Fluorodeoxyuridine Modulation of the Incorporation of Iododeoxyuridine into DNA of Granulocytes: A Phase I and Clinical Pharmacological Study

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ABSTRACT

The amount of iododeoxyuridine (IdUrd) incorporated into DNA determines the degree of radiosensitization. Fluorodeoxyuridine (FdUrd) has been shown to biochemically modulate IdUrd incorporation into DNA in vitro and in vivo. In this Phase I study, these drugs were coadministered to patients during 14-day continuous i.v. infusion periods in order to investigate whether the incorporation of IdUrd into DNA in vivo could be increased without increasing the dose of IdUrd. IdUrd plasma concentrations and incorporation of IdUrd into DNA of granulocytes were measured by high-performance liquid chromatography. Up to 8.9% substitution of thymidine by IdUrd was observed. Even at 3.5 mg/m²/day FdUrd for 14 days (78% of the maximum-tolerated dose as a single agent), no clinically relevant enhancement of incorporation of IdUrd into DNA of granulocytes was observed. Also, no changes in plasma levels of IdUrd were observed with escalating doses of FdUrd. Toxicity patterns (stomatitis, diarrhea, and bone marrow depression) and isobologram analysis suggested that IdUrd and FdUrd had additive, rather than synergistic, effects.

INTRODUCTION

Recently there has been a revival of interest in the clinical use of IdUrd⁴ and 5-bromo-2'-deoxyuridine (1, 2). The preclinical activity of these thymidine analogues was first reported in 1959 (3). IdUrd has been investigated both for its cytototoxic (4) and its radiosensitizing properties (5, 6). As the amount of thymidine replaced by the halopyrimidine increased, cells became more sensitive to radiation therapy (1, 6). In ascitic F388 leukemia in mice, substitution of only 2% produced a 2- to 4-fold potentiation of radiation effects (7).

Incorporation of IdUrd into DNA can be modulated in vitro and in vivo by prior or simultaneous exposure to agents such as fluorouracil (8), FdUrd (7–9), or methotrexate (10). FdUrd is phosphorylated to FdUMP, which blocks the de novo synthesis of dTMP by inhibiting TS (11). In vitro experiments with a cell line derived from human bone marrow showed that 0.02 μM FdUrd increased by a factor of 2 the X-ray sensitization observed after 4 days of incubation (3.3 cell divisions) with 2 μM IdUrd (12). In vivo experiments have shown that FdUrd can enhance the IdUrd incorporation in the mouse and rat (9) and has additive inhibitory effects on growth (4). On the other hand, IdUrd can potentiate FdUrd effects and toxicities in mice (11, 13).

IdUrd was introduced to clinical trials in the early 1960s (14–16) and occasionally marked regressions of solid tumors were observed. Although one study utilized infusions up to 17 days (17), IdUrd was generally administered as an infusion over 2 h or less. Our initial clinical studies with IdUrd focused upon lengthening the exposure period by administering IdUrd as a long-term infusion (18). For this schedule, the MTD was approximately 1000 mg/sq.m/day for 14 days.

The combination of FdUrd + IdUrd was evaluated in the clinic during the early 1960s (15, 16). Tumor regressions were observed, but toxicity was severe. Although it was concluded that no advantage resulted from combination therapy at the schedule and doses used (19), no rigorous Phase II studies were ever reported.

This paper reports an attempt to modulate IdUrd incorporation into cells with FdUrd in patients with malignancies who were also treated with radiotherapy. The purpose of this study was to determine the maximum achievable IdUrd incorporation into DNA during a 14-day infusion of FdUrd and IdUrd. The 14-day period of drug exposure was chosen to coordinate with radiotherapy and so that as many cells as possible of relatively slowly growing tumors such as glioblastoma multiforme (20) might be exposed to the drug during their S-phase. This design allowed incorporation of IdUrd into DNA before radiation started, and also incorporation into DNA of tumor cells recruited into DNA synthesis after radiation had been started (21).

Although plasma levels can sometimes be related to biological effects (22), intracellular interactions may occur which are unnoticed in extracellular fluids. Kriss observed rises in tissue levels of IdUrd in rats following FdUrd, while plasma levels were unchanged (9). Thus, we chose to monitor both plasma and intracellular levels. As a readily accessible cell population, circulating granulocytes served as an indicator for the IdUrd incorporation into DNA of normal host cells (23), and its possible modulation by FdUrd coadministration.

MATERIALS AND METHODS

Clinical. Patients with histological confirmation of malignancy for which there was no curative conventional therapy were eligible for this study. All patients gave informed consent for participation and the study was approved by the Institutional Clinical Research Committee.

Lyophilized IdUrd powder was supplied by the National Cancer Institute (NSC 39661) in 220-mg vials (which also contained NaOH) and reconstituted in water at 20–40 mg/ml. FdUrd was obtained from commercial suppliers. The two drugs were mixed together and placed in a portable infusion pump (model AS-2F; Auto-Syringe, Hookset, NH). The solution of FdUrd + IdUrd was infused through a silastic subclavian vein. All treatment was performed on an outpatient basis.

Two consecutive courses of FdUrd + IdUrd were administered on an overlapping schedule with radiation. The two infusion courses were separated by 3–4 weeks of rest. If the first full course was well tolerated,
the second course was given at the next higher dose level. At least three patients were treated at each dose level.

For the first part of the study, the dose of IdUrd was fixed at 200 mg/sq.m/day, approximately 20% of the MTD for the 14-day schedule (18). FdUrd was coadministered at a starting dose of 0.6 mg/sq.m/day, approximately 15% of its established MTD (24) for this schedule. While the dose of IdUrd was kept constant, the dose of FdUrd was increased until toxicity occurred. In the second part, the dose of FdUrd was kept constant at one dose level below the maximal dose achievable in the first part, while the dose of IdUrd was increased from 200 mg/sq.m/day until the new MTD of that combination had been reached. For the final part, the dose of FdUrd was fixed at 1.2 mg/sq.m/day and the dose of IdUrd was escalated.

Patients were followed closely for systemic toxicities. Blood samples were taken at least twice a week during the 14-day infusion. In cases of progressive decrease in the thrombocyte count, treatment was terminated a few days early. Following completion of the infusion, blood tests were taken at least twice weekly until all test results returned to the normal range.

Laboratory Studies. On Days 1 and 7, blood was collected in heparin- or EDTA-containing Vacutainer tubes (Becton Dickinson, Rutherford, NJ) and immediately placed on ice. After centrifugation, plasma was collected and stored at −20°C until analyzed. Determination of IdUrd in plasma by HPLC has been described previously (25).

On Day 14 or at the end of the infusion, granulocytes were isolated from peripheral blood, initially using the Ficoll-Hypaque technique as described previously (23). Subsequently, a Sepacell-Mn density gradient (Sepatech Inc., Oklahoma City, OK) was used to separate the granulocytes (26). Granulocyte suspensions were always more than 98% pure, as determined from microscopic preparations. Cells were resuspended in 1 ml of 0.9% NaCl solution, fixed by adding 3 ml of 95% ethanol and subsequently stored at −20°C until analyzed by HPLC, usually within 2 weeks. The percentage of IdUrd incorporated into DNA was determined using slight modifications of the method described previously (27).

RESULTS

Clinical Observations. The majority of patients entered on this study were male (21/27). The most frequent diagnosis was glioblastoma multiforme (16/27). Most patients had no prior treatment.

In the first part, dose escalations of FdUrd began at 0.6 mg/sq.m/day and reached 3.5 mg/sq.m/day, while the IdUrd dose was kept constant at 200 mg/sq.m/day. Side effects that preceded further FdUrd dose increments were thrombocytopenia, mucositis, diarrhea, and elevation of liver function tests (Table 1).

For the second part, at a fixed FdUrd dose of 2.4 mg/sq.m/day, IdUrd could only be increased from 200 to 450 mg/sq.m/day. Symptoms that precluded dosage escalation were predominantly severe diarrhea and thrombocytopenia (Table 1).

Since these symptoms resemble those seen with FdUrd monotherapy (24, 28), the dose of FdUrd was reduced to 1.2 mg/sq.m/day and then it was attempted to increase the dose of IdUrd again. Only one increment could be made, when toxicity occurred again, namely severe diarrhea and thrombocytopenia (Table 1).

For the first part of this study, 2/20 cycles were terminated early, on Days 10 and 13. In the second part, early stopping occurred for 3/10 cycles (Days 11, 11, and 12). In the last part, the frequency was 4/12 cycles (Days 11, 11, 11, and 13). In most cases, early stopping was due to decreasing thrombocyte counts.

When the above-mentioned toxicities were taken as endpoints of the different parts in this study, the different MTDs of IdUrd and FdUrd can be plotted in an isobologram (Fig. 1). The straight line between the MTDs of IdUrd and FdUrd administered as single agents reflects the case in which no synergism or antagonism occurs, but only additive effects exist. Since the dosage combinations for all three parts fall near the line, this suggests that the toxic effects of FdUrd + IdUrd are additive, at least on this schedule.

No clinical signs of iodism were observed. Transient minimal increases were noted in thyroid-stimulating hormone levels in 13/14 evaluable patients and in thyroxine levels in 15/17 evaluable patients on Day 14. All values returned to pretreatment levels 2 weeks later (data not shown).

Pharmacokinetics and Pharmacodynamics. Since the dose of IdUrd was constant in the first part of this study, measurements of IdUrd levels in plasma and granulocyte DNA (Table 2) provided the clearest indication of the presence or absence of biochemical modulation. At the lowest dose of FdUrd (0.6 mg/sq.m/day), plasma IdUrd concentrations were 0.3 ± 0.1 μM, and the IdUrd substitution was 3.1 ± 0.1%. At the highest dose of FdUrd (3.5 mg/sq.m/day), the values were essentially similar. No trend in increase of IdUrd substitution in granulocytes was observed during the increase of the dose of FdUrd.

In the second part, the plasma IdUrd concentrations rose proportionally with the increasing dose of IdUrd. Also, the percentage substitution increased to 8.1 ± 2.4% at 450 mg/sq.m/day (Table 2). In the third part, at 1.2 mg/sq.m/day FdUrd and 450 mg/sq.m/day IdUrd, a slightly lower percentage IdUrd incorporation was observed (5.7 ± 1.5%), compared with the 2.4 mg/sq.m/day dose (8.1 ± 2.4%), but this difference was not statistically significant. A further increase of the IdUrd dose (to 675 mg/sq.m/day) resulted in proportional increases in IdUrd and FdUrd were coadministered in two courses of 14-day continuous infusions, separated by 3-4 weeks of rest.

Table 1

<table>
<thead>
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<th>Doses (mg/sq.m/day)</th>
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<th>Marrow</th>
<th>GI#</th>
<th>Liver</th>
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<tr>
<td></td>
<td>Number of patients (courses)</td>
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<td>0 1 2 3</td>
<td>0 1 2 3</td>
</tr>
<tr>
<td>Part one</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
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<td></td>
<td>2 (3)</td>
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<td></td>
</tr>
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<td></td>
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<td>5</td>
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<td>4 0 2</td>
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<td>200</td>
<td></td>
<td>3 3 3 3</td>
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<td>3 3</td>
</tr>
<tr>
<td>Part two</td>
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</tr>
<tr>
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<td>2 (3)</td>
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<td></td>
</tr>
<tr>
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<td></td>
<td>1 1 1 1</td>
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<td>300</td>
<td></td>
<td>2 2 2 2</td>
<td>3 3 3</td>
<td>3 3</td>
</tr>
<tr>
<td>Part three</td>
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<tr>
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<td></td>
<td>1 1 1 1</td>
<td>3 3 3</td>
<td>1 1 1</td>
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</tbody>
</table>

* Toxicity rating scales as follows: Bone marrow: 1 = WBC, 1-2 million/ml; platelets, 100-150 million/ml. 2 = WBC, >0.5 million/ml; platelets, 50-100 million/ml. 3 = WBC, <0.5 million/ml; platelets, <50 million/ml. GI tract: 1 = erythema of oral mucosa, mild diarrhea; 2 = patchy mucositis, moderate diarrhea; 3 = confluent mucositis, severe diarrhea. Liver toxicity: 1 = 1-2x elevation of liver function tests (LFTs), primarily transaminases. 2 = 2-4x elevation of LFTs, primarily transaminases. 3 = >4x elevation of LFTs, primarily transaminases.

# GI, gastrointestinal.
MODULATION OF IdUrd INCORPORATION BY FdUrd

ISOBOLOGRAM

Fig. 1. Isobologram of the relationship between the MTDs of FdUrd and IdUrd administered as single agents or coadministered in different combinations as outlined in Table 1. Values on the line, additive effects; values below the line, synergistic effects; values above the line, antagonism. Dose-limiting toxicity is the endpoint.

Table 2 Plasma and cellular pharmacokinetic data for each dose combination

<table>
<thead>
<tr>
<th>Dose (mg/sq.m/day)</th>
<th>Plasma IdUrd</th>
<th>Substitution % in DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>IdUrd</td>
<td>FdUrd</td>
<td>µM</td>
</tr>
<tr>
<td>Part one</td>
<td></td>
<td></td>
</tr>
<tr>
<td>200</td>
<td>0.6</td>
<td>0.3 ± 0.1</td>
</tr>
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<td>200</td>
<td>1.2</td>
<td>0.5 ± 0.2</td>
</tr>
<tr>
<td>200</td>
<td>2.4</td>
<td>0.5 ± 0.1</td>
</tr>
<tr>
<td>200</td>
<td>3.5</td>
<td>0.4 ± 0.1</td>
</tr>
<tr>
<td>Part two</td>
<td></td>
<td></td>
</tr>
<tr>
<td>200</td>
<td>2.4</td>
<td>0.5 ± 0.1</td>
</tr>
<tr>
<td>300</td>
<td>2.4</td>
<td>0.8 ± 0.3</td>
</tr>
<tr>
<td>450</td>
<td>2.4</td>
<td>1.1 ± 0.3</td>
</tr>
<tr>
<td>Part three</td>
<td></td>
<td></td>
</tr>
<tr>
<td>200</td>
<td>1.2</td>
<td>0.5 ± 0.2</td>
</tr>
<tr>
<td>450</td>
<td>1.2</td>
<td>1.0 ± 0.3</td>
</tr>
<tr>
<td>675</td>
<td>1.2</td>
<td>1.8 ± 0.4</td>
</tr>
</tbody>
</table>

plasma IdUrd levels and the percentage incorporation (Table 2).

Our previous studies with single-agent IdUrd at 1000 mg/sq.m/day produced 11.1 ± 2.3% IdUrd substitution (23) at plasma concentrations of 3.3 ± 0.9 µM (25). Thus, with the schedule outlined above, no signs of clinically relevant biochemical enhancement of IdUrd incorporation were observed with coadministration of FdUrd, based upon monitoring of peripheral granulocytes and plasma.

An excellent correlation was observed between plasma IdUrd levels and the incorporation of IdUrd into DNA of granulocytes (Fig. 2). In only a few cases, the IdUrd incorporation in DNA was higher than expected from the measured plasma levels. This might reflect temporary disturbances in the flow rate of the infusion pump, or ex vivo degradation of IdUrd by phosphorylases in plasma.

Fig. 3 displays the relationship between incorporation of IdUrd into DNA of granulocytes and the absolute granulocyte and platelet counts. Myelosuppression (either granulocyte or platelet counts) produced by the combination of FdUrd + IdUrd does not correlate with incorporation of IdUrd into granulocyte DNA. This result is in contrast to the findings of our earlier study of IdUrd as a single agent on the same schedule (23).

DISCUSSION

The concept of biochemical modulation is attractive, but an objective evaluation of its utility requires well-defined end-points. With most combinations, the biochemical rationale is initially tested in cell culture. Although in vitro systems are excellent for testing biochemical hypotheses, they generally lack the constraints of host tissue toxicity. Thus, selectivity must be tested in a separate set of experiments in vivo. Testing in vivo provides a realistic challenge in terms of toxicity to host tissues. However, the translation of exposure conditions in vitro to studies in vivo is complicated by pharmacokinetic considerations such as the half-life, volume of distribution, and clearance.
Continuous infusions substantially reduce the difficulty of matching exposure conditions.

In cell culture, the addition of FdUrd to IdUrd has been shown to greatly increase the incorporation of IdUrd into DNA (8, 29). Unfortunately, it appears that most of these experiments in vitro have been performed at unrealistically high FdUrd concentrations, namely 0.4–10 μM. Although micro-molar levels of FdUrd can be generated for short periods of exposure (30), they are not tolerated for long-term infusions. FdUrd concentrations achieved during a 14-day infusion of 1-5 mg/sq.m/day are not precisely known, but are well below conventional detection limits (<0.1 μM) (31). Extrapolation from the data of Ensminger et al. (30) would suggest that 0.001 μM is a more likely range.

The optimization of drug delivery schedules is a subject which has attracted considerable clinical attention. Studies with FdUrd and 5-fluorouracil have demonstrated that the way a drug with a short half-life is administered may dramatically alter its activity and toxicity (28). Since IdUrd has a plasma half-life of less than 5 min (25), it seemed logical to administer this drug as a long-term infusion. Furthermore, the 14-day infusion period provided a convenient matching with radiotherapy, which was delivered in 2-week cycles (18). Finally, close monitoring of platelet counts provided an early warning of myelosuppression, which could then be minimized by stopping the infusion (18).

We found that various ratios of FdUrd + IdUrd can be safely delivered. The combination of reduced dosages of IdUrd and FdUrd caused toxic side-effects which were not observed at these dosages given as monotherapy. This has similarities with the previously reported exacerbation of the host toxicity of each drug by the coadministration of the other in mice (11, 13, 19) and in humans (15, 16).

Even in rapidly proliferating bone marrow we did not appear to achieve a substantial increase in IdUrd incorporation into DNA. The highest mean IdUrd incorporation into granulocyte DNA attained in this study was 8.8%, at a dose of 675 mg/sq.m/day FdUrd + 1.2 mg/sq.m/day IdUrd. This is 79% of the IdUrd incorporation (11.1%) observed for single-agent therapy at 1000 mg/sq.m/day IdUrd (23). However, with the drug combination, there was equal or more severity than for single-agent IdUrd.

The toxicity data in Table 1 show that dose-dependent myelosuppression is produced by both FdUrd and IdUrd. When IdUrd is used as a single agent, monitoring of IdUrd in granulocyte DNA is a reasonable predictor of myelosuppression (23). However, the data in Table 2 and Fig. 3 indicate that an alteration in the incorporation of IdUrd is not produced by FdUrd and does not explain the increased myelotoxicity of the combination. FdUrd appears to contribute to the myelotoxicity of the drug combination by some other mechanism. Thus, when FdUrd is added to IdUrd, monitoring of IdUrd in granulocyte DNA is no longer a useful tool for predicting myelosuppression.

When given as a 14-day continuous infusion, FdUrd is thought to act via inhibition of TS, which produces a decrease in dTMP levels. Such events should favor increased incorporation of IdUrd into DNA. Since increased incorporation did not occur, we might infer that FdUrd on this schedule does not substantially inhibit TS in bone marrow stem cells.

Since both FdUrd and IdUrd compete for activation by the same enzyme, TK, there may be antagonism if the enzyme is saturated. Deoxuryridine, which also competes for TK, may be generated secondary to an accumulation of deoxyuridylate. These TK-interactions could decrease the amount of IdUrd in DNA. Use of a folate analogue such as methotrexate could block TS without requiring activation by TK.

Using purified enzyme, IdUMP has been shown to inhibit TS (32). In intact cells and in cell lysates, we have verified that IdUrd/IdUMP does inhibit TS.6 Thus, it is possible that TS in marrow stem cells is already inhibited by IdUMP, after which further decreases in enzyme activity by FdUrd are unimportant.

Regardless of the mechanism, we must avoid a premature conclusion that the combination of FdUrd + IdUrd is not a fruitful one. Such a conclusion would be based solely upon evidence obtained from normal host tissues. A key remaining piece of information concerns the effect of the combination upon tumors. For most patients in this study, it is too early to evaluate antitumor response. A majority of patients entered had glioblastoma multiforme. In a previous Phase I/II study of continuous i.v. infusion of IdUrd alone, there appears to be an improvement in survival compared to radiation therapy alone (33). Until the information regarding the effect of the combination is available, it would be preferable to consider the efficacy of the drug combination an open question. A rigorous clinical assessment of the value of modulation would require randomized testing of the FdUrd + IdUrd combination versus IdUrd alone. Such a trial would require a large number of patients and would require several years to complete.

The same techniques which have been used to examine DNA from granulocytes can also be applied to any tissue in the body. Peripheral granulocytes have the advantage of ready accessibility, plus some relationship to target organ toxicity. To address the issue of tumor effects from a pharmacological perspective, we have designed a clinical trial which includes measurements of IdUrd incorporation into DNA obtained from tumor biopsies after an infusion of either IdUrd alone or FdUrd + IdUrd. Other than a randomized clinical trial, the granulocyte results reported in this paper plus the tumor results from the new clinical trial should provide the clearest evaluation of the utility of FdUrd modulation of IdUrd on a 14-day schedule.

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