Novel Marine-derived Anticancer Agents: A Phase I Clinical, Pharmacological, and Pharmacodynamic Study of Dolastatin 10 (NSC 376128) in Patients with Advanced Solid Tumors

Timothy Madden,2 Hai T. Tran, Debrah Beck, Reeves Huie, Robert A. Newman, Lajos Pusztai, John J. Wright, and James L. Abbruzzese2

Division of Pharmacy [T. M., H. T. T., R. H.], Clinical Investigation [D. B., R. A. N.], Breast Medical Oncology [L. P.], and Gastrointestinal Medical Oncology and Digestive Diseases [J. L. A.], the University of Texas M. D. Anderson Cancer Center, Houston, Texas 77030, and The Cancer Therapy Evaluation Program, National Cancer Institute, NIH, Bethesda, Maryland 20892 [J. J. W.]

ABSTRACT

Dolastatin (DOLA)-10 is a pentapeptide isolated from the mollusc Dolabella auricularia with clinically promising antitumor activity documented in various in vitro and in vivo tumor models. The objectives of this Phase I study were to determine the maximum tolerated dose, evaluate toxic effects, and document any antitumor activity of this novel agent. Using an electrospray ionization mass spectroscopy system, we also characterized the clinical pharmacokinetics, pharmacodynamics, and metabolism of DOLA-10. The maximum tolerated dose was reached at 300 μg/m2. Granulocytopenia, the dose-limiting toxicity, was documented in 33% of the patients treated at that dose level. There were no episodes of thrombocytopenia or severe anemia (Hgb < 8), and no major nonhematological toxicity was observed. Stabilization of tumor growth was observed in four patients, but no objective responses were seen. Whereas a two-compartment model described the DOLA-10 plasma concentration-time data reasonably well, a three-compartment model consistently performed better. After a rapid distribution phase, DOLA-10 plasma levels declined with mean β and γ half-lives of 0.99 and 18.9 h, respectively. Significant interpatient and intrapatient variability in DOLA-10 plasma clearances was observed. The mean area under the concentration-time curve increased proportionally as the dose was escalated, but there was significant overlap between dose levels. The area under the concentration-time curve and the percentage of decline in neutrophils were correlated. A single DOLA-10 metabolite was detected in five patients. Unlike the in vitro studies of DOLA-10, the principal metabolite detected was an N-demethyl derivative, confirmed by mass spectroscopy. In all five subjects, the concentration of this metabolite never exceeded 2% of the simultaneously measured parent drug concentration. The available preclinical, pharmacological, and clinical data suggest that further study of escalated DOLA-10 dosing with cytokine support is warranted.

INTRODUCTION

The DOLAs are small lipophilic polypeptides isolated from the mollusc Dolabella auricularia (1–3). The antitumor activity of this class of compounds was discovered in 1972 while screening extracts of D. auricularia against the NCI i.p. implanted P388 leukemia (4). After isolation and structural determination of the cytotoxic constituents, the most potent compound, DOLA-10, was identified as a linear peptide. Structurally, it consists of four amino acids (dolavaline, valine, dolisoleine, and dolaprole), three of which are unique, linked to an unusual primary amine (dolaphenine) at its COOH terminus (Fig. 1; Ref. 1). Subsequently, the absolute configuration of its nine asymmetric centers was established, and total synthesis was achieved (2).

DOLA-10 and a structurally related compound, DOLA-15, (Fig. 1), were evaluated in preclinical models, and DOLA-10 appeared to possess the most promising clinical features (5–8). Whereas both molecules are potent inhibitors of cell proliferation, DOLA-10, on average, appears to be nine times more active than DOLA-15 (IC50 values, 2.3 × 10−9 and 2.1 × 10−9 M, respectively) against ovarian, lymphoma, and colon carcinoma cell lines (9). DOLA-10 was more potent than paclitaxel or vinblastine as an antiproliferative agent and was also more active than DOLA-15 against ovarian carcinoma xenografts in a mouse tumor model (9).

Mechanistically, DOLA-10 is a highly lipophilic pentapeptide that interacts with tubulin. Its spectrum of action is typical of that of tubulin-binding agents. It inhibits tubulin polymerization and tubulin-dependent GTP hydrolysis and the binding of vinblastine, maytansine, and vincristine to tubulin. It stabilizes the colchicine binding of tubulin and, at higher drug concentrations, causes the formation of cold-stable tubulin aggregates

Received 6/28/99; revised 12/28/99; accepted 12/29/99.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 To whom requests for reprints should be addressed, at Division of Pharmacy, Box 90, The University of Texas M. D. Anderson Cancer Center, 1515 Holcombe Boulevard, Houston, Texas 77030. Phone: (713) 794-8000; Fax: (713) 745-2007 (T. M.) or Department of Gastrointestinal Medical Oncology and Digestive Diseases, Box 78, The University of Texas M. D. Anderson Cancer Center, 1515 Holcombe Boulevard, Houston, TX 77030. Phone (713) 792-2828; Fax: (713) 745-1163 (J. L. A.).

3 The abbreviations used are: DOLA, dolastatin; MTD, maximum tolerated dose; NCI, National Cancer Institute; MS, mass spectroscopy; DLT, dose-limiting toxicity; AUC, area under the concentration-time curve; ANC, absolute neutrophil count; G-CSF, granulocyte colony-stimulating factor; HPLC, high-performance liquid chromatography.
It inhibits GTP-dependent tubular polymerization and, at higher concentrations, induces GTP-independent tubulin aggregation. The binding site on tubulin for DOLA-10 is different than the vinca alkaloid- or colchicine-binding sites. DOLA-10 is a noncompetitive inhibitor of vinca alkaloid binding to tubulin but has no effect on colchicine binding. In chronic B-leukemia cell lines, the DOLAs are cytostatic rather than cytotoxic and cause accumulation in S phase and G2-M phase of the cell cycle (6). DOLA-10 also induces apoptosis (8) and down-regulation of the bcl-2 oncprotein in some human lymphoma cell lines (11). Up-regulation of the p53 protein was also observed in lymphoma cell lines after exposure to DOLA-10 (11). These latter data suggest that DOLA-10 produces cell-specific biochemical effects that may not be directly related to the antimitotic activity of this molecule.

The pharmacokinetics of DOLA-10 were investigated in mice after i.v. administration of 0.25 mg/kg [3H]DOLA-10 (12). The plasma drug concentration declined rapidly with a half-life of 5.6 h. The drug was highly protein bound (>80%), and urinary excretion was less than 2% of the administered dose. DOLA-10 appeared to undergo rapid metabolism such that 15 min after injection, only 10% of the total plasma radioactivity was attributable to the parent compound. Incubation of DOLA-10 with an activated S9 liver preparation revealed the rapid formation of up to three metabolites, suggesting that this compound may also undergo extensive hepatic transformation in vivo. Electron impact MS studies suggested that one of the microsomal metabolites of DOLA-10 is dihydroxylated. The antitumor activity of the metabolites is unknown, but this and other studies suggested that maximum antitumor activity would result from bolus dosing (12). In vitro toxicology data indicated that normal human (7, 13) and canine (13) hematopoietic progenitor cells are highly sensitive to the cytotoxic effects of DOLA-10. In vivo studies in rodents and dogs suggest that dogs are the most sensitive species, with a toxic dose low of 10 µg/kg (200 µg/m²). The DLT was bone marrow suppression.

Based on the preclinical studies outlined above, a Phase I trial was initiated in patients with advanced solid tumors to determine the MTD, evaluate the toxic effects, document the antitumor activity, and assess the clinical pharmacology and pharmacodynamic effects of DOLA-10 using a highly sensitive MS-based technique.

**PATIENTS AND METHODS**

**Patient Selection**

Adults (age ≥16 years) with pathologically documented advanced solid tumors were eligible for this study if their disease was refractory to standard chemotherapy agents. Patients may have received prior radiation or biologic therapy for their disease. However, no concurrent anticancer therapy was allowed, and patients must have recovered from surgery and acute toxic effects of prior chemotherapy. Patients with stable brain metastasis were allowed to participate. Other eligibility criteria included: (a) life expectancy of at least 12 weeks; (b) Zubrod performance status of ≤2; (c) measurable or evaluable disease; (d) signed informed consent; and (e) adequate bone marrow (ANC ≥ 1,500 cells/mm³ and platelets ≥ 100,000 cells/mm³), liver (total bilirubin ≤ 1.5 mg/dl and serum transaminases < 4 × the upper limit of normal), and renal (serum creatinine ≤ 1.5 mg/dl) functions. Patients with severe comorbid conditions, such as uncontrolled diabetes, unstable cardiovascular disease, and nursing mothers, were not eligible for this study.

**Study design**

This study used a modified Fibonacci strategy for dose escalation. A minimum of three patients without prior exposure
Table 2  Hematological toxicity of DOLA-10: first course of therapy

<table>
<thead>
<tr>
<th>Dose level</th>
<th>Dose (µg/m²)</th>
<th>No. of patients</th>
<th>No. of courses</th>
<th>Thrombocytopenia</th>
<th>Anemia</th>
<th>Granulocytopenia</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>65</td>
<td>4</td>
<td>4</td>
<td>0/4</td>
<td>0/4</td>
<td>0/4</td>
</tr>
<tr>
<td>1</td>
<td>100</td>
<td>3</td>
<td>3</td>
<td>0/3</td>
<td>0/3</td>
<td>0/3</td>
</tr>
<tr>
<td>2</td>
<td>200</td>
<td>3</td>
<td>3</td>
<td>0/3</td>
<td>0/3</td>
<td>0/3</td>
</tr>
<tr>
<td>3</td>
<td>300</td>
<td>9</td>
<td>9</td>
<td>0/9</td>
<td>0/9</td>
<td>0/9</td>
</tr>
</tbody>
</table>

*Value represents the number of patients with a given level of toxicity/number of patients at risk.

Table 3  Hematological toxicity of DOLA-10: all courses of therapy

<table>
<thead>
<tr>
<th>Dose level</th>
<th>Dose (µg/m²)</th>
<th>No. of patients</th>
<th>No. of courses</th>
<th>Thrombocytopenia</th>
<th>Anemia</th>
<th>Granulocytopenia</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>65</td>
<td>4</td>
<td>6</td>
<td>0/6</td>
<td>0/6</td>
<td>0/6</td>
</tr>
<tr>
<td>1</td>
<td>100</td>
<td>6</td>
<td>9</td>
<td>0/9</td>
<td>0/9</td>
<td>0/9</td>
</tr>
<tr>
<td>2</td>
<td>200</td>
<td>8</td>
<td>15</td>
<td>0/15</td>
<td>0/15</td>
<td>0/15</td>
</tr>
<tr>
<td>3</td>
<td>300</td>
<td>9</td>
<td>14</td>
<td>0/14</td>
<td>0/14</td>
<td>0/14</td>
</tr>
</tbody>
</table>

*Values represent the number of courses with a given level of toxicity/total number of courses administered at that dose level.

to DOLA-10 were treated at each dose level. Patients treated at doses without toxic effects were allowed to receive subsequent courses at higher doses as long as three previously untreated patients had completed one course of therapy without toxic effects. However, once any grade of toxicity (other than nausea or vomiting) was observed, intrapatient dose escalation ceased. At each dose level, all three patients were observed for 3 weeks (one course of therapy) before patients were escalated to the next dose level. The MTD was based only on a patient’s toxic effects during their first course of therapy. Near the MTD, additional patients were treated to more completely define acute and cumulative toxicities. Patients who tolerated therapy continued to receive treatment as long as their tumors showed no evidence of progression. All patients were evaluated for toxicity. Patients were formally evaluated for antitumor response after every two courses of therapy. For patients with measurable disease, standard response criteria were used (14). The criteria for removing patients from the study included disease progression, patient noncompliance, request to withdraw, and the development of unacceptable toxicity.

The MTD was defined as the dose of DOLA-10 that produced reversible grade 3 or 4 hematological toxicity lasting at least 5 days or reversible grade 3 nonhematological toxicity (grade 2 for neurotoxicity) in 33% of the patients during their first course of therapy. To be sure that the dose was not underestimated, additional patients were treated at the MTD. Toxicity was evaluated by clinical assessment at least once every 3 weeks and through serial laboratory studies. Complete blood counts were monitored twice weekly, and biochemical parameters (blood urea nitrogen, serum creatinine, electrolytes, transaminases, bilirubin, and alkaline phosphatase levels) were checked weekly. Urinalysis was performed weekly and before each course of therapy. Toxicity was graded according to the NCI Common Toxicity Criteria. G-CSF was not used to support hematological recovery in this study. All patients were evaluated for antitumor response with standard radiological, laboratory, and clinical evaluations after the initial two courses and at least every two courses thereafter.

Drug Administration

DOLA-10 was supplied by Cancer Therapy Evaluation Program, Division of Cancer Treatment (NCI) at 0.2 mg/ml concentration in sterile 0.01 potassium phosphate buffer and was administered once every 22 days by rapid i.v. push (<1 min) followed by a 50-ml normal saline wash-through. Because extravasation of DOLA-10 at the site of injection produced severe chronic inflammation and ulceration during preclinical studies in dogs, all patients were encouraged to use central venous access. Based on the existing preclinical pharmacokinetic and toxicology data, an i.v. bolus route of administration every 3 weeks was chosen at a starting dose level of one-third of the dog toxic dose low (i.e., 65 µg/m²), and doses were escalated to 100, 200, and 300 µg/m².

Plasma Pharmacology/Pharmacodynamics

Pharmacokinetic Sampling. All patients who were eligible for treatment were also eligible for pharmacokinetic studies. Blood samples (10 ml in heparinized tubes) were taken before and immediately after the administration of the drug and at 5, 10, 15, 30, 45, 60, and 90 min and 2, 3, 6, 8, and 12 h after the end of the drug administration. Additional samples were collected up to 48 h dose after administration, starting at the 65 µg/m² dose level. Samples were collected on ice into 10-ml heparinized glass tubes and centrifuged immediately after being drawn. Once obtained, plasma was kept frozen at −70°C until analysis.

DOLA Extraction and Analysis. After thawing at room temperature, a 1-ml aliquot of plasma was transferred into an 8-ml nonsilanized glass tube containing 20 µl of internal standard (DOLA-15, 0.25 mg/ml). n-Butyl chloride (5 ml) was then added, and the mixture was shaken for 1 h at 250 strokes/min. Tubes were then centrifuged at 1500 × g for 10 min and then
frozen in a dry ice bath for 30 min, followed by transfer of the organic phase to a clean 10-ml nonsilanized conical glass tube for evaporation under nitrogen at 40°C. The dried extract was then reconstituted in 20 μl of a 60:40 (v:v) acetonitrile/water and sonicated for 5 min, followed by vortexing for 5 min. Samples were centrifuged for 10 min before injection.

**HPLC-MS Conditions.** Plasma samples were analyzed using HPLC/electrospray ionization MS as described previously (15). DOLA-10 and DOLA-15 were obtained as dry powders from the NCI (Bethesda, MD). Clinical grade drug was supplied by the NCI as a sterile solution containing 200 μg/ml DOLA-10 in 100 mM potassium phosphate buffer (pH 7). Acetonitrile was HPLC grade and was purchased from Burdick and Jackson (Muskegon, MI). Quantitation of DOLA-10 and detection of its N-demethylated metabolite in human plasma were accomplished by injecting 5 μl of the extracted drug onto a microbore C\(_18\) reverse-phase column (Zorbax, 50 × 1 mm, 5-μm particle size; Micro-Tech Scientific; Sunnyvale, CA) at ambient temperature. Separation was achieved using a linear gradient with a mobile phase consisting of water and acetonitrile which ranged from 2–98% acetonitrile over 5.2 min with a constant flow rate of 75 μl/min. Under these conditions, DOLA-10 eluted at 3.8 min, followed by the DOLA-15 internal standard at 4 min. The inter- and intraday coefficients of variation for this assay were <10%, and the lower limit of quantitation was 25 pg/ml.

**Pharmacokinetic/Pharmacodynamic Analysis.** Relevant pharmacokinetic parameters were estimated by fitting two- and three-compartment models to the measured plasma concentration vs time data for each patient data set using maximum likelihood estimation. Individual observations were weighted by the inverse of the variance of the model prediction of the observation. All pharmacokinetic modeling was performed using the ADAPT II program, version 4.0 (16). Model selection was performed using Akaike’s information criteria. Population parameters were calculated statistically from the individual patient data. Some patients received dose escalations. Because of the small number of observations, these measures were included in the population parameter estimates.

**RESULTS**

**Phase I Trial**

Twenty-two patients with pathologically documented advanced solid tumors were enrolled. Nineteen patients actually received treatment with DOLA-10 and received a total of 44 treatment courses at four different dose levels. Three patients who were registered but were never treated (one patient each of three dose levels) were included in the analysis.

### Table 4  DOLA-10 pharmacokinetic parameters

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>i.v. drug dosage(^a)</th>
<th>(t_{1/2a})</th>
<th>(t_{1/2b})</th>
<th>(t_{1/2c})</th>
<th>AUC</th>
<th>(Cl_p)</th>
<th>(V_{ss})</th>
<th>(V_c)</th>
<th>(C_{max})</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>65</td>
<td>0.04</td>
<td>0.5</td>
<td>5.0</td>
<td>10.97</td>
<td>5.92</td>
<td>27.5</td>
<td>1.62</td>
<td>53.7</td>
</tr>
<tr>
<td>2</td>
<td>65</td>
<td>0.08</td>
<td>0.7</td>
<td>5.7</td>
<td>12.51</td>
<td>5.19</td>
<td>23.2</td>
<td>1.57</td>
<td>60.9</td>
</tr>
<tr>
<td>3</td>
<td>65</td>
<td>0.09</td>
<td>0.7</td>
<td>8.0</td>
<td>17.41</td>
<td>3.73</td>
<td>25.3</td>
<td>1.92</td>
<td>52.9</td>
</tr>
<tr>
<td>4</td>
<td>65</td>
<td>0.12</td>
<td>1.0</td>
<td>10.4</td>
<td>37.04</td>
<td>1.76</td>
<td>16.9</td>
<td>1.61</td>
<td>77.2</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>0.08 ± 0.03</td>
<td>0.7 ± 0.2</td>
<td>7.3 ± 2.4</td>
<td>19.48 ± 12.02</td>
<td>4.15 ± 1.84</td>
<td>23.2 ± 4.6</td>
<td>1.68 ± 0.16</td>
<td>61.1 ± 11.2</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>0.09</td>
<td>0.7</td>
<td>6.8</td>
<td>14.96</td>
<td>4.46</td>
<td>24.2</td>
<td>1.61</td>
<td>57.3</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>100</td>
<td>0.14</td>
<td>1.5</td>
<td>7.2</td>
<td>22.29</td>
<td>4.49</td>
<td>34.9</td>
<td>3.38</td>
<td>60.9</td>
</tr>
<tr>
<td>6</td>
<td>100</td>
<td>0.12</td>
<td>1.2</td>
<td>11.1</td>
<td>17.10</td>
<td>5.85</td>
<td>54.0</td>
<td>3.27</td>
<td>49.0</td>
</tr>
<tr>
<td>2</td>
<td>100</td>
<td>0.16</td>
<td>2.0</td>
<td>3.5</td>
<td>7.92</td>
<td>12.63</td>
<td>17.4</td>
<td>5.00</td>
<td>29.4</td>
</tr>
<tr>
<td>3</td>
<td>100</td>
<td>0.06</td>
<td>0.3</td>
<td>2.2</td>
<td>35.64</td>
<td>2.81</td>
<td>4.8</td>
<td>0.60</td>
<td>259.0</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>0.12 ± 0.04</td>
<td>1.3 ± 0.7</td>
<td>6.0 ± 4.1</td>
<td>20.74 ± 11.58</td>
<td>6.44 ± 4.31</td>
<td>27.8 ± 21.4</td>
<td>3.06 ± 1.82</td>
<td>99.6 ± 107.1</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>0.13</td>
<td>1.4</td>
<td>5.3</td>
<td>19.70</td>
<td>5.17</td>
<td>26.1</td>
<td>3.33</td>
<td>55.0</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>200</td>
<td>0.12</td>
<td>0.7</td>
<td>7.4</td>
<td>58.14</td>
<td>3.44</td>
<td>21.9</td>
<td>2.31</td>
<td>165.3</td>
</tr>
<tr>
<td>2</td>
<td>200</td>
<td>0.08</td>
<td>1.5</td>
<td>17.0</td>
<td>26.82</td>
<td>7.46</td>
<td>53.3</td>
<td>1.55</td>
<td>189.3</td>
</tr>
<tr>
<td>3</td>
<td>200</td>
<td>0.21</td>
<td>1.9</td>
<td>111.8</td>
<td>103.10</td>
<td>1.94</td>
<td>206.9</td>
<td>3.59</td>
<td>806.9</td>
</tr>
<tr>
<td>8</td>
<td>200</td>
<td>0.14</td>
<td>1.3</td>
<td>20.3</td>
<td>34.60</td>
<td>5.78</td>
<td>81.2</td>
<td>3.59</td>
<td>103.5</td>
</tr>
<tr>
<td>9</td>
<td>200</td>
<td>0.11</td>
<td>1.0</td>
<td>7.6</td>
<td>52.49</td>
<td>3.81</td>
<td>17.1</td>
<td>3.39</td>
<td>143.9</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>0.13 ± 0.05</td>
<td>1.3 ± 0.4</td>
<td>32.8 ± 44.5</td>
<td>55.03 ± 29.75</td>
<td>4.49 ± 2.15</td>
<td>76.1 ± 77.6</td>
<td>2.89 ± 0.92</td>
<td>137.8 ± 42.5</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>0.12</td>
<td>1.3</td>
<td>17.0</td>
<td>52.49</td>
<td>3.81</td>
<td>53.3</td>
<td>3.39</td>
<td>143.9</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>300</td>
<td>0.05</td>
<td>0.3</td>
<td>33.9</td>
<td>282.95</td>
<td>1.06</td>
<td>16.2</td>
<td>9.61</td>
<td>74.0</td>
</tr>
<tr>
<td>11</td>
<td>300</td>
<td>0.06</td>
<td>0.6</td>
<td>13.0</td>
<td>58.82</td>
<td>5.10</td>
<td>60.0</td>
<td>1.83</td>
<td>164.0</td>
</tr>
<tr>
<td>12</td>
<td>300</td>
<td>0.10</td>
<td>0.8</td>
<td>58.3</td>
<td>365.70</td>
<td>0.82</td>
<td>63.9</td>
<td>3.13</td>
<td>96.0</td>
</tr>
<tr>
<td>13</td>
<td>300</td>
<td>0.13</td>
<td>1.4</td>
<td>6.3</td>
<td>68.18</td>
<td>4.40</td>
<td>26.1</td>
<td>3.55</td>
<td>84.6</td>
</tr>
<tr>
<td>14</td>
<td>300</td>
<td>0.05</td>
<td>0.5</td>
<td>35.9</td>
<td>89.90</td>
<td>3.34</td>
<td>141.3</td>
<td>2.62</td>
<td>114.5</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>0.08 ± 0.04</td>
<td>0.7 ± 0.4</td>
<td>29.5 ± 20.6</td>
<td>173.11 ± 141.56</td>
<td>2.94 ± 1.94</td>
<td>61.5 ± 49.2</td>
<td>4.15 ± 3.12</td>
<td>106.6 ± 35.4</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>0.06</td>
<td>0.6</td>
<td>33.9</td>
<td>89.90</td>
<td>3.34</td>
<td>60.0</td>
<td>3.13</td>
<td>96.0</td>
<td></td>
</tr>
<tr>
<td>All dose levels Mean ± SD</td>
<td>0.10 ± 0.01</td>
<td>1.0 ± 0.2</td>
<td>18.9 ± 19.6</td>
<td>4.51 ± 1.17</td>
<td>47.1 ± 32.1</td>
<td>2.94 ± 1.27</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>0.10</td>
<td>0.99</td>
<td>11.9</td>
<td>4.14</td>
<td>39.7</td>
<td>3.23</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^a\)i.v. drug dosage, i.v. dosage of DOL-10 (μg/m²); \(t_{1/2a}\), \(t_{1/2b}\), and \(t_{1/2c}\) are the half-lives in hours; AUC, area under the DOL-10 plasma concentration-time curve (ng/liter h); \(Cl_p\), normalized plasma clearance of DOL-10 (liter/h/m²); \(V_{ss}\), normalized distribution volume of DOL-10 at steady state (liter/m²); \(V_c\), normalized distribution volume of DOL-10 for the central compartment (liter/m²); \(C_{max}\), maximum plasma DOL-10 concentration (ng/ml).
with metastatic colorectal cancer, metastatic leiomyosarcoma, and metastatic gastric cancer) developed bowel obstruction due to abdominal/peritoneal metastases while under evaluation. The patient characteristics of all 22 registered patients are presented in Table 1. The median age for all patients was 56 years. All had advanced solid tumors of different histological types, including four patients with sarcoma and two patients with melanoma. The majority of patients had a good Zubrod performance status on initial evaluation (86% had a Zubrod performance status of 2). All but one patient had received prior chemotherapy, and 17 of 20 (85%) treated patients were heavily pretreated (defined as receiving three or more prior chemotherapy regimens). Thirteen patients were also pretreated with radiation therapy. Treatment toxicity was evaluated in all treated patients; response could be evaluated in 18 patients.

**Hematological toxicity.** The hematological toxic effects are summarized in Tables 2 and 3. No hematological DLT was seen at dose levels below 300 μg/m². However, beginning at 200 μg/m², grade 2 leukopenia and anemia were observed in 66% of the patients at that dose level. At a dose of 300 μg/m², three of nine patients (33%) developed grade 4 granulocytopenia lasting at least 5 days. In each instance, granulocyte nadirs were observed on day 19 after drug administration, with recovery to >1000 granulocytes by days 22–27. One of these three patients required hospitalization and antibiotic coverage for culture-negative neutropenic fever. Two of these patients were subsequently treated at the 200 μg/m² dose level without a recurrence of granulocytopenia. The third patient was removed from the study because of progressive disease. No episodes of thrombocytopenia of any grade were observed. Episodes of grade 2 anemia were also encountered at the highest dose levels studied. At 200 μg/m², there was one first course of therapy with grade 2 anemia and five subsequent courses with grade 2 anemia. At 300 μg/m², four of the nine first courses of therapy were complicated by grade 2 anemia, however, in no instances were transfusions required.

**Nonhematological Toxicity.** The nonhematological adverse effects were generally mild and reversible. Thirteen of the 19 patients who received DOLA-10 experienced fatigue. Most events were of grade 1 or 2 in severity. In two patients, two courses were characterized by reversible grade 3 fatigue. Other common nonhematological toxic effects included nausea and vomiting (grade 1 and 2) in six patients and anorexia and constipation in seven patients. Two cases of phlebitis (grade 2) at the site of drug injection were encountered at the 65 μg/m² dose level. To avoid additional reactions, we used central venous access for patients treated at dose levels above 65 μg/m².

The most frequently noted laboratory abnormalities (60% of the patients) were grade 1–2 elevations of lactic dehydrogenase and alkaline phosphatase levels. However, in 70% of these cases, the abnormal liver function tests were present before entry on the study, and the enzyme levels did not change significantly during therapy. In three patients, liver functions progressively worsened on DOLA-10; however, these abnormalities coincided with progressing tumor, suggesting that they were secondary to disease progression. No changes in serum creatinine levels or routine urine chemistry were noted while on therapy, with the exception of one patient who experienced increasing serum creatinine levels coinciding with the progression of disease in his kidneys.

**Antitumor Effects.** Four patients had no change in tumor volume through four to six courses of therapy (two patients with colon adenocarcinoma and one patient each with bronchoalveolar lung carcinoma and leiomyosarcoma of the left forearm). Three of these four patients received escalated doses of DOLA-10 over the course of their therapy. However, all patients eventually had documented tumor progression, and no objective responses were seen.

**Plasma Pharmacology**

**DOLA-10 Pharmacokinetic Characteristics.** The 18 patients in this study received a total of 35 cycles of therapy. Pharmacokinetic data were obtained in the first cycle in each of four patients receiving 65 and 100 μg/m² and in each of five patients receiving 200 and 300 μg/m², respectively. Intratpatient dose escalation was permitted in this study, and pharmacoki-
netic assessments were performed in three patients, at three different dose levels. Data from all courses of therapy have been included in the population summary statistics. The individual and population pharmacokinetic parameters are summarized in Table 4.

Two- and three-compartment models were fitted to the DOLA-10 plasma concentration versus time data. Akaike’s information criteria, visual inspection of the data, and statistical assessment of the goodness of fit were used for model selection. Whereas a two-compartment model described the DOLA-10 plasma concentration-time data reasonably well, a three-compartment model consistently performed better. A typical plasma concentration versus time plot is shown in Fig. 2. After a rapid distribution phase, the DOLA-10 plasma concentration declined much less rapidly, with mean $\beta$ and $\gamma$ half-lives of 0.99 and 18.89 h, respectively. Samples beyond 12 h postdose were not planned until the 200 $\mu$g/m$^2$ dose level (Fig. 3) because preclinical studies in our laboratory suggested that the DOLA-10 plasma concentration would decline rapidly. However, subsequent analysis of samples from the first three patients dosed at 65 $\mu$g/m$^2$ suggested that DOLA-10 clearance was much less rapid in humans than in rodents. Subsequently, we observed that we were able to follow the plasma concentration-time curves, even at doses of 65 $\mu$g/m$^2$, until 48 h postdose. Mean peak concentrations increased after each dose escalation in a linear manner, with the exception of that at 300 $\mu$g/m$^2$, where the mean peak declined 23% compared to that at 200 $\mu$g/m$^2$ (Table 4).

Mean and median DOLA-10 plasma clearances for all dose levels were 4.51 and 4.14 liters/h/m$^2$, respectively, and did not vary significantly by dose (Fig. 4A). Intrapatient variance in DOLA-10 plasma clearance was substantial, ranging 15-fold from 0.82–12.63 liters/h/m$^2$. Intrapatient variability was also encountered. For example, patient 2 (Table 4) was treated with doses of 65, 100, and 200 $\mu$g/m$^2$. Normalized plasma clearance values in this patient ranged from 5.19 liters/m$^2$ to 12.63 liters/h/m$^2$. As expected, median DOLA-10 AUC increased as drug dose increased, but the heterogeneity in drug clearance resulted in a substantial overlap in AUC across dose levels (Fig. 4B).

The liquid chromatography/MS assay used was both selective and specific, allowing us to determine the metabolism of DOLA-10 in human subjects. Previous in vitro studies demonstrated that DOLA-10 was rapidly metabolized to a number of metabolites. In these in vitro studies using murine S9 fractions, up to 75% of the parent drug was converted in as little as 10 min to a dihydroxy DOLA-10 metabolite (15). Using the same chromatographic conditions described above for the analysis of human plasma, a single DOLA-10 metabolite was detected in samples obtained from five of the subjects in this study. Unlike the in vitro studies, the principal metabolite detected was not hydroxylated but was an N-demethyl derivative as confirmed by MS. In all five subjects, the concentration of this metabolite never exceeded 2% of the simultaneously measured parent concentration (Fig. 3).

Pharmacodynamic Analyses. The pharmacodynamics of DOLA-10 were also investigated. Relationships between DOLA-10 disposition (peak plasma concentration, plasma clearance, and AUC) and toxicities such as anemia, leukopenia,
neutropenia, and peripheral neuropathy were investigated. The only noteworthy predictive relationship observed was between DOLA-10 AUC and the percentage of decline in nadir neutrophil count.

Of the models tested, the one with the least bias and highest predictive value was a sigmoid E_{max} model incorporating DOLA-10 AUC and ANC depression. This model was reasonably predictive in determining the depth of nadir in ANC versus baseline ANC (Fig. 5). The estimated EC_{50} was a plasma DOLA-10 AUC of 91 μg/liter × h. Estimates of precision and bias for this particular model are not available due to the lack of observations, particularly at higher DOLA-10 exposures. However, even with these limited data, a pharmacodynamic model predictive of hematological toxicity can be developed. For example, an observed AUC of 89.9 μg/liter × h produced an observed nadir ANC (versus baseline) of 51.3%, whereas the model estimate was 49% (predicted; observed ratio, 0.955). At the high and low ranges of DOLA-10 exposure, an AUC of 365 μg/liter × h produced an observed:predicted ratio of 1.12 (93% versus 83%), whereas a DOLA-10 exposure of 7.9 μg/liter × h produced a ratio of 0.77 (13.1% versus 17%), respectively.

Although these limited data are insufficient for the development of a clinically dependable model, they suggest a clear, measurable relationship between DOLA-10 exposure and neutropenia, the observed DLT.

DISCUSSION

DOLA-10 is a linear pentapeptide initially isolated from the sea hare D. auricularia with promising antitumor activity in a variety of preclinical studies (9). In this trial, we evaluated the toxicity and pharmacokinetics of this molecule in patients with advanced solid tumors using a single rapid i.v. infusion repeated every 22 days. The rationale for this schedule was based on preclinical studies demonstrating that a single bolus administration of DOLA-10 was superior to split-dosing regimens. These observations were consistent with the preclinical pharmacokinetic studies of Newman et al. (12), who suggested that because of protein binding and extensive metabolism, bolus doses of DOLA-10 were more likely to achieve a critical threshold plasma level resulting in an antitumor effect. In this trial, DOLA-10 was well tolerated up to a dose level of 300 μg/m². The major DLT observed at this level was reversible granulocytopenia in one-third of the patients. Significant episodes of thrombocytopenia or anemia were not detected. Nonhematological toxic effects, including fatigue, nausea, and vomiting, occurred frequently, but these effects were easily managed and appeared to be independent of dose. Two patients treated at the starting dose of 65 μg/m² experienced grade 2 phlebitis thought to be due to unrecognized drug extravasation. Due to the fact that a similar episode was encountered during toxicology studies in dogs, central venous access was used in all subsequent patients treated in this trial. No objective anticancer responses were observed.

The plasma pharmacokinetic behavior of DOLA-10 was assessed using a very sensitive HPLC/electrospray ionization MS system developed previously by our group (15). Preclinical studies suggested that DOLA-10 would be rapidly metabolized, precluding measurement >12 h after bolus administration. Thus, the pharmacokinetic behavior of DOLA-10 in this clinical trial was unexpected. Even at the starting dose of 65 μg/m², it was possible to detect the drug for periods of >48 h after bolus administration. Compared to previous preclinical investigations (12), plasma clearance was significantly slower; therefore, drug exposure was considerably higher. Both interpatient and intrapa-
tient differences in drug plasma clearance were substantial. Consistent with the preclinical findings supporting the rapid metabolism of DOLA-10, an N-demethyl DOLA-10 derivative was detected in five patients. Other minor metabolites, including the dihydroxy species detected after in vitro incubation of DOLA-10 with rat liver, were not observed in this trial. Overall, after administration to patients, DOLA-10 appears to be rapidly metabolized to more polar products.

The clinical and pharmacological results of this trial were similar to the results recently reported by Pitot et al. (16) from the Mayo Clinic. In that study, DOLA-10 was also administered by rapid i.v. bolus every 22 days. The MTD and recommended Phase II dose reported by these investigators was 400 μg/m² for patients with minimal prior chemotherapy, but because of severe granulocytopenia, 325 μg/m² was recommended for those patients previously treated with ≥2 prior chemotherapy regimens. Our patient population was primarily composed of heavily pretreated patients (Table 1); thus, our MTD of 300 μg/m² is quite consistent with this previously reported experience.

The pharmacokinetic parameter values and variances described in this trial are also similar to those reported by Pitot et al. (16). These investigators also characterized DOLA-10 disposition using a three-compartment model. Clearance values were similar across dose ranges, with a mean value of 4.2 liters/h/m², which is very close to the reported mean value of 4.51 liters/h/m² for the current study. Likewise, terminal half-life and steady-state volume of distribution were also comparable. These data demonstrate that the different analytical methods (bioassay and LC/MS) used in these studies are both capable of quantifying DOLA-10 in complex matrices. The fact that different techniques resulted in the derivation of equivalent parameter estimates suggests that these reported values are true and accurate.

Examination of the relationships between drug exposure (AUC) and hematological toxicity revealed some correlation between DOLA-10 AUC and percentage decrease in granulocytes, but not between dose and granulocytopenia. Because the drug exposures of most of the patients in this trial clustered at the low end, information exists to create a meaningful pharmacodynamic model describing this relationship.

The lack of documented objective antitumor effects in this trial (even in the Phase I setting) was disturbing. Of even greater concern was the fact that both the available preclinical data and the experience with this trial suggest that the DOLA-10 drug exposures required for meaningful antitumor activity may not be achieved due to unacceptable granulocytopenia in the clinic. However, the absence of significant anemia, thrombocytopenia, or severe nonhematological toxic effects encountered in this or the Mayo Clinic trial (16) support the concept that higher doses of DOLA-10 could be safely administered with G-CSF. The rationale for such an approach is based on the following data. First, in the NCI human tumor cell line screen (48-h exposure), total growth inhibition generally required concentrations of 0.1–1.0 nM (~0.08–0.8 ng/ml; Ref. 17). Similarly, with continuous exposure to DOLA-10, 50% of the human cell lines tested in a human colony-forming assay were sensitive to a concentration of 1.3 nM (1 ng/ml; Ref. 7). Unfortunately, human hematopoietic progenitor cells were inhibited by considerably lower DOLA-10 concentrations (IC₅₀ = 0.13–1.3 pM; Ref. 13). Thus, to maximize the potential that DOLA-10 will have clinically meaningful antitumor activity, it appears that we will need to develop a strategy that will maximize the time that DOLA-10 plasma levels are above 1 ng/ml and simultaneously minimize the myelosuppressive effects of the drug. Optimally, based on the available preclinical data, the target duration above the 1 ng/ml target would be at least 24–48 h. Based on this rationale, we have designed a second Phase I trial that incorporates prophylactic G-CSF into the treatment plan to maximize the dose of DOLA-10 and achieve the target drug exposure outlined above.

In summary, DOLA-10 is well tolerated after bolus dosing, and reversible granulocytopenia is the DLT. An estimated MTD of 300 μg/m² was observed in this heavily pretreated patient population. DOLA-10 has a long terminal half-life in patients and is extensively metabolized to more polar derivatives. Phase II trials of this agent are currently in progress.

ACKNOWLEDGMENTS

We appreciate the thoughtful clinical care of many of the patients treated in this trial provided by Dr. Nikolaos Touroungiou. The nursing and data management assistance of Kathy Taebel is gratefully acknowledged. We thank Marilyn C. Thompson for skilled secretarial assistance in preparation of the manuscript.

REFERENCES


Novel Marine-derived Anticancer Agents: A Phase I Clinical, Pharmacological, and Pharmacodynamic Study of Dolastatin 10 (NSC 376128) in Patients with Advanced Solid Tumors

Timothy Madden, Hai T. Tran, Debrah Beck, et al.


Updated version
Access the most recent version of this article at:
http://clincancerres.aacrjournals.org/content/6/4/1293

Cited articles
This article cites 16 articles, 5 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/6/4/1293.full#ref-list-1

Citing articles
This article has been cited by 1 HighWire-hosted articles. Access the articles at:
http://clincancerres.aacrjournals.org/content/6/4/1293.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.