Bradykinin Antagonist Dimer, CU201, Inhibits the Growth of Human Lung Cancer Cell Lines in Vitro and in Vivo and Produces Synergistic Growth Inhibition in Combination with Other Antitumor Agents

Daniel C. Chan, Lajos Gera, John M. Stewart, Barbara Helfrich, Tom Linmin Zhao, Wan Yong Feng, Kenneth K. Chan, Joseph M. Covey, and Paul A. Bunn, Jr.

Lung Cancer Program of the University of Colorado Cancer Center [D. C. ., L. G., J. M. S., B. H., T. L. Z., P. A. B.] and the Departments of Medicine [D. C. C., B. H., T. L. Z., P. A. B.] and Biochemistry [L. G., J. M. S.], University of Colorado Health Sciences Center, Denver, Colorado 80262; College of Pharmacy, Ohio State University Comprehensive Cancer Center, Columbus, Ohio 43210 [W. Y. F., K. K. C.]; the National Cancer Institute, Bethesda, Maryland 20892 [J. M. C.]; and Carcinex Inc., Boulder, Colorado 80301 [D. C., L. G., J. M. S., P. A. B.]

ABSTRACT

Small cell lung cancers (SCLCs), many non-SCLCs, and other cancers have neuroendocrine features, including paracrine and autocrine growth stimulation by various neuropeptides. Interference with this pathway is an attractive target for novel therapies. We developed a novel bradykinin antagonist dimer, CU201 (B9870), that acts as a "biased agonist" for neuropeptides by blocking Gαq signaling and activating Gα12,13 signaling. CU201 induced apoptosis and complete growth inhibition in various lung cancer and other cancer cell lines. CU201 was 10-fold more potent than substance P derivatives and was stable in serum for >7 days. In this study, we evaluated the ability of CU201 to produce additive or synergistic growth inhibition in combination with various antitumor agents used in lung cancer therapy. We found that CU201 produced additive or synergistic growth inhibition when combined with doxorubicin, etoposide, cisplatin, vinorelbine, and paclitaxel for SCLC lines and with paclitaxel and ZD1839, an epidermal growth factor receptor tyrosine kinase inhibitor, for non-SCLC cell lines. Pharmacokinetic parameters associated with the i.v. administration of CU201 were evaluated in normal mice, and the effects of CU201 on the growth of human lung cancer xenografts were evaluated in athymic nude mice. In CD2F1 mice given an i.v. bolus infusion of 5 mg/kg, the t1/2 was 5773 ng/ml (5 μM), and the decay was biexponential. When fitted to a two-compartment model, the t1/2a was 14.4 min, and the t1/2b was 44.3 h, indicating a long terminal half-life consistent with the prolonged in vitro effects. CU201 inhibited the growth of human lung cancers in athymic nude mice by the intratumoral, s.c., and i.p. routes at a dose of 5 mg/kg/day. This dose is >10-fold less than the dose of substance P derivatives used to inhibit SCLC xenografts in nude mice. We conclude that CU201 should undergo further preclinical toxicity studies in its development as a novel targeted therapy for the treatment of lung cancers with neuroendocrine features. These studies are in progress through the NCI RAID mechanism.

INTRODUCTION

Many cancers, including lung cancers, have autocrine/paracrine growth pathways that are involved in their pathogenesis and progression (1–3). Interference with these pathways has led to cancer regressions in preclinical models and in cancer patients (1, 3). For example, the EGFR plays an essential role in cancer regressions in preclinical models and in cancer patients (1, 3). For example, the EGFR plays an essential role in cancer regressions in preclinical models and in cancer patients (1, 3). For example, the EGFR plays an essential role in cancer regressions in preclinical models and in cancer patients (1, 3). For example, the EGFR plays an essential role in cancer regressions in preclinical models and in cancer patients (1, 3).

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2 To whom requests for reprints should be addressed, at University of Colorado Cancer Center, Box B171, 4200 E. 9th Avenue, Denver, CO 80262. E-mail: Dan.Chan@UCHSC.edu.

3 The abbreviations used are: EGFR, epidermal growth factor receptor; SCLC, small cell lung cancer; NSCLC, non-SCLC; GRP, gastrin-releasing peptide; SPD, substance P derivative; BK, bradykinin; NCI, National Cancer Institute; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; CI, combination index.

4 Iressa is a trademark of the AstraZeneca group of companies.
However, 2A11, the first anti-GRP antibody tested, produced a complete response among 1 of 12 treated patients (19). This observation was the first proof of principle that interference with the neuropeptide pathway could produce antitumor effects in humans.

SPDs were the first reported compounds to inhibit signal transduction by multiple peptides and to inhibit the growth of many SCLC cell lines (20). Subsequent studies showed that SPDs induced apoptosis in SCLC cell lines (21, 22). We later showed that the SPDs blocked peptide-receptor activation of G<sub>aq</sub>, protein kinase C, and downstream events, leading to activation of c-jun and c-fos-induced proliferation (21). SPDs also stimulated G<sub>a12,13</sub> proteins and downstream events, including Jun kinases, caspase-3, and apoptosis (21, 22). The term “biased agonist” was defined by Jarpe et al. (21) to describe this phenomenon in which the agent (SPD) discordantly inhibited the G<sub>aq</sub> pathway on one hand and stimulated the G<sub>a12,13</sub> pathway on the other hand. Although SPDs inhibited the growth of SCLC cell lines, they did not inhibit the growth of NSCLC cell lines; in addition, the inhibition of SCLC lines required high concentrations, and the serum half-life was relatively short (20, 21, 23–25). Despite these limitations, the SPDs were introduced to clinical trials in humans, and a Phase I trial was completed (26).

The effectiveness of the SPDs in preclinical investigations led to the search for more potent and stable compounds. BK receptors were shown to be the most ubiquitously expressed receptors on both SCLCs and NSCLCs (9). Specific BK antagonist monomers failed to inhibit the growth of most SCLC cells (3, 9, 10). However, we showed that a novel BK antagonist dimer, CU201, inhibited the growth of both SCLC and NSCLC cell lines in vitro (25). Similar to SPDs, this peptide dimer acted as a biased agonist by inhibiting G<sub>aq</sub> activation and downstream events and by stimulating G<sub>a12,13</sub> and downstream events. The end result was induction of apoptosis and inhibition of growth (25). CU201 was 10-fold more potent than the SPDs in each of these actions, including the inhibition of growth, and its activity persisted for 7 days when incubated in human serum (25). In this study, CU201 inhibited a wide variety of SCLC cell lines (average IC<sub>50</sub> = 2.1 μM), including both “classic” and “variant” cell lines and SCLC cell lines that expressed multidrug resistance protein or were made drug resistant (25). CU201 also inhibited the growth of five NSCLC, five breast cell lines, and five prostate cancer cell lines with IC<sub>50</sub>s ranging from 2.6 to 4.1 μM (25). CU201 also inhibited the in vitro growth of NSCLCs and breast and prostate cancer cell lines in the NCI panel. The in vitro activity of CU201 led to the studies reported here, in which CU201 was combined with other cytotoxic agents and human lung cancers were treated with CU201 in vivo in athymic nude mouse xenograft studies. Analyses of the distribution and elimination of CU201 in normal mice are also reported.

MATERIALS AND METHODS

**Cell Lines and Culture Conditions.** The NSCLC line A549 was obtained from the American Type Culture Collection (Rockville, MD). Dr. Aurelio Koros (University of Pittsburgh, Pittsburgh, PA) kindly provided SHP-77, the SCLC cell line. These cell lines were selected from nine SCLC cell lines and five NSCLC cell lines studied in vitro (25) because they were representative of the in vitro sensitivity of all these cell lines, having neuroendocrine features, good growth in athymic nude mice, and having been frequently used in studies of other agents. Cell lines were maintained in RPMI (Life Technologies, Inc., Grand Island, NY) supplemented with 10% heat-inactivated fetal bovine serum (HyClone, Logan, UT). The cell lines were grown in 5% CO<sub>2</sub> at 37°C in incubators with 100% humidity.

**Chemicals.** Paclitaxel was kindly provided by Bristol Myers-Squibb (Princeton, NJ). Cisplatin and etoposide were purchased from Sigma Chemical Co. (St. Louis, MO). Vinorelbine was kindly provided by Glaxo-Wellcome (Research Triangle Park, NC.). AstraZeneca Inc. (London, Unite Kingdom) kindly provided the ZD1839. Doxorubicin was purchased from Cetus Oncology (Emeryville, CA). The CU201 (B201, B9870, NSC 710295) and its monomer CU202 (B202, B9430) were synthesized by two of the authors (L. G. and J. S.) according to previously described methods (27, 28). The chemical structures of CU201 and CU202 are shown below: CU202, D-Arg-Arg-Pro-Hyp-Gly-Igl-Ser-d-Igl-Oic-Arg-COOH; CU201, SUIM-[D-Arg-Apro-Pro-Hyp-Gly-Igl-Ser-d-Igl-Oic-Arg-COOH], where SUIM is a bifunctional suberyl imidate cross-linker with six carbon chains. For the mouse studies, the CU201 was diluted in normal saline.

**MTT Growth Assay.** Cell growth was assessed using a modified tetrazolium salt (MTT) assay (29). Briefly, 5,000–10,000 viable cells were plated in 100 μl in 96-well plates (Corning, Ithaca, NY). After an overnight incubation, peptides and various cytotoxic chemotherapy agents were added in various concentrations and incubated for 6 days. After the 6 days, the tetrazolium salt was added at a concentration of 0.4 mg/ml to each well. The microtiter plates were incubated with the salt for 4 h at 37°C. At 4 h, the medium was aspirated off, leaving the dark blue formazan product in the bottom of the wells. The reduced MTT product was solubilized by the addition of 100 μl of 0.2 N HCl in 75% isopropanol-23% MiliQ water to each well. The contents of each well were thoroughly mixed with a Titertek multichannel pipettor (Flow Laboratories). The absorbency of each well was measured using an automated plate reader (Molecular Devices, Sunnyvale, CA). When cell lines were incubated with combinations of BK antagonists and chemotherapeutic agents, the combination effects were assessed with the isobologram method of Chou and Talalay (30).

**Mouse Tumor Model.** NCI (nru) athymic nude mice 6 weeks of age were obtained from the NCI (Bethesda, MD) and maintained in pathogen-limited conditions. s.c. injections of 2 × 10<sup>6</sup> SHP-77 SCLC or 1 × 10<sup>6</sup> A549 NSCLC tumor cells in an equal volume of Matrigel (Collaborative Biomedical Products, Bedford, MA) were implanted into the mouse posterior flanks before the administration of CU201 by various routes. Tumor-bearing mice were randomly divided into five per group. The control group was treated with vehicle (saline solution), and the other groups were treated with various amounts of CU201 via specific routes as indicated in the text and figure legends. Bidimensional tumor measurements were made with calipers three times weekly until the tumors reached a volume of 3 cm<sup>3</sup>, at which time the mice were sacrificed. Tumor volume was calculated according to the formula: V = π(short diameter)<sup>2</sup> (long diameter)/6. All animal studies were conducted with a...
protocol approved by the University of Colorado Health Sciences Center Institutional Animal Care and Use Committee.

**Pharmacokinetic Study.** CD2F1 mice (Harlan, Indianapolis, IN) weighing 21.0–27.4 g were used in the pharmacokinetic study. CU201 in normal saline was injected into each mouse through the tail vein, producing an i.v. bolus dose of 5 mg/kg. The animals were anesthetized with CO2, and the blood was removed from the heart through a thoracic opening according to a typical schedule of 0 (predose), 5, 10, 15, 30, 60, 120, 180, 210, 240, 480, 720, 1260, 1440 min after dosing. Blood was collected from four animals at each time point. The blood kinetic study. CU201 in normal saline was injected into each mouse through the tail vein, producing an i.v. bolus dose of 5 mg/kg. The animals were anesthetized with CO2, and the blood was removed from the heart through a thoracic opening according to a typical schedule of 0 (predose), 5, 10, 15, 30, 60, 120, 180, 210, 240, 480, 720, 1260, 1440 min after dosing. Blood was collected from four animals at each time point. The blood samples were centrifuged at 1000 × g for 5 min, and the plasma for each was collected and kept at −20°C until analysis. One hundred-μl aliquots from each of four animals per time point were pooled. A 200-μl aliquot of each pooled plasma sample was analyzed for CU201 by a liquid chromatography-tandem mass spectrometry method reported previously (31). The pharmacokinetic parameters were calculated by fitting the plasma concentration-time data to a two-compartmental pharmacokinetic model with the WinNonlin computer software (Pharsight, Mountain View, CA; Ref. 32).

**Statistical Analysis.** In vivo tumor growth experiments were analyzed for statistical significance with a multivariate method described by Heitjan et al. (33). Tumor volumes and growth rates for each treatment were compared with those of the control groups and found to be statistically significant as determined by the Ps. No significant difference was found between the two treatment groups. All statistical analyses were carried out with the SAS MIXED procedure (SAS Software, Version 8.1; SAS Institute, Cary, NC).

**RESULTS**

**Effects of CU201 in Combination with Various Cytoxic Chemotherapeutic Agents and Targeted Therapies on the Growth of SCLC and NSCLC Cell Lines.** In the NSCLC cell line A549, we tested CU201 in combination with paclitaxel and ZD1839; the results are summarized in Fig. 1. A and B. The concentrations of CU201 (1–3 μM) and paclitaxel (6–10 nm) were chosen to include the IC50 and concentrations above and below the IC50. The CI was determined using the isobologram method of Chou and Talalay (30), in which a CI >1 indicates antagonism, a CI of ~1 indicates additive interactions, a CI <1 indicates synergy, and a CI ≤0.5 indicates strong synergy. The combination of CU201 with paclitaxel produced greater than additive or synergistic interactions at all concentrations of both agents. At paclitaxel concentrations ≥6 nm, synergy or strong synergy was noted (Fig. 1A). Additive or synergistic interactions were also observed at all concentrations of the combination of CU201 and ZD1839 (Fig. 1B). At the highest concentrations of CU201, these interactions were strongly synergistic with a CI ≤0.5 for both paclitaxel and ZD1839.

**Panels C–F** in Fig. 1 show the effects of CU201 in combination with paclitaxel, doxorubicin, etoposide, and vinorelbine on the in vitro growth of the SCLC cell line SHP-77. Slightly lower concentrations of CU201 (0.3–1.5 μM) were used because the IC50 as a single agent in the SHP-77 cell line is lower (1 μM). SHP-77 cells overexpress MDR1, MRP, and LRP phenotypes; therefore, high paclitaxel concentrations (40–80
nm) were required for 50% growth incubation compared with A549 cells. The combination of CU201 with paclitaxel produced additive interactions (CI = 0.7–1.1) at the lowest paclitaxel concentration of 40 nm (Fig. 1C). At paclitaxel concentrations ≥60 nm, synergistic interactions were observed. In combination with doxorubicin, additive (CI = 1.0) or synergistic (CI < 1) interactions were observed at all concentrations of both agents, with the interactions being strongly synergistic at low concentrations of both agents (Fig. 1D). Etoposide (average IC_{50} = 1.5 μM when given alone) in combination with CU201 was synergistic to strongly synergistic at all concentrations tested (Fig. 1E). The combination of CU201 with vinorelbine (average IC_{50} = 80 nm when used alone; Fig. 1F) produced synergy at the higher concentrations of CU201 (1 and 1.5 μM) and vinorelbine (80 and 100 nm). Interestingly, the combination of CU201 with cisplatin did not produce synergy at any concentration, and the lowest CI values (1.5) were observed at the lowest cisplatin concentration (10 μM; data not shown).

**Growth Rates of SHP-77 SCLC Cells in Athymic Nude Mice Treated with CU201 Administered by Direct Intratumor Injections.** In our first experiment, we administered the CU201 via the intratumoral route. A daily dose of 5 mg/kg was selected on the basis of a comparison of in vitro activity between SPD and CU 201. CU201 has an IC_{50} that is 10-fold lower than that of SPD for in vitro growth inhibition (24). A dose of 50–70 mg/kg SPD is required for in vivo efficacy in mice, so we chose 5 mg/kg for CU201. As shown in Fig. 2, control group (vehicle) and CU201 injections began 21 days after tumor heterotransplantation, when the tumors were ~200 mm^3 in size, and continued daily for 3 weeks. At day 45, the tumors had reached >3000 mm^3 in the control mice. CU201 produced a statistically significant growth delay (P = 0.025) relative to the control (vehicle). On day 45, the tumor volume was 1400 mm^3 in the treated mice compared with 3000 mm^3 in the controls. By the intratumor route, the doubling time was increased from 6.5 days to 7.6 days (Table 1), whereas the tumor growth rate was decreased from 155.5 mm^3/day to 79.5 mm^3/day, representing a 43% growth inhibition and a mean delay of 8.5 days (P = 0.025). There were no differences in the body weights of the treated or control groups (data not shown).

**Growth Rates of SHP-77 SCLC Tumors in Athymic Nude Mice Treated with CU201 Administered by Continuous s.c. Infusion via an Alzet Pump.** In this experiment CU201 was administered by constant s.c. infusion beginning on day 14, when the tumor size was <100 mm^3, and continued for 5 weeks (Fig. 3). The control mice were sacrificed on day 50 when the tumor volume reached 4500 mm^3. The CU201 treatment significantly reduced the tumor growth rate (P = 0.015). In the treated mice the tumor volume was 1200 mm^3 on day 45 and <1400 mm^3 on day 50. The continuous s.c. infusion of CU201 by Alzet pump at 5 mg/kg/day was slightly more effective than the intratumor route (Table 1). The tumor doubling time was increased from 6.5 days in the controls to 9 days in the CU201-treated animals, and the tumor growth rate decreased from 155.4 mm^3/day in the controls to 54.2 mm^3/day in the treated animals. This reduction in tumor growth represented a 58.7% growth inhibition and a tumor growth delay of 7 days (P = 0.015). There were no differences in the body weights of the CU201-treated or saline-treated groups (data not shown).

**Growth Rates of SHP-77 SCLC Tumors in Athymic Nude Mice Treated with CU201 Administered by the i.p. Route.** To evaluate the effects of CU201 administered by the i.p. route and to evaluate the effect of tumor size on CU201 effectiveness, we treated athymic mice bearing SHP-77 xenografts with 5 mg/kg daily starting on either day 2 or day 22 after tumor heterotransplantation, and continuing until day 48. As shown in Fig. 4, there was a significant reduction in the tumor growth rate for both treated groups. On day 50, the control tumors had an mean volume of 3700 mm^3 compared with 1500 mm^3 in the group starting treatment on day 22 and 1200 mm^3 in the group starting treatment on day 2. By day 52, the differences between the control and treated groups was even greater, with a tumor volume of 4600 mm^3 in the control and 1600 and 1800 mm^3 in the two treatment groups. In the control group, the doubling time was 5.3 days; this was increased to 6.8 and 5.7 days in the groups in which treatment was initiated on days 2 and 22, respectively. The tumor growth delay averaged 9.5 and 5.5 days, and the growth rates averaged 21.8 and 31.6 mm^3/day in the two treatment groups, respectively. Both treatment groups differed significantly from the control group (P = 0.015).

### Table 1: Effects of CU201 on SCLC and NSCLC tumor growth in athymic nude mice

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Route</th>
<th>Rx</th>
<th>DT (days)</th>
<th>GR (mm^3/day)</th>
<th>TGD (days)</th>
<th>Inhibition (%)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHP-77 i.t.</td>
<td>Control</td>
<td>6.5</td>
<td>155.5</td>
<td>0</td>
<td>0</td>
<td>0.025</td>
<td></td>
</tr>
<tr>
<td>SHP-77 i.t.</td>
<td>5 mg/kg</td>
<td>7.6</td>
<td>79.5</td>
<td>8.5</td>
<td>42.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SHP-77 s.c.</td>
<td>Control</td>
<td>6.5</td>
<td>155.4</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SHP-77 s.c.</td>
<td>5 mg/kg/d</td>
<td>9.0</td>
<td>54.2</td>
<td>7.0</td>
<td>58.7</td>
<td>0.015</td>
<td></td>
</tr>
<tr>
<td>SHP-77 i.p.</td>
<td>Control</td>
<td>5.3</td>
<td>65.3</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SHP-77 i.p.</td>
<td>5 mg/kg/day</td>
<td>6.8</td>
<td>21.8</td>
<td>9.5</td>
<td>67.5</td>
<td>0.010</td>
<td></td>
</tr>
<tr>
<td>SHP-77 i.p.</td>
<td>5 mg/kg/day</td>
<td>5.7</td>
<td>31.6</td>
<td>5.5</td>
<td>57.3</td>
<td>0.020</td>
<td></td>
</tr>
<tr>
<td>SHP-77 i.p.</td>
<td>Control</td>
<td>8</td>
<td>42.2</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SHP-77 i.p.</td>
<td>5 mg/kg/day</td>
<td>8.5</td>
<td>32.5</td>
<td>7.7</td>
<td>27.7</td>
<td>&lt;0.028</td>
<td></td>
</tr>
<tr>
<td>SHP-77 i.p.</td>
<td>20 mg/kg/day</td>
<td>9.7</td>
<td>18.8</td>
<td>13</td>
<td>45.8</td>
<td>0.009</td>
<td></td>
</tr>
<tr>
<td>A549 i.p.</td>
<td>Control</td>
<td>8.0</td>
<td>42.5</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A549 i.p.</td>
<td>5 mg/kg</td>
<td>12.3</td>
<td>14.4</td>
<td>9.0</td>
<td>46.2</td>
<td>0.001</td>
<td></td>
</tr>
</tbody>
</table>

*a Rx: treatment; DT, doubling time; GR, growth rate; TGD, tumor growth delay; i.t., intratumoral.
Starting at day 2.
Starting at day 22.
CU201 Inhibits Human Lung Cancer Cell Lines

Two treatment groups were not significant (P = 0.010 for day 2 and P = 0.020 for day 22). Although there was slower tumor growth and more growth delay in the treatment group starting on day 2 compared with day 22, the differences were not significant.

To evaluate the dose-response effect and to determine whether higher doses of CU201 would produce any overt signs of toxicity, we repeated this experiment using daily doses of 5 and 20 mg/kg starting on day 7 and continuing for 5 weeks until day 46. The results are summarized in Fig. 5 and Table 1. Both doses produced a significant tumor growth delay. On day 46, when the treatment was stopped, the tumor volume was 2000 mm$^3$ in the controls, 900 mm$^3$ in the 5 mg/kg/day group, and 600 mm$^3$ in the 20 mg/kg/day group. Both treatment doses increased the doubling time from the 8 days in the controls to 8.5 days at 5 mg/kg and to 9.7 days at 20 mg/kg. The tumor growth delay averaged 7.7 and 13 days in the two groups, respectively. The growth rate decreased from 42.2 mm$^3$/day in the control group to 32.5 mm$^3$/day in the 5 mg/kg group and to 14.4 mm$^3$/day in the 20 mg/kg group. The tumor growth delay averaged 7.7 and 13 days in the two groups, respectively. The growth rate decreased from 42.2 mm$^3$/day in the control group to 32.5 mm$^3$/day in the 5 mg/kg group and 18.8 mm$^3$/day in the 20 mg/kg group. The growth rates of both groups were significantly less than in controls (P = 0.028 for 5 mg/kg and P = 0.009 for 20 mg/kg). Although the 20 mg/kg group had the slowest growth rate, the differences between the two treatment groups were not significant (P = 0.98).

Mice in each group were weighed throughout the study period, and the mean weights ± SE are shown in Fig. 5B. There were no changes in mouse weights in either of the CU201 treatment groups compared with the saline control treatment group. There were also no changes in the activity levels of mice in any of the treatment groups.

**Growth Rates of A549 NSCLC Tumors in Athymic Nude Mice Treated with CU201 Administered by the i.p. Route.** The NSCLC cell line A549 has neuroendocrine features, including responsiveness to BK and bombesin. The growth of this cell line was not affected by SPD, but was inhibited by CU201 in vitro alone and in combination with paclitaxel and ZD1839 (Fig. 1). Thus, we evaluated the effects of CU201 on the growth of this NSCLC cell line in athymic mice. As shown in Fig. 6, CU201 at daily i.p. doses of 5 mg/kg/day produced a statistically significant growth delay in the A549 tumors. On day 45, when the daily treatments were stopped, the control group had a tumor volume of 1400 mm$^3$ compared with 600 mm$^3$ in the CU201-treated group. By day 50, the control group had a tumor volume of 1800 mm$^3$ compared with 1000 mm$^3$ in the CU201-treated group. The effects of CU201 in this NSCLC model were similar to those observed in the SHP-77 SCLC model (Table 1). The doubling time increased from 8 days in the control group to 12.3 days in the CU201 group. The mean tumor growth delay was 9 days, and the tumor growth rate decreased from 42.5 mm$^3$/day to 14.4 mm$^3$/day. This difference was highly significant (P = 0.001). There were no differences in the body weights of the CU201-treated or control groups (data not shown).

**Preliminary Pharmacokinetic Evaluation of CU201 in Mice.** Preliminary pharmacokinetic studies of CU201 in CD2F1 mice were carried out after an i.v. bolus dose of 5 mg/kg. Plasma concentration-time profiles showed a biexponential decay, and the concentrations were detectable up to 24 h. The concentration-time profiles were fitted to a two-compartment model via WinNonlin computer software; the relevant pharmacokinetic parameters are shown in Table 2. The $c_{max}$ of 5773 ng/ml (equivalent to 5 μM) exceeded the in vitro IC$_{50}$ for growth inhibition. The $t_{1/2a}$ of 14.4 min represents the distribution phase of the drug in the volume of distribution (631 mg/kg). CU201 has a long terminal half-life ($t_{1/2b}$) of 44.3 h. Additional pharmacokinetic profiles are being constructed for other routes of administration and with various doses in support of our NCI RAID project. The i.v. data suggest that daily i.p. administration should provide prolonged exposure to concentrations that are effective in vitro.
DISCUSSION

Neuropeptides play an important role in the autocrine/paracrine growth of SCLCs and other cancers with neuroendocrine features (2, 3, 11–17). CU201 is a novel targeted therapy that inhibits lung cancer growth by acting as a biased agonist (25). In this study we showed that CU201 induces additive or synergistic growth inhibition when combined with several standard chemotherapeutic agents and with ZD1839, an EGFR tyrosine kinase inhibitor. We showed that CU201 has a long serum half-life in normal mice consistent with its prolonged biological effects in serum. We also showed that CU201 inhibited the growth of human SCLC xenografts in athymic mice at a dose 10-fold or more lower than those required for SPDs. Finally, CU201 inhibited the growth of human NSCLC xenografts in athymic mice. Although only one SCLC and one NSCLC cell line were studied in the mouse experiments, the growth of a wide variety of SCLC and NSCLC cell were inhibited in vitro (25). No evidence of toxicity of CU201 was observed in the mice treated by any route or at any dose as evidenced by the lack of weight loss or changes in physical activity. These data suggest that further development of CU201 and related compounds should continue.

SCLCs have high initial response rates to various chemotherapeutic agents (8). Unfortunately, few patients are cured, and relapse with drug-resistant cells develops in nearly all cases (8). Thus, targeted agents with novel mechanisms of action are sorely needed. The neuroendocrine properties of SCLC tumors have been recognized for many years (2, 3, 8–11). These tumors produce a variety of neuropeptides and are growth stimulated by these peptides (8–11). Thus, the neuropeptides and their receptors are logical targets for novel therapeutic strategies. These observations led to the development of several specific peptide inhibitors, including monoclonal antibodies to the peptide, antibodies to the receptor, and peptide antagonists (2, 3, 18, 19). In many cases these specific peptide inhibitors were able to inhibit the growth of some SCLC cell lines in vitro and in athymic mice with human lung cancer xenografts (18, 19, 33). However, a single specific inhibitor inhibited a minority of cell lines. For example, a monoclonal antibody to GRP, 2A11, inhibited the growth of the SCLC cell line NCI-H345, which is very sensitive to GRP (18, 19). However, this antibody did not inhibit many additional SCLC cell lines. 2A11 was evaluated in 12 patients with advanced SCLC, and 1 complete response was documented in a patient whose tumor expressed high levels of GRP receptor (19). No response was noted in the other 11 patients.

SPDs were the first peptide inhibitors that blocked the effects of multiple peptides (20–25). SPDs also inhibited the growth of many SCLC, but not NSCLC cell lines (20–25). Initially, inhibition of proliferation was ascribed to the inhibition of binding of multiple peptides to their cognate receptors. Subsequent studies showed that induction of apoptosis was an essential characteristic of the SPD-induced growth inhibition.
CU201 Inhibits Human Lung Cancer Cell Lines

Table 2  Pharmacokinetic parameters associated with the i.v. administration of CU201 (5 mg/kg) to CD2F1 mice

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
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<tr>
<td>cmax (μg/ml)</td>
<td>5773</td>
</tr>
<tr>
<td>α (1/h)</td>
<td>2.85</td>
</tr>
<tr>
<td>t1/2α (min)</td>
<td>14.4</td>
</tr>
<tr>
<td>β (1/h)</td>
<td>0.0156</td>
</tr>
<tr>
<td>t1/2β (h)</td>
<td>44.3</td>
</tr>
<tr>
<td>A (ng/ml)</td>
<td>4030</td>
</tr>
<tr>
<td>B (ng/ml)</td>
<td>3.3</td>
</tr>
<tr>
<td>AUC(α) (ng · min/ml)</td>
<td>164,405</td>
</tr>
<tr>
<td>AUC(β) (ng · min/ml)</td>
<td>174,366</td>
</tr>
<tr>
<td>CLα (ml/min/kg)</td>
<td>0.72</td>
</tr>
<tr>
<td>Vdα (ml/kg)</td>
<td>631</td>
</tr>
</tbody>
</table>

* AUC, area under the curve; CL, clearance.

We showed that SPDs act as biased agonists for peptide receptor-G-protein interaction (21). In this way, SPDs blocked Gαq activation as demonstrated by inhibition of activation of phospholipase C-β and intracellular calcium flux in response to the peptides. SPDs stimulated Gαq,12,13 and downstream events, including activation of c-Jun kinase and apoptosis (21, 25). The clinical development of SPDs is hampered by their low potency and short half-life. In Phase I trials, a concentration of 25 μM (the IC50 for in vitro growth inhibition) could be reached, and these concentrations were well tolerated and prevented the response to exogenous substance P infusion. However, the effective serum concentration disappeared within 1 h.

We developed a series of peptide antagonist dimers that overcome many of the deficiencies of the SPDs (25). One of these dimers, CU201, was shown to act as a biased agonist for peptide receptor-G-protein interactions (25). We previously found that CU201 retained Iressa, an epidermal growth factor receptor tyrosine kinase inhibitor in combination with carboplatin and paclitaxel in previously untreated advanced non-small cell lung cancer. Proc. Am. Soc. Clin. Oncol., 20: 326a, 2001.

REFERENCES


Bradykinin Antagonist Dimer, CU201, Inhibits the Growth of Human Lung Cancer Cell Lines *in Vitro* and *in Vivo* and Produces Synergistic Growth Inhibition in Combination with Other Antitumor Agents


*Clin Cancer Res* 2002;8:1280-1287.