular DNA, RNA, and protein and characteristically forms covalent, bifunctional adducts between adjacent guanosine residues in DNA (17, 18). This alteration inhibits DNA replication and results in cell death. The mechanism of cytotoxic effect with relation to cell proliferation and cell cycle is poorly understood and has been the subject of contro-

![Graph showing cDDP dose-dependent in vivo clonogenic tumor-cell kill in combination with pretreatment (0.5 mg/kg/day for 3 days) of the Ro23-7553 (●) or cDDP alone (○). Points are means (bars, SD) of the surviving fraction for total clonogenic cells/gram of tumor (three to five mice per treatment group) from a representative experiment that was replicated two to three times. Values significantly different from cDDP alone are shown: *, P < 0.001 (ANOVA).](image1)

Continuous administration of Ro23-7553. SCCVII/SF tumor-bearing mice (day 9 postimplantation) were treated with Ro23-7553 administered continuously through the use of Alzet pumps. At the end of Ro23-7553 administration, cDDP was injected i.p. at 6 mg/kg. No-treatment or single-treatment animals were injected with vehicle (PBS) or implanted with sham pumps depending on the treatment group. As shown in Fig. 6, animals experienced a significant decrease in fractional tumor volume when pretreated with Ro23-7553 before cDDP as compared to treatment with either agent alone. When tumor regrowth delay (mean ± SD of the difference in time for treated and control tumors to reach four times pretreatment size; Fig. 6, horizontal line) was examined, a significant increase was observed in animals treated with Ro23-7553 plus cDDP as compared to either cDDP or Ro23-7553 alone (Table 1).

**DISCUSSION**

1,25-D$_3$, which is known for its role in regulation of bone and mineral metabolism (31, 32), has also been shown to modulate cell growth of several normal and malignant cell types. In *in vitro*, 1,25-D$_3$ inhibits cell proliferation and *in vivo*, it prolongs survival and inhibits tumor growth in a variety of rodent leukemia/tumor and human xenograft tumor model systems (1–12). Clinical applications of 1,25-D$_3$, however, have been limited due to hypercalcemia, the dose-limiting toxicity (33). As a result, 1,25-D$_3$ analogues such as Ro23-7553 have been developed that maintain antitumor activity without inducing hypercalcemia (8–10, 34–38). We have demonstrated that both 1,25-D$_3$ and Ro23-7553 are equally effective at inducing significant antiproliferative activity *in vitro* and *in vivo*; however, Ro23-7553 can be administered at higher doses than 1,25-D$_3$ due to the lack of a hypercalcemic effect with Ro23-7553 (12). In studies described

![Graph showing Surviving Fraction/gram tumor vs. D$_{16,23}$ (mg/kg/dx3d). Points are means (bars, SD) of the surviving fraction for total clonogenic cells/gram of tumor (three to five mice per treatment group) from a representative experiment that was replicated two to three times. Values significantly different from Ro23-7553 or cDDP alone are shown: *, P < 0.01 (ANOVA).](image2)
different from cDDP alone are shown: *, P < 0.001 (ANOVA). The horizontal line represents four times pretreatment control tumor size.

versus. Several authors have, however, reported an increased sensitivity of cells to cDDP treatment in the G1 phase of the cell cycle (24, 25). HeLa cells and quiescent fibroblasts that are stimulated to synchronously enter the cell cycle, are maximally sensitive to the cytotoxic effect of cDDP just prior to the onset of DNA replication in G1 (25). This sensitivity abates with cell phase progression into S phase with the onset of DNA synthesis. The studies described here have not demonstrated directly that cells arrested in G0-G1 are more sensitive to the cytotoxic effects of cDDP. We have demonstrated directly that cells arrested in G0-G1 are more sensitive to cDDP-mediated cell kill and that, if tumor cells are examined at the time of cDDP administration, a significant proportion of these cells would be found to be arrested in G0-G1. It is possible, however, that other mechanisms are involved. For example, epidermal growth factor has been shown to increase the sensitivity of cells to cDDP-mediated cell kill (39). Ro23-7553 could be involved in growth factor induction, thereby sensitizing cells to cDDP.

In both the murine SCC and rat Dunning prostate model systems, we have demonstrated significant in vitro and in vivo antiproliferative activity with both 1,25-D3 and the analogues, Ro23-7553 and Ro25-6760 (12). In both instances, the analogues have not been shown to be more active than the parent, but higher doses can be achieved with the analogues due to diminished toxicity. In the SCC model, in vitro activities with 1,25-D3 and Ro23-7553 are similar, with no significant difference observed in the IC50 for these agents. In vivo, when SCC tumor-bearing animals are treated three times per week with 1,25-D3 at 0.5 μg/dose, animals experience a significant antitumor response with a significant increase in serum calcium and the accompanying toxicities. In contrast, Ro23-7553 can be administered at doses up to 10–15 μg/dose three times per week with comparable antitumor activity without experiencing significant hypercalcemia. In the Dunning rat model, we used the MLL metastatic variant and examined the effect on tumor growth as well as on metastasis. The MLL cell line is an aggressive tumor that when injected s.c. metastasizes quickly to regional lymph nodes (40). In this model, treatment of animals with 1 μg/rat three times per week of 1,25-D3 or 5 μg/rat three times per week of Ro25-6760 results in an inhibition of tumor volume and a reduction in the number and size of lung metastases. Although the analogue Ro25-6760 at 5 μg/rat has similar antitumor activity to the 1 μg/rat 1,25-D3, both agents have been shown to result in slight hypercalcemia. In addition, both treated groups of rats have been shown to experience a 30% weight loss by the end of the 3-week treatment period.

Toxicity as measured by serum calcium has been shown to be much more severe when animals were treated long-term for 3 weeks (daily, every other day, or three times per week) with 1,25-D3 or even the analogues as compared to a single bolus injection daily for 3–4 days (data not shown). The rationale for long-term administration is based on the notion that to achieve an antitumor effect on the tumor, 1,25-D3 must be present to bind to its receptor and affect proliferation. To use 1,25-D3 as an agent to arrest tumor cells and thereby perhaps sensitize them to a cytotoxic agent such as cDDP, may not require such long-term treatment. If 1,25-D3 is administered with cDDP, for example, 1,25-D3 or Ro23-7553 would only have to be given 2–4 days before each cycle of cDDP. This regimen could potentially allow larger doses of 1,25-D3 or Ro23-7553 to be administered with less toxicity. In addition, we have also demonstrated enhanced antiproliferative activity with 1,25-D3 in combination with carboplatin. As a result, this schema has been incorporated into a Phase I trial currently planned to involve the s.c. administration of calcitriol (1,25-D3) daily for 5 days, with carboplatin administered on day 5 to patients with advanced solid tumors.

Regrowth-delay studies in animal tumor model systems are a measure of both tumor cell death and posttreatment growth rate (41). This measurement of antitumor activity in vivo may not correlate with median survival studies in which the end point is quite different. In many ways, a similar situation exists in the clinical evaluation of new treatment regimens, whereby drugs have been developed on the basis of their tumor response rates (complete or partial). In both the animal model and clinical settings, tumors respond only temporarily by regression or regrowth delay, which is usually followed by recurrence or a rebound of tumor growth. The studies described here focused on the effects of Ro23-7553 and cDDP on the initial events of clonogenic cell kill and tumor regrowth delay and demonstrate that pretreatment for 48–72 h before cDDP resulted in a significant increase in antitumor activity. Studies are in progress to determine whether further cycles of treatment can prolong this “regressed” state and ultimately result in an increase in median survival.

Table 1 Effect of Ro23-7553 and cDDP on tumor regrowth delay

<table>
<thead>
<tr>
<th>Treatment⁴</th>
<th>Tumor regrowth delay⁴</th>
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<tbody>
<tr>
<td>Ro23-7553</td>
<td>1.8 ± 0.8</td>
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<tr>
<td>cDDP (6 mg/kg)</td>
<td>4.4 ± 0.3</td>
</tr>
<tr>
<td>Ro23-7553/cDDP</td>
<td>7.7 ± 0.4</td>
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⁴ SCCVII/SF tumor-bearing mice (day 9; tumor volume, 150 ± 30 mm³) underwent implantation i.p. of Alzet pump 1007D for delivery of Ro23-7553 over a 7-day continuous infusion followed by cDDP (6 mg/kg) i.p. or Ro23-7553 or cDDP alone.

Mean ± SD (n = 4) of tumor regrowth delay (the difference in time required for treated and control tumors to reach 4 times pretreatment volume). Data are from a representative experiment that was replicated two to three times.

Significantly different from Ro23-7553 and cDDP treatment alone. P < 0.001 (ANOVA).
ence in cell viability as compared to control cells using a trypan blue exclusion method. This action effectively abrogated cell proliferation, with only 3% of cells undergoing synthesis of DNA after 48 h. Previous work in our laboratory demonstrates this effect to be reversible with the removal of Ro23-7553 or 1,25-D3 exposure to tumor cells resulting in the release of previously synchronized cells into S phase within 24 h (data not shown). The 20-epi-vitamin D3 analogue has been shown to be antiproliferative and induce differentiation as well as apoptosis in HL-60 cells (2). In these cells, the analogue has also been shown to decrease bcl-2 expression. The switch that tells vitamin D-analogue-treated cells to undergo apoptosis versus differentiation remains to be determined. Cells could be arrested in G0/G1 prior to traveling down either one of these pathways. Studies to examine these potential mechanisms are currently in progress.

These results form the basis to investigate this therapeutic approach in patients with advanced tumors in the Phase I setting. Further insight into the mechanism(s) involved in the interaction between vitamin D and/or its analogues will ultimately optimize additional treatment designs and will expand the potential utility of vitamin D in the therapy of solid tumors.

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