Blocking of glioma proliferation in vitro and in vivo and potentiating the effects of BCNU and cisplatin: UCN-01, a selective protein kinase C inhibitor

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Seven-hydroxystaurosporine (UCN-01) is a derivative of the nonselective protein kinase inhibitor staurosporine that exhibits significant selectivity for protein kinase C (PKC) in comparison to a variety of other intracellular kinases and appears to be well tolerated in vivo at concentrations sufficient to achieve effective inhibition of PKC. Because recent studies have indicated that the proliferation of malignant gliomas may result from activation of PKC-mediated pathways and, conversely, may be inhibited by blocking PKC, the authors examined the efficacy of this agent as an inhibitor of proliferation in three established and three low-passage malignant glioma cell lines in vitro. A striking inhibition of proliferation was produced by UCN-01 in each of the cell lines, with a median effective concentration of 20 to 100 nM, which correlated with the median in vitro PKC inhibitory concentration of 20 to 60 nM for this agent in the U-87 and SG-388 glioma cell lines. Inhibition–recovery studies of clonogenic activity indicated that UCN-01 had both cytostatic and cytotoxic effects on the treated cells. Proliferation resumed after short-term (6- and 24-hour) exposures to this agent; in contrast, with longer exposures, recovery of proliferative activity was severely compromised. In addition, UCN-01 enhanced the inhibition of glioma cell proliferation achieved with conventional chemotherapeutic agents, exhibiting synergistic effects with cisplatin and additive effects with 1,3-bis(2-chloroethyl)-1-nitrosourea. In vivo studies in which UCN-01 was administered by continuous intraperitoneal infusion in subcutaneous and intracranial intraparenchymal nude rat models demonstrated significant activity against U-87 glioma xenografts at dose levels that were well tolerated. It is concluded that UCN-01 is an effective agent for the inhibition of glioma proliferation in vitro and in vivo and has potential for clinical applicability in the treatment of human gliomas.

KEY WORDS • astrocytoma • glioma • growth inhibition • protein kinase C • signal transduction • 7-hydroxystaurosporine
Effect of UCN-01 on glioma proliferation

Materials and Methods

Cell Culture

We used the human malignant glioma cell lines A-172, T-98G, and U-87. Each of these cell lines was maintained in growth medium consisting of α-minimal essential medium (MEM) supplemented with 1-glutamine, ribonucleosides, deoxyribonucleosides, 10% fetal calf serum, and the following antimicrobial agents: 100 IU/ml penicillin, 100 μg/ml streptomycin, and 0.25 μg/ml amphotericin B. Cultures were established in 75-cm² flasks, maintained at 37°C in a humidified atmosphere with 5% CO₂ in air, and subcultured every 4 to 7 days with 0.25% trypsin in Hank’s balanced salt solution (HBSS).

Three low-passage malignant glioma cell lines (SG-388, SG-611, and H-7) were established at our institution from tumor specimens that had been transported directly from the operating room to the laboratory in α-MEM. In each case, the histopathological diagnosis was confirmed by a neuropathologist. These specimens were finely dissected to remove excess blood and necrotic material, cut into pieces approximately 1 mm² in diameter, and dissociated by incubation for 1 hour at 37°C in 0.25% trypsin and 0.02% deoxyribonuclease. Dissociated cells were filtered through 100-μm nylon mesh and then cultured for 48 hours in growth medium. Medium containing nonadherent cells was removed and fresh medium applied. Cells were subcultured after reaching confluence and studied in approximately the fifth passage in vitro.

Assay of Cell Proliferation

A colorimetric cell proliferation assay was used in each of the cell lines to assess the effect on cell proliferation of a range of concentrations of UCN-01. For these studies, 5 x 10⁴ cells were plated and grown for 12 hours in 100 μl of growth medium in 96-well microtiter plates and then treated for 4 days with various concentrations of UCN-01, which was prepared from a 1 mg/ml–stock solution dissolved in dimethylsulfoxide (DMSO). Control cells were treated with equivalent concentrations of DMSO without UCN-01. All studies were performed in triplicate.

After a 4-day incubation period, the number of viable cells was determined by measuring the bioreduction of the tetrazolium compound 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) by intracellular dehydrogenases in the presence of the electron coupling reagent phenazine methosulfate (PMS). We confirmed in preliminary studies that the quantity of the formazan product, measured spectrophotometrically, was proportional to the number of living cells in culture for cell counts between 2.5 x 10⁴ and 5 x 10⁴ in the cell lines included in this study. To perform the assay, 20 μl of combined MTS/PMS solution containing 2 mg/ml MTS and 150 μM PMS in buffer (0.2 g/L KCl, 8.0 g/L NaCl, 0.2 g/L KH₂PO₄, 1.15 g/L Na₂HPO₄, 133 mg/L CaCl₂·2H₂O, 100 mg/L MgCl₂·6H₂O, pH 7.35) was added to each well, and the mixture was incubated for 3 hours at 37°C in a humidified 5% CO₂ atmosphere. Absorbance at 490 nm was measured using an enzyme-linked immunosorbent assay microplate reader. Background absorbance of the medium was measured in a triplicate set of control wells that contained medium and the MTS/PMS solution without added cells and was subtracted from the absorbance measured in each of the sample wells to provide a corrected absorbance for each of the wells. Triplicate wells with predetermined cell numbers were subjected to the above assay in parallel with the test samples; this also provided internal confirmation that the assay was linear over the range of absorbances and cell numbers measured.

Direct Measurement of Cell Proliferation

For a more direct assessment of the effect of UCN-01 on cell proliferation and viability in the U-87 and SG-388 malignant glioma cell lines, we plated 2.5 x 10⁴ cells in 60-mm petri dishes in growth medium. The cells were then grown as described above with select concentrations of UCN-01 or vehicle. After a 4-day incubation, the cells were harvested by treatment with 0.25% trypsin in HBSS, pelleted by centrifugation, resuspended in serum-free medium, stained with trypan blue, and counted using a hemacytometer. All samples were tested in triplicate. Cell numbers were then plotted as a function of UCN-01 concentration.

Clonogenic Assay

An assessment of the effect of selected concentrations of UCN-01 on cell viability was performed using a clonogenic assay. For these studies, 10³ U-87 cells were plated and, after an overnight attachment period, grown as described above with selected concentrations of UCN-01 and with appropriate vehicle controls. In initial experiments, cells were exposed to selected concentrations of UCN-01 vehicle for 48-hour period. In subsequent experiments, we examined whether the effects of UCN-01 on glioma viability were critically dependent on the duration of treatment with this agent by using a series of shorter (6- and 24-hour) exposure times in addition to the 48-hour incubation. After treatment with UCN-01 or vehicle, the cells were washed with inhibitor-free medium and grown in serum-supplemented medium for an additional 2-week period. The plates were then stained with crystal violet, and colonies consisting of groups of approximately 50 or more cells were counted. All studies were performed in triplicate.

Measurement of PKC Activity

The PKC activity was measured 24 hours after treatment with selected concentrations of UCN-01 in an established glioma cell line (U-87) and in one of the low-passage cell lines (SG-388). Glioma cells (5 x 10⁵) were plated in 75-cm² flasks and grown for 12 hours in growth medium. Cells were then treated with various concentrations of UCN-01 or DMSO.

The technique used for PKC extraction was a modification of the procedure of Gschwendt, et al. Cell lysates were obtained by homogenization of cultured cells in 2.5 ml of extraction buffer (20 mM Tris, pH 7.5, 0.5 mM ethylenediamine tetracetic acid (EDTA), 0.5 mM ethyleneglycol bis (β-aminoethylthether) N, N’, N”-tetracetic acid (EGTA), 50 μg/ml phenylmethylsulfonate, 25 μg/ml leupeptin, 1 μg/ml pepstatin, 10 mM β-mercaptoethanol, and 0.5% Triton X-100) followed by stirring for 30 minutes at 4°C. Following centrifugation at 30,000 g for 60 minutes at 4°C, the supernatant was collected and subjected to DE52-cellulose chromatography. The PKC was eluted with 0.5 ml of 0.1 M NaCl in 20 mM Tris- HCl (pH 7.5), 0.25 M sucrose, 0.5 mM EDTA, 0.5 mM EGTA, and 10 mM β-mercaptoethanol. Protein concentrations within the samples were measured using the Bradford method.

The PKC activity was measured on the basis of phosphorylation of acetylated myelin basic protein peptide using γ[32P]-adenosine triphosphate (ATP) as a tracer. For these studies, 25 μl of each PKC preparation was incubated with 10 μg 12-O-tetradecanoylphorbol-13-acetate, 0.28 mg/ml phosphatidyl serine, 10% mixed micelle solution, 50 μM acetylated myelin basic protein peptide, 20 μM ATP, 1 mM CaCl₂, 20 mM MgCl₂, 20 mM Tris (pH 7.5), 1 μCi γ[32P]-ATP (3000 Ci/mmol stock solution) in a final volume of 50 μl. The reaction was allowed to proceed for 5 minutes at 30°C and was terminated by spotting a 25-μl aliquot onto phosphocellulose disks. The disks were quickly transferred to a tray containing 500 ml of 1% phosphoric acid to remove unreacted γ[32P]-ATP, washed for at least 5 minutes, and transferred to fresh phosphoric acid. The disks were then washed twice in water and placed in scintillation vials with 5 ml of Ecolite scintillation cocktail.

The level of nonspecific phosphorylation was determined in control samples that were preincubated with an excess of a PKC pseudosubstrate inhibitor peptide, which acts as a potent inhibitor of PKC activity for a variety of PKC substrates including myelin basic protein. For these specimens, 25-μl aliquots of the PKC preparation were preincubated at room temperature with 20 μM of the PKC pseudosubstrate inhibitor peptide for 20 minutes prior to addition of the other components of the reaction mixture. All samples were tested in duplicate. Incorporated radioactivity of samples and their controls was then measured. The PKC activity (sample minus control) was calculated as picomoles of peptide-incorporated phosphate per minute per microgram of partially purified cell lysate. The UCN-01 concentration–response curves were then plotted.
Interaction of UCN-01 With Conventional Chemotherapeutic Agents

Because previous studies have indicated that inhibitors of PKC may interact synergistically with conventional chemotherapeutic agents to inhibit tumor cell proliferation in vitro,\textsuperscript{2,3,7,21,22} we examined the effect in the U-87 and SG-388 cell lines of combining various concentrations of UCN-01 with a range of concentrations of cisplatin or BCNU, agents that each have some activity against malignant gliomas. First, concentration–response curves were established for each of the single agents using the MTS assay. Then selected concentrations of UCN-01 were combined with various concentrations of cisplatin or BCNU, and the cells were incubated as described for the MTS assay. All studies were performed in triplicate. Evaluation of the effects of drug combinations were performed as described by Chou and colleagues\textsuperscript{11–13} using commercially available software for combined drug-effect calculations and isobologram analysis.

Assessment of Antiproliferative Activity in a Nude Rat Subcutaneous Glioma Model

In vivo assessment of the effect of UCN-01 on glioma proliferation was initially performed using a nude rat subcutaneous model of the U-87 glioma cell line. This model was chosen so that tumor size could be easily followed serially in each animal. For these studies, 4-week-old nu/nu rats were injected in the right flank with $10^6$ tumor cells suspended in 100 μl of a 1:1 mixture of phosphate-buffered saline (PBS)/Matrigel. This amount of cells typically produced palpable tumors within 2 weeks. Animals were examined daily for evidence of tumor growth and treatment was initiated when tumors 0.5 cm in diameter were identified. All animal studies were approved by the Animal Research and Care Committee of the Children’s Hospital of Pittsburgh.

The UCN-01 was administered intraperitoneally in eight animals via an implanted osmotic infusion pump at a dose level of 1 mg/kg/day, one third of the maximum tolerated daily dose established in previous rodent toxicology studies (EA Sausville, personal communication, 1995) for a 7-day period. Four animals were subsequently treated with 2 mg/kg/day of UCN-01. Litter-matched control animals received vehicle (50% DMSO) alone. For the pump implantation, the animals were anesthetized with ketamine (100 mg/kg) and acepromazine (0.02 mg/kg). Under sterile conditions, a small transverse incision was made in the abdomen. The muscle layers and peritoneum were incised, the pump was inserted, and the wound was approximated in layers with absorbable sutures. Tumor dimensions were measured every other day using vernier calipers and animals were weighed twice weekly. Tumor volume was calculated as $L \times W^2 / 2$ (where $L$ is the longest diameter and $W$ is the diameter perpendicular to $L$). Comparisons between volumes at various time points were made using Student’s t-test.

Assessment of Antiproliferative Activity in a Nude Rat Intracranial Glioma Model

Animals were anesthetized as described above and immobilized in a stereotactic frame. An opening was made in the bone via a small right paramedian scalp incision, using a 20-gauge needle tip. We suspended $2 \times 10^5$ U-87 cells in a volume of 10 μl PBS and injected the suspension stereotactically into the right frontal lobe to a...
Effect of UCN-01 on glioma proliferation

depth of 3.5 mm using a Hamilton syringe. This amount of cells typically induces tumors that manifest with contralateral hemiparesis within 3 to 5 weeks.

Five days later, rats were chosen randomly to receive 2 mg/kg/day of UCN-01 for 7 days (eight animals) or vehicle (14 animals), with the doses being delivered by continuous intraperitoneal infusion. Animals were examined daily for evidence of neurological symptoms and were killed when they manifested severe hemiparesis. Their brains were examined histologically to confirm that the cause of hemiparesis was tumor growth (rather than infection) in each case. Survival curves for the UCN-01–treated and control animals were compared using a Wilcoxon rank sum test.

Sources of Supplies and Equipment

The nu/nu rats used in the experiment were obtained from the National Cancer Institute, Bethesda, MD; the cell lines A-172, T-98G, and U-87 were obtained from the American Type Culture Collection, Bethesda, MD. The UCN-01 was kindly provided by Peter Worland and Edward Sausville from the National Cancer Institute, Bethesda, MD. Growth medium components and basic protein and inhibitor peptides were supplied by Gibco, Grand Island, NY. Buffer used in the proliferation assay was supplied by Promega, Madison, WI. The γ[32P]-ATP was obtained from Amersham, Arlington Heights, IL. Computer software used in the drug-effect calculations and isobologram analysis was supplied by Elsevier Biosoft, Cambridge, UK. The osmotic infusion pump was obtained from Alzet, Palo Alto, CA.

Results

Effect of UCN-01 on Cell Proliferation and Viability

In each of the malignant glioma cell lines examined, UCN-01 produced concentration-dependent inhibition of cell proliferation as assessed by the MTS assay, with a median effective dose of 20 to 100 nM (Fig. 1). An 80% decrease in cell numbers was generally achieved with concentrations in the range of 200 nM. In contrast, no significant inhibition was seen in control cells that were treated with equivalent concentrations of vehicle (DMSO) in the absence of UCN-01.

Direct measurements of cell numbers after a 4-day exposure to UCN-01 in an established and a low-passage malignant glioma cell line using selected concentrations of the agent confirmed a dramatic inhibitory effect on cell proliferation (Fig. 2). As with the MTS assay, the median effective dose was in the range of 60 to 100 nM. Increasing concentrations of UCN-01 produced a progressive increase in the number of cells that were nonviable, as assessed by their inability to exclude trypan blue and by the development of morphological features of apoptosis, suggesting that the effect of this agent on glioma cells was not strictly cytostatic but also cytotoxic.

The cytotoxic effect of UCN-01 was confirmed using a clonogenic assay in the U-87 and SG-388 malignant glioma cell lines. A 4-day exposure to UCN-01 concentrations in excess of 20 nM produced a sharp drop in colony-forming activity (Fig. 3 upper). Concentrations in excess of 150 nM produced almost complete loss of colony-forming potential.

Recovery in Proliferative Activity After Exposure to UCN-01

Based on our observations, it was apparent that UCN-01 inhibited proliferation and also had an irreversible toxic effect on cells that had been exposed to this inhibitor for a 4-day period. To determine whether the inhibitory effects of UCN-01 on glioma proliferation and viability were reversible following shorter periods of exposure, we performed an inhibition recovery assay. These studies showed that exposure to UCN-01 concentrations of up to 200 nM, again correlating closely with the 80% decrease in cell numbers was generally achieved with concentrations in the range of 200 nM. In contrast, no significant inhibition was seen in control cells that were treated with equivalent concentrations of vehicle (DMSO) in the absence of UCN-01.

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Effect of UCN-01 on PKC Activity

The relationship between PKC activity and cell proliferation in the U-87 cell line is demonstrated in Fig. 3 lower. Comparable results were obtained with the SG-388 malignant glioma cell line. The UCN-01 produced a concentration-dependent decrease in PKC activity that paralleled the inhibitory effect of this agent on cell proliferation and viability. The IC50 for PKC inhibition of approximately 50 nM was comparable to the median effective concentration for inhibition of cell proliferation. An 80% inhibition of PKC activity was seen at a concentration of 200 nM, again correlating closely with the 80% decrease in cell proliferation and viability produced by this concentration of UCN-01 in the glioma cell lines.

Interaction of UCN-01 With Conventional Chemotherapeutic Agents

Concentration–effect plots of percent inhibition versus drug concentration for each of the individual drugs and for the UCN-01/drug combinations are provided in Fig. 4 upper and lower left. The median effective concentrations of cisplatin and BCNU were approximately 5 and 40 μM, respectively, in both the U-87 and SG-388 cell lines. The UCN-01 produced a statistically significant enhancement
in the inhibitory effects of both cisplatin and BCNU (Fig. 4 upper and lower left). To determine whether this enhancement reflected additive or synergistic interactions, we performed concentration–effect and isobologram analyses. The data from Fig. 4 upper and lower left were used to produce median effect plots, which relate log concentration to log (fraction affected (the degree of inhibition achieved)/fraction unaffected). These data were then applied to determine the combination index, which provides a semiquantitative assessment of the presence of additive, synergistic, or antagonistic interactions at different effect levels. The combination index is 1 for additive interactions, greater than 1 for antagonistic interactions, and less than 1 for synergistic interactions. Figure 4 upper and lower right illustrates plots of the combination index versus fraction affected for cisplatin and BCNU, respectively. The combination of UCN-01 with cisplatin produced synergistic inhibition, based on the observation that the combination index was substantially less than 1 for effect levels above 30% (Fig. 4 upper right). In contrast, the combination of UCN-01 with BCNU had additive effects (Fig. 4 lower right), with a combination index of approximately 1 at all effect levels. The synergistic and additive effects of UCN-01 with cisplatin and BCNU, respectively, were also apparent at the 50%, 70%, and 90% effect levels on isobologram analyses (data not shown). In parallel studies in the SG-388 cell line, UCN-01 was also effective in significantly potentiating the effects of both BCNU and cisplatin.

Assessment of Antiproliferative Activity in a Nude Rat Subcutaneous Glioma Model

Tumor growth curves for the animals treated with 1 mg/kg/day of UCN-01 and control animals treated with DMSO are shown in Fig. 5. Six of the eight animals that received UCN-01 had an objective reduction in tumor size during the first 10 days after treatment. One of the animals had complete tumor regression, five had a 25% to 75% decrease in tumor volume, and two had stable disease. The mean tumor sizes in these animals decreased from 0.12 ± 0.05 cm$^3$ to 0.06 ± 0.04 cm$^3$ by 10 days after the start of treatment (p < 0.05; two-tailed t-test). In contrast, all five control animals exhibited progressive tumor growth during the same interval. The differences in the mean tumor volumes between the UCN-01–treated and control animals were significant at 5, 10, and 15 days after the start of therapy (p < 0.05). Tumors in the UCN-01–treated animals resumed growth approximately 15 days after the start of the therapy, indicating that a single course of therapy at this dose level was unable to completely eradicate the tumor even among animals with apparently complete responses.

Four additional animals were subsequently treated with a higher dose of UCN-01 (2 mg/kg/day for 7 days). This produced complete tumor regression in two of the four animals, neither of which showed tumor recurrence at 21 and 28 days of follow-up review. These animals were killed and had no evidence of tumor on postmortem inspection. Partial tumor regression was achieved in two animals; however, both were ultimately killed because of recurrent tumor growth. In contrast, six litter-matched control animals treated with vehicle exhibited unremitting tumor growth.

Assessment of Antiproliferative Activity in a Nude Rat Intracranial Glioma Model

Based on the results of our subcutaneous animal model studies, a dose of 2 mg/kg/day of UCN-01 was selected for use in the intracranial animal model studies. One of the eight animals randomized to the UCN-01 treatment group died of peritonitis 3 days after pump implantation and was excluded from outcome statistics. Survival curves for the remaining UCN-01–treated and control animals are
shown in Fig. 6. Median survival was 35 days in the control group, whereas four of seven animals in the UCN-01 group were still alive at 90 days. Differences between the respective survival curves were statistically significant (p < 0.02, rank sum test). No adverse sequelae were apparent in the animals treated with UCN-01. Animals that died all had evidence of intracranial tumor on histopathological examination.

Discussion

We27–29 and others5,14,15 have reported that agents which inhibit PKC-mediated pathways can block the proliferation of malignant glioma cells in vitro. Unfortunately, many of the agents that have been used previously are too toxic at therapeutically effective concentrations to be useful clinically or insufficiently potent as PKC inhibitors to reliably block tumor growth at clinically achievable concentrations. Accordingly, we have focused our attention on examining the therapeutic efficacy of novel PKC inhibitors such as UCN-011,2,36,37 and CGP 41251,24 which are derivatives of staurosporine; and structurally related bisindolylmaleimide derivatives,9,18,41 which are highly potent and selective for PKC over a variety of other protein kinases, and are well tolerated in vivo.

The compound UCN-01 was chosen for detailed testing because of its practical advantages over a variety of the other selective PKC inhibitors: 1) UCN-01 is derived from a naturally occurring fungus and can be isolated in large quantities; 2) an assay for UCN-01 using high-per-
PKC activity and growth rate in malignant gliomas, we hypothesized that this potent PKC inhibitor would be particularly effective in blocking glioma proliferation in vitro. The results reported here confirm this hypothesis. The UCN-01 inhibited the proliferation of each of the glioma cell lines tested with a median effective concentration of 50 to 100 nM.1,35

However, each of the animals treated with this dose of UCN-01 ultimately exhibited recurrent tumor growth.

concentrations than those needed to block PKC activity (Pollack, et al., unpublished data), possibly reflecting the fact that this agent may be inhibiting different pathways in these tumors than in the malignant glioma cell lines. Similar results have been obtained in other tumor types: whereas UCN-01 inhibits certain tumor cell lines at concentrations in the range of 20 to 100 nM, other types of tumor-derived cell lines are inhibited at concentrations in the range of 100 to 300 nM.1,35

The effect of UCN-01 on the cell lines examined in this study was initially cytostatic, but with prolonged exposures direct cytotoxicity was apparent. This correlated with the induction of morphological and electrophoretic features of apoptosis (IF Pollack, et al., unpublished data), a genetically regulated “programmed” form of cell death. Coulldwell, et al.,15 have also noted that PKC inhibition using hypericin induced apoptosis in malignant glioma cell lines. The molecular pathway underlying this effect in cells treated with UCN-01 remains uncertain. Apoptosis is induced in a variety of tumor types following exposure to diverse stimuli, such as conventional chemotherapeutic agents and radiotherapy, or by withdrawal of critical cell survival factors.6,20,31,42 Because PKC has been reported to be an inhibitor of apoptosis in other cell types,15,30,31 it is not surprising that agents that inhibit PKC are capable of inducing apoptosis in glioma cells. An important implication of these results is that putative PKC inhibitors, such as UCN-01, may be used to achieve a cytotoxic (rather than strictly a cytostatic) effect on glioma cells, and thus may be used to stabilize disease and also to reduce tumor burden in vivo. However, it is apparent that for UCN-01 to have such cytotoxic effects, a prolonged period of exposure is required. Accordingly, optimum use of this agent in vivo must take this factor into account to incorporate dosing schedules that are sufficient to ensure maintenance of therapeutically effective drug concentrations in the tumor for periods in excess of 24 hours.

Another important observation of this study was the ability of UCN-01 to act in conjunction with conventional chemotherapeutic agents to inhibit glioma cell prolifer-
Effect of UCN-01 on glioma proliferation

ation. Previous studies have noted that a variety of PKC inhibitors potentiate the effects of platinum derivatives, nitrogen mustard, and mitomycin C, in a number of nonglial tumor types. Because BCNU and cisplatin are two of the most active conventional chemotherapeutic agents for human gliomas, the observation that UCN-01 could be effectively combined with these agents to potentiate therapeutic efficacy in vitro provides a strong rationale for evaluating the safety and efficacy of these combinations in animal tumor models in vivo.

The observation that UCN-01 had a synergistic effect with cisplatin but only an additive effect with BCNU is of mechanistic interest. One explanation for these results may be that UCN-01 and BCNU are interfering with unrelated intracellular targets, whereas UCN-01 is directly facilitating the effect of cisplatin by enhancing intracellular drug accumulation, by potentiating the toxicity of this agent, by interfering with cellular repair of cisplatin-induced damage, or by favoring cell cycle-specific DNA damage. In other cell types, PKC modulation has been found to enhance intracellular cisplatin concentration by increasing uptake or decreasing efflux. Alternatively, because UCN-01 appears to be capable of independently inducing DNA fragmentation, it is conceivable that this agent is directly potentiating the DNA damage caused by cisplatin.

In view of the encouraging results of our in vitro studies, we examined the effect of UCN-01 in vivo using the U-87 glioma cell line in a xenogeneic nude rat subcutaneous model. The UCN-01 consistently induced stabilization or regression of glioma xenografts at dose levels that were well tolerated. Subsequent studies in an intracranial intraparenchymal model confirmed a significant antitumor effect without apparent toxicity.

Together, our in vitro and in vivo studies with UCN-01 indicate that this selective PKC inhibitor holds promise as a therapeutic agent against malignant gliomas. In addition to its potential utility in single drug therapy, UCN-01 may also have applicability in combination chemotherapy regimens that incorporate platinum or nitrosourea derivatives, although the safety and efficacy of this approach will need to be explored further in animal glioma models.

References

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1031
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