ABSTRACT

Previously, 5-bromodeoxyuridine (BrdUrd) has been shown to be an effective radiosensitizing agent in rapidly dividing cells. As part of a Phase I/II study to evaluate BrdUrd as a radiosensitizer in gliomas, the pharmacology was studied in eight patients. BrdUrd was infused using an i.v. route as a 12-hr constant infusion each day for as long as 14 days. BrdUrd steady-state arterial levels are described for three different infusional rates: 1.6 μmol/sq m/min (350 mg/sq m/12 hr) produced a steady-state arterial level of 0.7 μM; 3.2 μmol/sq m/min (700 mg/sq m/12 hr) resulted in 2.1 μM; 5.9 μmol/sq m/min (650 mg/sq m/6 hr) showed a level of 3.9 μM. Because of myelosuppression, the highest tolerable dose for this intermittent long-term infusion therapy with BrdUrd appears to be 700 mg/sq m/12 hr. Contrary to the nonlinear pharmacokinetics of thymidine, 5-fluorouracil, and 5-fluorodeoxyuridine described previously, BrdUrd shows linear behavior in the range studied. BrdUrd still has promise as a radiosensitizer for gliomas in humans, but an alternative means of safe delivery into the carotid artery is needed. Because of an estimated 11- to 16-fold-higher local concentration, use of the intraarterial route could deliver optimum levels of BrdUrd to the tumor with minimal systemic toxicity.

INTRODUCTION

In vitro techniques have demonstrated that incorporation of halogenated pyrimidines into DNA induces radiosensitization (5, 8, 14, 15, 19, 26, 28). For BrdUrd, in vitro work has demonstrated a correlation between degree of radiosensitization and the amount of BrdUrd incorporated into DNA (6, 11, 19). Since neoplastic cells were assumed to have a higher turnover and, hence, a higher DNA synthesis rate than did surrounding normal tissue, a basis for selective tumor radiosensitization was envisioned. Two clinical trials using BrdUrd ensued (1, 6, 12, 13, 23, 24).

In the first study (12, 13, 23, 24), the drug was administered as a continuous intracarotid arterial infusion for up to 6 weeks in the treatment of gliomas. Since normal brain tissue in adults has a very low mitotic rate (21), it was thought that gliomas would preferentially incorporate BrdUrd. Improved survival without substantial toxicity to normal central nervous system tissues was reported. At the BrdUrd doses used, there were no reports of systemic toxicity. Drug concentration measurements were apparently not made. Ultimately, the trial was abandoned due to complications from the i.a. catheter.

The second trial (1, 6) also evaluated BrdUrd delivery via the carotid artery. This trial evaluated differences in response and survival using BrdUrd plus radiation versus radiation alone in advanced head and neck tumors. The addition of BrdUrd provided no advantage for local tumor control, and there were significant normal tissue toxicities. Additionally, there were serious catheter-related complications. No pharmacokinetic studies were reported. In retrospect, it would appear that the turnover rate of oral mucosa is comparable to or greater than that of many oral cancers. Thus, in contrast to the situation for gliomas, there was no selectivity in BrdUrd incorporation in head and neck disease.

In both trials, unacceptable complications with intracarotid catheters were encountered. Additionally, neither trial reported serious systemic toxicity. These trials utilized i.a. infusion based upon limited pharmacokinetic data which suggested rapid elimination of BrdUrd following bolus administration to animals and humans (17, 18). More recently, pharmacokinetic studies of prolonged infusions of dThd, FUra, and FdUrd have shown that their steady-state concentration obeys a nonlinear profile (4, 7, 25), a finding not appreciated in earlier bolus infusion pharmacokinetic studies.

Subsequent to these clinical trials, Brown et al. (3) showed in a rat model that i.a. infusion of 5-bromodeoxyuridine, a compound rapidly deaminated to BrdUrd and known to radiosensitize to an equivalent level of BrdUrd (8), was capable of sensitizing the KHT sarcoma with relative sparing of normal tissue. Furthermore, little advantage was seen when i.a. infusion was compared to a higher (5 times) i.v. infusion of 5-bromodeoxyuridine (10). Thus, a Phase I/II trial of i.v. BrdUrd was instituted because: (a) arterial catheter problems were limiting in previous studies; (b) the original pharmacokinetic rationale for not trying the i.v. route was not appropriate; and (c) animal models suggested that the i.v. route could be efficacious. At the National Cancer Institute, we have performed a Phase I/II trial of i.v. BrdUrd in combination with radiation therapy for patients with gliomas. Since glioma cells exhibit a cell-cycle time of 1 week or longer (9, 22, 27), we chose a schedule of 12-hr infusions daily for 10 to 14 days to provide the prolonged exposure required for BrdUrd incorporation. Clinical details have been reported separately (16). In this report, our focus is upon a comparison of the BrdUrd concentrations which can be achieved in vivo by the i.v. route with estimates of BrdUrd concentrations which were achieved by the previous i.a. study and also with BrdUrd concentrations required to sensitize cells in vitro.

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2 The abbreviations used are: BrdUrd, 5-bromodeoxyuridine; FUra, 5-fluorouracil; FdUrd, 5-fluorodeoxyuridine; dThd, thymidine; HPLC, high-performance liquid chromatography; i.a., intraarterial.

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MATERIALS AND METHODS

Clinical Studies. Patients studied had a diagnosis of primary brain tumor (gliomas, Grades III to IV, all histologically confirmed either by incisional biopsy or subtotal resection). In order to qualify for pharmacological studies, patients had to be able to cooperate to the extent of understanding and signing a consent form. All patients were also required to have normal liver function studies and normal cardiac and renal function, as well as normal complete blood counts and a life expectancy of at least 2 months. No patient had previous treatment with radiation or chemotherapy. Selected patients admitted to this study gave consent to have pre- and post-BrdUrd infusion iliac crest bone marrow aspirates to assess the degree of radiosensitization of normal marrow cells. BrdUrd (NSC 38297) was supplied by the Developmental Therapeutics Program of the National Cancer Institute in glass vials containing 500 mg of BrdUrd in lyophilized form. BrdUrd pharmacological studies were performed on the first day of entrance into the trial. After pharmacological studies, the patients had BrdUrd delivered via an automatic infusion pump (Auto-Syringe Model AS-2F, Auto-Syringe, Hocksett, NH) through a flexible silastic catheter (Centrisal; Travenol Laboratories) placed into the subclavian vein. All glioblastoma patients received 4000 to 4500 rads of whole-brain irradiation via opposed lateral fields with a 10 MeV linear accelerator at 180- to 200-rad fractions over 4.5 to 5 weeks. A cone-down volume predetermined by computerized axial tomography prior to commencement of treatment was given an additional 2200 to 2400 rads in 200-rad fractions. Although irradiation was continued throughout the 7 weeks of treatment, BrdUrd infusion was stopped after 10 to 14 days; a break period free of BrdUrd was imposed for 2 weeks (to minimize the frequency of once weekly. Liver function studies were followed weekly. Complete blood counts were checked 3 times/week for the first week; thereafter, daily blood counts were drawn for at least 5 to 7 days, or until blood counts had normalized. Dosage was started at 350 mg/sq m/day infused over 12 hr. After 3 patients had shown no toxicity at this schedule, the dosage was escalated to 700 mg/sq m/day infused over 12 hr. For pharmacological considerations, 3 patients received a single infusion of 700 mg/sq m/day over 6 hr.

Pharmacological Studies. BrdUrd was dissolved in 500 ml of 5% dextrose and infused into a peripheral vein. A central venous catheter was inserted prior to beginning the BrdUrd study. A radially arterial line was established and maintained for blood sampling purposes throughout the pharmacological study, after which the arterial line was discontinued and the central venous catheter was used for BrdUrd infusion. The BrdUrd was infused for 12 of every 24 hr. Five-mil samples were collected in heparinized tubes from the arterial line at 0, 0.5, 1, 2, 4, 8, and 12 hr. The arterial blood samples were kept in a refrigerator at 4° until they were processed together at the end of the collection period. The samples were centrifuged at 2500 rpm at 4° for 10 min. The plasma was collected and stored at -20° until it was readied for extraction and analysis. The extraction procedure consisted of placing 0.5 ml of plasma to be analyzed into a glass test tube. The plasma was then spiked with 50 µl of 1 × 10^-4 M 5-iododeoxyuridine (internal standard) and then 25 µl of 1 M potassium phosphate monobasic (pH 4.6), mixed by vortex apparatus; then, 4 ml of ethyl acetate (Burdick & Jackson Laboratories, Inc.; HPLC grade) were added. The sample was then mixed thoroughly with a vortex apparatus for 20 sec and then centrifuged at 2000 rpm for 10 min. The top layer (organic phase) was collected, transferred to another glass tube, and evaporated under a gentle nitrogen stream. The sample was then resuspended in 150 µl of the HPLC mobile phase. All solvents were filtered and degassed. Water was doubly distilled in glass, and methanol was obtained from Burdick & Jackson (HPLC grade). A Waters HPLC instrument equipped with a RCM(100) 10 µm C-18 column was used for separation. The mobile phase was an isocratic 0.01 M potassium phosphate with 9% methanol (adjusted to pH 3.5 with phosphoric acid) at a flow rate of 3 ml/min. The column was washed with methanol after each.

RESULTS

Serial plasma samples of BrdUrd were analyzed in 6 patients. Table 1 presents the measured Cm (i.v.) for the various i.v. infusion rates. At doses of 350 mg/sq m/12 hr (1.6 µmol/sq m/min), an average steady-state arterial plasma BrdUrd concentration of 0.68 to 0.98 µM was achieved. Chart 2 shows that...
shown to exhibit nonlinear pharmacokinetics (4, 7, 25); therefore, it was hypothesized that BrdUrd (an analogue of dThd) might behave similarly. However, it was found in this study that systemic toxicity prevails before any nonlinear behavior was demonstrable for BrdUrd. The linear behavior simplified pharmacokinetic analysis and allowed direct calculation of BrdUrd concentrations for a variety of infusion rates.

The pharmacokinetic studies reported in this paper were performed as part of a Phase I/II evaluation of i.v. BrdUrd as a radiosensitizer for gliomas. Some antitumor response was noted, but the overall efficacy was low (16). Myelosuppression was the dose-limiting toxicity. Although the i.v. route does not appear to be as feasible as does the i.a. route for the effective delivery of BrdUrd, a thorough description of the clinical pharmacology of BrdUrd is now available. It is now possible to estimate retrospectively the concentrations of BrdUrd attained in both the carotid artery and systemic sites during earlier clinical trials of intracarotid BrdUrd infusion. It may also be possible to optimize future reevaluations of i.a. BrdUrd.

Our clinical trial (16) demonstrated myelosuppression at plasma BrdUrd concentrations of 2.1 μM but not at 0.7 μM. Thus, the optimal BrdUrd delivery protocol must keep systemic plasma concentrations at or below 2.1 μM. Prior to and following the course of BrdUrd infusion, bone marrow samples were harvested from these patients from sites outside the field of radiation and evaluated for in vivo BrdUrd incorporation by comparison of in vitro radiation survival curves (20). There was no radiosensitization when the plasma BrdUrd concentration was 0.7 μM. However, when the plasma concentration was 2.1 μM, bone marrow samples exhibited a radiation enhancement factor as high as 2.2. These results are consistent with the in vitro work of Mahler and Elkind (19). Although the radiation enhancement factor for gliomas was not measured, these bone marrow results serve as a useful reference point.

The intracarotid BrdUrd infusion studies of Sano et al. (12, 13, 23, 24) can provide additional reference points. In their studies, an average of 450 mg/sq m/day (1 μM/min/sq m) of BrdUrd was infused through the carotid artery for 4 to 6 weeks. No plasma BrdUrd concentrations were measured, but the improved survival rates for high-grade gliomas suggested an enhancement of radiation effect with BrdUrd. No myelosuppression was reported. Retrospective calculations based upon the data in this study suggest that the intracarotid artery BrdUrd concentrations would have been approximately 4 μM in these earlier trials, while systemic plasma BrdUrd concentrations would have been approximately 0.4 μM.

Because of the systemic toxicity of BrdUrd, the use of a constant i.v. infusion does not appear to be as effective as is a direct i.a. infusion. However, the requirement for a long (>2 weeks) infusion of BrdUrd (into an artery) to facilitate incorporation into DNA may be too demanding for external pumps. Recently, Baker and Wheeler (2) have reported successful application of totally implanted pumps for long-term intracarotid infusions of FdUrd. Perhaps, new technology such as this can be used to test BrdUrd effectiveness in an optimal setting.

**REFERENCES**

Pharmacology of BrdUrd


Pharmacological Evaluation of Intravenous Delivery of 5-Bromodeoxyuridine to Patients with Brain Tumors

Angelo Russo, Luca Gianni, Timothy J. Kinsella, et al.


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