Phase I Trial of Intraperitoneal Iododeoxyuridine with and without Intravenous High-Dose Folinic Acid in the Treatment of Advanced Malignancies Primarily Confined to the Peritoneal Cavity: Flow Cytometric and Pharmacokinetic Analysis


ABSTRACT

In this Phase I study, the maximally tolerated doses (MTDs) of i.p. iododeoxyuridine (IdUrd) alone and in combination with i.v. calcium leucovorin (LV) were determined. The pharmacokinetics and pharmacological advantage of IdUrd were evaluated, and flow cytometric analysis allowed examination of the extent of incorporation of IdUrd into tumor cells with and without the addition of i.v. LV. Thirty-nine patients with advanced neoplasms primarily confined to the peritoneal space were enrolled in a dose-escalation trial using 4-h dwells of IdUrd administered i.p. daily for 4 days with and without an i.v. infusion of LV 500 mg/m²/day for 4.5 days. Twenty-three patients received single-agent therapy, and 13 patients received IdUrd in combination with LV. The MTD of single-agent IdUrd administered on this schedule was 4125 mg/m²/day for 4 days; and that of the IdUrd in combination was 3438 mg/m²/day. Dose-limiting toxicities were myelosuppression and stomatitis. During the period of the dwell, the peritoneal AUC (area under the curve) of IdUrd exceeded the plasma AUC of IdUrd by one or two orders of magnitude in all patients at all doses tested; there was a possible effect of LV on peritoneal AUC. The geometric mean pharmacological advantage (AUCₚ/AUCₚᵣ) was 181 at 625 mg/m²/day and 90 at 4538 mg/m²/day. Flow cytometric analysis suggests saturation of IdUrd measured in DNA at the 2500–3125 mg/m² dose level, without an increase after the addition of LV. Twelve patients received 4–12 courses of therapy. One patient with recurrent ovarian cancer who received 16 courses of therapy experienced complete resolution of her ascites, near normalization of CA-125 levels, and improved quality of life; two patients with high-risk tumors receiving “adjuvant” therapy are disease-free at 3 and 6 years after treatment; other patients experienced transient clearing of ascites. The recommended Phase II dose of i.p. IdUrd using a 4-h dwell daily for 4 days is 3750 mg/m²/day alone or 3125 mg/m²/day in combination with continuous i.v. LV at 500 mg/m²/day for 4.5 days. Although flow cytometric data suggest that DNA incorporation of IdUrd is not affected by the addition of LV, the cytotoxicity of the combination regimen may be increased due to i.v.-LV-related inhibition of thymidylate synthase. For this reason, we recommend that efficacy studies of the combination continue in parallel with studies of IdUrd alone.

INTRODUCTION

Steep dose-response relationships have been observed for epithelial neoplasms in the clinic and laboratory (1–3). Efforts to increase dose intensity include the i.p. delivery of chemotherapy, which enhances drug exposure in patients with peritoneal carcinomatosis, a common presentation of advanced ovarian and gastrointestinal malignancies (4–6). i.p. chemotherapy confers a pharmacological advantage (defined as the ratio of the peritoneal to the i.v. drug concentrations) compared with the administration of i.v. agents and may represent an advance in our ability to treat these neoplasms. Recently, the Gynecologic Oncology Group has documented an increased median survival and decreased toxicity in optimally debulked ovarian cancer patients treated with i.p. chemotherapy compared with a control group treated with standard i.v. chemotherapy using the same dose intensity (7).

IdUrd,3 a substituted pyrimidine, was initially synthesized in the late 1950s (8). This compound, which acts as an analogue of thymidine due to the similarity of the radius of the substituted halogen atom (9), has been found to be active as a radiation and chemotherapy sensitizer due to the incorporation of the molecule into DNA during the synthesis phase of cell replication (10–12). IdUrd has also been shown to be cytotoxic to a variety of solid tumors (13). Therapeutic effectiveness is proportional to the incorporation of the agent into tumor cell DNA (14–16). In cell culture, Greene and Collins (17) demonstrated that the addition of calcium LV to IdUrd increases the replacement of thymidine in DNA by 50–100% compared with IdUrd alone.

The clinical pharmacology of i.v. IdUrd administration has been evaluated in Phase I and II studies. The toxicities of i.v. infusion schedules include stomatitis, leukopenia, skin rash, and alopecia (13), with thrombocytopenia reported as the dose-limiting toxicity of IdUrd administered as daily 12-h infusions for 14 days (18). There are no reports documenting the pharmacokinetics or incorporation of IdUrd into DNA when IdUrd is administered i.p. Thus, we investigated the toxicity, DNA uptake, and clinical pharmacology of IdUrd administered i.p. with and without concomitant i.v. LV in patients with malignant neoplasms primarily confined to the peritoneal cavity.

PATIENTS AND METHODS

Patient Selection. Between February 1991 and June 1995, 39 patients with advanced malignancies, primarily confined to the peritoneal cavity, were entered into this Phase I trial. The diagnosis must have been histologically proven, the tumor must have been unresponsive to previous chemotherapeutic regimens, or have no defined "standard" chemotherapeutic regimen. Patients were required to have a Karnofsky performance status of ≥60%, age ≥18 years, and expected survival of at least 3 months. Adequate renal function was

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3 The abbreviations used are: IdUrd, iododeoxyuridine; LV, leucovorin; MTD, maximally tolerated dose; IUra, iodouracil; BndUrd, bromodeoxyuridine; AUC, area under the curve.
defined as serum creatinine ≤1.5 mg/dl or 24-h creatinine clearance of ≥60 ml/min. Adequate bone marrow function was defined as a total WBC ≥4000/mcL and platelet count ≥150,000/mcL. Adequate hepatic function was defined as serum bilirubin ≤1.5 mg/dl and aspartate aminotransferase and alanine aminotransferase twice the upper limit of normal. Prior radiation or chemotherapy must have been completed at least 4 weeks before beginning treatment on this protocol. There was no limit on the number of prior courses of chemotherapy. Female patients could not be pregnant. Patients must have been willing to undergo the necessary surgical procedure for insertion and removal of i.p. catheters. All patients gave their voluntary informed consent and signed a consent document that had been reviewed and approved by the City of Hope National Medical Center Institutional Review Board. This trial and signed a consent document that had been reviewed and approved by the City of Hope National Medical Center Institutional Review Board. This trial was also approved by the Cancer Therapy Evaluation Program, National Cancer Institute.

**Pretreatment Evaluation.** All patients had a complete history and physical examination including documentation of weight, Karnofsky performance status, presence of measurable or evaluable disease, a complete blood count with platelet count and differential, 18-channel blood chemistry analysis, CA-125 level, chest X-ray, electrocardiogram, urinalysis, pregnancy test if indicated, and computed tomographic scans of the chest, abdomen, and pelvis as needed to document measurable or evaluable disease. In addition, all patients underwent peritoneal fluid analysis for cell count, cytology, and culture for bacteria. Patients with measurable disease were required to have radiographic procedures for analysis of measurable disease repeated after every other cycle of therapy.

**Treatment Plan.** This study was performed in two phases: patients treated in the initial cohorts 1–6 (see Table 1 for dosage escalations) received IdUrd mixed in two liters of physiological saline instilled into the peritoneal cavity through an i.p. catheter, daily for 4 successive days. Treatment was repeated every 3 weeks. Patients were turned hourly to bathe the peritoneal cavity completely. All remaining fluid was drained after 4 h of daily treatment. Patients in cohorts 7–10 received a continuous i.v. LV infusion beginning 8 h before the first dose of i.p. IdUrd; the LV was continued for 12 h after the final dose of IdUrd (a total of 4.5 days).

The starting dose of IdUrd was determined by using a conservative estimate of the MTD of i.v. IdUrd of 1000 mg/m² daily for 10 days (total dose, 10,000 mg/m²; Ref. 18). The initial total dose (5000 mg/m²) was one-half the MTD, administered as 1250 mg/m²/day by i.p. infusion, once daily for 4 days, given 24 h apart. The dosage escalation was planned to follow the Fibonacci scheme for Phase 1 protocols. One patient in the first cohort experienced abdominal discomfort thought to be secondary to chemical peritonitis; the starting dosage was then decreased by 50% to ensure that this observation was not dose related; and following safe administration of IdUrd at this dose level, escalation was resumed. Following the establishment of the MTD of single-agent IdUrd, the IdUrd dose was deescalated by two dose levels to 2500 mg/m²/day, and LV was added to the treatment regimen. Due to excessive toxicity encountered at the 3750 mg/m²/day dose level in combination with LV, two intermediate dose levels were then examined to more accurately define the toxicity profile of the combination. Patients experiencing any grade 3 toxicity were retreated after a dose reduction of one level. A minimum of three patients were entered at each dose level. Dosage escalations were determined by the toxicity encountered after the first cycle of i.p. chemotherapy. If after one complete course of therapy there were no grade 3 or 4 toxicities observed in any member of the cohort, the dosage of IdUrd was escalated by one level. A single instance of grade 3 toxicity resulted in the accrual of three additional patients at that dose level. Dose escalation continued until grade 3 or 4 toxicity was observed. If no further grade 3 toxicities were observed in the additional patients, drug doses were escalated to the next level. A single instance of grade 4 toxicity or a second grade 3 toxicity in the additional three patients established the MTD. Standard response criteria were used in patients having measurable or evaluable disease (19). Toxicity was measured using the Common Toxicity Criteria of the National Cancer Institute. IdUrd was supplied by Cancer Therapy Evaluation Program, National Cancer Institute; LV was obtained commercially.

**Plasma Sampling.** Plasma samples were collected for pharmacokinetic analysis immediately before initiation of IdUrd administration and hourly for 4 h on the first and fourth days during the first course of treatment. Three ml of whole blood were collected in heparinized tubes and immediately placed on ice. The plasma samples were separated, labeled, and frozen at −70°C within 1 h of collection.

**Peritoneal Fluid Samples.** Peritoneal fluid samples were collected for IdUrd pharmacokinetics and flow cytometry immediately after the administration of IdUrd; fluid samples for pharmacokinetics were obtained hourly during the first and fourth doses of the first course. The pretreatment sample was collected after the administration of 500 ml of warmed saline into the peritoneal cavity through the peritoneal catheter, followed by immediate withdrawal of all possible fluid; then the sample was placed on ice. Ten ml of fluid were frozen at −70°C for pharmacokinetic analysis; the remainder was processed for flow cytometric analysis of IdUrd incorporation. Additional samples for pharmacokinetic analysis were collected hourly during the first and fourth doses by withdrawing 1 ml of peritoneal fluid.

**High-Performance Liquid Chromatography Analysis of IdUrd.** Standard curves of IdUrd and IUra were prepared in each patient's plasma and peritoneal fluid. BrdUrd was added to all samples as an internal standard. Plasma samples were filtered (0.22 mm) and then injected onto a 4.6 × 250-mm Semi-Permeable Surface 5-mm C8 high-performance liquid chromatography column (Regis) flowing 50 nm potassium phosphate buffer, pH 6, at 1 ml/min; then the plasma samples were quantified by UV absorbance at 310 nm by comparing the ratios of the peak areas, IdUrd/BrdUrd and IUra/IUra in the unknown samples to the standard curves. Peritoneal fluid samples were diluted 1:10 with water before injection and then quantified in the same manner as the plasma samples.

**Pharmacokinetic and Pharmacodynamic Calculations.** AUCs (µm × h) for peritoneal IdUrd, plasma IdUrd, and plasma IUra were approximated using the trapezoidal rule. The AUCs were estimated over the interval from the beginning of the fluid instillation until the fluid was removed at 4 h after the end of the instillation. The first interval was taken from the beginning of the fluid instillation to the time point 1 h after the end of instillation, which assumed that the concentrations increased linearly over this interval. Because fluid was removed at 4 h, the curves were not extrapolated beyond this time point. The pharmacological advantage (PA) was calculated as the IdUrd AUCceptive/AUCplasma.

**Flow Cytometric Analysis.** Peritoneal fluid was collected for flow cytometric analysis; 1 liter of fluid was aspirated via the i.p. portacath using a 20-ml syringe 4 h after the initial dose of IdUrd and at hour 8 on the second, third, and fourth days of therapy by infusing 1 liter of warmed saline into the peritoneal cavity and aspirating the fluid using 20-ml syringes. The samples were immediately placed on ice. The cells and tissue fragments were cellularly fixed and mixed in HCF fixative as described previously (20, 21). The tumor cell fraction of the cell suspension was identified by using pooled antibodies specific for human milk fat globulin, epithelial membrane antigen, TAG 72, or carcinoembryonic antigen, gating on the IdUrd-positive fraction of the cell suspension, and/or forward scatter gating to identify the larger cell population. Flow cytometric analyses were performed as described previously (22). For analysis of IdUrd incorporation, cells were stained using indirect immunofluorescent goat anti-mouse monoclonal antibody to IdUrd (Becton Dickinson, Mountain View, CA).

**Statistical Methods.** This study was designed as two linked sequential Phase 1 trials designed to establish the MTD and the dose-limiting toxicity of IdUrd administered alone and in combination with LV. The dosing schema are outlined in the treatment plan section. Statistical analyses of the pharmacoki-

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**Table 1 Summary of toxicity by dose level**

<table>
<thead>
<tr>
<th>Dose level</th>
<th>No. of episodes (all courses)</th>
<th>No. of patients</th>
<th>No. of courses</th>
<th>AGC (≥ Gr 3)</th>
<th>Plts (≥ Gr 3)</th>
<th>Stomatitis (≥ Gr 3)</th>
<th>Rash (any)</th>
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<td>4</td>
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<td>4125/0</td>
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<td>16</td>
<td>2</td>
<td>10</td>
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<td>3438/500</td>
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<td></td>
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<tr>
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</tr>
</tbody>
</table>

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*a* IdUrd (mg/m²/day)/Leucovorin (500 mg/m²/day × 4.5 days).

*b* AGC, absolute granulocyte count; Gr, grade; Plts, platelets.
planned four cycles of chemotherapy in the adjuvant setting. These patients included: one patient with ovarian carcinoma who had microscopic residual disease remaining at second-look surgery and remains disease-free at 76 months after treatment; one patient with Fallopian tube carcinoma incompletely debulked, with coelomic disease remaining after her initial surgery, whose CA-125 level did not normalize until after the fifth of six cycles of platinum-based chemotherapy and who remains disease-free at 36 months; and one patient with perforated appendiceal carcinoma who remained clinically and biochemically disease free until 12 months after IdUrd, when the carcinoembryonic antigen level rose (recurrent disease was documented at 22 months after IdUrd). Twelve additional patients received four or more cycles of chemotherapy (Table 3). Of these, one patient with recurrent ovarian carcinoma received 12 cycles of therapy; she presented with marked ascites and a CA-125 level of 830 units/ml, which declined to 40 units/ml by the fourth cycle of therapy. Additionally, she had complete clinical and radiological resolution of ascites. After 16 months of treatment, the CA-125 level increased, and the ascites recurred, indicating disease progression. Three patients received six or seven cycles of chemotherapy, one patient each with ovarian, gastric, and pancreatic cancers; the CA-125 levels decreased in the first two patients, whereas the ascites radiographically cleared transiently in the third patient. Of the five remaining patients receiving four cycles of chemotherapy, one experienced clinical and radiographic resolution of ascites but experienced progression of hepatic metastases; one patient with positive cytology at initiation of therapy had normal cytology after two cycles and recurrence of positive cytology following four cycles; another patient had “decreased nodularity” seen on CT scanning after two cycles of chemotherapy and definitive progression of disease following four cycles; two additional patients had stable disease after two cycles of therapy and progression after four cycles of therapy. No patient treated on this study experienced a confirmed objective response meeting defined criteria.

**Reasons for Discontinuation of Protocol Therapy.** Of the 36 patients treated in this study, three patients were treated in the “adjuvant” setting and were arbitrarily treated for four cycles and then observed. One patient experienced symptoms consistent with chemical peritonitis at the first dose level. The trial was subsequently amended to decrease the initial dose level to 625 mg/m²/day. No further episodes of chemical peritonitis were observed, and dosage escalations continued as described above. One patient refused further therapy after cycle one; the CA-125 level had decreased after that initial cycle. The remaining 31 patients ultimately progressed after 1–12 cycles of chemotherapy.

**Reasons for Incomplete Cycles.** Ten of 113 planned cycles of chemotherapy (9%) were not completed for the reasons delineated in Table 4. Peritoneal catheter malfunctions occurred in five patients: failure to infuse in three patients; erosion into the small bowel in one patient; and an infection in one patient. One patient with catheter failure during cycle one consented to undergo replacement and subsequently completed six cycles of i.p. chemotherapy. One patient developed culture-negative fever and abdominal pain during cycle 2 and experienced similar symptoms on retreatment. This was believed

**Table 2 Patient characteristics**

<table>
<thead>
<tr>
<th>Age (median, range)</th>
<th>54 (32-77)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Karnofsky performance status</td>
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</tr>
<tr>
<td>Sex F: 27 M: 12</td>
<td></td>
</tr>
<tr>
<td>Primary site (no. of patients)</td>
<td></td>
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<tr>
<td>Intraabdominal liposarcoma</td>
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<tr>
<td>Retroperitoneal</td>
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</tr>
<tr>
<td>Sarcoma</td>
<td>1</td>
</tr>
<tr>
<td>Mesothelioma</td>
<td>1</td>
</tr>
<tr>
<td>Colon/Rectum</td>
<td>13</td>
</tr>
<tr>
<td>Fallopian tube</td>
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</tr>
<tr>
<td>Ovary</td>
<td>13</td>
</tr>
<tr>
<td>Pancreas</td>
<td>2</td>
</tr>
<tr>
<td>Stomach</td>
<td>3</td>
</tr>
<tr>
<td>Unknown primary</td>
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<td>Appendix</td>
<td>1</td>
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<td>Gallbladder</td>
<td>1</td>
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</table>

**RESULTS**

**Patient Characteristics.** Of the 39 patients enrolled, 36 patients completed 103 courses of treatment (see Table 1). Three patients did not receive a complete cycle of therapy due to: rapid clinical deterioration in one patient, intolerance of therapy in a second patient, and i.p. catheter failure in the third patient. Twenty-three patients received single-agent IdUrd, and 13 patients were treated with combination therapy including i.v. LV. Twenty-seven patients were female; 12 patients were male (Table 2). The median age was 54 years (range, 32–77), and the median Karnofsky performance status was 80% (range, 70–100%). The predominant tumor types included: adenocarcinoma of the colon or rectum and other gastrointestinal primaries (20); ovarian or Fallopian tube cancer (15); and abdominal sarcomas (3). All patients had received prior surgery; 34 patients had received prior chemotherapy [median number of prior chemotherapy regimens: 1 (range, 0–3)]; and two patients had received prior radiation. Nine patients had received no prior chemotherapy or radiotherapy.

**Toxicities of Therapy.** Dosage escalation and toxicity suppression by dose level are summarized in Table 1. Myelosuppression was the dose-limiting toxicity of both single-agent i.p. IdUrd and IdUrd with i.v. LV. Dose-limiting toxicities of IdUrd alone included one episode of grade 4 neutropenia (absolute granulocyte count, <500/μl) after the first treatment cycle in cohort 6 (IdUrd dosage, 4538 mg/m²/day), establishing the MTD; one patient developed grade 3 neutropenia (absolute granulocyte count, 0.5–0.9/μl) after her third cycle of therapy after cycle one; the CA-125 level had decreased after that initial cycle. The remaining 31 patients ultimately progressed after 1–12 cycles of chemotherapy.

**Table 3 Number of courses completed**

<table>
<thead>
<tr>
<th>No. of courses</th>
<th>No. of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
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<td>1</td>
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<td>7</td>
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</tr>
<tr>
<td>12</td>
<td>1</td>
</tr>
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</table>

The dose-limiting toxicities of IdUrd in combination with i.v. LV were observed at dose level 10 with grade 3 neutropenia in each of two patients (IdUrd 3750 mg/m²/day and LV 500 mg/m²/day). At the intermediate dose level 9 (IdUrd 3438 mg/m²/day with LV 500 mg/m²/day), one episode of grade 3 neutropenia, and one episode of grade 3 stomatitis (fibrotic mucositis) were observed, thereby determining the MTD of combination therapy.

Other toxicities included mild cutaneous eruptions in several patients independent of dose level. One patient developed hand/foot syndrome with symptoms of nonpainful but pruritic desquamation on the hands, which healed and recurred during each of the four cycles of therapy. No significant emesis was noted at any dose level.

**Therapeutic Responses in Patients Receiving Four Cycles or More of Treatment.** Of the 39 patients entered into this study, 29 were evaluable for response, and three additional patients received a...
to represent a chemical irritation of the peritoneum, and further chemotherapy was not administered in this trial. One patient had a rapid clinical deterioration after enrollment, and one patient was intolerant of i.p. fluid; these patients did not finish any complete courses of therapy. One patient developed gastrointestinal bleeding due to disease progression during cycle 2 of therapy.

**Pharmacokinetics.** Plasma pharmacokinetics were available for 27 patients, 22 of whom had data on both days 1 and 4. Both plasma and peritoneal samples were available for 19 patients. Fig. 1a represents the plasma AUCs, which increased from 4.38 (1.93–6.97) μM × h to 139.03 (104.82–179.0) μM × h [geometric mean (range)] as the dose increased from 625-4538 mg/m²/day. Fig. 1b shows that the peritoneal AUCs increased similarly to a peak of 14,099 (11,892–21,400) μM × h [geometric mean (range)]. The relative pharmacological advantage decreased as the total dose increased, as shown in Fig. 2. The geometric mean pharmacological advantage was 181 at 625 mg/m²/day and was 90 at 4538 mg/m²/day. A linear regression of all of the data in the log-log plot predicted slightly lower values at these two doses, 175 and 65, respectively. For both plasma and peritoneal fluid, linear regressions adequately described the data in both linear plots (not shown) and log-log plots (Fig. 1). However, the slope of the linear regression on the log-log-transformed data for plasma AUC versus dose is greater than 1, indicating that doubling the dose more than doubled the plasma AUC. Fig. 3 illustrates that the extent of granulocytopenia is proportional to the systemic exposure to IdUrd, which corresponds to the observed dose-limiting toxicity of myelosuppression. Repeated measures analysis was used to model the pharmacological parameters on days 1 and 4 as a function of dose and presence of LV. Plasma levels of IdUrd were not statistically significantly affected by the addition of LV (P = 0.13, one-sided). A highly significant dose-response was noted (P < 0.0001). Additionally, plasma levels on day 4 were predicted to be ~16% higher than on day

<table>
<thead>
<tr>
<th>Dose (mg/m²/day)</th>
<th>Course</th>
<th>Reason</th>
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<tbody>
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<td>625</td>
<td>4</td>
<td>Peritoneal catheter erosion into small bowel</td>
</tr>
<tr>
<td>625</td>
<td>5</td>
<td>Decreased Karnofsky performance status</td>
</tr>
<tr>
<td>1250</td>
<td>7</td>
<td>Patient intolerance</td>
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<td>1250</td>
<td>3</td>
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<td>2500</td>
<td>2</td>
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<td>GIb bleeding secondary to disease progression</td>
</tr>
<tr>
<td>3438/200</td>
<td>1</td>
<td>Peritoneal catheter failure</td>
</tr>
</tbody>
</table>

*a* IdUrd (mg/m²/day)/Leucovorin (500 mg/m²/day × 4.5 days)

*b* GI, gastrointestinal.

Fig. 1. *a* and *b*, log-log plots of AUC\(_{\text{plasma}}\) and AUC\(_{\text{peritoneum}}\), respectively, as a function of dose. Both of the AUCs have a statistically significant dose effect. *b* indicates that the AUC\(_{\text{peritoneum}}\) appears lower in the LV-treated patients. ○, day 1 without LV; △, day 4 without LV; □, day 1 with LV; ▲, day 4 with LV.
Fig. 2. Pharmacological advantage (AUC$_{\text{peritoneal}}$/AUC$_{\text{plasma}}$) as a function of dose. Note that the pharmacological advantage in the LV-treated patients is lower, primarily due to lower peritoneal concentrations of IdUrd. O, day 1 without LV; △, day 4 without LV; ●, day 1 with LV; ●, day 4 with LV.

1, and levels in patients treated with LV were ~14% higher; however, this effect was not statistically significant. Analysis of AUC$_{\text{peritoneal}}$ and AUC$_{\text{plasma}}$ and their ratio suggest a statistically significant ($P = 0.0001$) LV effect on the ratio due to lower peritoneal levels in the LV-treated patients. Plasma IUra levels were quantified and determined to be ~10-fold greater than the plasma IdUrd levels at the end of the dwell. Plasma and peritoneal fluid levels of IUra were approximately equal; however, the peritoneal levels could not be accurately quantified due to interference from the extremely high IdUrd peak.

**Flow Cytometric Analysis.** Cell samples were analyzed for IdUrd incorporation. Adequate peritoneal fluid sampling was obtained from 26 of the 36 patients. Table 5 summarizes the fluid sample characteristics. The fluid in 16 patients was cytologically positive for malignant cells using standard cytological analysis under light microscopy. These cells were identified in 13 of these patients using specific antibodies. In 12 of these patients, the same cell populations were also identified using forward scatter to isolate the larger cell population. Fig. 4 represents these patients: the IdUrd uptake represented by fluorescence intensity in millibead equivalents plotted on the vertical axis versus the administered dose of IdUrd in mg/m$^2$/day on the horizontal axis. Triangles represent the relative peak fluorescence intensity of the cell population positive for specific antibodies in patients with cytologically positive peritoneal washings, whereas the circles represent the same cell populations separated by the use of forward scatter. There is no significant difference between the data points ($P = 0.17$); thus, the two methods of separating the tumor cells in these mixed cell populations are identifying the same tumor cell population.

Fig. 5a represents the effect of IdUrd dose on the incorporation of IdUrd into the DNA in tumor cells of the 16 patients with cytologically positive peritoneal washings; Fig. 5b represents the IdUrd incorporation in all 26 patients from whom samples were obtained. These figures illustrate that with the best-fitting linear, quadratic, and piecewise linear models, the overall DNA incorporation increases with dose ($P = 0.001$); however, there is evidence of curvature (quadratic, $P = 0.04$), suggesting saturation of the DNA at higher doses. The addition of i.v. LV does not significantly alter the incorporation profile ($P = 0.4$).

**DISCUSSION**

Increased dose intensity of chemotherapeutic agents results in improved response rates with steep dose-response relationships being demonstrated in many neoplasms (1-3). Tumors that are predominantly confined to the peritoneal cavity allow a unique opportunity to
deliver increased doses of active agents directly to the area of the
greatest tumor bulk. Tumor exposures of 50–1000 times the concen-
trations possible by the i.v. route are possible by i.p. drug delivery
(24–26). The utility of this approach has been demonstrated in pa-
sients suffering from advanced ovarian carcinoma in a recent random-
ized trial comparing i.v. versus i.p. cisplatin (7) in combination
therapy. In this study, increased survival and decreased toxicity were
reported to achieve a median survival of 38 months in patients with
minimal residual ovarian cancer after second-look laparotomy (27).

IdUrd has been reported to have therapeutic antineoplastic activity
in a wide variety of solid tumors (13); however, because of rapid
first-pass dehalogenation in the liver, this drug has been used primarily
as a radiosensitizer when administered i.v.. The current study was
designed to determine the maximally tolerated dose of IdUrd agent
delivered directly into the peritoneal cavity. We have determined that
the pharmacological advantage of IdUrd is comparable with other
halogenated pyrimidines (4, 28); the mean value for days 1 and 4 in
individual patients ranges from 181 to 90, with a trend toward a lower
pharmacological advantage at higher IdUrd doses. Nonetheless, both
the systemic and the i.p. exposure increase throughout the tested
dosage range while maintaining a pharmacological advantage within
the peritoneal space. The decrease in the pharmacological advantage
at higher doses is due to the more than doubling of the plasma AUC
with a doubling of the dose. Saturable elimination of IdUrd from the
plasma could cause this result, but the pharmacokinetics of IdUrd is
reported to be linear with i.v. infusions, which result in comparable
plasma concentrations of IdUrd (29). An alternate explanation is that
at higher doses, more of the IdUrd that leaves the peritoneum reaches
the plasma intact, possibly due to saturation of first-pass metabolism
in intervening tissues. The extent of granulocytopenia is proportional,
as expected, to the plasma AUC.

Statistical analysis of the effect of LV on the plasma and peritoneal
levels of IdUrd shows a significant effect of this agent on the phar-
macological advantage, primarily due to lower measured levels of IdUrd
in the peritoneal fluid. The reason for this effect is not imme-
diately clear. Due to observed toxicity, the dosage range of LV-treated
patients in this study is limited, and broader dosage ranges and
increased numbers of patients would be required for further evaluation
of this effect.

Tumor cell kill by IdUrd is at least partially determined by the
extent of incorporation of IdUrd into tumor cell DNA. High dose
folinic acid (LV) has been reported to increase the cellular uptake and
DNA incorporation of IdUrd when cells are exposed to this agent in
vitro (17); however, McGinn et al. (30) have recently reported in an
in vivo Phase I trial using sequential cycles of i.v. IdUrd with and
without i.v. LV that IdUrd incorporation into peripheral blood gran-
ulocytes was not increased with the addition of LV. Our study
supports this in vivo observation in tumor cells harvested from the
peritoneal cavity. This may be due to saturation of the tumor cell DNA
by IdUrd during the exposure time, an effect that is dose dependent.
In spite of this finding, however, the MTD of IdUrd in combination
with LV is 25% lower than single-agent therapy, possibly due to
 toxicity from increased inhibition of thymidylate synthase.

Body fluids of patients with peritoneal carcinomatosis contain
mixed populations of cells representing multiple lineages including
hematopoietic, mesothelial, and tumor cell populations. Cytological
evaluation by light microscopy is the usual diagnostic standard for the
presence of tumor cells in peritoneal fluid or washings. Flow cytom-
etry offers the possibility of examining individual characteristics of
large numbers of cells in a mixed cell population; however, major problems remain in differentiating between the various populations of cells to be certain that the characteristic under evaluation is that of the population of cells in question. This is particularly true when the light-microscopic evaluation of the cells is cytologically negative for malignancy. Separation of cell populations can be accomplished, however, using specific characteristics including antigenic expression on the cell population in question, the absence of antigen expression, or by using the characteristic light scatter properties of different cell populations to differentiate between the cell types.

We performed flow cytometric analysis on patients with both cytologically positive and negative peritoneal fluids. In 12 patients with cytologically positive fluids, gating analyses were performed to separate the individual cell populations: using tagged antibodies to tumor membrane antigens in one series of experiments and subsequently using the light scatter characteristics of the same mixed tumor population. Our data indicate that these markers isolate the same population of tumor cells present in the peritoneal fluid (Fig. 4). Further analysis of all patients, both cytologically positive and negative, shows that the accumulation of IdUrd measured by flow cytometry reached a plateau (Fig. 5). We have interpreted the plateau to indicate saturation of the accumulation of IdUrd in the DNA, but it could represent saturation of the detection method despite the fluorescence intensities being within the linear range of the instrument. The plateau is not affected by the addition of i.v. LV to the therapeutic regimen.

Patient tolerance of i.p. IdUrd was excellent with reasonable toxicity. In our patient population, two patients with high-risk tumors remain disease-free at 3 and 6 years after treatment, and one patient with progressive recurrent ovarian cancer was palliated with complete resolution of symptomatic ascites for 16 months. These responses suggest that this agent may be useful in the therapeutic management of i.p. malignancies as a single agent, in combination, or as a chemoradiotherapy sensitizer. Future Phase II studies of IdUrd in combination with LV are warranted to test the advantage of the enhanced cytotoxicity expected from the inhibition of thymidylate synthase. The recommended doses are IdUrd 3125 mg/m\(^2\)/day daily for 4 days by IP infusion in combination with LV 500 mg/m\(^2\)/day for 4.5 days. Further studies are planned to determine the utility of combining this agent with i.p. radioimmunotherapeutic and chemotherapeutic agents.

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Phase I Trial of Intraperitoneal Iododeoxyuridine with and without Intravenous High-Dose Folinic Acid in the Treatment of Advanced Malignancies Primarily Confined to the Peritoneal Cavity: Flow Cytometric and Pharmacokinetic Analysis
