The Cyclin-Dependent Kinase Inhibitor UCN-01 Plus Cisplatin in Advanced Solid Tumors: A California Cancer Consortium Phase I Pharmacokinetic and Molecular Correlative Trial

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Abstract

Background: UCN-01 (7-hydroxy-staurosporine) is a novel antineoplastic agent targeting cyclin-dependent kinases, which shows potent in vitro and in vivo activity against a broad range of tumor types. Our group has previously shown that UCN-01 potentiates the apoptotic response of agents such as cisplatin in vitro by preventing sequence-specific abrogation of G2 arrest caused by DNA-damaging chemotherapies.

Patients and Methods: This National Cancer Institute–sponsored phase I trial was designed to determine the safety, maximum tolerated dose, and pharmacokinetics of escalating doses of cisplatin in combination with UCN-01 in patients with advanced malignant solid tumors, as well as to do molecular correlative studies on tumor specimens. Cisplatin was infused over 1 hour before UCN-01 (45 mg/m2/d) given as a 72-hour continuous infusion. Escalation of cisplatin was planned through five dose levels at 20, 30, 45, 60, and 75 mg/m2.

Results: Ten patients were accrued. Accrual was halted at dose level 2 (cisplatin, 30 mg/m2) due to dose-limiting toxicities consisting of grade 5 sepsis with respiratory failure associated with grade 3 creatinine (one patient) and grade 3 atrial fibrillation (one patient). Plasma and salivary pharmacokinetics of UCN-01 were unaffected by cisplatin. Pretreatment and posttreatment tumor biopsies showed that UCN-01 was active against a key molecular target, the checkpoint kinase Chk1.

Conclusions: This phase I trial failed to achieve targeted therapeutic dose levels of cisplatin when combined with prolonged infusion UCN-01. However, because preclinical data indicate that UCN-01 potentiates response to platinum, further studies with alternative dose schedules of the combination, or with other platinum analogues, are warranted.

UCN-01 (7-hydroxystaurosporine, NSC 638850; Fig. 1) is a staurosporine analogue isolated from Streptomyces and is a potent inhibitor of protein kinase C (1). This compound exhibits potent in vitro and in vivo anticancer activity against a broad range of murine tumors, human cancer cell lines, and xenografts (2–5). Initial studies have indicated that UCN-01 treatment results in modulation of cell cycle–associated factors, including inhibition of cyclin-dependent kinase (CDK) activity, p27 induction, hypophosphorylation of retinoblastoma, and accumulation of cells in the G1 phase (6–10).

In a National Cancer Institute (NCI)–sponsored phase I single-agent trial, UCN-01 was delivered initially as a 72-hour continuous infusion every 2 weeks but was later revised to a 36-hour infusion every 4 weeks (in the second and later cycles) because of an unexpectedly prolonged terminal half-life exceeding 300 hours (11). The observed maximum tolerated dose in that study was 45 mg/m2/d with dose-limiting toxicities of pulmonary toxicity, lactic acidosis with hyperglycemia, nausea/vomiting, and transaminitis. Preliminary results of a 3-hour infusion schedule designed by Japanese investigators have also been reported (12). In this schedule, no dose-limiting toxicities have been observed at the 51.1 mg/m2 dose level. Furthermore, unbound levels of the drug were detected in plasma at levels over 12.8 mg/m2 and were maintained for over 1 month following a single 3-hour administration.

UCN-01 has been shown to potentiate the anticancer activity of cisplatin, radiation, and mitomycin (13–15). This is attributed to the ability of UCN-01 to abrogate the S and G2 arrest checkpoints following DNA damage thus limiting repair and increasing toxicity in cycling cells. Previous work by our group showed potent growth inhibitory effects of single-agent UCN-01 on human cancer cell lines characterized by differential abnormalities in cell cycle regulatory genes. Single-agent activity seems dependent on an intact RB pathway and is associated with G1 growth arrest, retinoblastoma hypophosphorylation, and induction of p21, a CDK inhibitor (16, 17). In combination with cisplatin, UCN-01...
therapy resulted in dose-, time-, and sequence-dependent potentiation of cisplatin independent of RB status, suggesting a mechanism distinct from UCN-01’s single-agent activity (18). Specifically, the sequence of cisplatin preceding UCN-01 was synergistic. The reverse sequence was found to be antagonistic. This is consistent with a UCN-01-mediated G1 arrest protecting cells from subsequent cisplatin treatment, whereas cisplatin-mediated cell cycle arrest can be abrogated by subsequent UCN-01.

We therefore conducted a phase I pharmacokinetic and molecular correlative trial of UCN-01 in combination with cisplatin in patients with advanced solid tumors, employing the schedule determined to be most optimal in our preclinical studies (i.e., cisplatin infused before UCN-01). In addition, tumor tissue was collected serially in selected patients based on the hypothesis that laboratory correlative studies can identify intermediate molecular markers that will predict activity of the combination at the cellular level. A starting dose of 20 mg/m² of cisplatin followed by 45 mg/m² of UCN-01 was delivered over 72 hours for the initial cycle, then over 36 hours for subsequent cycles, based on the initial NCI phase I trial schedule.

**Patients and Methods**

**Patient selection.** Patients with advanced, recurrent, or metastatic malignant tumors, deemed incurable or refractory to therapy, were eligible. Measurable disease was not required. Fresh tumor tissue, paraffin tumor tissue, or unstained slides were requested at protocol entry for the proposed correlative studies. Patients with brain metastases were ineligible, unless asymptomatic and controlled (i.e., after surgical resection or radiotherapy). Because of potential issues in assessing neurologic toxicities, patients with brain metastases, with or without surgery or radiation therapy to measurable lesions. A central indwelling venous catheter was required.

Because of the antiproliferative activity of UCN-01 and its unknown effects on the developing fetus or nursing infant, pregnant or lactating women were excluded from this trial and appropriate contraceptive practices were required for all patients while on protocol therapy.

Initially, the protocol required the availability of serially biopsiable tumor sites for the correlative studies. Because of poor patient acceptance, serial biopsies were later revised to become optional.

**Baseline evaluation.** Before study entry, all patients underwent a complete history and physical examination. Baseline imaging studies of all known sites of disease were obtained within 4 weeks of initial therapy. Laboratory studies included a complete blood count with differential and platelet count and a comprehensive metabolic panel (which includes electrolytes, serum creatinine, total bilirubin, and aspartate transaminase).

**Dose-limiting toxicity.** Dose-limiting toxicity in a given patient was defined as any grade 3 or 4 nonhematologic toxicity (except nausea, vomiting, and alopecia), grade 3 or 4 thrombocytopenia, grade 4 neutropenia, or neutropenic fever. Dose-limiting toxicity was based on the first cycle of treatment. Toxicity was graded according to the NCI Common Toxicity Criteria version 2.0. The length of a treatment cycle was 28 days. To be evaluable for toxicity, a patient must have received at least one course of treatment and be observed for at least 28 days after the previous cycle or have experienced dose-limiting toxicities. All patients not evaluable for toxicity were to be replaced.

**Maximum tolerated dose and moderate toxicity definitions.** The maximum tolerated dose was defined as the highest dose level in which six patients have been evaluated for toxicity with no more than one patient experiencing dose-limiting toxicities attributable to the New York Heart Classification. No more than two prior chemotherapy regimens were allowed and patients must have recovered completely from all toxicities. Additionally, a minimum of 4 weeks must have elapsed because the completion of prior chemotherapy (6 weeks for prior mitomycin or nitrosourea) to be eligible for this study. There must be no plans for the patient to receive concurrent hormonal, biological, or radiation therapy to measurable lesions. A central indwelling venous catheter was required.

Because of the limited data on the toxicity profile of this compound in the pediatric age group, patients must have been at least age 18 before protocol entry. It was anticipated that pediatric patients would be enrolled in future trials of this agent once safety data were confirmed.

All patients were required to be informed of the investigational nature of the study and must have signed and given written informed consent in accordance with institutional and federal guidelines. The NCI and the institutional review boards of the participating institutions all approved the trial before patient entry.

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study drugs, during the first course of therapy. The maximum tolerated dose was one dose level below that associated with unacceptable toxicity; that is, the dose tested in which two or more patients experienced dose-limiting toxicities attributable to the study drug(s). At least six patients were to be treated at the maximum tolerated dose. Moderate toxicity was defined as any grade ≥2 nonhematologic toxicity (excluding nausea, vomiting, and alopecia) or any grade ≥3 hematologic toxicity that does not qualify as a dose-limiting toxicity.

Rules for dose escalation. The study was initially designed to proceed in two phases: an accelerated phase, which planned to enroll one patient per cohort to the first two dose levels and a standard phase, which planned to enroll three patients per cohort, beginning with dose level 3 unless moderate toxicity was encountered at a lower dose (Table 1).

During the accelerated phase, one patient was to be enrolled per dose level and evaluated for toxicity before the next dose level was opened. If the patient experienced moderate toxicity on the first course, or if two patients experienced moderate toxicity on any course, the accelerated phase would end, and the current dose level was expanded to a total of three patients who would constitute the first cohort of patients in the standard phase. If a patient experienced dose-limiting toxicities on the first course during the accelerated phase, then the accelerated phase would end, and the current dose level was expanded to three patients, who would constitute the first cohort of the standard phase. Because dose-limiting toxicity was observed in the first patient entered in this study, the accelerated phase ended early.

During the standard phase, three patients were treated at each new dose level. If zero of three patients experienced dose-limiting toxicities, three patients were treated at the next dose level. If dose-limiting toxicity attributable to the study drug(s) was experienced in exactly one of three patients, three more patients (for a total of six) were to be treated at that dose level. If no additional dose-limiting toxicity was observed at the expanded dose level (i.e., one of six with dose-limiting toxicities), the dose was escalated. Escalation terminated as soon as two or more patients experienced any dose-limiting toxicity attributable to the study drug(s), at a given dose level. The phase I trial was closed when six patients were treated at the next lower dose level and no more than one of six patients experienced dose-limiting toxicities. If more than one of six patients experienced dose-limiting toxicities, the next lower dose was expanded. Up to six patients were allowed to be added at maximum tolerated dose to further define toxicity and potential efficacy in this subgroup.

All patients who have not experienced any dose-limiting toxicity must have been observed for a minimum of 28 days after the start of the first course (i.e., the length of the treatment cycle) before the next dose level was escalated. Patients were to receive a minimum of one treatment cycle and a maximum of six cycles. There was no intrapatient dose escalation for patients enrolled in this study.

Treatment plan. Study drugs were to be given once during each 4-week treatment cycle. Each cycle of treatment consisted of dosing on day 1 with follow-up through day 28. The treatment plan consisted of six cycles. If administration of UCN-01 was delayed for >8 weeks, the subject would discontinue treatment and be removed from the study. Subjects with progressive disease at any time during the study were removed from the study. Antiemetic premedications were given. For all dose levels, UCN-01 and cisplatin must be given in a fixed-time relationship where cisplatin was infused over 2 hours followed 22 hours later by the UCN-01 infusion. In the absence of progressive disease, treatment was continued for up to six cycles provided that criteria for treatment continuation are met and the patient has not received a cumulative dose of cisplatin of >500 mg/m².

Patients receiving UCN-01 had blood pressure and pulse rate measurements every 6 hours during the infusion. Blood pressure was taken in both the supine and standing positions to determine orthostatic changes. Patients with clinically significant orthostatic hypotension were treated with i.v. saline or other treatments at the discretion of the investigator. UCN-01 was temporarily held, if clinically indicated, and restarted once hemodynamic stabilization was achieved.

Off-treatment criteria were defined as death, progressive disease, unacceptable toxicity, intercurrent medical problems interfering with completion of protocol treatment, noncompliance, voluntary withdrawal, and completion of treatment.

Toxicity assessment and dose modifications. Patients were examined and any toxicities were graded on day 1 of each treatment cycle. Dose delays or adjustments were based on treatment day hematologic variables and interim nonhematologic toxicities. Patients who experienced grade ≥3 nonhematologic toxicity must return to baseline status before a new cycle of treatment is started. In the event of treatment delays, both cisplatin and UCN-01 were delayed to maintain concurrent cyclic therapy. All dose reductions were permanent. No intrapatient dose escalation was allowed. All severe and unexpected adverse events were reported to the NCI and the appropriate regulatory bodies. The study used the NCI Common Toxicity Criteria version 2.0 for toxicity and adverse event reporting.

Criteria for evaluation. All patients who are registered were to be accounted for in the report of the results. Dose escalation rules and toxicity summaries were to include those patients who completed one course of therapy and who were followed up a minimum of 4 weeks after the start of the first course or who experience dose-limiting toxicities. Patients who complete two courses of treatment were evaluable for response. Treatment response was assessed using standard WHO criteria.

Molecular correlative studies. In selected patients, biopsies were done before initiation of treatment and following completion of cisplatin/UCN-01 infusion. For patients 2 and 3, the second biopsy was collected at the end of the 72-hour UCN-01 infusion. For patient 1, the second biopsy was collected 3 days after the end of the UCN-01 infusion. A portion of each biopsy was snap-frozen in liquid nitrogen and stored at −80°C. Frozen specimens were minced in a liquid nitrogen–cooled pulverizer immediately before lysis and protein quantitation. Western blot analyses of Cyclin B, G2/M Kinase-1, Chk1, Cdc25C, and p27KIP1 were conducted as previously described, using β-actin as an endogenous standard for loading control (18).

Pharmacokinetics. Plasma and salivary pharmacokinetics of UCN-01 were evaluated in all patients during cycle 1 of therapy. Serial blood, obtained from a peripheral venous site (not from the central indwelling catheter), and saliva samples were obtained at the following times: pretreatment, 24, 48, 72, 168 (1 week), 336 (2 weeks), 504 (3 weeks), and 672 hours (4 weeks) after the start of the first 72-hour UCN-01 infusion. Blood samples were collected in green top (sodium or lithium heparin) tubes and kept on ice until processing, which consisted of separation of plasma from the whole blood by centrifugation at 1,500 × g for 10 minutes. Saliva samples (3-5 mL) were collected directly into labeled polypropylene tubes. Plasma and saliva samples were stored frozen at −70°C until analysis.

UCN-01 in plasma and saliva was measured using a validated reverse-phase liquid chromatography/tandem mass spectrophotometric assay done on a Agilent Technologies LC 1100 series system (Palo Alto, CA) interfaced with a Micromass Quattro Ultima Triple Quadrupole Mass Spec.
Spectrometer (Waters Corp., Milford, MA). Separation was achieved across a Phenomenex Synergi Hydro-RP 4 μ, 80 A, 150 × 2.0 mm column proceeded by a Phenomenex C18 guard column (Torrance, CA). Column temperature was maintained at a constant 25°C, and the flow rate was 0.2 ml/min. The isocratic mobile phase consisted of 32% acetonitrile (high-performance liquid chromatography grade, Fisher Scientific, Fair Lawn, NJ), 0.1% formic acid (88%, Mallinckrodt Baker, Inc., Phillipsburg, NJ) in water. The injection volume was 20 μL and the total run time was 9 minutes. Under these conditions, UCN-01 eluted at 4.8 minutes and internal standard (staurosporine) at 6.5 minutes. The electrospray ionization source of the mass spectrometer was operated in the positive ion mode with a cone gas flow of 190 L/h and desolvation gas flows of 500 L/h. The capillary voltage was set to 4.1 kV. Cone and collision cell voltages were optimized separately for UCN-01 (40 V and 17 eV) and internal standard (47 V and 18 eV). The source temperature was 125°C. The desolvation temperature was 300°C. The precursor → product ion combinations monitored for UCN-01 and internal standard were 483 → 130 and 467 → 338 m/z, respectively. All chromatographic data acquisition and analysis were done using MassLynx version 3.5. The lower limit of quantitation for the assay is 3 nmol/L, with inter-day and intra-day precision and accuracy of <10% and >93%, respectively.

**Results**

**Patient demographics.** Ten patients with advanced malignant solid tumors were accrued. Patient characteristics are outlined in Table 2. The median age was 59 years, seven patients were female, and all patients had an acceptable performance status (i.e., three patients had Zubrod 0, whereas the remaining had either Zubrod 1 or 2). The mean number of prior chemotherapy regimens was 2. The tumor types represented in this trial included non–small cell lung cancer (n = 2 patients), soft tissue sarcoma (n = 2), esophageal cancer (n = 1), head and neck cancer (n = 1), pancreatic cancer (n = 1), malignant melanoma (n = 1), gastric cancer (n = 1), and adenocarcinoma of unknown primary (n = 1).

**Dose escalation and toxicities.** The first patient in dose level 1 experienced grade 3 hypoxia probably related to UCN-01. This finding ended the accelerated phase of the study. This dose level was expanded to enroll six additional patients; one of these patients had progressive brain metastases before completion of cycle 1 and was therefore determined to be nonassessable for toxicity or response. No further dose-limiting toxicities were observed in the five remaining patients treated in this expanded cohort. Dose level 2 was then opened with the nearly simultaneous enrollment of three patients. Dose-limiting toxicities consisting of grade 5 sepsis with respiratory failure associated with grade 3 creatinine (one patient) and grade 3 atrial fibrillation (one other patient) were observed at this dose level. Attributable grade ≥3 toxicities are summarized in Table 3. The maximum tolerated dose was therefore determined to be cisplatin, 20 mg/m² and UCN-01, 45 mg/m².

**Efficacy.** Although response rate was only a secondary objective in this phase I trial, responses were recorded. One patient with metastatic adenocarcinoma of unknown primary had a partial response. This patient had predominantly skin metastases that partially regressed during protocol therapy. Six patients had progressive disease as the best response. Three patients were not assessable for response due to receipt of less than two treatment cycles before protocol discontinuation.

**Pharmacokinetics.** Pharmacokinetic variables for plasma and salivary UCN-01 are summarized in Table 4 and graphically illustrated in Fig. 2. Average peak plasma concentrations at the end of a 72-hour continuous infusion of 45 mg/m²/d were 34 ± 14 μmol/L. As shown in the figure, plasma UCN-01 displays a prolonged elimination phase, with a mean elimination t1/2 of 680 ± 334 hours following the end of the 72-hour infusion. Thus, the mean plasma CL is 0.03 ± 0.03 L/h, and the mean area under the curve is >25,000 μmol/L × hours.

Salivary levels of UCN-01 were ~0.1% of the corresponding plasma concentration measured at each time point. For instance, mean end of infusion salivary UCN-01 levels were 0.06 ± 0.08 μmol/L and represented 0.18% of the mean plasma concentration. Elimination of UCN-01 from the saliva was similarly delayed, with a mean elimination t1/2 of 288 ± 108 hours, reflecting the prolonged plasma levels. The mean salivary UCN-01 area under the curve was 20.5 ± 16 μmol/L × hours, which was 0.1% of the mean plasma area under the curve.

**Molecular correlates.** Western blot analyses of pretreatment and posttreatment tumor biopsies were done in three patients, using the A549 non–small cell lung cancer cell line as a control. Treatment of A549 cells with UCN-01 (200 nmol/L for 24 hours) resulted in a substantial loss of Chk1 and Cdc25C protein expression, associated with reduced levels of Cyclin B1 and loss of Cdk1 phosphorylation (Fig. 3). UCN-01 also induced expression of p27KIP1, as shown previously (2, 4). Treatment of A549 cells with cisplatin (20 μmol/L for 3 hours followed by 21 hours in fresh medium) resulted in increased phosphorylation of Cdk1 and an induction of Cyclin B1, consistent with a DNA damage-induced G2 arrest as previously observed in this cell line (18). All three patients tested showed decreased levels of both Chk1 and Cdc25C (Fig. 3). Patient 1, who was responsive to treatment, showed an increase in expression of p27KIP1. In one patient whose disease progressed rapidly (patient 2), dramatically increased levels of cyclin B and moderately increased Cdk1 phosphorylation in the posttreatment sample were indicative of a cisplatin-induced G2 arrest and failure of UCN-01-induced G2 abrogation.

![Click to view larger version](Image)
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and UCN-01 would be a critical factor, as shown by
cisplatin, UCN-01-mediated inhibition of the checkpoint
kinase Chk1 restricts DNA damage repair, thereby increasing
platinum-induced cytotoxicity (22, 23). We hypothesized that
UCN-01 will overcome cisplatin-mediated cell cycle arrest in
the tumor at concentrations that will be tolerated by the
patient. We anticipated that a plasma concentration of cisplatin
sufficient to induce DNA damage and cell cycle arrest would be
required for this interaction.
This phase I study showed the difficulty of achieving
established therapeutic doses of cisplatin (>60 mg/m²) in
combination with prolonged infusion UCN-01. Dose-limiting
toxicities resulting in sepsis and respiratory failure (and
subsequently death) occurred in one patient, whereas grade 3
atrial fibrillation occurred in another at the second dose level.
These toxicities, although surprisingly severe, are consistent
with the pleiotropic effects of UCN-01 on biological systems.

We also hypothesized that the timing of administration of
cisplatin and UCN-01 would be a critical factor, as shown by
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that when UCN-01 preceded cisplatin, growth inhibition was
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analysis (18). In contrast, when non–small cell lung cancer
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cells were treated with cisplatin followed by UCN-01, the
combination was synergistic. In this treatment sequence, a
decrease in the proportion of cells at the G2 checkpoint was
confirmed by reduced expression of cyclins A and B and
activation of Cdk1. Abrogation of the G2 DNA damage
checkpoint and apoptosis were prevalent only in cell populations
exposed to cisplatin followed by UCN-01 and was
markedly enhanced in the cell lines with disrupted p53. These
data provided the basis for the sequencing of the UCN-01
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tration.

Unfortunately, the sequence specificity of cisplatin and
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half-life; thus, the optimal effects of sequencing cisplatin before
UCN-01 probably occurred during the initial cycle of treatment.
Whether the lingering effects of UCN-01 negatively affected
platinum efficacy in subsequent cycles cannot be determined
from this study. Whereas plasma levels of UCN-01 diminished
only slightly before cycle 2, saliva levels (expected to be more
representative of intratumoral concentration) decreased pro-
portionally faster, although complete clearance was not
achieved before administration of cisplatin in cycle 2 (Fig. 2).

In three patients, tumor biopsies were procured before
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molecular targets of UCN-01. To determine whether UCN-01
treatment resulted in abrogation of cisplatin-induced G2 arrest,
analysis of Chk1, Cdc25C, Cyclin B, and Cdk1 was conducted.
The checkpoint protein Chk1 mediates G2 arrest in response to
DNA damage by inactivating the phosphatase Cdc25C via
phosphorylation and cytoplasmic sequestration. Inactivation of
Cdc25C results in accumulation of inhibitory phosphorylation
on Cdk1, preventing cell cycle progression. In vitro treatment
with UCN-01 results in inhibition of both Chk1 and Chk2,
leading to abrogation of DNA damage-induced G2 arrest and

Discussion
UCN-01 inhibits a number of molecular targets, including
CDKs, protein kinase C, and the AKT pathway, making it an
attractive anticancer agent (19–21). In combination with
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![Table 3. Toxicities attributable to treatment (grade ≥3)](https://www.aacrjournals.org/doi/fig/canres/11/12/4448/figure/fig1)
The human pharmacology and clinical use of UCN-01 has been complicated by its high affinity for α-1-acidglycoprotein (11, 29, 30). Previous clinical studies have shown that volume of distribution of UCN-01 is very small, likely because very little circulating UCN-01 is unbound and available for uptake into tissues (28). Thus, clinically achievable UCN-01 doses were much higher than originally predicted by preclinical models. Because of its high degree of protein binding and the potential for serious drug-drug interactions if UCN-01 were to be displaced from α-1-acidglycoprotein, we conducted correlative pharmacokinetic investigations in conjunction with this phase I clinical trial. Both cisplatin and UCN-01 are strongly associated with plasma proteins; however, when given together, the plasma pharmacokinetics of UCN-01 are remarkably similar to those reported for single agent UCN-01 (11). Furthermore, salivary levels of UCN-01 measured on the current study are also quite similar to single agent data. Because salivary levels have been proposed to be a surrogate for free drug concentrations, our data suggest that UCN-01 protein binding is unaffected by concomitant administration of cisplatin.

We therefore conclude that with a prolonged infusion schedule of UCN-01, targeted therapeutic dose levels of cisplatin (maximum tolerated dose: cisplatin, 20 mg/m² plus UCN-01, 45 mg/m²) is not achievable. Nevertheless, because of its novel mechanisms of action and promising preclinical effects, further studies of UCN-01 and platinum with alternative dose schedules and/or use of other platinum analogues are warranted.

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References


Fig. 3. Western blot analysis of tumor biopsies. Western blot analyses of pretreatment and posttreatment tumor biopsies were done in three patients, using the A549 NSCLC cell line as a positive control. Treatment of A549 cells with UCN-01 (200 nmol/L for 24 hours) resulted in a loss of Chk1 and Cdc25C protein expression, with reduced levels of Cyclin B1 and loss of Cdk1 phosphorylation. Treatment with cisplatin (200 nmol/L for 3 hours followed by 21 hours in fresh medium) resulted in increased phosphorylation of Cdk1 and induction of Cyclin B1. All three patients tested showed decreased levels of both Chk1 and Cdc25C. In one patient whose disease progressed rapidly, increased levels of cyclin B and increased Cdk1 phosphorylation were seen.

Following therapy, both Chk1 and Cdc25C protein levels seemed reduced in all three patients, with the caveat that one patient did not show consistent levels of β-actin used as an endogenous standard for loading. Although it was anticipated that these proteins would undergo changes in phosphorylation state, reduced protein levels are consistent with previous reports and with the results of the A549 cells used as a control (Fig. 3; refs. 27, 28).
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