In Vivo Antitumor Activity of Two New Seven-substituted Water-soluble Camptothecin Analogues

David L. Emerson, Jeffrey M. Besterman, H. Roger Brown, Michael G. Evans, Peter P. Leitner, Michael J. Luzzio, Joel E. Shaffer, Daniel D. Sternbach, David Uehling, and Alain Vuong

ABSTRACT

The development of camptothecin-like compounds as inhibitors of topoisomerase I for the treatment of resistant tumors has generated clinical excitement in this new class of drugs. We have developed two novel water-soluble camptothecin analogues which are specific inhibitors of topoisomerase I and are potent cytotoxins with significant antitumor activity. We added water-solubilizing groups off position 7 in the B ring of either 10,11-ethyleneoxy- or 10,11-methyleneoxy-20(S)-camptothecin. These water-soluble camptothecin analogues were demonstrated to be nanomolar inhibitors of the topoisomerase I enzyme in the cleavable complex assay. The compounds, GI147211 [7-(4-methylpiperazinomethyl)-10,11-ethylenedioxy-20(S)-camptothecin], and GI149893 [7-(4-methylpiperazinomethyl)-10,11-methyleneoxy-20(S)-camptothecin], were compared to topotecan, a known water-soluble inhibitor of topoisomerase I. Both GI compounds were found to be slightly more potent than topotecan as inhibitors of topoisomerase I in the cleavable complex assay and were 1.5–2 times more soluble. Tumor cell cytotoxicity assays using 5 separate cell lines demonstrated that both GI compounds were 5–10 times more potent than topotecan, although by comparison all three topoisomerase I inhibitors were unaffected by the multidrug resistance P-glycoprotein. The antitumor activity of all three topoisomerase I inhibitors was compared concomitantly in two human colon xenograft models. In both models, GI147211 and GI149893 were able to induce regression of established HT-29 and SW-48 colon tumors by as much as 60%. The antitumor activity of both compounds was also demonstrated in the MX-1 and PC-3 xenografts. Microscopic examination of selected tissues indicated that drug-induced toxicity was primarily limited to the gastrointestinal tract and was comparable among the three compounds. Further clinical development of this class of compounds is ongoing.

INTRODUCTION

The ability of DNA topoisomerase enzymes to facilitate the winding and unwinding of supercoiled DNA has led researchers to investigate the consequences of inhibiting these enzymes as an approach to the development of novel antitumor agents. The human topoisomerase I enzyme is a M, 100,000 monomeric protein encoded by a single copy gene located on chromosome 20. The topoisomerase I enzyme interacts with certain consensus sequences of DNA, catalyzing the complete relaxation of both positively and negatively supercoiled DNA without energy dependence (1). The mechanism by which topoisomerase I induces topological changes in DNA is apparently via a breakage-rejoining reaction involving single strand breaks accompanied by a covalently linked topoisomerase I-DNA complex. Upon completion of unwinding, the enzyme induces a rejoining of the DNA. Topoisomerase I inhibitors apparently interfere with the breakage-rejoining interaction and generate intermediate forms of drug-enzyme-DNA, “cleavable complexes.” Accumulation of such complexes may lead to cell cytotoxicity.

Inhibitors of topoisomerase I have demonstrated potent antitumor activity in both preclinical and clinical trials (2–7). Earlier studies with the potent topoisomerase I inhibitor, camptothecin, have indicated that inhibition of this enzyme leads to DNA breaks and eventual cell death. However, the early clinical studies with the sodium salt of camptothecin resulted in unacceptable toxicities in humans, including severe hemorrhagic cytisits and unpredictable myelosuppression which limited further development (8, 9). Further understanding of the mechanism of action and structure activity relationships of camptothecin allowed investigators to introduce A-ring modifications into the camptothecin backbone which greatly improved water solubility and reduced protein binding (10). There are currently two camptothecin analogues, topotecan and irinotecan, in early clinical development.

We have synthesized several analogues of camptothecin where water-solubilizing groups were introduced at position 7 of the B ring. These compounds were evaluated in several in vitro assays including a cell cytotoxicity and a cleavable complex assay, and antitumor activity was demonstrated using the HT-29 and SW-48 colon tumor xenograft model.

MATERIALS AND METHODS

Materials. Calf thymus topoisomerasases I and II were purified by column chromatography as described previously (11, 12). The ([γ-32P]ATP was purchased from New England Nuclear (Boston, MA) and pBR322 plasmid, proteinase K, and Klenow polymerase were obtained from Promega (Madison, WI). Autoradiography was performed with XAR-5 films (Eastman Kodak Company, Rochester, NY). The cell lines of HT-29 and SW-48 colon carcinoma and T47D breast carcinoma were obtained from the American Type Culture Collection (Rockville, MD), and the Lox melanoma cell line and the PC3 and MX-1 tumors were a kind gift from Southern Research Institute (Birmingham, AL). The SKO3V ovarian adenocarcinoma and SKVLB multidrug-resistant ovarian adenocarcinoma (13) were provided by Dr. V. Ling, Ontario Cancer Institute (Toronto, Ontario, Canada). The synthesis of camptothecin analogues have been reported previously (14, 15) (Table 2 for compound identification). The synthesis required either 4,5-methyleneoxy-α-chloroacetophenone or 4,5-ethylenedioxy-α-chloroacetophenone combined with the triyclic ketone which was synthesized previously by Wani et al. (16, 17), in an acid-catalyzed Friedlander condensation which lead to the 7-chloromethylcamptothecin derivatives. The respective chlorides were displaced with 4-methylpyrazine to provide the corresponding tertiary amines. The final compounds were isolated as their trifluoroacetate salts. All in vivo studies used female nu/nu mice from Harlan Sprague Dawley (Madison, WI).

Topoisomerase Inhibition Assays. The ability of camptothecin analogues to inhibit topoisomerasases I and II were quantified in the cleavable complex assays as described previously (18–20). All reactions were carried out in 10-μl volumes of reaction buffer (50 mM Tris-HCl, pH 7.5-100 mM KCl, 10 mM MgCl2, 0.5 mM EDTA-30 μg/ml BSA) in microtiter plates. For routine analysis of compounds, stock solutions of the camptothecin analogues were dissolved in DMSO at 10 mg/ml and serially diluted in 96-well microtiter plates to which the 32P-end-labeled pBR322 DNA and topoisomerase enzyme were added. The reaction mixture was incubated at room temperature for 30 min and then stopped by adding 2 μl of a mixture of sodium dodecyl sulfate and proteinase K (Boehringer Mannheim, Indianapolis, IN) (1.6% and 0.14 mg/ml final concentrations, respectively). The plates were heated at 50°C for 30 min and samples were then loaded onto agarose gels. For topoisomerase I assays 10 μl

Received 8/5/94; accepted 11/21/94.

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 Department of Pharmacology, Glaxo Research Institute, 5 Moore Drive, Research Triangle Park, NC 27709.
of 0.45 N NaOH were added to the stop mixture in order to generate single stranded DNA, and samples were electrophoresed in 1.5% agarose gel in Tris-buffered EDTA, while samples for topoisomerase II assays were electrophoresed in 1.1% agarose gels in Tris-buffered EDTA at 30 V overnight. Gels were blotted on nitrocellulose paper (Bio-Rad, Richmond, CA), dried, and exposed to X-ray film. The units of cleavage were calculated from the autoradiographs and plotted against the log drug concentration using the Nonlin84 software package from SCI Software (Lexington, KY). The IC_{50}^2 for each drug were determined from three separate topoisomerase-mediated cleavable complex experiments; values are expressed as mean ± SE.

**Solubility Determinations.** Compounds were assayed for solubility using a HPLC method which separated the lactone and carboxylate forms. A BDS Hypersil C_{18} column, 250 × 4.6 mm, 5 μm particle size, was used with a mobile phase typically consisting of 40% organic/60% aqueous with 0.005 M tetrabutylammonium phosphate added as a competing base. The solubilities were determined at pH 5.0 in 0.1 M sodium acetate buffer, by equilibrating an excess of solid compound in 0.25 ml buffer at 25°C for 24 h. The samples were filtered through a 0.22 μm Millipore (Milllex GY4) filter unit and injected on the column. Calibration curves were obtained by plotting the peak areas of standards as a function of drug concentration.

**Cell Culture Cytotoxicity Assays.** The cytotoxicities of compounds were determined using a microculture tetrazolium assay (21). All cell lines used for the cytotoxicity assay were grown under identical conditions in a-MEM containing 15% heat-inactivated fetal bovine serum, 2 mM L-glutamine, 1 mM sodium pyruvate, 0.1 unit/ml insulin, nonessential amino acids, and 0.5 mg/ml gentamicin (Gibco BRL, Grand Island, NY). All cell lines were grown at 37°C in an atmosphere of 5% CO_{2} in air. Tumor cells were plated in 96-well microtiter plates and allowed to adhere overnight. Cells were incubated with compound dissolved in culture media for 48 h, and then with fresh media for 48 h. The drugs were tested over a 0.17 nM to 10 μM range, in quadruplicate at each concentration tested. Following a 4-h incubation of treated cells with MTT, the reduced dye product was extracted from the cells with DMSO and quantitated spectrophotometrically. Assay data for each compound were fitted using a four-parameter logistic equation to obtain IC_{50}s.

**Xenograft Studies.** Female nu/nu mice (18–24 g, 10–14 weeks old) were obtained from Harlan Sprague Dawley, housed in microisolator filtration racks, and maintained with filtered acidified water and sterile laboratory chow ad libitum. Mice were allowed to acclimate to their new environment for 1 week prior to testing. Prior to initiating treatment of tumor-bearing animals with test compounds, a dose range study of compound was performed in naive mice to determine the highest dose for the different dose schedules used. The test compounds were dissolved in 0.1 M sodium acetate buffer, pH 5.0. The different dose schedules were: a single divided high dose with injections given over three 4-h intervals over the course of a single day (MTD3); and twice a week with test compounds, a dose range study of compound was performed in naive mice. Mice were allowed to acclimate to their new environment for 1 week. The animal body weight was monitored twice weekly. The average IC_{50} ± SE were determined for each compound in three separate experiments and were: GI149893, 0.23 ± 0.03 μM; GI147211, 0.42 ± 0.06 μM; topotecan, 1.02 ± 0.13 μM.

Table 1 Cytotoxicity of topoisomerase 1 inhibitors toward cultured tumor cells

<table>
<thead>
<tr>
<th>Tumor cell line</th>
<th>GI147211</th>
<th>GI149893</th>
<th>Topotecan</th>
<th>Doxorubicin</th>
</tr>
</thead>
<tbody>
<tr>
<td>SKOV3</td>
<td>15</td>
<td>4</td>
<td>44</td>
<td>3</td>
</tr>
<tr>
<td>SKVLB</td>
<td>99</td>
<td>6</td>
<td>149</td>
<td>873</td>
</tr>
<tr>
<td>Lox</td>
<td>1</td>
<td>0.5</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>T47D</td>
<td>40</td>
<td>27</td>
<td>100</td>
<td>44</td>
</tr>
<tr>
<td>HT-29</td>
<td>6</td>
<td>2</td>
<td>30</td>
<td>43</td>
</tr>
</tbody>
</table>

The data were plotted as the percentage of change in mean values of tumor volume and body weight for each group. The overall growth of tumors was expressed as a ratio of T/B. Thus any tumor group which did not respond to treatment and grew over the course of the experiment displayed a T/B ratio of >1, and treatment groups where tumors regressed displayed T/B ratios of <1. Tumor morphology was evaluated microscopically and compared between treatment groups. Selected tissues were also collected from terminally necropsied animals and paraffin sections were prepared and stained with hematoxylin and eosin for microscopic examination.

**RESULTS**

**Topoisomerase Inhibition and Tumor Cell Cytotoxicity.** The ability of GI147211, GI149893, and topotecan to inhibit calf thymus DNA topoisomerase I was determined in the cleavable complex assay as demonstrated in Fig. 1. All three compounds were demonstrated to be potent inhibitors of topoisomerase I. In addition, these compounds were selective for topoisomerase I compared to topoisomerase II (IC_{50} > 10,000 nm). Both GI147211 and GI149893 were more potent topoisomerase I inhibitors than topotecan as determined by their IC_{50}s.

The difference in potency...
ranged from a factor of 2.5 for GI147211 to a factor of 7 for GI149893. The cytotoxicity toward cultured human tumor cell lines was determined using the MTT microculture assay and are reported in Table 1. GI147211 is 3–5-fold more potent than topotecan and GI149893 was 3–10-fold more potent as well. However, all three topoisomerase I inhibitors demonstrated little susceptibility to multidrug resistance as determined by comparing the IC\textsubscript{50}s obtained using the MDR1-positive SKVLB cell line to that ob-

Fig. 2. Growth curve and body weight graphs of HT-29 tumor xenografts treated with GI147211, GI149893, and topotecan. The three compounds were dosed contemporaneously with a vehicle control group, twice weekly for 5 weeks. The initial mean tumor volume (mm\textsuperscript{3}) and SE (bars) for each group (n = 6) are: GI147211, 228.5 ± 31, 162 ± 18, 171 ± 20, and 169 ± 53 for the 2.1-, 4.2-, 6.3-, and 8.3-mg/kg dose groups, respectively; GI149893, 193 ± 28, 185 ± 41, 153 ± 19, and 194 ± 28 for the 1-, 2-, 3-, and 4-mg/kg dose groups, respectively; topotecan, 114 ± 15, 124 ± 23, 204 ± 39, and 218 ± 36 for the 4.6-, 6.5-, 8.3-, and 10.3-mg/kg dose groups, respectively; vehicle control group, 173 ± 24.
Table 2  Antitumor activity of topoisomerase I inhibitors toward human colon tumors

<table>
<thead>
<tr>
<th>Compound</th>
<th>Solubility (mg/ml) (pH = 5.0)</th>
<th>Efficacy (T/B)</th>
<th>BW loss* (%)</th>
<th>Dose (mg/kg)</th>
<th>Deaths</th>
<th>Regrowth (T/B at 4 wk)</th>
<th>Efficacy (T/B)</th>
<th>BW loss* (%)</th>
<th>Dose (mg/kg)</th>
<th>Deaths</th>
<th>Regrowth (T/B at 4 wk)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GI147211</td>
<td>5.77</td>
<td>5.5</td>
<td>8</td>
<td>3</td>
<td>0</td>
<td>3.7</td>
<td>15</td>
<td>3</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.6</td>
<td>5</td>
<td>6</td>
<td>0</td>
<td>1.4</td>
<td>17</td>
<td>6</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.8</td>
<td>7</td>
<td>9</td>
<td>0</td>
<td>0.9</td>
<td>9</td>
<td>9</td>
<td>0</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.4</td>
<td>22</td>
<td>12</td>
<td>2</td>
<td>0.6</td>
<td>12</td>
<td>12</td>
<td>0</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>GI149893</td>
<td>4.47</td>
<td>6.4</td>
<td>11</td>
<td>1</td>
<td>0</td>
<td>4.4</td>
<td>10</td>
<td>1</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.7</td>
<td>8</td>
<td>2</td>
<td>0</td>
<td>1.6</td>
<td>14</td>
<td>2</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.9</td>
<td>12</td>
<td>3</td>
<td>0</td>
<td>1.5</td>
<td>24</td>
<td>3</td>
<td>0</td>
<td>3.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.6</td>
<td>20</td>
<td>4</td>
<td>0</td>
<td>0.8</td>
<td>35</td>
<td>4</td>
<td>0</td>
<td>0.8</td>
<td></td>
</tr>
<tr>
<td>Topotecan</td>
<td>1.02</td>
<td>8.6</td>
<td>15</td>
<td>5</td>
<td>0</td>
<td>4.3</td>
<td>9</td>
<td>5</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>7</td>
<td>11</td>
<td>7</td>
<td>0</td>
<td>2</td>
<td>21</td>
<td>7</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.3</td>
<td>17</td>
<td>9</td>
<td>2</td>
<td>3.1</td>
<td>23</td>
<td>9</td>
<td>0</td>
<td>8.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.9</td>
<td>23</td>
<td>11</td>
<td>0</td>
<td>2</td>
<td>33</td>
<td>11</td>
<td>0</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Vehicle control</td>
<td>12.1</td>
<td>11% gain</td>
<td>0</td>
<td>&gt;18</td>
<td></td>
<td>20</td>
<td>5% gain</td>
<td>0</td>
<td>&gt;37.6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* BW, body weight.

In Vivo Efficacy of Topoisomerase I Inhibitors in Xenograft Models. The ability of all three topoisomerase inhibitors to effect the growth of the HT-29 and SW-48 human colon xenografts were assessed by monitoring tumor growth kinetics over a 5-week dosing period. Once drug dosing was complete, the two most efficacious dose groups were monitored for an additional 4 weeks in order to study the regrowth kinetics of the treated tumors. The body weight and tumor size were determined for each group on Mondays and Thursdays, and drugs were dosed every Tuesday and Friday. All three drugs were able to inhibit tumor growth in a dose-dependent fashion (Fig. 2). In contrast, both GI compounds were able to shrink tumors to less than 50% of their original size, whereas topotecan was only able to slow tumor growth relative to control (Table 2). To ensure an equal comparison, all compounds were dosed to the limit of toxicity as determined by body weight loss approaching 30%. Throughout the 5-week dosing period it was apparent that the maximum weight loss occurred within the first 2-3 weeks followed by no additional weight loss. However, the weight loss was reversible upon withdrawal of drug. After completion of the 10th dose of drug, one-half of the remaining animals in the highest dose group of each test compound were sacrificed and necropsied. The remaining animals were allowed to recover and further tissue samples were prepared from animals in the second highest dose group at day 12 post-drug treatment. The tissues which were analyzed for evidence of drug induced toxicity included tumor, brain, heart, lung, spleen, kidney, jejunum, ileum, colon, and pancreas. Microscopic evaluation of formalin-fixed hematoxylin and eosin-stained sections taken from control HT-29 tumors and tumors treated with GI147211 or GI149893 or topotecan revealed tumor cell necrosis and reduced cellularity of all treated tumors as compared to controls. Cellularity was most consistently reduced in the GI147211- and GI149893-treated tumors and persisted into a 12-day recovery period, whereas continued tumor necrosis was not observed with topotecan during the recovery period. Mild toxicity was noted in the small and large intestine of animals treated with all three compounds. The predominant finding was an increase in individual crypt or epithelial cell loss (apoptosis), mild inflammatory infiltrates, and a compensatory hyperplasia of the crypt epithelium. Additionally, cecal erosion or ulceration was noted in one topotecan-treated mouse (Fig. 3). The toxic differences seen between GI147211, GI149893, and topotecan are summarized in Table 3. The only other notable toxicity was a reduction of germinal centers in the spleen and minimal centrilobular hypertrophy in the livers of some treated mice. The overall effect on tumor necrosis and the ongoing inflammatory response in the large bowel and cecum were most notable in the mice examined.

Both GI147211 and GI149893 were evaluated in the 5-week HT-29 xenograft model a total of 4 times and in the 5-week SW-48 xenograft model twice, with similar results. The GI compounds were evaluated against two additional tumors, the MX-1 breast tumor and the PC-3 prostate tumor using the MTD3 dose schedule (Figs. 4 and 5). Both compounds demonstrated activity using the MTD3 dose schedule, with the MX-1 tumor being the more sensitive with complete regression relative to vehicle controls. These data clearly demonstrate that alternate dose schedules with these compounds confirm the antitumor
Fig. 3. Hematoxylin and eosin-stained tissue sections from xenograft studies. A, vehicle control-treated tumor demonstrating healthy appearance of confluent sheets of epithelial cells separated by thin bands of fibrovascular tissue. Note occasional mucus-secreting signet (s) cells, and rare acinus (a) formation. Arrowheads, mitotic figures with abnormal metaphase. Original magnification, × 400. B, GI147211-treated HT-29 tumor, demonstrating cell death and dissolution and replacement by amorphous (r) areas and fibrous connective tissue (f). Necrotic cells (n) undergoing nuclear pyknosis and karyorrhexis as well as cytolysis are found throughout the treated tumor. An apparent increase in the ratio of mucus-secreting signet (s) cells to nonmucus-producing cells is also seen. Original magnification, × 200. C, cecum with apoptotic bodies (a) in the neck of the crypt glands (g) and within the luminal epithelium from a nude mouse treated with GI147211. Original magnification, × 200. D, low power micrograph of a cecal erosion occurring in a nude mouse treated with topotecan. The area of erosion (e) is characterized by loss of glands and surface epithelium, lymphoid hyperplasia (arrowheads), and dilated vessels in the submucoa (d). Original magnification, × 45.

activity seen with the 5-week dose schedules using the colon tumor xenografts.

DISCUSSION

The purpose of this study was to evaluate the water-soluble topoisomerase I inhibitors in both in vitro enzyme assays and cytotoxicity assays and to compare their relative antitumor effectiveness in human colon xenograft models. The basic criteria for complete evaluation of synthesized compounds were dependent on the water solubility and topoisomerase I-inhibitory activity in the cleavable complex assay. Both the GI compounds described demonstrated superior solubility and enzyme-inhibitory activity (Table 2). Since topotecan is an active topoisomerase I inhibitor and active in vivo, we utilized it as a compound for comparison of our compounds. Both GI147211 and GI149893 were determined to be more potent inhibitors in the cleavable complex assay and considerably more soluble than topotecan. When assessed in the MTT cell cytotoxicity assay both GI compounds demonstrated significantly greater potency in 4 of the 5 cell lines tested compared to topotecan. All three topoisomerase I inhibitors were less potent against the MDR1+ SKVLB ovarian cell line but were less affected compared to doxorubicin, a clinically useful chemotherapeutic agent. Other studies have demonstrated similar differences in intracellular accumulation of topotecan in MDR1+ tumor cells (22, 23). Nevertheless, existing clinical data suggest that topoisomerase I inhibitors may prove to be useful in multidrug-resistant tumors (24). When the antitumor activities of GI147211 and GI149893 were compared to that of topotecan in two separate human colon tumor xenograft models, both GI compounds were able to induce tumor regression at the high dose whereas topotecan was able
The outstanding *in vitro* and *in vivo* antitumor activity reported for 10,11-methylenedioxy(20RS)-camptothecin focused our attention on this camptothecin derivative as a template for generating water-soluble camptothecin-based compounds (27–29). The 10,11-ethylenedioxy(20S)camptothecin series was investigated contemporaneously to circumvent possible metabolism problems encountered for the 10,11-methylenedioxy series. The 7 position of the B ring was demonstrated to be an accessible position for the addition of water-solubilizing groups, without significant loss of topoisomerase I-inhibitory activity. For these reasons, a number of derivatives of 10,11-methylenedioxy- and 10,11-ethylenedioxy camptothecin were prepared and evaluated for biological activity. Complete details of other derivatives prepared in these series will appear elsewhere.

The only known naturally occurring topoisomerase I inhibitor, camptothecin, was found to have potent antitumor activity in preclinical models and was progressed to clinical trials in the 1960s (30–32). However, due to the poor solubility of camptothecin in aqueous systems, the water-soluble sodium salt of the lactone hydrolysate of camptothecin was used in the clinic. Although some objective tumor responses were observed, unpredictable myelosuppression and hemorrhagic cystitis deemed the lactone hydrolysate to be too toxic for

to slow tumor growth only relative to control. The loss in animal body weight and number of toxic drug deaths was comparable among the three drugs tested; hence the differences seen in efficacy are not due to dose differences. The dose schedule of twice weekly for 5 weeks was chosen since previous studies with camptothecins have demonstrated that optimal efficacy was achieved with a low dose given over several weeks (2, 25). However, these were insoluble drugs formulated as suspensions and injected i.m., and thus cannot be compared with the same schedule and route of administration used for the water-soluble drugs. In fact, recent data have been presented that clearly demonstrate GI147211 to be more active when administered at higher doses using an every 4 days for a total of 3 doses schedule (26). The differences in *in vivo* efficacy between topotecan and GI147211 and GI149893 reported here may reflect dose schedule differences, and additional studies will address this issue. However, these data coincide with the relative differences seen in potency in the cleavable complex assay and the MTT assay. Additional efficacy testing of these drugs against multidrug-resistant tumors will be required to determine if any significant differences exist in drug susceptibility.
clinical use, and further development was halted (8, 9). Further interest in this structural chemical class was rejuvenated when the mechanism of action was determined in the early 1980s. The use of the cleavable complex assay together with in vitro cell cytotoxicity assays allowed structure activity studies to be performed on camptothecin analogues. The two analogues currently in clinical development, topotecan and irinotecan, are chemically quite different. Topotecan is a semisynthetic camptothecin analogue which incorporates a stable basic side chain at position 9 of the A ring of 10-hydroxycamptothecin. Irinotecan (7-ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyloxycamptothecin) is a synthetic prodrug analogue of camptothecin which requires hydrolysis to generate the active metabolite SN-38 (7-ethyl-10-hydroxycamptothecin) (33). We have generated a number of active camptothecin analogues by adding B-ring substituents off the 10,11-ethyleneoxy- or 10,11-methylenedioxy[20S]-camptothecin (14, 15). These compounds have proven to be potent, selective inhibitors of topoisomerase I and potent cytotoxins toward human tumor cells in vitro. In addition both GI147211 and GI149893 effectively caused inhibition or tumor regression of human colon tumor xenografts in nude mice. The overall toxicities of GI147211 and GI149893 are comparable to that of topotecan and also display significant antitumor activity. These results support the further development of these camptothecin analogues as chemotherapeutic agents for use in the treatment of cancer in humans.

ACKNOWLEDGMENTS

The authors gratefully acknowledge Dallas Croom, Francis Sun, Neil Jones, and Margaret McIntyre for their efforts with the xenograft studies; Gordon McIntyre, Joanne Bixler, and Julie Yates in performing MTT assays; Wei-Quin Tong and John Sisco for performing solubility studies; and Albert Cadogan for histology efforts. Furthermore, we thank Michael Peel for preparation of GI149893 and Francis Fang for synthesis of topotecan. We also thank Alan Menius for statistical analysis and James Gill and Eric Hallman for configuration of computer software used in data collection of xenograft studies.

REFERENCES

In Vivo Antitumor Activity of Two New Seven-substituted Water-soluble Camptothecin Analogues

David L. Emerson, Jeffrey M. Besterman, H. Roger Brown, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/55/3/603

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.