Enhanced Antitumor Activity of 6-Hydroxymethylacylfulvene in Combination with Irinotecan and 5-Fluorouracil in the HT29 Human Colon Tumor Xenograft Model

Carolyn D. Britten, Susan G. Hilsenbeck, S. Gail Eckhardt, Jennifer Marty, Gina Mangold, John R. MacDonald, Eric K. Rowinsky, Daniel D. Von Hoff, and Steve Weitman

ABSTRACT

6-Hydroxymethylacylfulvene (MGI-114) is a semisynthetic analogue of the toxin illudin S, a product of the Omphalotus mushroom. MGI-114 induces cytotoxicity in a variety of solid tumors in vivo, including the refractory HT29 human colon cancer xenograft. In this study, the potential application of MGI-114 in the treatment of colon cancer was further explored by evaluating the activity of MGI-114 in combination with irinotecan (CPT-11) and 5-fluorouracil (5FU). Groups of 9 nude mice bearing HT29 xenografts were treated with either single agent MGI-114, CPT-11, or 5FU, or MGI-114 in combination with CPT-11 or 5FU. MGI-114 was administered at doses of 3.5 and 7 mg/kg i.p. daily on days 1 through 5, and CPT-11 and 5FU were administered at doses of 50 and 100 mg/kg i.p. on days 1, 12, and 19. In the single agent studies, MGI-114, CPT-11, and 5FU all resulted in decreased final tumor weights compared with vehicle-treated controls (P < 0.05), but only MGI-114 at 7 mg/kg produced partial responses. When MGI-114 at 3.5 mg/kg was combined with CPT-11, significant decrements in final tumor weights occurred compared with monotherapy with the same doses of MGI-114 and CPT-11 (P ≤ 0.001). Also, administration of the low-dose combination (MGI-114 at 3.5 mg/kg and CPT-11 at 50 mg/kg) resulted in final tumor weights similar to those achieved after administration of high-dose MGI-114 as a single agent. Moreover, the combination of MGI-114 and CPT-11 produced partial responses in nearly all of the animals, with some animals achieving complete responses. The outcome with the combination of MGI-114 and 5FU was less striking, with fewer partial responses and no complete responses. These results suggest enhanced activity when MGI-114 is combined with CPT-11, and clinical trials to further evaluate this combination regimen are planned.

INTRODUCTION

MGI-114 is a semisynthetic analogue of the toxin illudin S, a natural product of the Omphalotus mushroom. After uptake into sensitive tumor cells, the illudins damage DNA in a unique manner that appears to be repaired by DNA helicases. Although the precise mechanism of MGI-114 cytotoxicity has not been elucidated entirely, recent studies have suggested that MGI-114 inhibits DNA synthesis, arrests the cell cycle in S phase, and induces apoptosis.

The illudins are preferentially cytotoxic to a variety of leukemia and solid tumor cell lines, including those with multidrug resistant phenotypes. Human MCF7 mammary carcinoma, HT29 colon carcinoma, and MV522 lung carcinoma cells are all sensitive to MGI-114 at nanomolar concentrations. In addition, PRs and CRs have been seen following the administration of MGI-114 to nude mice bearing human MX1 breast, HT29 colon, and MV522 lung carcinoma xenografts.

The activity of MGI-114 in HT29 xenografts is particularly notable inasmuch as CPT-11, dacarbazine, carbamustine, doxorubicin, and methotrexate, do not typically cause tumor reduction in this model. Chemoresistance in HT29 colon cancer cells is at least in part due to mutated p53 and the attendant loss of p53-dependent apoptosis. Whereas the incidence of p53 mutations in colorectal cancer is between 70 and 80%, the HT29 cell line frequently serves to characterize the activity of drugs for use in colorectal cancer.

Previous studies have explored the activity of both the antimetabolite 5FU and the topoisomerase I inhibitor CPT-11 in the HT29 model. Neither agent produces responses in HT29 xenograft tumors, although both agents inhibit tumor growth. In patients with metastatic colorectal cancer, this has translated to single-agent response rates of 8–85% and 17.7–36.4%, respectively, for 5FU and CPT-11. Despite the low response rate for CPT-11, the treatment of 5FU-resistant metastatic colorectal cancer with CPT-11 confers a survival benefit. Nevertheless, colon cancer remains the second leading cause of cancer death in the United States.

Given the unique activity of single agent MGI-114 in the HT29 colon carcinoma xenograft model, the efficacy of this compound was examined in combination with 5FU and CPT-11. The potential for enhanced activity with MGI-114 in combination with CPT-11 was realized after an earlier study that demonstrated synergistic activity in vitro between MGI-114 and topotecan, another topoisomerase I inhibitor. In the present study, MGI-114 was administered alone and in combination with either 5FU or CPT-11 to nude mice bearing HT29 xenograft tumors. Although substantial antitumor activity was observed after treatment with single agent MGI-114, there were no CRs in this setting. When MGI-114 was combined with a relatively ineffective dose of CPT-11, however, CRs were observed. This pattern of activity suggests that the combination of MGI-114 and CPT-11 may result in super-additive antineoplastic activity.

MATERIALS AND METHODS

Cytotoxic Drugs. MGI-114 was synthesized using illudin S from still cultures of Omphalotus illudens. The vehicle for MGI-114 was 1% ethanol in 5% dextrose in water. CPT-11 and 5FU were both obtained from Pharmacia & Upjohn (Kalamazoo, MI) and diluted in 5% dextrose in water.

In Vivo Evaluation in Human Tumor Xenograft Models. HT29 human colon carcinoma cells were obtained from the American Type Culture Collection (Rockville, MD). Nude mice (Harlan Sprague Dawley, Inc., Indianapolis, IN) were implanted s.c. by trocar with fragments of HT29 human colon tumors harvested from s.c. growing tumors in nude mice. When tumors were approximately 5 mm × 5 mm in size (usually about 12 days after inoculation), the animals were pair-matched into treatment and control groups (day 1). Each
ENHANCED ACTIVITY WITH MGI-114 AND CPT-11

The maximum percentage of animal weight loss, an indicator of toxicity, was calculated for individual animals as:

\[
\text{% animal weight loss} = \frac{\text{Day 1 weight} - \text{Minimum weight on study}}{\text{Day 1 weight}} \times 100\%
\]

Statistics. Parameters including actual tumor weight at study conclusion, % tumor reduction, and % animal weight loss were summarized using descriptive statistics. In addition, statistical analysis examined the actual tumor weight at study conclusion and time-to-tumor doubling. Actual tumor weight was analyzed using ANOVA after transformation to log(tumor weight + 1) [natural logarithm] as indicated by a Box-Cox analysis (19). One-way ANOVA with multiple range tests was used to determine which groups were different from each other. Survival, or time-to-tumor doubling, was determined based on the calculated tumor weights. Time-to-tumor doubling was analyzed using Kaplan-Meier methods and compared using log-rank tests (20). In the “multiple range” analyses of survival, pair-wise log-rank tests were computed, and then the P values were adjusted using the “step-down” Bonferroni method (21).

RESULTS

Single Agent Studies. Tables 1 and 2 summarize the efficacy studies of MGI-114, CPT-11, and 5FU in HT29 xenografts. There were no spontaneous tumor regressions in the control group. Single agent MGI-114 given daily for 5 days produced a TGI of 69.5% at the 3.5 mg/kg dose level and 92.3% at the 7 mg/kg dose level. The final tumor weights in MGI-114-treated animals were significantly decreased compared with controls (P ≤ 0.05). Moreover, the percentage of tumor reduction in 7 of the 9 animals treated with MGI-114 at 7 mg/kg averaged (± SE) 60.5 ± 7.9%. Fig. 2 illustrates the mean tumor weight approaching zero after treatment with the higher dose MGI-114 (7 mg/kg).

In comparison, CPT-11 and 5FU administered as single agents had minimal activity against HT29 xenografts whether administered at doses of 50 or 100 mg/kg i.p. Treatment with single agent CPT-11 resulted in a substantial decline in final tumor weights compared with controls, whereas the TGI was 36.9% at 50 mg/kg and 44.4% at 100 mg/kg. CPT-11-treated tumors continued to grow at a rate somewhat slower than controls, as shown in Fig. 2. 5FU also produced substantial decreases in final tumor weights, with TGI of 32.2% at 50 mg/kg and 50.0% at 100 mg/kg. There were no PRs or CRs with either single agent CPT-11 or 5FU.

Combination Studies. The combination of MGI-114 and CPT-11 produced notable activity against the HT29 human colon tumor xeno-
nografts, as outlined in Table 1. At all of the dosages, PRs occurred in nearly all of the mice. In addition, CRs were observed in one animal treated with MGI-114 at 3.5 mg/kg and CPT-11 at 50 mg/kg. Treatment with MGI-114 at 3.5 mg/kg together with either dose of CPT-11 resulted in remarkable decre-

Table 2: MGI-114 and 5FU in the HT29 human colon tumor xenograft

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Dose and route</th>
<th>Schedule</th>
<th>Actual final tumor weight (mg)</th>
<th>% TGI</th>
<th>Mice with tumor response</th>
<th>% tumor reduction (mean ± SE)</th>
<th>Maximum % animal weight loss (mean ± SE)</th>
<th>N of toxic deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>9</td>
<td>Vehicle</td>
<td></td>
<td>905.2 ± 63.6</td>
<td>0 ± 0</td>
<td>0</td>
<td>1.4 ± 1.4</td>
<td>6.3 ± 1.8</td>
<td>0</td>
</tr>
<tr>
<td>MGI-114</td>
<td>9</td>
<td>7 mg/kg i.p.</td>
<td>Daily × 5</td>
<td>47.8 ± 18.7</td>
<td>92.3</td>
<td>0</td>
<td>60.5 ± 7.9</td>
<td>25.6 ± 2.8</td>
<td>0</td>
</tr>
<tr>
<td>MGI-114</td>
<td>9</td>
<td>3.5 mg/kg i.p.</td>
<td>Daily × 5</td>
<td>318.2 ± 43.9</td>
<td>69.5</td>
<td>0</td>
<td>10.2 ± 3.1</td>
<td>18.8 ± 3.2</td>
<td>0</td>
</tr>
<tr>
<td>5FU</td>
<td>9</td>
<td>100 mg/kg i.p.</td>
<td>Days 1, 12, 19</td>
<td>484.4 ± 48.8</td>
<td>50.0</td>
<td>0</td>
<td>17.4 ± 3.2</td>
<td>32.0 ± 3.1</td>
<td>0</td>
</tr>
<tr>
<td>MGI-114 + 5FU</td>
<td>9</td>
<td>7 mg/kg i.p.</td>
<td>Daily × 5</td>
<td>633.4 ± 70.8</td>
<td>32.2</td>
<td>0</td>
<td>17.4 ± 3.2</td>
<td>32.0 ± 3.1</td>
<td>0</td>
</tr>
<tr>
<td>MGI-114 + 5FU</td>
<td>9</td>
<td>7 mg/kg i.p.</td>
<td>Days 1, 12, 19</td>
<td>13.1 ± 1.9</td>
<td>7 ± 1</td>
<td>1</td>
<td>77.5 ± 4.5</td>
<td>32.0 ± 3.1</td>
<td>1</td>
</tr>
<tr>
<td>MGI-114 + 5FU</td>
<td>9</td>
<td>3.5 mg/kg i.p.</td>
<td>Daily × 5</td>
<td>54.8 ± 12.4</td>
<td>96.1</td>
<td>5</td>
<td>58.1 ± 11.4</td>
<td>16.3 ± 2.7</td>
<td>0</td>
</tr>
<tr>
<td>MGI-114 + 5FU</td>
<td>9</td>
<td>7 mg/kg i.p.</td>
<td>Days 1, 12, 19</td>
<td>13.7 ± 0.4</td>
<td>9</td>
<td>0</td>
<td>75.8 ± 2.8</td>
<td>27.3 ± 1.8</td>
<td>0</td>
</tr>
<tr>
<td>MGI-114 + 5FU</td>
<td>9</td>
<td>3.5 mg/kg i.p.</td>
<td>Daily × 5</td>
<td>253.9 ± 42.2</td>
<td>77.2</td>
<td>0</td>
<td>10.6 ± 3.5</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* The term TGI does not apply here because all of the animals in this group experienced either death, PRs, or CRs.

** The term TGI does not apply here because all of the animals in this group developed PRs.

DISCUSSION

The illudins are a unique class of agents that are cytotoxic against a variety of refractory xenograft models (2, 22). Although the parent compounds produce substantial toxicity, the derivative MGI-114 has a favorable therapeutic index (1, 23). MGI-114 has previously demonstrated significant antitumor activity against HT29 human colon cancer (6), and this study explores the interaction of MGI-114 with 5-fluourouracil and CPT-11 in the HT29 xenograft model.

Before the commencement of combination studies, MGI-114, CPT-11, and 5FU were evaluated as single agents. Treatment with MGI-114 at 7 mg/kg produced 7 PRs at the expense of significant weight loss (25.6%). This activity was comparable to that previously seen with MGI-114 at doses of 6 mg/kg (TGI 68%, 1 CR) and 4 mg/kg (TGI 41%, no PRs or CRs; Ref. (6)). The activity of single agent CPT-11 and 5FU against the HT29 model was modest (TGI 32–50%, no PRs or CRs) as expected from previous studies (8, 12, 13).

The combination of MGI-114 and CPT-11 resulted in tumor reduction at all of the doses. At the highest dose level (MGI-114 7 mg/kg and CPT-11 100 mg/kg), all of the animals developed PRs, although this was associated with considerable weight loss. When the doses of both agents were halved, activity was maintained with reduced toxicity. The substantial activity of this low-dose combination was greater than predicted from the activity of low-dose MGI-114 and CPT-11 monotherapy. This augmented activity in vivo was consistent with the synergy demonstrated in vitro in pediatric tumor cell lines between MGI-114 and another camptothecin, topotecan (18).

One possible explanation for the enhanced activity of MGI-114 and
CPT-11 is an increase in apoptosis when the two agents are combined. CPT-11 inhibits topoisomerase I by trapping the enzyme and DNA, forming a ternary complex (15, 24). As DNA synthesis proceeds, this cleavable complex collides with an advancing replication fork, resulting in a double-strand break and subsequent apoptosis (15, 24). The activity of CPT-11 may not be entirely S-phase dependent, inasmuch as the camptothecins have been shown to induce apoptosis in terminally differentiated cells that are not synthesizing DNA (25). Apoptosis seems to be crucial to CPT-11 cytotoxicity, and studies in leukemia cells have demonstrated that CPT-11 cytotoxicity is dependent on the propensity of a cell to undergo apoptosis (26). MGI-114 may thus augment the activity of CPT-11 through its ability to induce apoptosis (4).

Cell cycle control may also be instrumental in producing the enhanced activity of CPT-11 and MGI-114 in combination. DNA strand breaks, such as those produced by CPT-11, activate wild-type p53 leading to either apoptosis or G1 arrest (27). The delay of entry into S phase after DNA damage allows for DNA repair. However, in the mutant p53 HT29 model, the potential for p53-dependent G1 arrest is lost, and other checkpoints become crucial for cell survival after DNA damage (28). At nanomolar concentrations, CPT-11 delays cell cycle progression through S phase, suggesting an S-phase checkpoint (29). Camptothecins also arrest cells in G2, an effect that has been attributed to cyclin B/cdc 2 kinase inactivation (30). The S-phase and G2 checkpoints have previously been implicated as determinants of CPT-11 activity in the HT29 model (28, 29), and MGI-114 may potentiate CPT-11 cytotoxicity by abrogating these checkpoints.

Alternatively, the enhanced activity of MGI-114 and CPT-11 in combination may result from decreased DNA repair. Illudin-induced DNA damage seems to be dependent on functional DNA helicase for repair (2, 3). Helicase disrupts the hydrogen bonds between the two strands of the DNA double helix, creating torsional tension. Topoisomerase may relax helicase-induced supercoiling by producing transient DNA strand breaks (31). As the two classes of enzymes may function in concert during DNA repair and replication (31), the direct inhibition of one class could result in decreased activity of the other. In this manner, the repair of illudin-induced DNA damage could be diminished by topoisomerase inhibitors.

Further delineation of the mechanism of action of MGI-114 should clarify the nature of the interaction between MGI-114 and CPT-11. Meanwhile, their super-additive activity presents a unique opportunity for the treatment of colon cancer. Previously, even the most active agents against this malignancy, including 5FU, CPT-11, and oxaliplatin, failed to produce CRs in nude mice bearing HT29 colon carcinoma xenografts (8, 12, 13). In the current study, combining a moderately effective dose of MGI-114 (3.5 mg/kg) with a relatively ineffective dose of CPT-11 (50 mg/kg) resulted in both PRs and CRs. This enhanced activity in the HT29 model awaits confirmation in clinical trials, and a phase I trial of MGI-114 and CPT-11 in combination is planned.
REFERENCES


Enhanced Antitumor Activity of 6-Hydroxymethylacylfulvene in Combination with Irinotecan and 5-Fluorouracil in the HT29 Human Colon Tumor Xenograft Model

Carolyn D. Britten, Susan G. Hilsenbeck, S. Gail Eckhardt, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/59/5/1049

Cited articles
This article cites 27 articles, 9 of which you can access for free at:
http://cancerres.aacrjournals.org/content/59/5/1049.full#ref-list-1

Citing articles
This article has been cited by 13 HighWire-hosted articles. Access the articles at:
http://cancerres.aacrjournals.org/content/59/5/1049.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.