Pharmacokinetic and Biochemical Studies on Acivicin in Phase I Clinical Trials

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ABSTRACT

Acivicin pharmacokinetics were studied in Phase I patients receiving i.v. treatment on single-dose or daily ×5 (daily times five doses) regimens repeated every 3 weeks. In 14 patients, the time course of plasma concentrations was characterized by a biexponential equation with a terminal (elimination-phase) half-life of 9.92 ± 3.91 h (mean ± SD), distribution phase half-life of 0.32 ± 0.28 h, total body clearance of 1.69 ± 0.48 liters/h/m², and volume of distribution of 21.79 ± 2.94 liters/m². Acivicin kinetics appeared to be dose-independent over the range of 8.5–150 mg/m²/day. Urinary excretion of intact acivicin in nine patients ranged from 2–42% in the first 24 h following administration; interpatient variability in urinary excretion was large, but daily urinary recovery within patients on the daily ×5 schedule was quite consistent.

Measurements of acivicin effects on the activity of carbamyl phosphate synthetase II (CPS II) were conducted using leukocytes and/or malignant ascites of three colon cancer patients. Acivicin given to one patient at 8.5 mg/m²/day on the daily ×5 schedule caused a 70% reduction in leukocyte CPS II activity within 5 h after therapy was initiated. Leukocyte CPS II activity remained suppressed at this level over the 5-day dosing regimen. In this patient, CPS II activity in malignant ascitic cells had decreased by 75% on day 4 of the daily ×5 regimen. On the single-dose schedule, treatment of two patients with 100 mg/m² caused leukocyte CPS II activity to decrease by >90% within 4 h of treatment with gradual recovery over the next 2 days.

INTRODUCTION

Acivicin [[αS,βS]-α-amino-3-chloro-2-isoxazoline-5-acetic acid; NSC 163501; AT-125] is a fermentation-derived amino acid antitumor agent currently being evaluated in Phase II clinical trials (1). Acivicin is a glutamine antagonist and is thought to kill cells through inhibition of several glutamine-dependent amidotransferases, including cytidine triphosphate synthetase and CPS II (2–4), key enzymes in purine and pyrimidine biosynthesis. A preliminary report of objective antitumor activity against human non-small cell lung cancer has appeared (5). No objective responses were noted in a Phase II study of acivicin on the ×5 schedule in 20 previously untreated patients with advanced colorectal carcinoma (6).

Phase II studies on acivicin were conducted on ×1, ×5, 24-h infusion, and 72-h infusion schedules. Toxicity was found to be schedule-dependent, with myelosuppression being dose-limiting on the ×5 schedule and CNS disturbances predominating on ×1, 24-h infusion, and 72-h infusion schedules (7–11). The pharmacokinetics of acivicin in patients treated on the two infusion schedules were described previously (10, 11). We report here the pharmacokinetic data from patients treated on ×1 and ×5 schedules in four institutions (five patients from the University of Kansas, seven from City of Hope National Medical Center, three from the M. D. Anderson Hospital and Tumor Institute, and six from Mount Sinai Medical Center).

MATERIALS AND METHODS

Clinical Methods. Pharmacokinetic and biochemical studies were conducted in advanced solid tumor patients receiving acivicin in Phase I clinical trials conducted under the auspices of the Investigational Drug Branch, Cancer Therapy Evaluation Program, Division of Cancer Treatment, National Cancer Institute. Previous reports have described the treatment protocols and results pertaining to efficacy and toxicity observed in studies conducted at the University of Kansas Medical Center (7), Mount Sinai School of Medicine (8), and the M. D. Anderson Hospital and Tumor Institute (9).

Methods were similar at City of Hope Hospital, where a total of 12 adult patients (5 male, 7 female) with advanced solid cancers refractory to conventional treatment received a total of 15 courses. Eight patients were treated on the ×1 schedule (two at 40, one at 60, three at 100, and two at 150 mg/m²), and four patients were treated on the ×5 schedule (one at 5.4 mg/m²/day and three at 8.5 mg/m²/day). All patients had received prior chemotherapy, and four had received prior radiation therapy.

Patients received acivicin as an i.v. infusion over periods ranging from 1 min to 1.75 h. For pharmacokinetic studies, serum or plasma was separated from blood samples collected at intervals post-infusion and frozen. Urine was also collected over defined intervals. The urinary volume was recorded, and aliquots were frozen. Plasma and urine specimens were shipped on dry ice to The Upjohn Company for assay. In addition, the effect of acivicin treatment on the activity of CPS II was studied in the leukocytes and/or ascitic cells of three colon cancer patients at City of Hope National Medical Center.

Serum chemistry values (serum bilirubin, serum glutamic-oxaloacetic transaminase, serum glutamic-pyruvate transaminase) were used to classify the degree of hepatic dysfunction as: none (enzymes or bilirubin < 1.25 times normal limits), mild (enzymes or bilirubin 1.25–2.50 times lab normal), moderate (enzymes or bilirubin 2.50–5.00 times lab normal), severe (enzymes or bilirubin > 5.00 times lab normal). No patients studied had compromised renal function based on serum creatinine and blood urea nitrogen values.

Analytical Methods. Plasma and urine specimens were assayed for acivicin by a microbiological method using Bacillus subtilis UC-902.
method was described previously in detail (12). Patients were not concurrently treated with antibiotics which could interfere with the microbiological assay. Whenever possible, sufficient pretreatment serum or plasma and urine were obtained for preparation of standard curves using each patient's own fluids. Otherwise, plasma or urine from normal human subjects was used in preparation of standards. Each sample was assayed at two dilutions, and the results were averaged. Analytical sensitivity was approximately 0.05 µg/ml in plasma and 0.8 µg/ml in urine.

Also, as described previously (12), bioautographic studies were conducted on selected plasma and urine samples to determine the specificity of the microbiological assay for unchanged acivicin. In agreement with previous studies (10, 11), only one zone of inhibition per sample was observed in the bioautographic studies, and, in each case, the Rf of the zone corresponded to that of intact acivicin.

As an additional check on assay specificity, selected urine specimens from acivicin-treated patients were assayed both by the microbiological method and by a murine L1210 cell tube-dilution growth inhibition assay (13). This was done to determine if urine contained substances in addition to acivicin which would inhibit the growth of mammalian cells while allowing the growth of the bacterial assay organism. On four urine samples containing between 12 and 39 µg/ml acivicin bioequivalents based on the B. subtilis assay, values determined by the L1210 assay differed by −8 to +14%, suggesting that the microbiological assay accurately reflected the concentration of substances toxic to mammalian cells. This finding combined with the bioautographic results suggested that only intact acivicin was measured, and all pharmacokinetic calculations were done under that assumption.

Pharmacokinetic Analysis. Acivicin plasma concentration-time data from all patients but one were described adequately by a biexponential equation. In one patient (No. 4), a monoexponential equation yielded a elimination phase of plasma level decline. (15). ADC was computed from the parameters of the equations of best-fit from all patients but one were described adequately by a biexponential cells. This finding combined with the bioautographic results suggested that only intact acivicin was measured, and all pharmacokinetic calculations were done under that assumption.

Pharmacokinetic Analysis. Acivicin plasma concentration-time data from all patients but one were described adequately by a biexponential equation. In one patient (No. 4), a monoexponential equation yielded a better fit to the data. Computer fitting was done with the nonlinear parameter estimation program, NONLIN (14), on an IBM digital computer using routines for the one- or two-compartment open models for drug disposition with zero-order input. The duration of zero-order input was varied according to individual patient infusion lengths. The data points were weighted using the factor 1/y2 in the fitting procedure. Goodness of fit was determined from correlation coefficients (r²), residual sums of squares, standard deviations of estimated parameters, and from visual inspection of plots of the predicted and observed concentrations versus time. Pharmacokinetic parameters were calculated by standard methods (15). AUC was computed from the parameters of the equations of best-fit (15). CLv was calculated as dose/AUC, and Varea was calculated as CLv/β, where β is the first-order rate constant describing the terminal elimination phase of plasma level decline.

Biochemical Methods. CPS II activity was measured at intervals in the leukocytes of two patients receiving acivicin on the x1 schedule. In one patient treated on the x5 schedule, CPS II activity was measured in the leukocytes at intervals over 5 h during the first day of treatment and then prior to drug administration on days 2, 3, 4, and 5. In the latter patient, malignant ascites was also collected for measurement of CPS II activity prior to treatment on day 1 (baseline) and day 4. Approximately 10 ml of blood or ascitic fluid were withdrawn at each time for CPS II measurements. A buffy coat was isolated from heparinized whole blood or ascitic fluid following low-speed centrifugation (150 × g, 5 min). Contaminating erythrocytes were removed by brief hypotonic shock and recentrifugation (16). The leukocyte or ascitic cell pellets were immediately homogenized by hand in 1 volume of a buffer consisting of 30% dimethyl sulfoxide, 5% glycine, 0.1 M Tris-HCl (pH 8.4), 0.1 M KCl, 0.2 mM EDTA, and 0.2 mM dithiothreitol. After centrifugation for 3 min at 12,000 × g, supernatant fractions were immediately assayed for CPS II activity by determination of 14C incorporation from NaH14CO3 into N-[14C]carbamyl-L-aspartate (17).

RESULTS

Clinical Observations. Toxic effects of acivicin observed in patients treated at City of Hope Hospital were consistent with results reported previously from the University of Kansas (7), Mt. Sinai Medical Center (8), and M. D. Anderson Hospital and Tumor Institute (9). Mild to moderate CNS toxicity was observed on both x1 (8 of 8 patients) and x5 schedules (1 of 3 patients) and consisted of lethargy, weakness, disorientation, somnolence, confusion, vivid dreams, and hallucinations. The onset of these effects was usually the second day after initiation of therapy. Toxicity persisted for 2–3 days after therapy ended. Myelosuppression consisting of mild thrombocytopenia was observed in only one patient treated on the x1 schedule (100 mg/m²), in agreement with previous results (7). Myelosuppression was not observed in x5 patients receiving 5.8 or 8.5 mg/m²/day. This was consistent with results from the x5 study conducted at Mt. Sinai Medical Center, which showed myelosuppression (leukopenia and neutropenia) to be significant at ≥12 mg/m²/day and to be the dose-limiting toxicity at 20 mg/m²/day (8). No partial or complete responses were noted in the City of Hope Hospital study. One patient with adenocarcinoma of the large bowel had stable disease and a reduction in carcinoembryonic antigen levels from 150 to 60 ng/ml upon treatment with 8.5 mg/m²/day ×5.

Acivicin Pharmacokinetics in Plasma. In patients receiving doses less than 8.5 mg/m²/day, plasma concentrations could be quantitated through only 6 h or less, and these data are not included. Table 1 shows individual and mean pharmacokinetic parameters in 15 patients treated with doses ranging from 8.5–150 mg/m². Chart 1 shows the time course of acivicin concentrations in plasma in three patients (Nos. 02, 04, and 17). A biexponential equation adequately described the time course of post-infusion plasma concentrations in 14 of 15 patients; a distribution phase was not detectable in patient 4's plasma concentrations, which declined monoexponentially (earliest sampling time was 5 min post-infusion; Chart 1). The initial distribution phase of plasma pharmacokinetics is less evident in patients receiving infusions as opposed to bolus injections (15); however, the infusion duration was only 15 min in patient 4, and this is not believed to be the explanation for the lack of a detectable α-phase in this patient. Values of t½ varied considerably between patients but were brief compared to t½ in all patients. Values of t½ ranged from 4.28–17.97 h with a mean ± SD of 9.92 ± 3.91 h. CLv (calculated as dose/AUC) was constant among patients (mean ± SD = 1.69 ± 0.48 l/h/m²), reflecting the direct proportionality between dose and AUC (r² = 0.8507). Varea showed little interpatient variability (21.79 ± 2.94 liters/m²). The magnitude of Varea approximated total body water, suggesting that acivicin was distributed throughout the body but was not highly bound in tissues. Acivicin pharmacokinetics appeared to be dose-independent over the range of 8.5–150 mg/m².

Patient 6, who had the longest t½ value reported to date, had acivicin plasma levels of 0.15, 0.16, and 0.17 µg/ml just prior to dosing on days 3, 4, and 5 of the 5-day regimen. Patients 13 and 14 had no measurable drug accumulation between days 1 and 5.

At the recommended Phase II dose of 15 mg/m²/day on the daily ×5 schedule (8), peak plasma levels of approximately 1 µg/ml were achieved (Chart 1). The maximum tolerated dose of 150 mg/m² given on the x1 schedule yielded peak levels of approximately 10 µg/ml (Chart 1). The latter schedule was not recommended for Phase II studies because of CNS toxicity (7).

Urinary Excretion. Table 2 shows the daily percent of dose excreted in the urine as intact acivicin by patients on x1 and x5
### ACIVICIN PHARMACOKINETICS AND BIOCHEMICAL EFFECTS

#### Table 1
Pharmacokinetic parameters in acivicin-treated patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>Degree of hepatic dysfunction</th>
<th>Treatment Schedule</th>
<th>Dose (mg/m²/day)</th>
<th>Infusion length (h)</th>
<th>t₁/₂ (h)</th>
<th>t₁/d (h)</th>
<th>CLₘ (liters/h/m²)</th>
<th>Vₘ (liters/m²)</th>
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<tr>
<td>6</td>
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<td>x5</td>
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<td>17.75</td>
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<td>16</td>
<td>Mild</td>
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<td>0.14</td>
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<td>13</td>
<td>Moderate</td>
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<td>12</td>
<td>0.417</td>
<td>0.14</td>
<td>9.68</td>
<td>1.59</td>
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<tr>
<td>14</td>
<td>Moderate</td>
<td>x5</td>
<td>5</td>
<td>12</td>
<td>0.417</td>
<td>0.38</td>
<td>10.89</td>
<td>1.18</td>
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<tr>
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<td>3</td>
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<td>0.250</td>
<td>0.86</td>
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<tr>
<td>7</td>
<td>Severe</td>
<td>x1</td>
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<td>0.44</td>
<td>7.53</td>
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<tr>
<td>5</td>
<td>Mild</td>
<td>x1</td>
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<td>80</td>
<td>0.250</td>
<td>0.11</td>
<td>8.99</td>
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<tr>
<td>8</td>
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<td>x1</td>
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<td>100</td>
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<td>7.50</td>
<td>1.77</td>
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<tr>
<td>9</td>
<td>None</td>
<td>x1</td>
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<td>0.250</td>
<td>0.24</td>
<td>5.95</td>
<td>2.45</td>
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Mean ± SD.

#### Table 2
Urinary excretion of acivicin

<table>
<thead>
<tr>
<th>Patient</th>
<th>Schedule</th>
<th>Dose (mg/m²/day)</th>
<th>% of daily dose in 24-h urine on day:</th>
</tr>
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<tbody>
<tr>
<td>19</td>
<td>x5</td>
<td>1.5</td>
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<tr>
<td>20</td>
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<td>1.5</td>
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<td>21</td>
<td>x5</td>
<td>6.8</td>
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<tr>
<td>16</td>
<td>x5</td>
<td>9</td>
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<td>x5</td>
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<td>80</td>
<td>—</td>
</tr>
<tr>
<td>5</td>
<td>x1</td>
<td>80</td>
<td>—</td>
</tr>
<tr>
<td>2</td>
<td>x1</td>
<td>150</td>
<td>—</td>
</tr>
</tbody>
</table>

Table 2 also shows that daily recoveries were quite consistent within patients studied on successive days on the x5 schedule.

#### Effect of Hepatic Disease on Acivicin Pharmacokinetics.
When pharmacokinetic indices of drug elimination (t₁/₂, CLₘ, extent of urinary drug excretion) were related to the degree of hepatic dysfunction (Table 1), no consistent relationships were noted. However most patients studied had no or mild hepatic impairment, and no firm conclusions can be drawn about liver function effects on acivicin disposition.

#### Biochemical Effects of Acivicin Treatment.
Biochemical studies were carried out in three colon cancer patients who had leukocyte and/or malignant ascites collections before and after treatment. Chart 2 shows the time course of CPS II activity of the leukocytes and malignant ascites in a patient who received 8.5 mg/m²/day x5. Both leukocytes and ascitic cells demonstrated a similar baseline activity of 800 pmol/h/mg protein. During the first 5 h of the study there was a 70–75% reduction in the specific activity of CPS II, which remained suppressed for

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**Chart 1.** Time course of acivicin plasma concentrations in patients 2 (O, 150 mg/m² x1), 4 (X, 80 mg/m² x1), and 17 (A, 15 mg/m²/day x5). The lines were drawn using the computer-fitted pharmacokinetic parameters. Considerable variability between patients was noted, with percentages ranging from 2–42% of the daily dose. These values are generally consistent with urinary recoveries reported previously in 24-h infusion and 72-h infusion studies (10, 11).
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5 days in both normal and neoplastic cells. CPS II activity was not followed long enough for observation of recovery. The initial carcinomogenic antigen of this patient was 150 ng/ml which with treatment decreased to 60. Chart 2 also shows the time course of leukocyte CPS II activity in two patients who received acivicin at 100 mg/m² x1. There was >90% reduction in the specific activity of CPS II 4 h after administration of drug with gradual restitution of activity to baseline over the next 2 days. Comparison of leukocyte CPS II inhibition demonstrates a more prolonged inhibition with the x5 schedule but a more pronounced inhibition at the much higher dose given on the x1 schedule.

DISCUSSION

These studies demonstrated that acivicin pharmacokinetics in patients treated on x1 or x5 schedules are consistent with previous findings on 24- and 72 h-infusion schedules (10, 11). Total body clearance on the 72-h infusion schedule was approximately 1.9 liters/h/m² (11), as compared to approximately 1.7 liters/h/m² in these studies. Values of t½ (means of 7–10 h) and t½, (0.3–0.5 h) were also similar on the four schedules tested to date.

Acivicin appears to be cleared primarily by non-renal mechanisms as shown by the percent recovery of intact drug in the urine (Table 2). This finding suggests that hepatic metabolism could be an important mechanism for acivicin disposition. However too few patients with moderate to severe liver dysfunction were studied for conclusions to be drawn about effects on acivicin disposition.

Acivicin pharmacokinetics appeared to be dose-independent over the dose range tested. Considerable variability was noted between patients in extent of urinary excretion (Table 2), but daily renal excretion was consistent within patients on the multiple dosing regimen. No significant accumulation of drug was noted in patients treated on the x5 schedule.

Significant biochemical effects on both normal and neoplastic cells were demonstrated by serial measurements of CPS II activity (Chart 2) in three patients. Acivicin blocks purine and pyrimidine biosynthesis by inhibition of glutamine-dependent amidotransferases such as CPS II and cytidine triphosphate synthetase (3, 4), and this has been suggested as the mechanism of cytotoxic action. However the lack of objective therapeutic responses in patients exhibiting substantial biochemical changes in these studies suggests that mechanisms of drug resistance, possibly nucleoside salvage pathways, were operative in tumor cells. Fischer et al. (18) and Zhen et al. (19) have demonstrated recently that dipryramidole, an inhibitor of nucleoside salvage, can enhance the cytotoxicity of acivicin in vitro.

The studies reported here provide no information on the nature of potential acivicin metabolites. The bioautographic studies and the corroboration of the bacterial assay by the mammalian L1210 assay ("Materials and Methods") suggest that acivicin metabolites do not possess cytotoxic activity. However compounds with structures similar to acivicin, such as ibotenic acid and muscimol, are known to cause CNS effects similar to those reported for acivicin (20, 21). Thus acivicin itself or a metabolite could cause CNS toxicity directly; alternatively, the effects could be related to drug- or metabolite-induced biochemical changes. In the latter regard it is interesting that the onset and duration of behavioral disturbances is comparable to the time course of CPS II inhibition.

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