Leucovorin Modulation of 5-Iododeoxyuridine Radiosensitization: A Phase I Study

Cornelius J. McGinn, Keith A. Kunugi, Kendra D. Tutsch, Christine Feierabend, Dona Alberti, Mary J. Lindstrom, George Wilding, Rhoda Z. Arzoomanian, and Timothy J. Kinsella
Department of Human Oncology, University of Wisconsin Medical School, Madison, Wisconsin 53792

ABSTRACT
Evidence for clinically significant radiosensitization by the halogenated pyrimidine 5-iododeoxyuridine (IdUrd) continues to accumulate. In vitro radiosensitization has been demonstrated in human colon tumor cell lines following exposure to 1–10 μM. Coadministration of leucovorin (LV) increases radiosensitization, which correlates directly with increased IdUrd DNA incorporation. Clinical data regarding proliferation rates and thymidine kinase levels in tumors versus normal tissues suggest selective incorporation of IdUrd into gastrointestinal tumors may occur. The objectives of this Phase I study were: (a) to assess the feasibility of LV modulation of IdUrd radiosensitization by determining the maximum tolerated dose (MTD) of IdUrd plus LV; and (b) to perform correlative laboratory studies to investigate the potential of IdUrd plus LV to increase radiosensitization in vivo. Seventeen patients with unresectable or recurrent gastrointestinal adenocarcinomas received a 14-day course of continuous i.v. infusion of IdUrd prior to initiation of radiotherapy. Two additional 14-day infusions of IdUrd with LV were given during the course of radiotherapy (60 Gy in 6 weeks). The initial dose of IdUrd was 250 mg/m²/day and was escalated in subsequent patients to 400 and 600 mg/m²/day. The LV dose remained fixed at 250 mg/m²/day. Leukopenia was the dose-limiting toxicity, and 400 mg/m²/day was the MTD for this trial. At the MTD, the mean ± SD steady-state plasma concentration of IdUrd during the infusion, measured by high-performance liquid chromatography, was 0.66 ± 0.23 μM. There was no significant influence of LV on IdUrd DNA incorporation in peripheral blood granulocytes as measured by high-performance liquid chromatography. Based on toxicity data and correlative laboratory studies, a meaningful increase in radiosensitization would not be achieved with the IdUrd infusion schedule and dose of LV investigated compared with IdUrd alone.

INTRODUCTION
Cancers of the GI tract result in 124,000 deaths annually in the United States (1). The vast majority of these deaths can be attributed to unresectable and recurrent primary tumors or hepatic metastases. Additional gains in tumor control at these sites may have a significant impact on overall survival. It has been estimated that an additional 17,000 survivors per year could result if local and regional failures were eliminated in patients with stage M0 colorectal cancer (2). Effective management of hepatic metastases may also prolong survival in patients with colorectal cancer. This view is supported by autopsy data on patterns of failure (3) and surgical series in which 25–40% 5-year survival has been achieved following surgical resection of solitary or a limited number of multiple metastases (4). Indeed, a new paradigm of cancer dissemination proposed by Hellman and Weichselbaum (5) suggests that liver metastases may not necessarily represent widespread dissemination and that a subpopulation of these patients may be cured with appropriate therapy.

The use of conventional radiotherapy in patients with unresectable or recurrent GI cancers has met with limited success. This has been attributed to the intrinsic radioresistance of these tumors, particularly pancreatic adenocarcinoma. However, the improved local control and survival reported in many GI sites with the use of FU and radiation therapy suggest that radiosensitization may play a significant role in the treatment of these tumors (6). Delivery of a tumorcidal radiotherapy dose to patients with hepatic metastases has been limited by normal tissue toxicity. Recent advances in three-dimensional radiation treatment-planning techniques have been applied in this setting (7) and may serve to reduce this toxicity, thereby allowing a higher total dose. This Phase I trial was initiated in attempt to investigate the role of halogenated pyrimidine radiosensitization in patients with GI cancers, considering the success of fluoropyrimidine radiosensitization, while taking advantage of three-dimensional treatment planning in selected cases to limit normal tissue toxicity.

IdUrd, a halogenated thymidine analogue, has been investigated as a radiosensitizer since the early 1960s (8). Evidence for clinically significant radiosensitization with halogenated pyrimidines continues to accumulate, primarily in patients with...
IdUrd was selected for investigation in the present trial considering preclinical data on its mechanism of action. In vitro studies have demonstrated radiosensitization in human colon tumor cell lines following exposure to 1–10 μM IdUrd (12, 13). Furthermore, radiosensitization was noted to be greatest in the more radioresistant line (HT-29; Ref. 12). In these studies and many others, the degree of radiosensitization is directly related to the extent of thymidine replacement by IdUrd in DNA. This incorporation into DNA occurs through the thymidine salvage pathway, in which thymidine and its analogues enter the DNA precursor pools after phosphorylation to nucleotides by TK (Fig. 1A). However, the extent of thymidine replacement is not simply a function of competition within the salvage pathway, because the preferred mechanism for thymidine incorporation is through the de novo synthesis of pyrimidine nucleotides. TS, which converts dUMP to dTMP, is a critical enzyme in this pathway. It is known that the monophosphate form of IdUrd is a weak inhibitor of TS (Fig. 1B; Ref. 14). The ability of LV to increase this inhibition, thereby increasing IdUrd DNA incorporation, has been investigated, considering its ability to increase FU-mediated TS inhibition (15). These in vitro studies, in the HT-29 cell line, have demonstrated higher IdUrd DNA incorporation following exposure to IdUrd and LV compared with IdUrd alone (16, 17). As expected, this results in a direct linear increase in radiosensitization (17).

The therapeutic index was also considered in addition to these preclinical data on IdUrd radiosensitization. Two lines of evidence suggest that selective tumor sensitization may occur in patients with GI cancers. The first relates to TK, the enzyme required for activation of IdUrd, and may be important for a broad group of patients with GI cancers. Levels of this enzyme have been reported to be higher in GI tumors than in corresponding normal tissue, which may result in higher incorporation into tumor cells (18). The second line of evidence relates to the selective incorporation of IdUrd into cells undergoing DNA synthesis and may be significant for patients with liver metastases. Indeed, it has been shown that relevant levels of IdUrd or bromodeoxyuridine incorporation into hepatic metastases can occur without significant incorporation into normal surrounding parenchyma (19, 20).

This article reports the first attempt to evaluate the feasibility of LV modulation of IdUrd radiosensitization in patients with GI cancers. In this trial, each patient received a 14-day infusion of IdUrd alone, followed by two 14-day infusions of IdUrd and LV. The objectives were: (a) to assess the feasibility of LV modulation of IdUrd radiosensitization by determining the MTD of IdUrd when combined with LV; and (b) to perform correlative laboratory studies to investigate the potential of this combination to increase radiosensitization in vivo.

PATIENTS AND METHODS

Patient Eligibility and Pretreatment Evaluation. Patients with histological or cytological confirmation of unresectable or recurrent GI adenocarcinoma originating from the stomach, pancreas, gallbladder, biliary tree, colon, or rectum were entered onto protocol. Eligible patients were required to be ≥18 years old with normal renal (creatinine, ≤2.0 mg/dl) and hepatic (bilirubin, ≤1.5 mg/dl) function, adequate bone marrow reserve (hematocrit, ≥25%; WBC, ≤4000/mm³; and platelets, ≤100,000/mm³), expected survival >6 months and Eastern Cooperative Oncology Group performance status of ≤2. Patients who received prior systemic chemotherapy were eligible if they received no chemotherapy for 1 month prior to entry. Patients who received prior radiotherapy to involved sites of disease were not eligible. Metastases beyond the planned irradiation field were not considered in determining eligibility. Signed informed consent on the experimental nature of this study was obtained from all patients before entry, in accordance with National Cancer Institute and institutional guidelines. An indwelling central venous catheter was required for drug infusion. A complete history, physical examination, and laboratory evaluation were obtained within 2 weeks of patient registration. A radiological evaluation was obtained within 4 weeks of study entry and included a chest X-ray and CT of the abdomen and pelvis.

Study Design. The treatment plan is shown in Fig. 2. Patients received IdUrd as a 24-h continuous infusion for 14 consecutive days prior to initiation of radiotherapy. Two additional 14-day infusions of IdUrd were delivered concurrent with LV during the course of radiation therapy. Each 14-day infusion was separated by 1 week. The initial dose of IdUrd was 250 mg/m²/day, which is 25% of the MTD when given alone as a
continuous 14-day infusion (21). This dose was escalated in subsequent patients following an evaluation of toxicity at the preceding level, according to a standard Phase I schedule in which a minimum of three patients is evaluated at each dose level. The dose of LV remained fixed at 250 mg/m²/day. Patients were evaluated on a weekly basis for signs and symptoms of toxicity. This evaluation included a complete blood count, chemistry panel, and electrolytes. Significant toxicity for IdUrd dose escalation was defined as grade 3 or 4 toxicity using the common toxicity criteria from the National Cancer Institute. Patients were required to recover from severe toxicity prior to retreatment with IdUrd. For patients who developed acute radiation-induced toxicity requiring a treatment break, IdUrd and LV were withheld until radiation therapy was resumed. Patients were removed from the trial if progressive disease was noted, general or specific changes in the patients' conditions rendered them unacceptable for further treatment in the judgment of the study chairman, severe toxicity developed that did not return to grade 2 or less, or they elected to withdraw from the study.

Drug Administration. IdUrd was supplied from the National Cancer Institute as a sterile lyophilized powder in 200-mg vials. IdUrd was reconstituted in sterile water at a concentration of 20 mg/ml, with sodium hydroxide to adjust to pH 9–10. The IdUrd dose was then diluted in 120 ml DSW and infused at a rate of 5 ml/h for 48 h. For outpatient infusions, a portable battery-operated pump was used. The bag was changed by nursing staff every 48 h; tubing was changed weekly. LV was diluted and infused along with the IdUrd. When IdUrd with or without LV was not being infused, the patency of the catheter was maintained by daily flushes with heparinized solution.

Radiation Therapy. Radiation therapy commenced immediately following the first 14-day IdUrd infusion and was given with a linear accelerator using 6–24-MV photons. The dose to the initial treatment field was 45 Gy, followed by a 15-Gy boost to a reduced volume, for a total dose of 60 Gy. The initial treatment field included the tumor as well as regional lymphatics if there was potential or proven nodal involvement, with a minimum of a 1-cm margin. The boost field included only the tumor volume with at least a 1-cm margin. Treatment was delivered with two or more fields per day in 1.8–2.0-Gy fractions 5 days/week. Pretreatment planning CT was obtained on all patients for target volume localization and isodose computation. Patients with pelvic tumors were treated in the prone position with a small-bowel displacement device in an attempt to reduce toxicity (22). A three-dimensional treatment planning system was used to design fields for the majority of patients with intrabdominal tumors.

Correlative Laboratory Studies. Venous blood samples were obtained at the time of routine weekly phlebotomy for IdUrd incorporation studies and pharmacokinetic analysis. Granulocyte isolation for IdUrd incorporation into DNA was accomplished using a Histopaque (Sigma Chemical Co., St. Louis, MO) 1077/1119 gradient, based on a technique described previously (23). Following digestion of DNA to nucleosides, the percentage IdUrd DNA incorporation was determined by high-performance liquid chromatography using a modification of the method of Kunugi et al. (24). The percentage of thymidine replacement was calculated as: nmoles IdUrd/nmoles IdUrd + nmoles deoxythymidine), as determined using authentic nucleoside standards. A rank-based ANOVA was used to determine the effect of dose level and the addition of LV at each dose level on the percentage of thymidine replacement. Plasma IdUrd and IUra levels were measured using a high-performance liquid chromatographic method described previously (25).Css are reported as the mean of all weeks in the study when samples were drawn during the IdUrd infusion. Clearance of IdUrd was calculated for each patient as: (IdUrd infusion rate)/mean IdUrd Css). The molar ratio of IUra:IdUrd is reported to show the extent of IdUrd metabolism.

Patient Follow-Up. Patients were interviewed and examined 4 weeks following completion of therapy. This evaluation included routine laboratory studies as obtained during the course of therapy as well as reevaluation of disease status with appropriate imaging studies. Evaluation of acute toxicities was finalized 8 weeks after completion of treatment. Subsequent follow-up or additional therapy were at the discretion of the study chairman.

RESULTS

Seventeen patients were treated between February 1992 and February 1995 (Table 1). Table 2 outlines the primary tumor site, region irradiated, and course of therapy for each patient. Inability to complete therapy was attributed to toxicity (n = 4), progression of disease (n = 2), patient refusal following toxicity of grade 2 or less (n = 2), and clinical deterioration without specific toxicity or evidence of progression (n = 1). Hepatic dysfunction was the most common toxicity encountered, as seen in Table 3. Patients were considered to have experienced this toxicity unless unequivocal radiological or surgical findings of disease progression were noted. However, progression of disease was suspected in several instances when these criteria were not met. Evaluation of hepatotoxicity was further complicated by one patient with radiation-induced hepatitis (discussed be-
Leukopenia (<2000/mm³) was noted in two of three patients at the third dose level (600 mg/m²/day) and was considered the dose-limiting toxicity. The pattern of myelosuppression during the course of therapy for all patients is shown in Fig. 3. The additional depression during weeks 7–9 (i.e., following the first IdUrd and LV infusion) suggests that potentiation of leukopenia by LV may have occurred. The second dose level (400 mg/m²/day) was considered the MTD for this trial.

The majority of patients (n = 16) received drugs via a Hickman catheter. Occlusion of the catheter was noted in 7 of these patients and was attributed to thrombosis. As a result, interruption of drug infusion occurred in 5 patients. Among these 5 patients, 2 required a second Hickman catheter, and 2 patients ultimately withdrew from the protocol, having refused additional line placement. One of the patients who withdrew from the protocol (patient 9) experienced diffuse thrombophlebitis, which contributed to his clinical deterioration and death 16 days after treatment was discontinued.

An additional four patients died <8 weeks following completion (or early termination) of treatment. Postmortem examination was performed in two patients and revealed progression of disease. However, the possibility of radiation toxicity could not be ruled out in one (patient 1) who died of a massive bleed from a duodenal ulcer at the site of a necrotic tumor in the head of the pancreas. Clinical and radiographic evidence of progression was noted in the remaining two patients.

One patient (patient 15) developed radiation hepatitis 1 month following 25.2 Gy to the whole liver with a boost to 59.6 Gy to multiple discrete metastases. Radiation portals for the boost field were created on a three-dimensional treatment-planning system, and a dose volume histogram was reviewed prior to initiation of the boost. During the sixth week of treatment, she experienced grade 3 leukopenia and thrombocytopenia, which prevented her final course of IdUrd and LV from being delivered. One month following completion of radiotherapy, radiation hepatitis was suspected based on the development of ascites and markedly elevated serum bilirubin and alkaline phosphatase. An abdominal ultrasound failed to reveal any evidence of intrahepatic or extrahepatic ductal dilatation, and there was no evidence of malignant cells on cytological examination of fluid obtained at paracentesis. CT of the abdomen revealed only a mild interval increase in the size of the liver metastases, which had now become centrally hypodense with rim enhancement. Despite diuretics, multiple paracenteses, and the placement of a Denver shunt, the patient died 2.5 months following completion of radiotherapy. Postmortem examination of the liver revealed evidence of veno-occlusive disease, consistent with radiation hepatitis. In addition, viable tumor (as evidenced by multiple mitotic figures) was noted to be involving extensive areas of the liver. As a result of this event, the mean dose to the patient’s liver and the probability of developing radiation hepatitis were determined based on the method of Lawrence et al. (26) in an attempt to evaluate the influence of IdUrd and LV on this radiation toxicity. This method uses a dose volume histogram generated for the normal liver (total liver volume minus the tumor volume) and the normal tissue complication probability model with new parameters estimated from clinical data. This analysis revealed a mean dose to the liver of 42.2 Gy with a probability of developing radiation hepatitis of 0.67, suggesting that the radiation dose alone was sufficient to account for this fatal event.

Although response to treatment was not a primary end point of this trial, it was evaluated in eight patients who were able to complete the course of therapy. Four patients experienced partial responses with durations of 5, 9, 10+, and 15 months. Three patients were judged to have stable disease, and one patient’s tumor progressed while on treatment. The longest response was noted in patient 2, who remained stable for 15 months without additional treatment. He then developed progressive disease and died 2.75 years following IdUrd and LV.

IdUrd pharmacokinetic data are shown in Table 4. The mean overall clearance of IdUrd was 1.08 ± 0.34 liters/min/m². Plasma concentrations of IdUrd increased in a linear fashion over the limited dose range studied (P = 0.0086). Neither the clearance of IdUrd nor the molar ratio of IUra:IdUrd was dose dependent. The IdUrd plasma levels, high clearance of IdUrd, and high relative concentrations of IUra are consistent with previous reports of IdUrd pharmacology for continuous infusions (27, 28). However, the dose level (600 mg/m²/day) that achieved concentrations required for radiosensitization in vitro was not tolerated secondary to leukopenia.

The percentage of thymidine replacement by IdUrd in peripheral blood granulocytes is shown in Fig. 4. The percentage of thymidine replacement increased significantly from level 1 to level 2 (P = 0.0491). The percentage of thymidine replacement for level 3 was not significantly different for either level 1 or 2 (P = 0.0911), but the data from this dose level are limited, because only one patient completed the course of therapy (see Table 2). There was no significant influence of LV on IdUrd incorporation (compare weeks 3 and 6).

**DISCUSSION**

The primary objective of this trial was to determine the feasibility of LV modulation of IdUrd radiosensitization in a
in vitro (diosensitization concentration of IdUrd is below the range associated with ra-
correlative laboratory studies, is that a meaningful increase in
influence of LV. concentration (19) and is an appropriate end point to assess the
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granulocytes as a surrogate for tumor cell incorporation, gran-
cancers confined to the peritoneal cavity received i.p. IdUrd for
day, which resulted in plasma levels of 0.66 ± 0.23 μM. This
sion schedule and dose of LV investigated, compared with
radiosensitization would not be achieved with the IdUrd infu-
clinical setting. Our conclusion, based on toxicity data and
corelative laboratory studies, is that a meaningful increase in
radiosensitization would not be achieved with the IdUrd infusion schedule and dose of LV investigated, compared with
IdUrd alone. The MTD of IdUrd in this trial was 400 mg/m²/day, which resulted in plasma levels of 0.66 ± 0.23 μM. This
concentration of IdUrd is below the range associated with
radiosensitization in vitro (1–10 μM). It is also substantially less
than plasma levels obtained in trials using the MTD of IdUrd alone (3.2 ± 0.5 μM and 1000 mg/m²/day, respectively; Ref. 27)
with a similar infusion schedule, trials that have reported favor-
able outcomes suggesting radiosensitization (10). Furthermore,
there was no significant influence of LV on the percentage of
IdUrd DNA incorporation in peripheral blood granulocytes. Although there are limitations in the use of peripheral blood
granulocytes as a surrogate for tumor cell incorporation, gran-
uocyte incorporation has been correlated with IdUrd plasma
centration (19) and is an appropriate end point to assess the
influence of LV.

To our knowledge, there has been only one other clinical
trial evaluating LV modulation of IdUrd incorporation. In this
trial, reported by Morgan et al. (29), patients with advanced
cancers confined to the peritoneal cavity received i.p. IdUrd for
96 h with or without concomitant i.v. LV (500 mg/m²/day for
120 h). As in our trial, the addition of LV reduced the tolerable
dose of IdUrd. In addition, LV failed to increase IdUrd incor-
poration, based on flow cytometric analysis of tumor cells
obtained for the peritoneal cavity. Biochemical modulation of
IdUrd incorporation has also been attempted using FdUrd, based
on its ability to inhibit TS and increase IdUrd DNA incorpora-
tion in vitro and in vivo (13). In a trial reported from the
National Cancer Institute (30), IdUrd (200–675 mg/m²/day)
delivered as a 14-day continuous infusion concurrent with
FdUrd (0.6–3.5 mg/m²/day). Again, with the addition of FdUrd
as a modulating agent, there was no clinically relevant enhance-
ment of IdUrd DNA incorporation. The inability of our regimen
to increase incorporation may relate to the response of intracel-
lar TS levels to inhibition by IdUMP. It has been demonstrated
in both clinical and experimental settings that fluoropyrimidine
therapy results in a rapid increase in TS levels (31, 32). This
effect may have occurred following the initial exposure to IdUrd
in our trial, negating the subsequent influence of LV. The lack
of stomatitis and diarrhea, as seen with FdUrd, also suggested
that significant TS inhibition was not achieved.

Despite these results, the potential value of this approach
remains intriguing, considering current investigations into the
modulation of FU (33, 34). These studies may provide critical
information regarding the optimal means to enhance IdUrd-
medicated TS inhibition with LV or related compounds. Alter-
natively, the use of recently developed, specific inhibitors of TS
may be considered as a means to increase IdUrd DNA incorpo-
ration (35–37). However, it will be important to consider that
efforts to increase IdUrd DNA incorporation will only influence
the radiosensitivity of tumor cells that are exposed to IdUrd.
This distinction between cellular radiosensitization and tumor radiosensitization suggests that the infusion schedule of IdUrd may be of equal or greater importance for clinical radiosensitization (38, 39).

The larger issue that remains unanswered concerns the role of halogenated pyrimidine radiosensitization in GI cancers. Although several patients in this trial experienced radiation-induced GI toxicity, which may have been increased by IdUrd, the dose-limiting toxicity was systemic. The ability of patients to tolerate concurrent IdUrd and abdominal or pelvic radiotherapy has also been demonstrated in a recent trial for patients with retroperitoneal sarcomas (40). Nonetheless, the rate of proliferation within the mucosa of the GI tract suggests that radiosensitization within the mucosa of the GI tract suggests that radiosensitization (40).

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