Measurement of Thymidine Replacement in Patients with High Grade Gliomas, Head and Neck Tumors, and High Grade Sarcomas after Continuous Intravenous Infusions of 5-Iododeoxyuridine


ABSTRACT

Based upon the radiation sensitization properties of the halogenated pyrimidines, 5-iododeoxyuridine (IdUrd) and 5-bromodeoxyuridine, long term i.v. infusions of halogenated pyrimidines in conjunction with fractionated radiation therapy have been evaluated in the treatment of a variety of human malignancies. While clinical studies have attempted to measure the halogenated pyrimidine incorporation, few have successfully related tumor response to the incorporation of IdUrd by the tumor. The present study reports the continuous IdUrd labeling index (number of cells labeled) and the IdUrd corrected replacement (percentage of thymidine replacement in the labeled cells of the population) from 17 patients who received continuous infusions of IdUrd (1000 mg/m²/24 h). The tumors treated included four high grade gliomas, five head and neck tumors, four high grade sarcomas, and five other tumors of varying types. Less than 25% of the cells in three of four gliomas incorporated IdUrd after 5–7-day IdUrd infusion time. Corrected replacement for the gliomas ranged from 0 to 4%. In contrast, 63–85% of the cells in the head and neck biopsies were labeled with IdUrd after 3–7-day IdUrd infusions suggesting that these large tumors (3–12 cm diameter) have a high fraction of dividing cells. Corrected replacements values for the head and neck tumor patients ranged from 2.9 to 26.3%. The high grade sarcomas also demonstrated a high percentage of labeled cells (57–79%) with three patients having corrected replacements of 7.5–14.2%. The continuous labeling and thymidine replacement data for four patients from whom serial biopsies were taken during IdUrd infusion demonstrated both an increasing IdUrd replacement and continuous labeling index with an increasing duration of IdUrd infusion. The clinical response of both the high grade glioma and head and neck tumor patients indicate that the IdUrd replacement and labeling data may provide some important predictive information with regard to the successful use of the halogenated pyrimidines in clinical radiation trials.

INTRODUCTION

The use of the HPs,2 IdUrd and BrdUrd, as radiosensitizers in the treatment of human cancers has received renewed interest in recent years (1–4). Because of complications associated with the use of i.a. catheter infusion of BrdUrd (5, 6) early clinical trials using BrdUrd were discouraging; however, in certain head and neck tumors impressive tumor responses were noted (5). The finding that i.v. continuous infusion of HPs is an effective and safe method of drug delivery provided the impetus for reinvestigating the use of the HPs in conjunction with radiation treatments (7). Thus, in the 1980s several clinical trials were initiated using either IdUrd or BrdUrd in tumors which were considered clinically radioresistant and difficult to control locally (3, 4).

In 1967 Bagshaw et al. (8) reported a 4.6% BrdUrd incorporation into a single H/N tumor biopsy after 4 days of continuous arterial BrdUrd infusion. However, initial HP uptake studies were limited because it was necessary to use 14C-labeled BrdUrd and, furthermore, it was not possible to determine the percentage of tumor cells labeled with BrdUrd. Two techniques now have been developed which greatly enhance the quantification and localization of HP’s in tumor specimens (9–12): (a) analytical HPLC methods can detect and quantify HP incorporation into DNA, thus avoiding the use of radioactive compounds (9, 10); and (b) monoclonal antibodies capable of recognizing HPs incorporated into DNA can be used to determine the percentage of cells in a tumor specimen that are labeled with the HPs (by either flow cytometry or microscope based systems) (11, 12).

Speth et al. (13), using both HPLC and the anti-BrdUrd monoclonal antibody technique, have reported the incorporation and labeling results in metastatic liver tumors from 5 patients receiving continuous i.v. infusions of IdUrd. After continuous infusion with 1000 mg/m²/day IdUrd for 3 days, approximately 3% thymidine replacement was achieved in 32% of the tumor cells (13). The influence of IdUrd on the radiation tumor response was not reported.

Phase II clinical trials using IdUrd in nonresectable high grade sarcomas and high grade gliomas have been reported (14, 15). IdUrd infusion of nonresectable high grade sarcomas in conjunction with high dose radiotherapy produced a local control rate of 60%, a response rate considered highly encouraging (14). In contrast, the use of IdUrd for gliomas has been disappointing (15); however, in the glioma and sarcoma protocols neither replacement nor labeling was measured. Thus, whether the glioma patients failed to respond because of the lack of IdUrd uptake is unknown, and on the other hand whether the sarcoma patients responded because of sufficient IdUrd uptake is also unknown.

We present IdUrd incorporation and labeling data obtained from 17 patients undergoing continuous i.v. infusions of IdUrd. In addition to the quantification of the IdUrd incorporation and labeling, the clinical response of the tumors is documented and correlated with the incorporation and labeling of each tumor. The results demonstrate the importance of obtaining both incorporation and labeling data, inasmuch as complex changes may occur in tumors as a function of IdUrd infusion times. Finally, the clinical results suggest that increased incorporation and labeling may correspond to increased tumor response.

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2 The abbreviations used are: HP, halogenated pyrimidine; IdUrd, 5-iododeoxyuridine; BrdUrd, 5-bromodeoxyuridine; i.a., intrarterial; H/N, head and neck; HPLC, high performance liquid chromatography; HBSS, Hanks' balanced salt solution; PBS, phosphate buffered saline; FITC, fluorescein isothiocyanate; PI, propidium iodide.
MATERIALS AND METHODS

Animal Studies. The KHT cell line was kindly provided by Dr. Dietmar Sieman (University of Rochester). KHT cells (10⁶) were injected s.c. into the right flank of C3H/HeNCR mice and allowed to grow for 8–12 days (0.8–1 cm in diameter). Research was conducted according to the principles outlined in Ref. 16.

Tissue and Cell Processing. Tumors were removed from animals, cut into 2–3-mm pieces, and incubated with 0.25% DNase (Sigma Chemical Co., St. Louis, MO) and 0.15% collagenase (Sigma) for 20–30 min. Cells were removed from digest buffer, centrifuged, and placed into an RBC lysing buffer for 3–5 min to remove RBC. The cells were then placed into HBSS containing 5% fetal calf serum, counted, and aliquoted for further studies.

DNA Isolation and Preparation. The nucleoside isolation procedure was modified from that of Gehrke et al. (10). Cells (3–5 × 10⁶) or tissue (1–10 mg) were incubated in 2 ml porcine pepsin (0.4 mg/ml, in 0.1 N HCl) (Calbiochem, La Jolla, CA) for 30–60 min at 37°C. At the end of the pepsin incubation time PBS containing 5% fetal calf serum was added to stop the pepsin action and the nuclei were isolated by filtering through a 60 µm nylon mesh. The nuclei were then treated with 0.2% DNase I (Sigma). The nuclei were then sheared through a 19-gauge needle and the lysate was incubated for 2 h at 37°C to remove DNA stabilizing proteins. The DNA was precipitated with ice-cold ethanol and isolated by centrifugation. The DNA was redissolved in 0.2 M sodium acetate buffer (30 mM sodium acetate, 0.5 mM zinc sulfate, pH 5.2) containing 20 µl of endonuclease P₁ (150 units/ml) (Sigma) and 20 µl of bacterial alkaline phosphatase (100 units/ml) (Sigma) and 140 µl of sodium acetate buffer (30 mM sodium acetate, 0.5 mM zinc sulfate, pH 5.2) containing 20 µl of endonuclease P₁ (150 units/ml) (Sigma) and 20 µl of bacterial alkaline phosphatase (100 units/ml) (Sigma) were added to cleave the DNA. The enzyme mixture was incubated at 37°C for 2 h at which time 25 µl of 0.5 M Tris buffer were added to increase the pH to 7.5 to allow the alkaline phosphatase to function. The mixture was incubated for an additional 2 h at 37°C. The enzyme mixture was then centrifuged through a Amicon M, 30,000 cutoff microcentrifuge tube (Amicon, Danvers, MA) to remove the majority of the large proteins present and the purified nucleosides were then stored at −20°C until HPLC analysis.

Clinical Radiation Protocol and Tissue Acquisition. All patients were simulated with computerized tomography based imaging for radiation treatments. The H/N patients were treated to three field head and neck regions (two lateral fields, one anterior suprACLavicular field). At 4500 cGy a cone down was made on all H/N patients and the isolated nucleosides were then stored at −20°C until HPLC analysis.

Clinical Radiation Protocol and Tissue Acquisition. All patients were carefully monitored to document tumor response after IdUrd and radiation treatments. The initial tumor diameters were as follows: (a) H/N tumors, 3–15 cm; (b) gliomas, 2.5–12 cm; (c) sarcomas, 5–35 cm. A complete response was noted if no discernible disease remained 1 month after treatment. A partial response identifies tumors in which the cross-product of the two largest diameters shrank by 50% in size, a minor response is one for tumors which shrank <50% in size, and no response indicates that there was no difference in tumor size 1 month after treatment.

Tumor tissue for the measurement of continuous IdUrd labeling and replacement was obtained by surgical resection in the patients with gliomas and by local biopsy for the patients with sarcomas and H/N tumors.

Flow Cytometry Analysis. The KHT cells (2 × 10⁶) or the human tumor tissue (5–10 mg) were centrifuged out of HBSS with 5% serum and resuspended into 2 ml HBSS. To the 2 ml cell sample 5 ml of ice-cold ethanol was added while vortexing and the samples were kept at 4°C until analyzed. Cells were centrifuged out of the 70% ethanol and resuspended in 2 ml of PBS, and the nuclei isolated as described above. The isolated nuclei 2 n HCl in PBS was added for 15 min to partially denature the cellular DNA. Cells were centrifuged out of the HCl and resuspended in borate buffer, pH 8.0, to neutralize any residual HCl. The cells were then incubated in 1 ml of PBS and 1 µg of an anti-BrdUrd/IdUrd monoclonal antibody (Becton Dickinson, Mountain View, CA) for 1 h at room temperature. Cells were washed once with PBS with 0.1% Triton X-100 and incubated with 20 µg of a FITC linked anti-mouse IgG secondary antibody (Sigma) for 45 min at room temperature. The cells were then washed and incubated with 10 µg/ml PI (Sigma) to label the total DNA. Samples to be used as unlabeled controls were processed in a manner similar to that described above except that the primary antibody (anti-BrdUrd/IdUrd antibody) was not used.

All samples were processed using an Epics V cell sorter (Coulter Electronics Inc., Hialeah, FL). Both the FITC secondary and PI were excited with the 488 nm line of the argon ion laser and the cellular fluorescence between 518–532 nm (IdUrd) and >630 nm (PI) was detected, electronically digitized, and stored for further processing. Stored 2-dimensional histograms of the IDUrd labeling versus the DNA labeling were analyzed to identify the percentage of labeled cells with the unlabeled controls being used to set the lower level discriminator.

Frozen Section Analysis. Small (2–5-mm) pieces of tumor tissue were frozen down in OCT embedding medium (Miles Inc., Elkhart, IN) and stored at −70°C. Sections (10 µm) were taken from the −70°C freezer and immediately fixed in ice-cold acetone for 10 min for staining. The sections were first incubated with 0.15 M HCl at room temperature for 15 min. After the HCl the sections were washed 3 times in a PBS + 0.2% Triton X-100 buffer and incubated with 1 µg/ml of an anti-BrdUrd monoclonal antibody at room temperature for 45 min. The sections were washed 3 times in buffer and incubated with 1 µg/ml of an biotinylated anti-mouse secondary antibody (Amersham, Arlington Heights, IL) at room temperature for 30 min. Again the sections were washed 3 times in buffer and incubated with 5 µg/ml FITC linked streptavidin (Amersham) at room temperature for 25 min. Finally the sections were incubated 5–7 min with PI to stain the DNA/RNA. The sections were mounted in a 90%/10% glycerol/water medium containing 1 mg/ml p-phenylenediamine (Sigma) as an antifading agent.

All images of the IdUrd labeled tumor sections were obtained using the Zeiss confocal laser scanning system (Carl Zeiss, Thornwood, NY). The FITC-streptavidin (IdUrd) was excited at 488 nm with an air cooled argon ion laser and the fluorescence was detected using a 525 band pass filter (Omega Optical, Inc., Brattleboro, VT). The PI was excited using the 488 nm line and the fluorescence was detected using a 530 nm long pass filter (Omega). The 2 black and white images of the FITC and PI labeled slides were overlaid and a pseudo-color image was obtained with the red representing the PI stained regions (nucleus) and the green/yellow representing the FITC labeled regions (IdUrd).

HPLC Analysis. Samples were processed using a Waters HPLC system (Waters, Bedford, MA). Nucleosides were separated using a Supelco C₁₈ LC-18-DB column (Bellefonte, PA) under isocratic conditions with the buffer containing 10 mM phosphate and 7% acetonitrile, pH 6.0. The major nucleosides were detected with 254 nm absorption while IdUrd was detected at 290 nm. Using this HPLC method thymidine eluted at 5 min while the IdUrd eluted at 12 min. Nucleoside identification was verified by known nucleoside standards (Sigma). To determine the thymidine replacement, the thymidine:IdUrd peak area ratio (R) was first calculated for several known thymidine and IdUrd concentrations and used in the formula:

(IdUrd/R)/(thymidine + IdUrd/R)
RESULTS

Single Cell Suspensions versus Tissue Pieces. Prior to the human tumor studies, the percent replacement of thymidine by IdUrd in in vitro cell lines was determined using single cell preparations. Because the amount of tissue available from biopsy material is often limited, studies were done to compare replacement results obtained from single cell suspensions versus whole tissue pieces. Mice bearing KHT tumors were given injections of 2 mg IdUrd every 3 h for 12 h (4 injections total) and 24 h after the last injection the tumors were removed and processed either to isolate single cells or multiple 2–3-mm pieces were used for determination of IdUrd replacement. Fig. 1 demonstrates results for the single cells and the whole tumor pieces. The tumor pieces gave a more variable IdUrd replacement when compared to the replacement obtained using the single cell suspension (range for single cells, 1.7–2.2%; range for tumor pieces, 1.2–3.2%); however, when multiple pieces for all 3 tumors were averaged and compared to the single cell results then no statistically significant differences were observed (single cells, 2.02%; tissue pieces, 2.06%).

Human IdUrd Data. Tumor specimens obtained from patients receiving 1000 mg/m²/day IdUrd were obtained and processed according to the procedure in Fig. 2. Tumor pieces were processed by flow cytometric analysis for the number of cells labeled, and by HPLC analysis, to determine the percent thymidine replacement by IdUrd. The HPLC and flow cytometry results were then combined to give the corrected replacement value, which is the average replacement of labeled cells within the cellular population (Fig. 2). Patients included in the IdUrd studies received continuous infusions of IdUrd for various times before biopsy or surgical resection.

Fig. 3 demonstrates the fluorescent staining pattern of three patients analyzed using the anti-BrdUrd/IdUrd monoclonal antibody on frozen tumor sections. Fig. 3A is from glioma patient 1761 and demonstrates the general finding that relatively few tumor cells were labeled with IdUrd in the brain patients. Fig. 3B is from patient 1721, a patient with esophageal squamous cell cancer, and is representative of sections in which about 50% of the cells were labeled. Finally, Fig. 3C is from patient 1680 and demonstrates the very high IdUrd labeling obtained from the H/N patients.

Table 1 contains the results for 17 IdUrd treated patients. Three groups of tumors are currently entered into the Radiation Oncology Branch IdUrd radiation protocol: (a) high grade gliomas; (b) head and neck tumors; and (c) high grade sarcomas. In 3 of 4 (patients 1604, 1761, and 1837) glioma patients, a continuous labeling index less than 21% was found (Table 1). Thymidine replacement for the glioma patients varied from 0 to 4%.

Four patients with head and neck tumors biopsied after 3–7-day infusions had more than 60% of their cells incorporating some IdUrd and in several cases over 80% were labeled (Table 1). In 3 of 5 patients studied with H/N tumors (patients 1680, 1817, and 1910) corrected replacement values of 5% or more occurred and a large percentage of the cells were labeled with IdUrd (63–80%).

In 3 of the 4 high grade sarcomas biopsied >50% of the cells were labeled with IdUrd (57–79%). In the 2 patients in whom sufficient tissue was available for replacement determination, replacement in excess of 7% was measured. One patient (patient 1792) had a corrected replacement of 14.2%; however, the percentage of labeled cells was only 12%

Four patients, 2 with H/N tumors and 2 with esophageal cancers, were biopsied serially during the IdUrd infusion (Table 2). In 3 of these 4 patients increased duration of infusion clearly produced elevated corrected replacement (patient 1680, from 4.4% to 26.3%; patient 1721, from 4.3% to 14.5%; patient 1910, from 3.0% to 5.2%). Corrected replacement in patient 1817 did not increase with increased infusion time; however, a greater number of labeled cells was observed with the increased IdUrd infusion interval (from 63% to 75%). In fact, all of the patients who received multiple biopsies had an increased number of IdUrd labeled cells with longer IdUrd infusions; however, while patient 1680 had an increased number of labeled cells going from the 3rd to the 6th day, a decrease in the number of labeled cells occurred with increased infusion time (from 80% on day 6 to 41% on day 10).

Fig. 4 demonstrates the importance of quantifying IdUrd labeled cells within the tumor. For these 3 patients the DNA profiles were aneuploid; hence, it was possible to identify the labeled aneuploid cells from other labeled cells with a normal DNA profile.
DNA content. In patient 1721 virtually 100% of the aneuploid tumor cells had incorporated some IdUrd (Fig. 4B). The aneuploid cells from patient 1680 were also predominantly labeled; however, a small percentage of aneuploid cells (10–15%) were unlabeled (Fig. 4A). It appeared that patient 1792 had a majority of labeled cells (>80%) with a normal diploid content of DNA and thus suggests that few if any of the aneuploid tumor cells actually incorporated IdUrd (Fig. 4C).

**Clinical Results.** The outcome of grade IV gliomas treated in the Radiation Oncology Branch has not improved using IdUrd and hyperfractionated irradiation (median survival, 11 months; 2-year survival, 8%) (15). In contrast, in metastatic and primary sarcomas the local control of massive disease (inoperable tumors larger than 10 cm diameter) has been excellent (14). In the head and neck patients, when the IdUrd labeling has been better than 50–60% and the replacement has ranged from 2.9 to 26.3%, the tumor response has been excellent; several tumors completely disappeared by 4000 cGy. There have been 5 of 5 complete remissions with only one local recurrence thus far.

**DISCUSSION**

The successful clinical use of IdUrd/BrdUrd should depend on both the extent of thymidine replacement by these sensitizer into the tumor DNA and the number of cells in the tumor mass which take up the IdUrd/BrdUrd (17–19). Fig. 4 demonstrates the complexity of IdUrd incorporation into human tumor populations and stresses the need to gather both flow cytometric and HPLC data. Fig. 4, B and C, illustrates flow IdUrd tumor data from 2 patients demonstrating vastly different uptake of IdUrd. Approximately 100% of the aneuploid cells in patient 1721 were labeled (Fig. 4B) in contrast to only 3–4% in patient 1792 (Fig. 4C). The corrected replacement listed for patient 1792 was 14%; this corrected replacement was heavily influenced by the very low IdUrd labeling (12%). Since only 3–4% of the total cells labeled were aneuploid, it might be expected that IdUrd would have little impact in this tumor, assuming all the aneuploid cells were cancerous in origin. In addition, all tumors demonstrated labeled cells with normal DNA content (possibly leukocytes), several with 40% or more. We did not attempt to modify the corrected replacement values for the presence of labeled normal cells, because it was not always possible to identify aneuploid cells in the tumor specimens. One concern is that the IdUrd replacement increases seen with many of the multiply biopsied patients (Table 2) may not represent increased tumor cell incorporation but rather increases in labeled normal cells present in the tumor biopsy. However, for patient 1680, in which aneuploid cells were easily distinguished from the normal cells in all 4 biopsies, there was not a substantial increase in the percentage of labeled normal cells compared to labeled tumor cells (data not shown). Nevertheless, the exact role of labeled normal cells in influencing the corrected replacement values is still unknown and should receive increased attention not only with regard to IdUrd incorporation but also with regard to tumor cell uptake of various chemotherapy drugs.

With the high grade glioma patients (grade III and IV) IdUrd incorporation after 3–7 days of infusion was the lowest of the 3 tumor types studied (2.6% gliomas, 5.6% H/N, 10.4% sarcomas) (Table 1). Although patient 1656 had corrected thymidine replacement of 4% and a high continuous labeling index (65%) the major feature of the other brain tumors was the very limited labeling of the tumor cells (<25%). In another study, 2 patients with gliomas receiving i.a. BrdUrd underwent surgical treatment after 4 weeks of continuous BrdUrd infusion and 2 weeks after a 6-week continuous BrdUrd infusion, respectively, and the BrdUrd incorporation was determined (20). After these long infusion times the percentage of thymidine replacement ranged from 2.2 to 5.6%; however, the number of labeled cells was not quantified (20). Our data, which indicate both low incorporation and labeling in gliomas, suggests that IdUrd, and

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possibly BrdUrd, in these patients will not greatly enhance the radiation induced damage to brain tumors and hence would not be expected to produce significant increases in survival. Indeed, in clinical trials at the Radiation Oncology branch at the National Cancer Institute (15), 45 glioma patients given BrdUrd and hyperfractionation radiation treatments (150 cGy twice a day up to 7300 cGy) had no significant increase in survival. Indeed, the same group reported no increased survival when BrdUrd and radiation was used for glioblastoma multiforme (21). This might be due to the greater radiation sensitivity of grade III anaplastic gliomas as compared to the more radioresistant grade IV gliomas (22) or differences in uptake and incorporation of BrdUrd and BrdUrd by gliomas and astrocytomas may play an important role.

The results in the four patients with head and neck tumors demonstrate IdUrd uptake into 63–85% of the cells (Table 1). This is the first determination of the continuous labeling index with IdUrd in head and neck tumors. A surprising feature of the H/N patients was the very high continuous labeling index (>60%) found for tumors which varied from 3 to 15 cm in diameter. One criticism of the H/N data is that the biopsied material may not reflect the entire tumor population. Indeed, in one H/N case, patient 1680, multiple pieces (3 or more) were available from several locations in the tumor and variation in the continuous labeling index was found (mean, 64%; range, 26–88%). However, additional biopsies from this patient were not available from several locations in the tumor and variation in the continuous labeling index was found (mean, 64%; range, 26–88%). However, additional biopsies from this patient showed less variability (Table 2). Thus, although additional data must be collected, we believe that the continuous labeling data are representative of the entire tumor. The majority of the cells in the H/N tumors appear to be progressing through cell cycle which suggests that H/N tumors might be more sensitive to agents that specifically target cycling cells. Furthermore, the H/N patients responded very briskly to the irradiation showing partial responses by 3500-5000 cGy (tumor mass completely resolving). Encouraging clinical responses using BrdUrd in head and neck tumors were documented in studies by Bagshaw et al. (8); however, the BrdUrd treatment was abandoned because of severe mucosa reactions with i.a. delivery of the drug.
THYMIDINE REPLACEMENT IN HUMAN CANCERS AFTER i.V. IdUrd

7: 10'

DNA (channel number)

Fig. 4. Two dimensional flow histograms of IdUrd labeling versus DNA content for 3 different human tumors. A, patient 1680, squamous cell carcinoma of the cheek; B, patient 1721 (8-day IdUrd infusion), esophageal carcinoma; C, patient 1792, high grade sarcoma. The histograms have been divided into 4 regions: Box 1, labeled normal cells; Box 2, labeled tumor cells; Box 3, unlabeled tumor cells; Box 4, unlabeled normal cells. For each histogram 20,000 cells were collected and analyzed.

8). In contrast, the patients with H/N tumors in this study had manageable normal tissue complications and were able to complete the full radiation treatment (7500 cGy total dose).

One of our H/N patients (patient 1680) presented with a massive stage IV squamous cell tumor of the cheek and was considered to have disease too extensive for a bi- or tri-modality approach. The patient was infused with IdUrd for 10 days and achieved a 26% corrected thymidine replacement prior to radiation treatment. This patient had a dramatic tumor response by 4000 cGy but later died of metastatic disease. Overall, although our patient numbers are small and it is too early to indicate the overall effect of IdUrd in achieving local control, the clinical results are encouraging with 5 of 5 H/N patients showing complete responses; 4 of these patients were locally controlled until death by metastatic disease.

The clinical results for patients with nonresectable sarcomas treated with IdUrd and aggressive radiation treatments at the National Cancer Institute have been positive with local control rates of 60% in a nonrandomized trial involving 36 patients (14). The basis for the use of IdUrd in these high grade sarcoma patients is indicated by the results in Table 1 in which 57–79% of the tumor cells were identified as IdUrd positive and 2 patients had IdUrd incorporation of 8–10% after 6- and 14-day infusions, respectively. Our sarcoma results are consistent with the single sarcoma datum reported by Speth et al. (13), which had labeling of 60–65% and corrected replacements of 1.5–4.0%.

The tumor radioresponsiveness of the sarcomas and gliomas poorly correlate with long term tumor control (23). In the case of the large sarcomas, partial responses may not be associated with radioresistance because sarcomas can produce a large acellular matrix occupying a large portion of the observable tumor mass (24). To date, 3 of the 4 sarcoma patients have shown no evidence of disease progression, while 5 of the 5 glioma patients over time have shown evidence of recurrent disease in the treatment field. Whether or not IdUrd is solely responsible for increasing local control in the sarcomas is unknown because the fractionation schedule has been altered as well (14). A randomized clinical trial is currently under way to clarify the role of IdUrd in the treatment of nonresectable sarcomas.

Of the several patients who received serial biopsies, 3 of 4 clearly demonstrated that longer infusion times produce higher corrected replacement values (Table 2). In addition, all patients analyzed showed an increasing number of IdUrd labeled cells with increasing infusion times (although patient 1680 did show an eventual reduction in labeled cells with further IdUrd infusion time). Because the effect of radiation on the uptake of IdUrd by human tumors is unknown, these data suggest that IdUrd infusions between 3 and 7 days may optimally balance the need for high labeling and incorporation with the problem of normal tissue toxicity (low platelet counts).

The amount of sensitizer (either IdUrd or BrdUrd) incorporation necessary to achieve a clinical response is still unknown. In addition to our data, work by Phillips et al. (19), where sensitizer enhancement ratios have been determined for different levels of IdUrd incorporation in V79 cells, indicate that at least 10–12% thymidine replacement is necessary to produce sensitizer enhancement ratio of 1.5. Speth et al. (13) hypothesize that 10–14-day arterial IdUrd infusions of metastatic disease in the liver could achieve replacements ≥30%. In one of our head and neck patients (patient 1680) i.v. IdUrd infusion for 10 days achieved a corrected replacement of 26% (Table 2). However only 40% of the cells in the tumor biopsy were labeled. Thus, even these high replacements may not prove to be clinically useful.

In conclusion, we biopsied and analyzed 17 different human tumors for IdUrd labeling and incorporation from patients receiving a continuous i.v. infusion of IdUrd. Although the number of patients biopsied and studied is small, the data suggest that IdUrd may have useful clinical possibilities with head and neck tumors and high grade sarcomas and offer a potential explanation for its lack of efficacy in high grade gliomas.
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