Phase I Pharmacokinetic Trial and Correlative in Vitro Phase II Tumor Kinetic Study of Apomine (SR-45023A), a Novel Oral Biphosphonate Anticancer Drug

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ABSTRACT

Purpose: To study the human pharmacokinetics and in vitro cytotoxicity of Apomine, an p.o. administered, nonmyelosuppressive agent that selectively inhibits cell proliferation and induces tumor cell apoptosis through the farnesoid X receptor.

Experimental Design: Seven solid cancer patients who participated in an ongoing Phase I study of Apomine and received the starting dose level of 125 mg/m²/day × 14 days every 3 weeks underwent a pharmacokinetic study on day 14 of the first course. Plasma concentrations of Apomine were assayed with a Hewlett Packard gas chromatograph using a nitrogen phosphorus detector and HP-5 15m column. Fresh human ovarian cancer tumor samples were obtained during initial exploratory laparotomy from 35 chemotherapy-naive, advanced stage epithelial ovarian cancer patients. Tumor samples were tested for sensitivity to Apomine, carboplatin, cisplatin, paclitaxel, and topotecan using an in vitro clonogenic [³H]thymidine end point assay.

Results: Pharmacokinetic analysis revealed a mean Apomine plasma Cmax of 16.4 ± 9.1 µg/ml (29.1 µM), a mean plasma AUC0–12h of 173.4 ± 105 µg · h/ml (308 µM · h), and a mean t½ (24–192 h) of 156.2 ± 42.9 h. In vitro assay results showed that 63 and 91% of the ovarian cancers were sensitive (i.e., >70% inhibition of tumor cell growth) to Apomine at concentrations of 10 and 20 µM. The sensitivity rates were 91% for carboplatin (270 µM), 88% for cisplatin (33 µM), 41% for paclitaxel (5.9 µM), and 85% for topotecan (2.2 µM).

Conclusions: These in vitro assay results, taken together with our preliminary plasma pharmacokinetic data, suggest that Apomine should be clinically active at the 125 mg/m² dose level.

INTRODUCTION

The natural history of ovarian cancer has been improved dramatically with the advent of the platinum compounds, cisplatin and carboplatin, and the taxanes, paclitaxel and Taxotere (1–4). In relatively recent Phase III clinical trials, the median survival for patients treated with i.v. cisplatin/paclitaxel with stage III suboptimal and optimal surgical cytoreduction were 38 and 53 months, respectively (2, 4). Although clinical complete response rates to standard chemotherapy regimens approach 50%, approximately half of advanced ovarian cancer patients with suboptimal disease die within 2.5 years of diagnosis, and the 5-year survival rate is relatively low (2). Thus, there is a continuing need for the development of well-tolerated, nonmyelosuppressive cytotoxic agents to combine with platinum/taxane regimens for the first-line treatment of stage III and IV disease.

Apomine (SR-45023A), a 1,1-biphosphonate ester (Fig. 1), is a novel, p.o.-active, apoptosis-inducing agent recently entered into Phase I clinical trials in cancer patients (5, 6). It is especially interesting because it was proven to be nonmyelosuppressive in preclinical models and possesses unique mechanisms of action. It appears to act via a farnesol mimetic mechanism with specific agonistic interaction with the nuclear receptor farnesoid X receptor and involves intracellular signaling and transcriptional control including: (a) suppression of the activity of HMG CoA reductase; (b) regulation of cell proliferation; and (c) induction of apoptotic cell death (5).

Because of its unique mechanisms of action, nonmyelosuppressive activity, and oral route of administration, Apomine could prove to be an extremely valuable drug for the management of ovarian cancer. We report here the results of an in vitro Phase II study of Apomine, carboplatin, cisplatin, paclitaxel, and topotecan against the growth of fresh human ovarian tumor samples obtained from chemotherapy-naive patients, along with the preliminary analysis of an ongoing Phase I and pharmacokinetic study of Apomine in patients with advanced solid cancers.

MATERIALS AND METHODS

Tumor Samples. Solid tumor biopsy specimens were obtained from 35 previously untreated patients with epithelial ovarian cancer (Fédération Internationale des Gynaecologistes et Obstetrices stage III-IV) during their initial exploratory lap-
The abbreviations used are: FBS, fetal bovine serum; AUC, area under the curve; FFA, free fatty acid; M, molecular weight; MCA, malignant cutaneous angiofibroma; MOC, malignant orbital cutaneous angiofibroma; N, number; NCCN, National Comprehensive Cancer Network; NA, not available; NCI, National Cancer Institute; NS, not significant; OA, osteosarcoma; OVC, ovarian ovarian carcinoma; PBI, percent body index; PBS, phosphate-buffered saline; PC, primary cutaneous; PCOS, polycystic ovary syndrome; PMCA, peripheral malignant cutaneous angiofibroma; RBC, red blood cell; T-DMAB, triethylboron dimethylamide; TPA, tumor promoter; TPA-1, tumor promoter-1; TP-1, tumor promoter-1; U, unit; V, volume; WBC, white blood cell; X, number of experiments; Y, number of samples; Z, number of patients.

arotomy and tumor debulking surgery. Specimens were transferred aseptically to the Arizona Cancer Center’s Human Tumor Cloning Assay laboratory within 24 h of surgery. Solid tumor specimens were transported in medium (McCoy’s 5A; Irvine, Santa Ana, CA) supplemented with 10% FBS, 100 units/ml penicillin, 100 mg/ml streptomycin, and 2 mM L-glutamine (Irvine), and topotecan (clinical sample; SmithKline Beecham, Philadelphia, PA) were reconstituted in distilled water. All drugs then were substituted in low concentrations of ethanol. All drugs were then assayed in batch mode with a Hewlett Packard gas chromatograph using a nitrogen phosphorus detector and an HP-5 15 m × 0.32 mm column. To a 250-μl sample of plasma, 10 μl of a methanol solution of the internal standard SR-9223n (n-propyl phosphonate analogue of Apomine) was added and mixed by vortexing. Each sample was then extracted with 0.5 ml of methyl tertbutyl ether, separated, and dried under nitrogen. The extract was then reconstituted in 250 μl of methyl tertbutyl ether, of which 3 μl were injected for analysis with an autosampler. For each patient, the plasma samples were analyzed during the same run along with standard plasma samples with spiked Apomine and SR-9223n for quantification purposes. Standard drug amounts added to control plasma gave a

Fig. 1 Chemical structure of Apomine.

3 The abbreviations used are: FBS, fetal bovine serum; AUC, area under the plasma concentration-time curve.
The percentage of recovery of the peak area ratio between Apomine and internal standard added to control plasma and extracted compared with a standard solution was 98.6%. During the assay of these samples, the intrarun SD was 0.06, and the interrun SD was 0.1. All of the samples were prepared in duplicate and injected twice. The limit of quantification was 1 ng/ml in plasma. The detector was linear between 0.001 and 50 μg/ml, and the variation of retention time was 0.01 min.

The pharmacokinetic parameters determined include maximum observed plasma concentration on day 14 ($C_{\text{max}}$) and time to reach it ($t_{\text{max}}$), the apparent terminal elimination half-life ($T_{1/2}$), which was calculated as 0.693/β, where β was the apparent terminal elimination rate constant derived from the exponential regression of the concentration-time curve between $T = 24$ h and $T = 192$ h, $y = a \exp (-βx)$. The apparent elimination half-life following the $C_{\text{max}}$, $T_{1/2\alpha}$, was calculated as 0.693/α, where α was the apparent elimination rate constant derived from the exponential regression of the concentration-time curve between $T_{\text{max}}$ and $T = 6h$, $y = a \exp (-αx)$. The AUC after the morning dose $[AUC_{0–12 \text{ h}}]$ on the 14th day and from 0 to 192 h $[AUC_{0–192 \text{ h}}]$ was calculated using the FigP software. Without knowledge of the fraction of dose absorbed F, only $C_{\text{f}}/F$ was estimated as $C_{\text{f}}/F = \text{Dose}/[AUC_{0–12 \text{ h}}]_{14\text{th day}}$.

RESULTS

**In Vitro Drug Sensitivity Data.** Table 1 shows the in vitro chemosensitivity data (expressed as percentage of growth compared with control plates) for each of the 32–35 ovarian cancers exposed separately to one concentration of the standard cytotoxic agents (i.e., clinically achievable concentrations of carboplatin, cisplatin, paclitaxel, and topotecan) and up to three different concentrations of Apomine. Table 2 displays the in vitro activity of each of the drugs. With sensitivity defined as <30% tumor cell growth as compared with control, the vast majority (89–91%) of the ovarian cancers were sensitive to Apomine, at both the 40 and 20 μM concentrations, and carbon-
platinum (91%), cisplatin (88%), and topotecan (85%). In contrast, 63% of tumors were sensitive to Apomine at a concentration of 10 μM, and only 41% of tumors were sensitive to paclitaxel, using the above criteria. Additionally, the following percentages of tumors exhibited intermediate (i.e., 30–50% growth inhibition) sensitivity to 40 μM Apomine (11%), 20 μM Apomine (9%), 10 μM Apomine (14%), carboplatin (9%), cisplatin (6%), topotecan (9%), and paclitaxel (34%). Tumor sensitivity to Apomine appeared to plateau at a concentration near 20 μM because there was not increased activity above this level.

In Vivo Phase I and Pharmacokinetic Data. Seven cancer patients registered into the Arizona Cancer Center’s ongoing Phase I trial of Apomine at the initial dose level (125 mg/m²/day) underwent pharmacokinetic studies after the morning dose (75 mg/m²) on day 14 of their first treatment cycle. None of the patients experienced more than mild toxicity (i.e., grade 1; WHO and Southwest Oncology Group common toxicities). All of the patients were evaluable for pharmacokinetics (i.e., 75 mg/m² on day 14 of their first treatment cycle). In the ongoing Phase I trial of Apomine at the initial dose level (125 mg/m²/day), patients experienced stable disease for 100% of the patients. An additional patient with metastatic melanoma experienced non-cross-resistant to paclitaxel. Seven of 32 tumors were sensitive (i.e., >30% of control; resistant, >50% of control).

### Table 2 In vitro clonogenic [³H]thymidine end point assays of apomine and standard anticancer drugs against fresh human ovarian cancers

<table>
<thead>
<tr>
<th>Drug</th>
<th>n</th>
<th>% sensitive</th>
<th>% intermediate</th>
<th>% resistant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apomine 10 μM</td>
<td>35</td>
<td>63</td>
<td>14</td>
<td>23</td>
</tr>
<tr>
<td>Apomine 20 μM</td>
<td>34</td>
<td>91</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>Apomine 40 μM</td>
<td>19</td>
<td>89</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td>Carboplatin 270 μM</td>
<td>34</td>
<td>91</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>Cisplatin 33 μM b</td>
<td>33</td>
<td>88</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Paclitaxel 5.9 μM b</td>
<td>32</td>
<td>41</td>
<td>34</td>
<td>25</td>
</tr>
<tr>
<td>Topotecan 2.2 μM b</td>
<td>33</td>
<td>85</td>
<td>9</td>
<td>6</td>
</tr>
</tbody>
</table>

a Sensitive, <30% of control; intermediate, 30–50% of control; resistant, >50% of control.
b Carboplatin 270 μM = 100 μg/ml; cisplatin 33 μM = 10 μg/ml; paclitaxel 5.9 μM = 5 μg/ml; topotecan 2.2 μM = 1 μg/ml.

Although the present in vitro data must be viewed conservatively, the high degree of Apomine activity against fresh ovarian cancers at clinically achievable plasma concentrations suggests that this drug should be active in the setting of our ongoing Phase I study (i.e., twice daily × 14 days every 3 weeks dosing schedule). In fact, one study patient with previously treated, drug-refractory ovarian cancer received eight cycles of Apomine and experienced a reduction of serum CA-125 level from 3013 units/ml to 554 units/ml with no progression of disease. An additional patient with metastatic melanoma experienced stable disease for >20+ months of therapy with no adverse effects to Apomine, except reversible fatigue during the final days of each 14-day Apomine course.

In the present study, there was evidence that Apomine is non-cross-resistant to paclitaxel. Seven of 32 tumors were insensitive (>50% cell growth compared with control) to paclitaxel. Of these 7 tumors, 100% were at least moderately sensitive (≥50% cell growth compared with control) to Apomine at concentrations of 40 and 20 μM, and 4 of 7 (57%) were at least moderately sensitive to Apomine at a concentration of 10 μM. There were too few cases of resistance to carboplatin (0 of 34 cases), cisplatin (2 of 33 cases), and topotecan (2 of 33 cases) to study patterns of cross-resistance to these three drugs.

The in vitro anti-ovarian cancer activity of Apomine must be viewed with caution, because in vivo it is highly protein bound, and the in vitro culture medium used contains only 10% FBS (i.e., only 10% serum protein versus 100% in vivo). On the other hand, we presume that the observed in vitro sensitivity to Apomine does represent loss of cell survival because in vitro apoptosis through a farnesoid X receptor nuclear receptor mechanism has been documented (5). Of course, validation of these in vitro chemosensitivity assay data awaits the results of Phase II clinical trials of Apomine in patients with recurrent ovarian cancer.

### REFERENCES


### Table 3 Summary of apomine pharmacokinetics after morning dose on day 14 in seven patients receiving apomine 125 mg/m²/day (75 mg/m² twice per day) × 14 days

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean value (± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cmax (μg/ml)</td>
<td>16.4 (± 9.1)</td>
</tr>
<tr>
<td>tmax (h)</td>
<td>3.1 (± 2.3)</td>
</tr>
<tr>
<td>[AUC0–12 h] (μg·h/ml)</td>
<td>173.4 (± 105)</td>
</tr>
<tr>
<td>t1/2(24–92 h) (h)</td>
<td>16.6 (± 16.6)</td>
</tr>
<tr>
<td>t1/2(12–24 h) (h)</td>
<td>14.4 (± 4.2)</td>
</tr>
<tr>
<td>t1/2(24–92 h) (h)</td>
<td>156.2 (± 42.9)</td>
</tr>
</tbody>
</table>

a 29.1 μM.
b 308 μM/h.

DISCUSSION

We performed an “in vitro Phase II” evaluation of Apomine against 35 fresh ovarian cancers and discovered that at clinically achievable concentrations (i.e., 10, 20, and 40 μM), Apomine proved remarkably active (i.e., 63–91% of ovarian tumors were sensitive). This activity level was comparable with that determined for cisplatin, carboplatin, and topotecan and was considerably higher than that for paclitaxel (at clinically achievable concentrations). Although the 41% rate of paclitaxel chemosensitivity (i.e., <30% of control) found in the present study is comparable with our previous in vitro experience with this agent and to recently reported phase III single-agent activity (i.e., 42% response rate in a Gynecologic Oncology Group study in previously untreated, advanced ovarian cancer; Ref. 8).


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