Marked Antitumor Activity of a New Potent Acronycine Derivative in Orthotopic Models of Human Solid Tumors

Nicolas Guilbaud,1 Laurence Kraus-Berthier, Florence Meyer-Loanic, Virginie Malivet, Christine Chacun, Michel Jan, François Tillequin, Sylvie Michel, Michel Koch, Bruno Pfeiffer, Ghanem Atassi, John Hickman, and Alain Pierré


ABSTRACT

S 23906-1 is a novel acronycine derivative selected on the basis of its potency in vitro. We investigated the antitumor activity of S 23906-1 against several murine transplantable tumors (C38 colon carcinoma, P388 leukemia, B16 melanoma, and Lewis lung carcinoma) and in orthotopic models of human lung (NCI-H460 and A549), ovarian (IGROV1 and NIH:OVCAR-3), and colorectal cancers (HCT116 and HT-29). Against established C38 colon carcinoma, S 23906-1 administered twice i.v. from 1.56–6.25 mg/kg markedly inhibited tumor growth. Treatment at the optimal dose (6.25 mg/kg) induced tumor regression in all of the mice. Acronycine was 16-fold less potent and only moderately active at the maximum tolerated dose, 100 mg/kg. Against other murine tumors of the former National Cancer Institute panel, S 23906-1 was either only moderately active or totally inactive. When evaluated in human orthotopic models, S 23906-1 given p.o. or i.v. demonstrated a marked antitumor activity against human carcinomas. In the two human lung cancer models, S 23906-1 increased the survival of the animals in a dose-dependent manner and induced treated versus control values of 162% (NCI-H460) and 193% (A549). Vinorelbine was less active, with treated versus control values of 119% and 174%, respectively. A significant survival benefit was also observed against the two i.p. ovarian tumors in which S 23906-1 was as active as paclitaxel, inducing 80% long-term survivors in the NIH:OVCAR-3 model. Lastly, S 23906-1 inhibited the growth of primary HT-29 and HCT116 colon tumors grafted onto the cecum as efficiently as irinotecan and eradicated the formation of lymph node, hepatic, and pulmonary metastases in the aggressive HCT116 model. The novel spectrum of activity of S 23906-1 compared with existing anticancer agents warrants further preclinical investigation.

INTRODUCTION

Cytotoxic drugs remain the mainstay of cancer chemotherapy and are being administered with novel types of therapy such as inhibitors of signaling. It is therefore important to discover novel cytotoxic agents with spectra of activity and toxicity that differ from those of current agents.

Acronycine is an acridone alkaloid that was first isolated from the bark of Acronychia baueri in 1948 (1). This cytotoxic agent with poor water solubility and low potency was subsequently found to be active in vivo against several murine experimental tumor models including Sarcoma 180 and AKR sarcomas, X-5563 myeloma, S-115 carcinoma, and S-91 melanoma (2). Based on this broad spectrum of activity against murine tumors that are considered today to be rather sensitive to chemotherapy (3), acronycine was formulated in oral capsules and evaluated in a limited Phase I-II clinical trial (4). In this study, one partial response was observed in a patient with multiple myeloma. However, the drug was associated with significant gastrointestinal and neurological toxicities (4). These observations, as well as more recent results (5, 6), confirmed that despite its low potency and the difficulties encountered for its formulation, acronycine exhibits moderate but interesting anti-tumor properties. Its precise mechanism of action has not yet been clearly identified at either the cellular or molecular level, although some experiments have suggested DNA binding properties for this alkaloid (5).

A new series of diester derivatives of 1,2-dihydroacronycine were designed recently to develop analogues with higher potency and improved antitumor activity (7, 8). One of the most potent analogues in these series, S 23906-1, was selected on the basis of an irreversible arrest in the S-phase of cell cycle accompanied by apoptosis (9) and a marked antitumor activity in vivo against murine C38 colon carcinoma. Interestingly, activity was less pronounced against P388 leukemia (8).

To complete the pharmacological evaluation of S 23906-1 on murine transplantable tumors, we investigated its antitumor activity against B16 melanoma and Lewis lung carcinoma. We found these two tumors to be resistant to S 23906-1 in contrast to colon C38. However, the use of the murine tumors of the NCI2 panel has recently been questioned due to their apparent...
MATERIALS AND METHODS

Mice and Tumor Models. B6D2F1 (C57Bl/6 × DBA2) mice were used in murine tumor models. Nude female congenic athymic mice of Swiss and BALB/C strains homozygous for the nude gene (nu/nu) or CB 17 SCID mice (scid/scid) were used in human orthotopic tumor models. They were 4–6 weeks old and weighed 18–20 g at the start of the experiments. Mice received proper care and maintenance in accordance with institutional guidelines. All murine tumors were provided by the Division of Cancer Treatment, Tumor Repository, NCI (Frederick, MD). Human tumor cell lines of lung (NCI-H460 and A549), ovarian (IGROV1 and NIH:OVCAR-3), and colorectal cancers (HCT116 and HT-29). S 23906-1 exhibited better activity against these human metastatic tumors than it did against the classical murine tumors. When administered i.v., acronycine itself was found to be marginally active at a higher dosage. In contrast, S 23906-1 was at least as efficient as the best reference anticancer drugs in the orthotopic models (vinorelbine, paclitaxel, and irinotecan in lung, ovarian, and colon cancers, respectively). Lastly, considering the potential of the oral route for antitumor agents in the clinic, we investigated this mode of administration for S 23906-1.

Evaluation of Antitumor Activity. Mice bearing s.c. implanted colon C38 adenocarcinoma were treated 11 days after the graft, when tumors were established. Tumors were measured twice a week and tumor volumes \( V_t \) were calculated using the following formula: length (mm) × width ² (mm ²)/2. The relative tumor volume was expressed as the \( V_t/V_0 \) index, where \( V_t \) is the tumor volume on a given day of measurement, and \( V_0 \) is the volume of the same tumor at the start of the treatment. Results are expressed as median T/C; median relative tumor volume of treated animals/median relative tumor volume of control animals × 100. The therapeutic index of a compound given following a definite schedule and route of administration, the MTD was defined as the highest dose determined on the basis of a body weight loss of <20% and an absence of early death. In each experiment, S 23906-1 was administered at three dose levels MTD, MTD/2, and MTD/4. The optimal dose was defined as the most active nontoxic dose.

Drugs. Irinotecan (CamptoR) was provided by Bellon (Aventis, France), vinorelbine (NavelbineR) was provided by Pierre Fabre Oncology (Bouloge, France), and paclitaxel was provided by Sigma Chemical Co. (L’Isle d’Abeau Chesnes, France). Immediately before administration, irinotecan and vinorelbine were dissolved in sterile distilled water at concentrations corresponding to the required dose administered in a volume of 0.1 ml/10 g body weight. Paclitaxel was first suspended at 80 mg/ml in ethanol, and 1 volume of suspension was mixed with 1 volume of polyoxy ethylated castor oil (chremophor EL; Sigma Chemical Co.) and then diluted in sterile distilled water as described previously. Acronycine and S 23906-1 were dissolved in DMSO (final concentration, 3.5%) and then diluted in saline containing 6.5% Tween 80. For oral administration, which necessitates higher drug concentrations, S 23906-1 was prepared as a suspension in 10% Solutol (17) in sterile water. Drugs were administered i.v. or p.o. to tumor-bearing mice following the indicated schedules. The lack of toxicity and/or antitumor activity of the corresponding vehicle was verified under similar experimental conditions. The doses and the schedules of administration of reference drugs were based on previous studies (10, 11). An optimal 10-day rest between two administrations of S 23906-1 was chosen, based on preliminary toxicity studies (data not shown). For a compound given following a definite schedule and route of administration, the MTD was defined as the highest dose determined on the basis of a body weight loss of <20% and an absence of early death. In each experiment, S 23906-1 was administered at three dose levels MTD, MTD/2, and MTD/4. The optimal dose was defined as the most active nontoxic dose.

Effect of S 23906-1 on Tumor Growth and Dissemination. The percentage of animals for which the tumor was successfully implanted, when tumors were established. Tumors were measured twice a week and tumor volumes \( V_t \) were calculated using the following formula: length (mm) × width ² (mm ²)/2. The relative tumor volume was expressed as the \( V_t/V_0 \) index, where \( V_t \) is the tumor volume on a given day of measurement, and \( V_0 \) is the volume of the same tumor at the start of the treatment. Results are expressed as median T/C; median relative tumor volume of treated animals/median relative tumor volume of control animals × 100. The therapeutic index of a compound given following a definite schedule and route of administration, the MTD was defined as the highest dose determined on the basis of a body weight loss of <20% and an absence of early death. In each experiment, S 23906-1 was administered at three dose levels MTD, MTD/2, and MTD/4. The optimal dose was defined as the most active nontoxic dose.

For orthotopic colon models, tumor-bearing animals were sacrificed on day 29 (HT-29) or on day 37 (HCT116). Tumors were removed from the cecum and weighed. Organs and tumors were fixed and embedded in paraffin. Sections of 5 µm were stained with hematoxylin and fixed for microscopic evaluation, which was subsequently performed in a blind manner by an anatomopathologist. The percentage of animals for which the cecum submucosa had been invaded by the tumor was determined in each group, and the results were expressed as follows: 
\( a \) +, <50%; (b) + +, 50–75%; and (c) ++ +, >75%.

For lung and ovarian orthotopic tumor models, mice were
treated when the tumors were established as indicated. In these models, as well as in the murine P388 leukemia, B16 melanoma, and Lewis lung carcinoma, mice were sacrificed by CO₂ inhalation when they became moribund, and the day of sacrifice of the animal was considered its survival time. The MST (in days) of each group was calculated, and results were expressed in terms of both the percentage T/C value and the number of LSTs. Median percentage of T/C = (MST of treated group/MST of control group × 100), and LSTs were defined as surviving animals sacrificed at the end of the experiment for which no tumor could be detected by macroscopic examination.

For the measurement of serum tumor marker on day 35 of the experiment, control animals or animals bearing NIH:OVCAR-3 tumors were anesthetized, and blood samples were obtained by jugular vein puncture. CA125 serum levels were determined using an immunoassay (Boehringer Mannheim, Meylan, France; Ref. 18).

Statistical Methods. A comparison of the survival curves between each treatment and control group was performed with a log-rank test. The significance threshold was 5%. If several doses of treatment were tested, a log-rank test was assessed on the overall treatment (all doses and the control). Then, if the log-rank χ² was significant (P ≤ 0.05), the pairwise comparisons of each dose to the control were done with a log-rank test, followed by a Holm’s adjustment to control the overall risk at 5%.

The effect of the reference drug on the tumor weights was compared with that of the control by using Student’s t test. If several doses of treatment were tested, a one-way ANOVA was assessed, followed, in case of significance of the overall analysis, by a Newman-Keuls test for pairwise comparisons. All significance thresholds were 5%.

RESULTS
Murine Tumor Models. The antitumor activity of S 23906-1 was first evaluated against the s.c. implanted murine colon C38 adenocarcinoma, a model that was previously found to be sensitive to acronycine and its derivatives (6–8). As can be seen in Fig. 1, acronycine administered i.v. to mice 11 days and 21 days after the graft of tumor fragments inhibited tumor growth at only one dose, 100 mg/kg, which is its MTD, and induced a T/C value of 29% on day 36. Under the same conditions, S 23906-1 was much more potent and demonstrated a marked antitumor effect because when it was administered i.v. from 1.56–6.25 mg/kg, it induced T/C values of 13% and 1%, respectively. S 23906-1 was superior to the reference drug 5-fluorouracil administered at the optimal dose, 80 mg/kg, which achieved a T/C value of 25%. Moreover, in the group of six mice treated with 6.25 mg/kg S 23906-1, two complete and three partial tumor regressions were induced, which lasted for the duration of the experiment (43 days). These results reflected the better efficiency and the wider therapeutic index of S 23906-1 as compared with acronycine, which was 16-fold less potent in vivo and only effective at the MTD. It should be noted that the striking antitumor effects of S 23906-1 following this schedule were associated with dose-dependent weight losses that were maximum 3 days after the second treatment and represented 12% of the weight of mice treated at 6.25 mg/kg. A complete recovery of body weight was observed 10 days after administration of the drug.

When tested in other murine tumor models, S 23906-1 was only moderately active against the P388 leukemia, inducing a 78% increase of survival of the animals at 12.5 mg/kg (Table 1), which is the MTD for one i.v. dose; the dose of 6.25 mg/kg was inactive (data not shown). Against the B16 melanoma and Lewis lung carcinoma tumors, S 23906-1 was devoid of antitumor activity following a two-administration schedule (Table 1).

Human Orthotopic Tumor Models. The antitumor efficacy of S 23906-1 was then investigated in models that had been designed in our laboratory to mimic the development of human cancers and measure their response to chemotherapy (Table 2). Among the clinically used anticancer agents that were previously evaluated against ovarian carcinoma models, paclitaxel was the most efficient in prolonging the survival of IGROV1 or NIH:OVCAR-3 tumor-bearing mice (11). In the IGROV1 tumor model, S 23906-1 administered at 6.25 mg/kg in an intermittent schedule consisting of two i.v. administrations separated by a 10-day rest induced a T/C of 193% and was as active as paclitaxel (T/C = 199%), whereas acronycine at 100 mg/kg was only slightly active (T/C = 120%). When compared in NIH:OVCAR-3 ovarian carcinoma, in which disease development is less rapid than that in the IGROV1 model, S 23906-1 and paclitaxel administered at their MTDs in an intermittent schedule again demonstrated a comparable antitumor efficacy and resulted in 80% LSTs (four of five mice; Table 2; Fig. 2). Interestingly, these results were reflected in the levels of serum CA125 in tumor-bearing animals on day 35. The mean value was 2979 units/ml (range, 1205–5400 units/ml) for control mice, whereas the corresponding mean values for S 23906-1 (6.25 mg/kg)- and paclitaxel-treated mice were 1.2 units/ml (range, 1.0–1.6 units/ml) and 1.9 units/ml (range, 0.1–5.9 units/ml), respectively. Serum CA125 levels decreased with S 23906-1 treatment in a dose-dependent manner, corresponding with the observed increase in survival.
Preclinical Evaluation of a New Acronycine Derivative

Antitumor activity of S 23906-1 was also noted in two metastatic models of human non-small cell lung carcinoma, NCI-H460 large cell carcinoma and A549 adenocarcinoma, established by injecting tumor cells in the pleural space of nude mice (Table 2). In these experiments, treatment was initiated when the disease was established (on day 6 for NCI-H460, when mediastinal invasion could be observed; and on day 14 for A549, when tumor nodules could be detected on the lungs of the animals [12]). Against NCI-H460 and A549, S 23906-1 administered at 6.25 mg/kg significantly enhanced the survival of the mice, inducing T/C values of 162% and 193%, respectively. However, treatment did not result in any LTS. Under the same experimental conditions, vinorelbine at 10 mg/kg was significantly more active, with a T/C of 191%, and was significantly superior to S 23906-1 administered at the MTD (P < 0.005).

Two models of human orthotopic colon cancers in nude mice were designed to evaluate the growth inhibitory efficacy of S 23906-1 and its effect on the local invasion of colon tumors as well as the formation of distant metastases. For this purpose, tumor tissues prepared from s.c. growing HT-29 and HCT116 tumors were surgically grafted onto the cecum serosa of anesthetized immunodeficient mice.

The suture of HT-29 tumor fragments onto the cecum of sutured immunodeficient mice led to the development of a primary tumor that progressively invaded the different layers of the cecal wall and metastasized to the lymph nodes, but rarely metastasized to other organs such as the liver or the lungs (data not shown). Forty days after the graft, the animals were necropsied, and the

### Table 1  Antitumor activity of S 23906-1 in murine models

<table>
<thead>
<tr>
<th>Cancer model</th>
<th>Tumor/site of implantation</th>
<th>Route</th>
<th>Schedule (days)</th>
<th>Optimal dose (mg/kg)</th>
<th>T/C (%)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>LTS&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Score&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukemia</td>
<td>P388/f.p.</td>
<td>i.v.</td>
<td>1</td>
<td>12.5</td>
<td>178</td>
<td>0</td>
<td>+&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Lung</td>
<td>Lewis lung carcinoma/s.c.</td>
<td>i.v.</td>
<td>3 and 13</td>
<td>6.25</td>
<td>115</td>
<td>1/7</td>
<td>−&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Melanoma</td>
<td>B16/f.p.</td>
<td>i.v.</td>
<td>2 and 12</td>
<td>6.25</td>
<td>108</td>
<td>0</td>
<td>−&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>Colon</td>
<td>C388/s.c.</td>
<td>i.v.</td>
<td>11 and 21</td>
<td>6.25</td>
<td>1&lt;sup&gt;g&lt;/sup&gt;</td>
<td>0/5</td>
<td>+&lt;sup&gt;h&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Results from survival studies, except tg (tumor growth).
<sup>b</sup> LTSs scored on day 60 (P388) or day 90 (Lewis lung carcinoma and B16).
<sup>c</sup> NCI score criteria (19).
<sup>d</sup> T/C ≥ 175, no LTS.
<sup>e</sup> T/C < 125.
<sup>f</sup> T/C < 125.
<sup>g</sup> T/C < 10%.
<sup>h</sup> T/C < 10%.

### Table 2  Antitumor activity of S 23906-1 administered by i.v. route in human orthotopic models

<table>
<thead>
<tr>
<th>Model</th>
<th>Tumor/site of implantation</th>
<th>MST (range) (days)</th>
<th>Drug</th>
<th>Schedule</th>
<th>Optimal dose (mg/kg)</th>
<th>T/C (%)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>P&lt;sup&gt;α&lt;/sup&gt;</th>
<th>LTS&lt;sup&gt;β&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ovary</td>
<td>IGROV1/f.p.</td>
<td>31 (29–32)</td>
<td>S 23906-1</td>
<td>days 3 and 13</td>
<td>6.25</td>
<td>193</td>
<td>0.002</td>
<td>0/5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Acronycin&lt;sup&gt;c&lt;/sup&gt;</td>
<td>days 3 and 13</td>
<td>100</td>
<td>120</td>
<td>0.002</td>
<td>0/5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Paclitaxel</td>
<td>days 3, 13, and 20</td>
<td>40</td>
<td>199</td>
<td>0.03</td>
<td>0/5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>S 23906-1</td>
<td>days 7 and 16</td>
<td>6.25</td>
<td>&gt;371</td>
<td>0.005</td>
<td>4/5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Paclitaxel</td>
<td>days 7, 16, and 26</td>
<td>40</td>
<td>&gt;371</td>
<td>0.002</td>
<td>4/5</td>
</tr>
<tr>
<td>NIH:OVCA-3/</td>
<td></td>
<td>48.5 (38–65)</td>
<td>S 23906-1</td>
<td>days 6 and 16</td>
<td>6.25</td>
<td>162</td>
<td>0.001</td>
<td>0/6</td>
</tr>
<tr>
<td>f.p.</td>
<td></td>
<td></td>
<td>Acronycin&lt;sup&gt;c&lt;/sup&gt;</td>
<td>days 6 and 16</td>
<td>100</td>
<td>107</td>
<td>0.04</td>
<td>0/6</td>
</tr>
<tr>
<td>Lung</td>
<td>NCI-H460/intrapleural</td>
<td>27 (25–28)</td>
<td>S 23906-1</td>
<td>days 6 and 13</td>
<td>10</td>
<td>119</td>
<td>0.005</td>
<td>0/6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Vinorelbine</td>
<td>days 6 and 13</td>
<td>10</td>
<td>119</td>
<td>0.005</td>
<td>0/6</td>
</tr>
<tr>
<td>A549/intrapl</td>
<td></td>
<td>37 (24–47)</td>
<td>S 23906-1</td>
<td>days 14 and 21</td>
<td>6.25</td>
<td>193</td>
<td>0.005</td>
<td>0/5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Vinorelbine</td>
<td>days 14, 21, and 28</td>
<td>10</td>
<td>174</td>
<td>0.002</td>
<td>0/5</td>
</tr>
</tbody>
</table>

<sup>α</sup> The survival of each treatment group was compared with that of the control group by the log-rank test.
<sup>β</sup> LTSs scored on day 120 (IGROV1 and NCI-H460) or day 180 (NIH:OVCA-3 and A549).
<sup>c</sup> S 23906-1, acronycin, paclitaxel, and vinorelbine were administered i.v.
presence of tumors within the cecum and peritoneal carcinomatosis could be reproducibly observed in a majority of untreated
animals, and the mean value of the tumor weights was 1.24 g in
this experiment (Table 3). Moreover, the presence of lymph
node metastases was observed by microscopic analysis in every
untreated animal. After treatment with S 23906-1 at 3.12 mg/kg
i.v., a 56% decrease in mean tumor weight was observed com-
pared with controls (\(P\) \(<\) 0.001). This effect was comparable to
that obtained with the reference drug, irinotecan, administered at
40 mg/kg i.v. (61% inhibition). The two drugs at their MTDs
similarly affected both peritoneal dissemination and the forma-
tion of distant metastases, inducing a 50% decrease in lymph
node secondary lesions as compared with the controls.

In the HCT116 model, tumor fragments grafted into SCID
mice resulted in highly invasive tumors that metastasized to indi-
vidual organs such as the liver, lungs, and mesenteric lymph nodes.
On day 37 of the experiment, the weights of the tumors resected
from animals treated with 3.12 mg/kg S 23906-1 were inhibited by
83% compared with controls, whereas those treated with 40 mg/kg
irinotecan were inhibited by 63%. A statistical analysis was per-
formed and showed that the tumor growth inhibition caused by
S 23906-1 was significantly greater than that caused by irinotecan
(\(P\) \(<\) 0.001). The histological analysis indicated a similar rank
order of efficiency of the two drugs in terms of tumor dissemina-
tion. Evaluation of the number of metastatic foci as well as the
incidence of peritoneal carcinomatosis showed that S 23906-1 at
3.12 mg/kg totally inhibited local tumor invasion and the formation
of metastases in the liver, lungs, and lymph nodes as opposed to
irinotecan, which was less efficient in this very aggressive colon
cancer model (Fig. 4; Table 3).

DISCUSSION
S 23906-1 was selected from a new series of diester deriv-
atives of 1,2-dihydroacronycine recently optimized by the ad-
dition of an aromatic ring fused to the acronycine skeleton (8).
Table 3 Antitumor activity in orthotopic models of human colon cancer

<table>
<thead>
<tr>
<th>Tumor model</th>
<th>Drug</th>
<th>Dose (mg/kg)</th>
<th>Schedule</th>
<th>Mean tumor weight (range) (grams)</th>
<th>Invasion of cecum submucosa</th>
<th>Lymph node metastasis</th>
<th>Hepatic metastasis</th>
<th>Lung metastasis</th>
<th>Abdominal carcinomatosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>HT-29</td>
<td>S 23906-1</td>
<td>1.56</td>
<td>Days 7 and 17</td>
<td>1.24 (0.83–1.71)</td>
<td>+ + +</td>
<td>7/7</td>
<td>ND</td>
<td>ND</td>
<td>7/8</td>
</tr>
<tr>
<td></td>
<td>S 23906-1</td>
<td>3.12</td>
<td>Days 7 and 17</td>
<td>0.79 (0.44–1.46)</td>
<td>&lt;0.01</td>
<td>+ + +</td>
<td>5/6</td>
<td>ND</td>
<td>4/6</td>
</tr>
<tr>
<td></td>
<td>Irinotecan</td>
<td>40</td>
<td>Days 10–14</td>
<td>0.55 (0.43–0.66)</td>
<td>&lt;0.001</td>
<td>+</td>
<td>3/6</td>
<td>ND</td>
<td>3/6</td>
</tr>
<tr>
<td>HCT116</td>
<td>S 23906-1</td>
<td>1.56</td>
<td>Days 7 and 17</td>
<td>0.48 (0.34–0.77)</td>
<td>&lt;0.001</td>
<td>+</td>
<td>3/6</td>
<td>ND</td>
<td>1/6</td>
</tr>
<tr>
<td></td>
<td>S 23906-1</td>
<td>3.12</td>
<td>Days 7 and 17</td>
<td>0.29 (0.08–0.40)</td>
<td>&lt;0.01</td>
<td>+</td>
<td>2/6</td>
<td>1/6</td>
<td>0/5</td>
</tr>
<tr>
<td></td>
<td>Irinotecan</td>
<td>40</td>
<td>Days 10–14</td>
<td>0.64 (0.49–0.84)</td>
<td>&lt;0.05</td>
<td>+ + +</td>
<td>3/6</td>
<td>0/6</td>
<td>2/6</td>
</tr>
</tbody>
</table>

On day 0, HT-29 or HCT116 tumor fragments were implanted onto the cecum of Swiss or SCID mice, respectively. S 23906-1 and irinotecan were administered i.v. On day 40 (HT-29) or on day 37 (HCT116), animals were sacrificed, and the tumors were excised and weighed. P is calculated for tumor weight in treated versus control animals. Histological analysis was performed. +, ≥50% of tumor-bearing mice with cecal submucosa invasion; + +, 50–75% of tumor-bearing mice with cecal submucosa invasion; + + +, ≥75% of tumor-bearing mice with cecal submucosa invasion; ND, not determined.

The results presented here demonstrate that S 23906-1 exhibits markedly improved antitumor properties as compared with acronycine, the parent compound, in terms of potency, activity, and therapeutic index. We confirmed the previously reported (6) low potency and weak antitumor activity of acronycine in one murine model and two human orthotopic models. Effectively, this compound, administered at 100 mg/kg, was only moderately active against colon C38 adenocarcinoma and was marginally active against IGROV1 and NCI-H460 solid tumors grafted orthotopically in nude mice. In contrast, S 23906-1 was at least 16-fold more potent in vitro than acronycine and displayed a wider therapeutic index against the colon C38 adenocarcinoma. A similar difference in cytotoxicity was found in vivo, with S 23906-1, on average, 24-fold more potent than acronycine when evaluated on five tumor cell lines (9). Surprisingly, S 23906-1 proved to be either only moderately active or inactive against other murine tumors of the former NCI panel (e.g., P388 leukemia, B16 melanoma, and Lewis lung carcinoma), whereas it was found to be at least as active as clinically used drugs in a panel of six orthotopic models of human ovarian, lung, and colon cancers. These striking differences in sensitivity of murine and human cancer cells in vivo confers a novel spectrum of activity for this drug as compared with existing antitumor agents. It is not clear to date whether it could be due to a higher sensitivity of the human tumor cells as compared with their murine counterparts because the results obtained using classical proliferation assays did not show any clear differences in this respect (9). However, recent results obtained in our laboratory with a clonogenic assay after a short-duration exposure (1 h) of the tumor cells to S 23906-1 indicated that the cytotoxicity measured with such an assay could be more predictive for its antitumor activity. Experiments aimed at comparing the sensitivity of human versus murine murine cancer cells with the clonogenic assay are now in progress. The marked effects observed in vivo after only two i.v. administrations of S 23906-1 corroborate these results and suggest that repeated administrations are not necessary to obtain an optimal effect of the cytotoxic drug against solid tumors.

The precise molecular mechanism of action of S 23906-1 has not yet been elucidated. Cell cycle analysis by flow cytometry has clearly demonstrated that this drug acts through irreversible inhibition of DNA synthesis followed by apoptotic cell death (9). This inhibition of DNA synthesis is also effective in vivo and could be responsible for the antitumor effects of S 23906-1 in orthotopic models because a significant decrease of bromodeoxyuridine incorporation has been measured in tumors 24 h after its administration by i.v. route to animals (data not shown). Ongoing biochemical investigations as well as comparison of the in vivo antitumor profile of S 23906-1 with that of other antitumor agents will help to define more precisely the novel mechanism of action of this cytotoxic compound.

Recent reviews on anticancer drug evaluation methods have emphasized the pertinence of models of human cancers that more accurately represent the human diseases and could thus help to predict the activity of investigational drugs in the clinic (13, 14). We have developed human orthotopic models that can be considered more realistic when compared with s.c. xenografts because they exhibit several important characteristics of metastatic disease (11, 12). In these models, treatments were initiated several days after tumor transplantation, corresponding to the time at which an established disease could be identified by histological analysis. Moreover, we have used clinical end points complementary to the simple measure of tumor growth to assess the effect of investigational drugs on the progression of the disease and to compare their antitumor activity with that of clinically used anticancer drugs. These end points include the dosage of tumor markers such as serum levels of CA125 in treated animals, whose decrease reflects the response to chemotherapy in our models and in the clinic (11, 20), the increase in survival of treated animals, and the inhibition of metastasis development in organs that are actually affected in the human pathology. The analysis of all these parameters clearly demonstrates the potential of S 23906-1 in orthotopic models of human cancers. In the two lung cancer models, it significantly increased the survival of animals in a dose-dependent manner and compared favorably with vinorelbine, one of the last compounds to be developed for the treatment of non-small cell lung cancers (21, 22). Against two ovarian tumors, a significant survival benefit for the animals was also observed, and S 23906-1 was at least as active as paclitaxel, which is currently one of the two most active drugs for the treatment of this disease (23). The fact that S 23906-1 and paclitaxel, despite a likely different mech-
anism of action, exhibit the same pattern of activity against the two ovarian tumors is particularly interesting, and experiments are under way to confirm this observation with other ovarian xenografts. Lastly, S 23906-1 markedly inhibited the growth of primary tumors in two orthotopic colon models and eradicated the formation of lymph node, hepatic, and pulmonary metastases in the aggressive HCT116 model. Because metastatic spread of colon adenocarcinoma to these organs is frequently seen in human disease, these results are again very encouraging for further development of S 23906-1.

Recently, the oral route for administration of cytotoxic agents has received considerable attention (24). However, several limitations, such as low bioavailability of drugs and inter-patient/intrapatient variability, often preclude its current use in the clinic. The fact that a marked antitumor activity of S 23906-1 was maintained on oral administration at doses 2-fold or 4-fold higher than those used for i.v. administration suggests a relatively good bioavailability. A further argument for possible oral administration of S 23906-1 is that this drug is still active against pure multidrug-resistant tumor cell lines and is therefore probably poorly recognized by P-glycoprotein (9), a membrane protein being known to limit the intestinal absorption of drugs (25).

Despite all these desirable properties, e.g., in vivo potency, apparent selectivity against human tumors, and marked antitumor activity by oral route, the determination of the benefit:risk ratio of S 23906-1, as for any new cytotoxic agent, will help to determine the clinical potential of this molecule. Experiments

Fig. 4  Histological analysis of HCT116 tumor-bearing animals treated with S 23906-1 i.v. on days 7 and 16 at 3.12 mg/kg (b, d, and f) or control (a, c, and e). a and b, (×40) mesenteric lymph node; arrow, metastasis. c and d, (×40) liver; arrow, metastasis. e and f, (×100 and ×40) lung; arrow, metastasis.
are now being conducted to examine the toxicological profile and the pharmacokinetic parameters of S 23906-1 to complete its preclinical evaluation. The data obtained from these studies will be of particular importance to consider an eventual development of this new anticancer agent in the clinic.

In conclusion, our results demonstrate that S 23906-1 is a new acronicine derivative with very impressive broad antitumor efficacy when evaluated on aggressive orthotopic models of human ovarian, lung, and colon cancers, with comparable and/or better activity than clinically used anticancer drugs. Moreover, a marked antitumor activity was maintained after oral administration, supporting further preclinical development of an oral formulation of S 23906-1.

ACKNOWLEDGMENTS

We are grateful to Dr. Jean-François Boivin for clear histological interpretation and Dr. Catherine De Montrieu and colleagues for determination of serum CA125 marker and preparation of histological slides. The skillful technical assistance of Marie-Hélène Rouillon, Muriel Bernigole, and Michael Burbridge in pharmacological studies is also acknowledged.

REFERENCES
