Kinetics of Bromodeoxyuridine Elimination from Human Colon Cancer Cells in Vitro and in Vivo

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ABSTRACT

We have previously shown that the thymidine analogue radiation sensitizer bromodeoxyuridine (BrdUrd) is incorporated into human tumors to a greater extent than into the livers of athymic mice bearing these tumors as xenografts. However, incorporation into the intestine and bone marrow exceeds that of the tumor (T. S. Lawrence, M. A. Davis, J. Maybaum, S. K. Mukhopadhyay, P. L. Stetson, P. E. McKeever, and W. D. Ensminger, Cancer Res., 52: 3698–3704, 1992). We hypothesized that the ratio of tumor incorporation to intestinal or bone marrow incorporation might increase during a period of drug elimination following the termination of an infusion. To test this hypothesis, we infused athymic mice bearing HT29 human colon cancer xenografts with BrdUrd and measured incorporation in the tumor and normal tissues up to 7 days after the infusion was discontinued. In addition, we assessed the effect of exposure to BrdUrd on subsequent incorporation in vitro and in vivo through the use of a stable isotope of BrdUrd (“isotopic BrdUrd”), which could be differentiated from normotopic BrdUrd using the gas chromatographic-mass spectrometric assay. We found a significant increase in the ratio of BrdUrd in the tumor compared to bone marrow and intestine during the drug elimination period. We also found that BrdUrd incorporation slowed the kinetics of subsequent BrdUrd incorporation and elimination. These findings suggest that when the radiation dose-limiting organ is rapidly proliferative, such as the intestine or bone marrow, delivering radiation during a drug elimination period may improve the therapeutic index.

INTRODUCTION

Although the thymidine analogue radiation sensitizers IdUrd and BrdUrd show promise, their application has been limited chiefly to tumors which are hypothesized to proliferate rapidly compared to the surrounding normal tissue (e.g., malignant gliomas, intrahepatic cancers) (1–4). However, in the case of tumors such as recurrent rectal, pancreatic, or cervical cancer, the tumor is adjacent to normal intestine which would be anticipated to incorporate analogues efficiently. Using athymic mice bearing human colon cancer xenografts, we confirmed that at the end of a continuous infusion, incorporation of BrdUrd into the tumor was significantly greater than incorporation into the liver (5). We also found that BrdUrd incorporation in the normal intestine and bone marrow exceeded that in tumor. Therefore, whereas concurrent irradiation and BrdUrd infusion is a rational strategy for the treatment of intrahepatic tumors, it could increase toxicity in patients whose intestines are in the radiation portal.

We hypothesized that the fraction of dThd replaced by BrdUrd in tumors might exceed that of highly proliferative normal tissues after discontinuation of the infusion. This would occur if the normal tissues continued to proliferate more rapidly than the tumor, since the fraction of dThd replaced by BrdUrd would be decreased by newly incorporated dThd.

To begin to assess this hypothesis, we carried out a two-part study. First, we assessed incorporation of BrdUrd into HT29 human colon cancer xenografts and normal tissues of athymic mice after the completion of an infusion. In agreement with our hypothesis, we found that after the infusion was discontinued, BrdUrd incorporation decreased significantly more rapidly from normal tissues than it did from the tumor. Second, we wished to understand better the kinetics of BrdUrd incorporation and elimination during continuous drug exposure. To perform these studies, we used an isotope of BrdUrd that is chemically identical to “normotopic” BrdUrd but could be differentiated by a GC/MS assay for incorporation (see below). We found that BrdUrd infusion significantly retards BrdUrd elimination from tumors and, to a lesser extent, normal tissues.

MATERIALS AND METHODS

Cell Culture. Human colon carcinoma cells (HT29) were cultured as described previously (6). BrdUrd-containing medium was changed daily. Experiments were performed in dim yellow incandescent light.

Flow Cytometry. Cells were trypsinized, washed, and resuspended in PBS; fixed by dropwise addition of 2.5 volumes of cold 70% ethanol; and stored at 4°C until the day of analysis. They were then washed with PBS and suspended in 1 ml of PBS containing 16.7 μg/ml of propidium iodide and 40 μg/ml of RNase A. Samples were analyzed on an EPICS C flow cytometer (Coulter Electronics). For two-parameter flow, cytometry cells were processed for immunoassay with the anti-BrdUrd antibody (Becton Dickinson) followed by fluorescein isothiocyanate-goat anti-mouse IgG (Sigma Chemical Co.) (7).

Infusion of Nude Mice Bearing Xenografts with Halogenated Pyrimidines. Female athymic nude mice (CD-1) were housed, prepared with tumors, and infused with BrdUrd ±FdUrd using s.c. implanted osmotic pumps as described previously (8). Pumps were changed after 7 days for the 14-day infusions. Animals were handled in accordance with established procedures of the University of Michigan Unit for Laboratory Animal Medicine.

GC/MS Analysis of BrdUrd Incorporation. BrdUrd incorporation into DNA was measured as described previously (9, 10). Briefly, cells or tissues were lysed in a buffer containing 10 mm Tris, 10 mm EDTA, and 0.6% sodium dodecyl sulfate (pH 8.0). DNA was isolated from the lysate by proteinase and RNase digestion, followed by ethanol precipitation. Halouracil and thymine were liberated from the DNA by hydrolysis with DNase I, phosphodiesterase, alkaline phosphatase, and thymidine phosphorylase. Chlorouracil was added as an internal standard, and the bases were extracted into ethyl acetate and derivatized with N,O-bis(trimethylsilyl)trifluoroacetamide. The derivatized products were measured with a Hewlett-Packard 5987A GC/MS in selected ion-monitoring mode. Samples were assessed in triplicate. BrdUrd incorporation is expressed as the percentage of dThd replaced.

Some experiments were performed using a stable isotope of BrdUrd, (+)-5-bromo-2-deoxyuridine-2-13C,15N2 (MSD Isotopes, Montreal, Quebec, Canada). Isotopic BrdUrd contained one 13C at the ring 2 position and both ring nitrogens were 15N. The resulting molecular weight of the isotopic bromouracil base was, therefore, increased by 3 over that of the normotopic species, which was sufficient to differentiate the isotopes using the GC/MS assay (<1% cross-contamination).

Statistical Analysis. Unless otherwise indicated, data are presented as the mean ± SE of at least 3 independent experiments or at least 3 animals. Means

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3 The abbreviations used are: IdUrd, 5-ido-2'-deoxyuridine; BrdUrd, 5-bromo-2'- deoxyuridine; dThd, thymidine; PBS, phosphate-buffered saline; GC/MS, gas chromatographic mass spectrometric; FdUrd, 5-fluoro-2'-deoxyuridine.
were compared using the Student t test for either paired samples or pooled variances. Statistical significance is defined as a difference of $P < 0.05$ (two-tailed).

**RESULTS**

We had previously shown that under a wide variety of infusion conditions, BrdUrd incorporation into the DNA of HT29 human colon cancer xenografts is exceeded by incorporation into the intestine and bone marrow (5). As described above, we hypothesized that after an infusion was discontinued, BrdUrd might be retained in the tumor significantly longer than in rapidly proliferative normal tissues. This hypothesis was investigated under three different infusion conditions: 100 mg/kg/day for 14 days; 200 mg/kg/day for 4 days; and 300 mg/kg/day for 4 days. The first two conditions were chosen to compare long and short infusions which produced similar, tolerable weight loss, while the latter condition represented the maximum dose that could be administered (in that it produced substantial weight loss). At the end of the infusion period, the pumps were removed. Three days later, the animals were sacrificed and both tumors and normal tissues were assessed for BrdUrd incorporation. There was a significantly longer incorporation in animals given 100 mg/kg/day for 14 days (Table 1). We had previously shown that under all infusion conditions, BrdUrd (200 mg/kg/day) and the higher dose rate of FdUrd (0.3 mg/kg/day) increased both tumor and normal tissue incorporation 3 days after the infusion was stopped, thus reducing the therapeutic index achieved with BrdUrd alone (Table 2).

To understand the basis for this prolonged elimination of BrdUrd from tumors compared to normal tissue, we performed experiments both in vitro and in vivo aimed at obtaining a more complete understanding of the kinetics of incorporation and elimination during continuous BrdUrd infusion. These studies relied on the use of two isotopes of BrdUrd, the standard normotopic BrdUrd and isotopic BrdUrd (see “Materials and Methods”), which can be differentiated by the GC/MS assay. On the basis of our previous observation that the population-doubling time of HT29 cells significantly increases after a 2-day exposure to 10 μM BrdUrd (11), we hypothesized that exposure to BrdUrd for 2 days would decrease the rate of incorporation of subsequent BrdUrd. To test this hypothesis, HT29 cells were incubated with 10 μM normotopic BrdUrd for 2 days (the population-doubling time of HT29 cells is approximately 19 h), followed by exposure to 10 μM isotopic BrdUrd for 4 additional days (Fig. 2). We compared the incorporation of isotopic BrdUrd (after previous normotopic BrdUrd) to (normotopic) BrdUrd incorporation in cells without previous drug exposure. We found that exposure to a high concentration of BrdUrd consistently decreased the rate of incorporation of subsequent BrdUrd (Fig. 2).

![Fig. 1. Elimination of BrdUrd in vivo after the discontinuation of an infusion. Athymic nude mice bearing HT29 human colon cancer xenografts were infused with BrdUrd (200 mg/kg/day) for 4 days. The infusion pumps were then removed (day 0), and the tumor and normal tissues were assessed for BrdUrd incorporation at the indicated times. * Incorporation into tumor significantly exceeds incorporation into the small intestine; columns, mean; bars, SE.](image-url)

<table>
<thead>
<tr>
<th>Tissue</th>
<th>100 mg/kg/day, 14 days</th>
<th>200 mg/kg/day, 4 days</th>
<th>300 mg/kg/day, 4 days</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Incorporation</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tumor</td>
<td>4.7 ± 0.4</td>
<td>5.9 ± 0.9</td>
<td>5.9 ± 0.9</td>
</tr>
<tr>
<td><strong>Liver</strong></td>
<td>1.3 ± 0.2</td>
<td>1.8 ± 0.5</td>
<td>2.3 ± 0.4</td>
</tr>
<tr>
<td><strong>Ratio</strong></td>
<td>4.5 ± 0.8</td>
<td>4.3 ± 1.0</td>
<td>2.4 ± 0.5</td>
</tr>
<tr>
<td><strong>Small intestine</strong></td>
<td>22.2 ± 2.0</td>
<td>7.7 ± 0.9</td>
<td>23.0 ± 1.1</td>
</tr>
<tr>
<td><strong>Ratio</strong></td>
<td>0.2 ± 0.1</td>
<td>0.8 ± 0.1</td>
<td>0.3 ± 0.2</td>
</tr>
<tr>
<td><strong>Bone marrow</strong></td>
<td>10.7 ± 1.3</td>
<td>3.4 ± 0.6</td>
<td>15.0 ± 2.1</td>
</tr>
<tr>
<td><strong>Ratio</strong></td>
<td>0.5 ± 0.1</td>
<td>1.6 ± 0.2</td>
<td>0.4 ± 0.1</td>
</tr>
</tbody>
</table>

* Percentage of dThd replaced by BrdUrd.

**RESULTS**

**Table 1 Influence of dose rate and duration on BrdUrd incorporation in tumor and normal tissues 3 days after discontinuation of drug infusion**

- Liver: Incorporation 1.3 ± 0.2, Ratio 4.5 ± 0.8
- Small intestine: Incorporation 22.2 ± 2.0, Ratio 0.2 ± 0.1
- Bone marrow: Incorporation 10.7 ± 1.3, Ratio 0.5 ± 0.1

- Liver: Incorporation 1.8 ± 0.5, Ratio 4.3 ± 1.0
- Small intestine: Incorporation 7.7 ± 0.9, Ratio 0.8 ± 0.1
- Bone marrow: Incorporation 3.4 ± 0.6, Ratio 1.6 ± 0.2

- Liver: Incorporation 2.3 ± 0.4, Ratio 2.4 ± 0.5
- Small intestine: Incorporation 23.0 ± 1.1, Ratio 0.3 ± 0.2
- Bone marrow: Incorporation 15.0 ± 2.1, Ratio 0.4 ± 0.1

- Liver: Incorporation 1.7 ± 0.4, Ratio 4.9 ± 1.7
- Small intestine: Incorporation 1.7 ± 0.3, Ratio 3.1 ± 1.0
- Bone marrow: Incorporation 4.4 ± 1.4, Ratio 1.3 ± 0.5

- Liver: Incorporation 3.7 ± 0.4, Ratio 2.0 ± 0.4
- Small intestine: Incorporation 25.1 ± 6.3, Ratio 4.4 ± 1.4
- Bone marrow: Incorporation 29.7 ± 1.4, Ratio 6.6 ± 0.4

- Liver: Incorporation 1.7 ± 0.1, Ratio 0.8 ± 0.1
- Small intestine: Incorporation 3.7 ± 0.4, Ratio 3.7 ± 0.4
- Bone marrow: Incorporation 5.9 ± 0.9, Ratio 5.9 ± 0.9

- Liver: Incorporation 0.8 ± 0.1, Ratio 0.8 ± 0.1
- Small intestine: Incorporation 1.3 ± 0.4, Ratio 1.3 ± 0.4
- Bone marrow: Incorporation 5.9 ± 0.9, Ratio 5.9 ± 0.9

- Liver: Incorporation 3.1 ± 0.5, Ratio 4.4 ± 1.4
- Small intestine: Incorporation 15.0 ± 2.1, Ratio 15.0 ± 2.1
- Bone marrow: Incorporation 29.7 ± 1.4, Ratio 29.7 ± 1.4

**KINETICS OF BrdUrd ELIMINATION**

- Liver: Incorporation 10.7 ± 1.3, Ratio 0.5 ± 0.1
- Small intestine: Incorporation 3.4 ± 0.6, Ratio 1.6 ± 0.2
- Bone marrow: Incorporation 10.7 ± 1.3, Ratio 0.5 ± 0.1
KINETICS OF BrdUrd ELIMINATION

Table 2 Effect of FdUrd on BrdUrd incorporation in tumor and normal tissues 3 days after discontinuation of drug infusion

<table>
<thead>
<tr>
<th>Tissue</th>
<th>200 mg/kg/day BrdUrd for 4 days</th>
<th>0.1 mg/kg/day FdUrd</th>
<th>0.3 mg/kg/day FdUrd</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 4</td>
<td>Day 7</td>
<td>Day 4</td>
</tr>
<tr>
<td>Tumor Incorporation</td>
<td>4.3 ± 0.4</td>
<td>3.2 ± 0.6</td>
<td>7.3 ± 0.8</td>
</tr>
<tr>
<td>Liver Incorporation</td>
<td>1.9 ± 0.3</td>
<td>0.3 ± 0.3</td>
<td>1.0 ± 0.3</td>
</tr>
<tr>
<td>Ratio</td>
<td>4.3 ± 1.8</td>
<td>3.4 ± 0.5</td>
<td>6.6 ± 1.3</td>
</tr>
<tr>
<td>Small intestine Incorporation</td>
<td>23.5 ± 3.5</td>
<td>2.2 ± 0.3</td>
<td>21.4 ± 3.7</td>
</tr>
<tr>
<td>Ratio</td>
<td>0.2 ± 0.1</td>
<td>1.6 ± 0.5</td>
<td>0.4 ± 0.1</td>
</tr>
<tr>
<td>Bone marrow Incorporation</td>
<td>22.0 ± 2.4</td>
<td>3.9 ± 0.5</td>
<td>22.5 ± 1.9</td>
</tr>
<tr>
<td>Ratio</td>
<td>0.2 ± 0.1</td>
<td>0.9 ± 0.2</td>
<td>0.3 ± 0.1</td>
</tr>
</tbody>
</table>

* Percentage of dThd replaced by BrdUrd.
* Tumor > normal tissue (P < 0.05).
* Incorporation in tumor = incorporation into liver, small intestine, and bone marrow (respectively) of same animal.

To assess the effect of continuous exposure to BrdUrd on the elimination of previously incorporated BrdUrd, cells were incubated with 10 μM normotopic BrdUrd for 2 days, followed for up to 4 days by either medium alone or 10 μM isotopic BrdUrd (Fig. 3). The fraction of dThd replaced by normotopic BrdUrd during days 3–6 represents the analogue remaining in DNA after (normotopic) BrdUrd has been removed from the incubation medium. We found that although there was little change in the fraction of dThd replaced by normotopic BrdUrd during the first 2 days after discontinuing normotopic BrdUrd, by the fourth day the presence of isotopic BrdUrd significantly slowed the elimination of normotopic BrdUrd. These data show that exposure to a high concentration of BrdUrd decreases the rate of elimination of previously incorporated BrdUrd.

We had previously shown that the increased population-doubling time of HT29 cells after exposure to BrdUrd is accompanied by a modest increase in the fraction of cells in the G1 phase of the cell cycle and a modest decrease in S-phase cells (11). To determine if subpopulations of cells with different proliferative capacities existed during this drug elimination period, two-parameter flow cytometry was performed in which BrdUrd and DNA content were separately assessed. If a highly proliferative subpopulation were present, a significant fraction of cells which did not contain BrdUrd would exist 4 days after removal of BrdUrd from the media. We found that after 24 h of BrdUrd exposure, virtually all cells demonstrated incorporation (not shown) and that incorporation was maintained up to 4 days after a 2-day exposure to 10 μM BrdUrd (Fig. 4). This suggests that no significant subpopulations of highly proliferative cells existed.

Because of these findings in cell culture experiments, we assessed the effect of a continuous BrdUrd infusion on the kinetics of BrdUrd incorporation and elimination in nude mice bearing HT29 xenografts. After a 4-day infusion with normotopic BrdUrd (200 mg/kg/day), the pumps were removed and replaced with pumps delivering the same dose rate of isotopic BrdUrd for up to 3 additional days. During these additional 3 days of infusion, relatively little isotopic BrdUrd was incorporated into the tumor and liver compared to the rate of incorporation into the small intestine and bone marrow (Fig. 5A). Conversely, during the 3 days of isotopic infusion, there was a rapid loss of normotopic BrdUrd from the intestine and bone marrow but relatively little loss from the tumor and liver (Fig. 5B). The infusion of isotopic BrdUrd during days 5–7 significantly retarded the elimination of normotopic BrdUrd from the tumor compared to rate of elimination.

Fig. 2. Effect of exposure to BrdUrd on subsequent incorporation of (isotopic) BrdUrd in vitro. Cultured HT29 cells were exposed to (normotopic) BrdUrd for 1–4 days (B) or normotopic BrdUrd (10 μM) for 2 days followed by isotopic BrdUrd for 1–4 days (E). Cells were then assessed for the incorporation of normotopic BrdUrd (B) or isotopic BrdUrd (E). * Incorporations differ significantly; columns, mean; bars, SE.

Fig. 3. Effect of exposure to BrdUrd on elimination of (normotopic) BrdUrd in vitro. Cultured HT29 cells were exposed to normotopic BrdUrd (10 μM) for 2 days followed by either medium alone or 10 μM isotopic BrdUrd for 1–4 days. Cells were assessed for the incorporation of normotopic BrdUrd. * Incorporation of normotopic BrdUrd in cells cultured in isotopic BrdUrd exceeds normotopic BrdUrd in cells cultured in medium alone; columns, mean; bars, SE.

Fig. 4. Presence of BrdUrd after a drug elimination period in vitro. Cultured HT29 cells were exposed to medium alone (A) or to BrdUrd (10 μM) for 2 days followed by medium for 4 days (B). Cells were then processed for two-parameter flow cytometry as described in "Methods and Materials." Contour plot shows cell number according to shading scheme in key.
Bone marrow incorporation and significantly exceeded small intestine incorporation during the drug elimination period. This improvement in the therapeutic index is probably due to the more rapid proliferation of cells (not containing BrdUrd) and loss of intestinal and bone marrow cells (containing BrdUrd) compared to the proliferation and loss of tumor cells. Since previous work from our laboratory and others has shown that the fraction of dThd replaced by BrdUrd is closely correlated with radiosensitization (12–14), these data suggest that an improved therapeutic index should result from delivering radiation during the drug elimination period for tumors in which the intestine or bone marrow is the dose-limiting organ. These findings are in agreement with earlier less quantitative studies in which radio-labeled IdUrd was injected into tumor-bearing mice (15, 16). Another potential advantage of using a drug elimination approach comes from the fact that stem cells in the intestine and bone marrow produce daughter cells. Therefore, in the case of the intestine, although at the end of the infusion crypt cells would contain most of the BrdUrd, during a drug elimination period the BrdUrd would tend to be contained in mature villus cells. Radiosensitization of these terminally differentiated cells should produce less organ toxicity than sensitizing the stem cell compartment.

A limitation of this study is that the kinetics of incorporation into mouse intestine (17) is more rapid than in human intestine, although there is evidence that for colonic epithelium, cell proliferation is increased in patients with cancer (18, 19). Therefore, it is unlikely that the differences observed in this study between the tumor and normal tissues will be identical to the analogous differences in the tumor and normal tissues of patients under treatment. However, we have preliminary data from a clinical protocol that this analogue elimination strategy may be effective in patients. In this protocol (for the treatment of locally advanced and retroperitoneal soft tissue sarcomas), patients undergo bone marrow and rectal mucosa biopsies both at the end of a 5-day infusion of IdUrd (day 5) and after a 3-day period of drug elimination (day 8). We have found that bone marrow incorporation and rectal crypt cell incorporation decrease significantly between days 5 and 8 (20) and that there has been no evidence of radiosensitization of the normal intestine (21). Furthermore, tumor incorporation does not appear to change significantly during the 3-day drug elimination period. Thus, these preliminary data with IdUrd support the use of a drug elimination strategy as a method of decreasing the morbidity of thymidine analogues when the tumor is surrounded by rapidly proliferating normal tissues.

The second major finding of this study comes from the use of isotopic BrdUrd, which permitted the evaluation of the effect of BrdUrd on its own incorporation and elimination. In both cultured cells and tumors, we found that incorporation retarded both further BrdUrd incorporation into and elimination from DNA. Since tumor cells vary widely in their ability to incorporate BrdUrd (22), it seems unlikely that all tumors will show the same kinetic changes. Some others has shown that the fraction of dThd replaced by BrdUrd is closely correlated with radiosensitization (12–14), these data suggest that an improved therapeutic index should result from delivering radiation during the drug elimination period for tumors in which the intestine or bone marrow is the dose-limiting organ. These findings are in agreement with earlier less quantitative studies in which radio-labeled IdUrd was injected into tumor-bearing mice (15, 16). Another potential advantage of using a drug elimination approach comes from the fact that stem cells in the intestine and bone marrow produce daughter cells. Therefore, in the case of the intestine, although at the end of the infusion crypt cells would contain most of the BrdUrd, during a drug elimination period the BrdUrd would tend to be contained in mature villus cells. Radiosensitization of these terminally differentiated cells should produce less organ toxicity than sensitizing the stem cell compartment.

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### Table 3 Total and normotopic BrdUrd incorporation after a 4-day infusion with normotopic BrdUrd and a 1- or 3-day infusion with isotopic BrdUrd

<table>
<thead>
<tr>
<th>Tissue</th>
<th>BrdUrd incorporation (1 day isotopic)</th>
<th>% normotopic</th>
<th>BrdUrd incorporation (3 days isotopic)</th>
<th>% normotopic</th>
</tr>
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<tr>
<td>Tumor</td>
<td>7.6 ± 1.1</td>
<td>89 ± 1</td>
<td>9.5 ± 1.2</td>
<td>70 ± 2</td>
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<tr>
<td>Liver</td>
<td>1.8 ± 0.2</td>
<td>94 ± 1</td>
<td>2.0 ± 0.3</td>
<td>76 ± 4</td>
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<tr>
<td>Small intestine</td>
<td>17.3 ± 3.1</td>
<td>64 ± 4</td>
<td>21.4 ± 3.1</td>
<td>21 ± 1</td>
</tr>
<tr>
<td>Bone marrow</td>
<td>11.5 ± 1.9</td>
<td>69 ± 5</td>
<td>23.7 ± 4.2</td>
<td>36 ± 7</td>
</tr>
</tbody>
</table>

* BrdUrd infusion rate of 200 mg/kg/day.

* Percentage of dThd replaced by BrdUrd.

after a 4-day infusion of (normotopic) BrdUrd alone (compare Figs. 1 and 2B).

Another method of expressing the elimination of normotopic BrdUrd during a continuous infusion is to determine the fraction of the total BrdUrd which is normotopic BrdUrd (Table 3). For both 1 and 3 days after the BrdUrd was changed from normotopic to isotopic (total duration of infusion, 5 and 7 days, respectively), a significantly greater fraction of tumor (and liver) BrdUrd was normotopic compared to the intestine and the bone marrow.

**DISCUSSION**

We had previously reported that although BrdUrd incorporation into xenografted colon cancer tumors at the end of a continuous infusion exceeded that of the liver, incorporation into the intestine and bone marrow was significantly greater than into the tumor. In this study, we found that there was a significant increase in the ratio of BrdUrd in the tumor compared to bone marrow and small intestine after a BrdUrd infusion is discontinued. In the nude mouse human tumor xenograft system which we used, tumor incorporation equaled bone marrow incorporation and significantly exceeded small intestine incorporation during the drug elimination period. This improvement in the therapeutic index is probably due to the more rapid proliferation of cells (not containing BrdUrd) and loss of intestinal and bone marrow cells (containing BrdUrd) compared to the proliferation and loss of tumor cells. Since previous work from our laboratory and others has shown that the fraction of dThd replaced by BrdUrd is closely correlated with radiosensitization (12–14), these data suggest that an improved therapeutic index should result from delivering radiation during the drug elimination period for tumors in which the intestine or bone marrow is the dose-limiting organ. These findings are in agreement with earlier less quantitative studies in which radio-labeled IdUrd was injected into tumor-bearing mice (15, 16). Another potential advantage of using a drug elimination approach comes from the fact that stem cells in the intestine and bone marrow produce daughter cells. Therefore, in the case of the intestine, although at the end of the infusion crypt cells would contain most of the BrdUrd, during a drug elimination period the BrdUrd would tend to be contained in mature villus cells. Radiosensitization of these terminally differentiated cells should produce less organ toxicity than sensitizing the stem cell compartment.

A limitation of this study is that the kinetics of incorporation into mouse intestine (17) is more rapid than in human intestine, although there is evidence that for colonic epithelium, cell proliferation is increased in patients with cancer (18, 19). Therefore, it is unlikely that the differences observed in this study between the tumor and normal tissues will be identical to the analogous differences in the tumor and normal tissues of patients under treatment. However, we have preliminary data from a clinical protocol that this analogue elimination strategy may be effective in patients. In this protocol (for the treatment of locally advanced and retroperitoneal soft tissue sarcomas), patients undergo bone marrow and rectal mucosa biopsies both at the end of a 5-day infusion of IdUrd (day 5) and after a 3-day period of drug elimination (day 8). We have found that bone marrow incorporation and rectal crypt cell incorporation decrease significantly between days 5 and 8 (20) and that there has been no evidence of radiosensitization of the normal intestine (21). Furthermore, tumor incorporation does not appear to change significantly during the 3-day drug elimination period. Thus, these preliminary data with IdUrd support the use of a drug elimination strategy as a method of decreasing the morbidity of thymidine analogues when the tumor is surrounded by rapidly proliferating dose-limiting normal tissues.

The second major finding of this study comes from the use of isotopic BrdUrd, which permitted the evaluation of the effect of BrdUrd on its own incorporation and elimination. In both cultured cells and tumors, we found that incorporation retarded both further BrdUrd incorporation into and elimination from DNA. Since tumor cells vary widely in their ability to incorporate BrdUrd (22), it seems unlikely that all tumors will show the same kinetic changes. Some investigators have advocated the use of computer models based on pretreatment tumor kinetics to suggest optimal methods of BrdUrd administration (23). The current study suggests that it may be difficult to predict incorporation under therapeutic conditions based only on pretreatment kinetic parameters. It should be noted that since normotopic and isotopic BrdUrd are equally radiosensitizing (data not

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advanced soft tissue sarcomas, we are currently accruing patients to clinical protocols using BrdUrd, administered according to a drug elimination design, as a radiation sensitizer in the treatment of pancreatic and cervical cancers. These protocols, along with the more mature protocol for soft tissue sarcomas, should begin to permit us to assess the clinical efficacy of this rational, unique radiation and sensitization schedule.

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Kinetics of Bromodeoxyuridine Elimination from Human Colon Cancer Cells \textit{in Vitro} and \textit{in Vivo}

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