Phase I Clinical and Pharmacological Studies of Benzylacyclouridine, a Uridine Phosphorylase Inhibitor

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ABSTRACT

Benzylacyclouridine (BAU, IND 039655) is a potent and specific inhibitor of uridine phosphorylase (UrdPase; EC 2.4.2.3). This enzyme plays a major role in regulating uridine homeostasis and also catalyzes the conversion of fluoropyrimidine nucleosides to their respective bases. Inhibition of UrdPase enzyme activity 18-24 h after 5-fluorouracil (5-FU) administration increased plasma levels of uridine and enhanced the therapeutic index of 5-FU by rescuing normal tissues. Moreover, in vitro preclinical studies have also shown that inhibiting UrdPase enzyme activity by BAU prior to administration of 5-FU increased cytotoxicity in a number of human cancer cell lines. A series of preclinical studies was performed in dogs and pigs to evaluate the pharmacological and pharmacodynamic properties of BAU. These data showed a sustained elevation in plasma uridine concentration in both animal models. The rapid degradation of a tracer dose of uridine into uracil was virtually arrested by BAU administered both p.o. or i.v. The t1/2 of BAU was 1.8-3.6 h in dogs, with bioavailability levels of 85% (30 mg/kg) and 42.5% (120 mg/kg). In pigs, the half-life varied from 1.6 to 2.3 h, with a bioavailability of 40% at 120 mg/kg. The drug was distributed into most tissues with a tissue:plasma ratio of approximately 0.7. On the basis of these preclinical studies, we performed a Phase I clinical trial of BAU in patients with advanced cancer. Patients received 200, 400, 800, and 1600 mg/m² BAU as a single oral dose.

Toxicities included grade 2 anemia, grade 1 fever, grade 1 constipation, grade 1 elevation in alkaline phosphatase; none of these toxicities were observed to be dose dependent. The maximum tolerated dose and dose-limiting toxicity were not reached at the doses given. BAU plasma concentrations and area under the curve correlated linearly with the oral dose level. The pharmacokinetics of BAU were consistent with a first-order clearance, with average peak concentrations ranging from 19 μM (200 mg/m²) to 99 μM (1600 mg/m²) and t1/2 ranging from 3.0 to 3.9 h at the four dose levels. Compared with baseline plasma uridine, treatment of patients with 200, 400, 800, and 1600 mg/m² BAU increased peak uridine concentrations by 120, 150, 250, and 175%, respectively. On the basis of this clinical study, the suggested Phase II starting dose of BAU in combination with 5-FU is 800 mg/m². Studies combining BAU with 5-FU and incorporating appropriate molecular and biochemical end points to assess the effects of this drug combination on tumor and/or surrogate tumor tissue are under way.

INTRODUCTION

UrdPase (EC 2.4.2.3) is a cytosolic enzyme that catalyzes the reversible phosphorylisis of uridine and, to a much lesser degree, thymidine. It also cleaves pyrimidine 2'- and 5'-deoxyribosides but at a much slower rate (1-5). UrdPase is present in most tissues and in tumors, where its activity is generally elevated (1, 3, 6). Furthermore, UrdPase plays an important role in the homeostatic regulation of uridine concentrations in plasma (7-10) and affects the activation and catabolism of fluoropyrimidines as well (11-12). The short half-life of uridine in plasma (10-15 min) coupled with its rigorous homeostasis at 2-4 μM in human plasma suggested that factors affecting its catabolism also could have a major effect on its tissue concentration with potential differences between normal and malignant cells. Several investigators have demonstrated that administration of exogenous uridine can modulate therapy with 5-FU to reduce toxicity (7, 10). However, the large doses of uridine necessary to obtain clinically relevant concentrations of the pyrimidine have not been well tolerated. We have shown that an inhibitor of UrdPase, BAU (4), in animal models elevates urino-

The abbreviations used are: UrdPase, uridine phosphorylase; AUC, area under the curve; BAU, benzylacyclouridine; Cmax, peak plasma concentration; DLT, dose-limiting toxicity; 5-FU, 5-fluorouracil; FdUMP, 5-fluoro-2'-deoxyuridine-5'-monophosphate; FUTP, 5-fluorouridine-5'-triphosphate; MED, minimum effective dose; MTD, maximum tolerated dose; Vss, volume of distribution (steady state); HPLC, high-performance liquid chromatography.

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dine concentrations both in plasma and tissues of mice and protects the host from 5-FU toxicity (7) without nullifying the antitumor effect. We initiated preliminary clinical studies of BAU to conserve endogenous uridine as a potentially more selective approach because of differences in the transport of uridine between normal and tumor tissues (13) and better tolerated means of elevating uridine pools.

A number of clinical trials have been conducted to determine the clinical pharmacology of uridine in patients (14, 15) and also to examine the ability of "uridine rescue" to prevent toxic effects of 5-FU alone or in combination with methotrexate and PALA, and to allow dose escalation of 5-FU (16). These studies indicate that patients tolerated combination therapy with uridine up to a dosage of 750 mg/m² of 5-FU, with 25% experiencing moderate toxicity (mucositis). Unfortunately, uridine is difficult to administer p.o., due to a very low bioavailability (7-12%) and dose-limiting diarrhea. Prolonged i.v. infusions of uridine were associated with high fevers, phlebitis, cellulitis, and, in some patients, superior vena cava syndrome. A recent Phase I clinical trial of an acetylated prodrug of uridine (PN401) also resulted in the elevation of the MTD of 5-FU to 1000 mg/m², with almost a complete elimination of the nonhematological toxicities (17).

BAU was originally synthesized as a potential antiviral agent (3, 4, 8) but was shown to be inactive. Subsequently, it was shown to be a potent inhibitor (Ki = 0.1 μM) of UrdPase (3, 4), and potential antineoplastic activity in combination with FdURD (18) was demonstrated. We extended these results to a murine in vivo model and demonstrated the ability of BAU to cause a 5–50-fold expansion of plasma and tissue uridine (19, 20). Treatment with BAU 18–24 h after 5-FU increases tissue pools of uridine and enhances the therapeutic index of 5-FU in mice bearing murine colon 38 tumors (13). In addition, treatment with BAU increases the duration and elevation of plasma and tissue uridine levels after oral uridine, dramatically decreasing the dose of oral uridine required to rescue mice from toxic concentrations of 5-FU (10). Thus, BAU may be able to minimize the amount of exogenous uridine required for rescue therapy by preventing uridine phosphorolysis and thus decreasing uridine catabolism.

However, BAU may also prove to be clinically useful in enhancing fluoropyrimidine cytotoxicity. Pretreatment with BAU significantly potentiated 5-FU cytotoxicity both in vitro and in vivo against a number of human cancer cell lines, including pancreas (DAN), lung (LX-1), prostate (PC-3), colon (HCT-8), and murine models (18, 21, 22). Incorporation of 5-FU metabolites into RNA and DNA and inhibition of TS enzyme activity were all significantly increased with BAU pretreatment, suggesting that a possible mechanism for the enhanced cytotoxicity is increased formation of critical cytotoxic 5-FU metabolites, including FdUMP, FdUTP, and FUTP, after UrdPase inhibition (20).

Preclinical studies were performed to evaluate the pharmacological properties of this agent and determine the biochemical role of BAU in combination with 5-FU. These studies demonstrated that BAU significantly enhanced the efficacy of 5-FU in vivo, with a 2- to 5-fold increase in tumor growth inhibition compared to 5-FU alone (18, 19). The combination of BAU and 5-FU was also associated with a decrease in the dose-limiting toxicities of 5-FU, such as diarrhea, mucositis, and nausea.

Fig. 1 Plasma concentration versus time of BAU administered p.o. (Os.) and i.v. at 120 mg/kg in dogs. Dogs were given 120 mg/kg of BAU by i.v. bolus or p.o. as gelatin capsules, blood was collected at indicated times from the femoral vein, and samples were analyzed by HPLC.
MATERIALS AND METHODS

by HPLC on a Rainin Microsorb C18 column

extraction and stored at -20°C until it was analyzed for uridine

mixed or homogenized in 2 volumes of ice-cold 15% TCA. The

under sterile conditions for blood sampling, and 2 mCi of

ducted in two pigs, except that a venous catheter was inserted

distribution studies.

samples of liver, kidney, gut, spleen, lung, heart, pancreas. and

were injected, and blood was collected as described previously.

were anesthetized with pentobarbital, 2 mCi of [6-3H]uridine

plewere collected as described above. One h later, the dogs

were treated with BAU after an overnight fast, and blood sam-

30, 60, 90, 120, 240, 480, 720, and 1440 min from the femoral

mg/kg) administered as gelatin capsules after an overnight fast

5, then collected at 0 (control), 2, 10, 30, 60, and 120

BAU was synthesized as described previously (8). The drug was
dissolved in ethanol and diluted 1:10 in 0.85% saline before iv.

[6-3H]Uridine was purchased from Moravek Biochemicals (Brea, CA). All other chemicals were purchased from Sigma (St. Louis, MO) or Schwarz-Mann (Cleveland, OH).

Animal Experiments. Six dogs were used to determine BAU pharmacokinetic, biochemical effects on plasma uridine and uridine clearance. Animals were injected with 1 mCi of [6-3H]uridine (1 ml) via the femoral vein. One ml of blood was then collected at 0 (control), 2, 5, 10, 20, 40, 80, 120, and 240 min. One day later, the dogs were treated with BAU (30 or 120 mg/kg) administered as gelatin capsules after an overnight fast or by iv. bolus injections. One ml of blood was collected at 15, 30, 60, 90, 120, 240, 480, 720, and 1440 min from the femoral vein, and the plasma samples were evaluated for both BAU and uridine content by HPLC methods. Seven days later, the dogs were treated with BAU after an overnight fast, and blood samples were collected as described above. One h later, the dogs were anesthetized with pentobarbital, 2 mCi of [6-3H]uridine were injected, and blood was collected as described previously. At appropriate time points after drug administration, biopsy samples of liver, kidney, gut, spleen, lung, heart, pancreas, and muscle were taken and rapidly frozen in liquid nitrogen for drug distribution studies.

A similar procedure was adopted also for the study conducted in two pigs, except that a venous catheter was inserted under sterile conditions for blood sampling, and 2 mCi of [6-3H]uridine were injected for uridine clearance studies.

Uridine Determination. Plasma and tissues were either mixed or homogenized in 2 volumes of ice-cold 15% TCA. The acid-soluble fraction was neutralized by Trioctylamine-Freon extraction and stored at -20°C until it was analyzed for uridine by HPLC on a Rainin Microsorb C18 column (25 cm × 4.6

Table 1 Pharmacokinetic parameters of BAU after administration to dogs

<table>
<thead>
<tr>
<th></th>
<th>30 mg/kg</th>
<th></th>
<th>120 mg/kg</th>
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<tbody>
<tr>
<td></td>
<td>i.v. (n = 3)</td>
<td>p.o. (n = 3)</td>
<td>i.v. (n = 3)</td>
</tr>
<tr>
<td>AUC0-∞ (mg · h/liter)</td>
<td>105.5 ± 15.2</td>
<td>90.4 ± 11.8</td>
<td>988.9 ± 42.1</td>
</tr>
<tr>
<td>t1/2 (min)</td>
<td>2.32 ± 0.33</td>
<td>105 ± 7</td>
<td>2.58 ± 0.59</td>
</tr>
<tr>
<td>t1/2 (min)</td>
<td>110 ± 16</td>
<td>110 ± 16</td>
<td>194 ± 11</td>
</tr>
<tr>
<td>Cmax (mg/liter)</td>
<td>105.4 ± 17.8</td>
<td>21.3 ± 4.1</td>
<td>470.1 ± 70.9</td>
</tr>
<tr>
<td>Cl (ml/h/kg)</td>
<td>284.4 ± 13.9</td>
<td>121.3 ± 15.1</td>
<td>284.7 ± 37.3</td>
</tr>
<tr>
<td>Vd (ml/kg)</td>
<td>470.1 ± 70.9</td>
<td>61.9 ± 11.6</td>
<td></td>
</tr>
</tbody>
</table>

*The values represent the mean ± SD obtained from three animals evaluated for each dose regimen.

* Values in parentheses indicate % of oral bioavailability.

Values in parentheses indicate t_{max}.

Effects in dogs and pigs. Subsequently, a Phase I clinical trial of BAU in cancer patients was conducted to define: (a) the MTD of BAU given as a single oral dose; and (b) the MED of BAU required to elevate uridine plasma levels above pretreatment (baseline) levels.

MATERIALS AND METHODS

Drugs and Chemicals for Pharmacological Studies. BAU was synthesized as described previously (8). The drug was dissolved in ethanol and diluted 1:10 in 0.85% saline before i.v. administration. [6-3H]Uridine was purchased from Moravek Biochemicals (Brea, CA). All other chemicals were purchased from Sigma (St. Louis, MO) or Schwarz-Mann (Cleveland, OH).

Animal Experiments. Six dogs were used to determine BAU pharmacokinetic, biochemical effects on plasma uridine and uridine clearance. Animals were injected with 1 mCi of [6-3H]uridine (1 ml) via the femoral vein. One ml of blood was then collected at 0 (control), 2, 5, 10, 20, 40, 80, 120, and 240 min. One day later, the dogs were treated with BAU (30 or 120 mg/kg) administered as gelatin capsules after an overnight fast or by i.v. bolus injections. One ml of blood was collected at 15, 30, 60, 90, 120, 240, 480, 720, and 1440 min from the femoral vein, and the plasma samples were evaluated for both BAU and uridine content by HPLC methods. Seven days later, the dogs were treated with BAU after an overnight fast, and blood samples were collected as described above. One h later, the dogs were anesthetized with pentobarbital, 2 mCi of [6-3H]uridine were injected, and blood was collected as described previously. At appropriate time points after drug administration, biopsy samples of liver, kidney, gut, spleen, lung, heart, pancreas, and muscle were taken and rapidly frozen in liquid nitrogen for drug distribution studies.

A similar procedure was adopted also for the study conducted in two pigs, except that a venous catheter was inserted under sterile conditions for blood sampling, and 2 mCi of [6-3H]uridine were injected for uridine clearance studies.

Uridine Determination. Plasma and tissues were either mixed or homogenized in 2 volumes of ice-cold 15% TCA. The acid-soluble fraction was neutralized by Trioctylamine-Freon extraction and stored at -20°C until it was analyzed for uridine by HPLC on a Rainin Microsorb C18 column (25 cm × 4.6

mm), eluted at 1 ml/min with 10 mM H₃PO₄ containing 30 μM heptanesulfonic acid, pH 3.1, at 8°C (19).

BAU Determination. Plasma and tissues were processed as described above. The neutralized extracts were analyzed by HPLC (19). The samples were injected in a Rainin Microsorb C18 column (25 cm × 4.6 mm), maintained at 20°C, and eluted with 25% methanol in water at 1 ml/min.

BAU Pharmacokinetics. AUC, t_{1/2}, C_{max}, Cl, and V_d were determined using the PCNONLIN software package (Clin-Trials, Lexington, KY) for the analysis of nonlinear models and standard pharmacokinetic equations.

Clinical Trial. An open-label, single-arm study was performed at the Rhode Island Hospital (Brown University) and Yale New Haven Hospital (Yale University School of Medicine). The study was opened for patient enrollment in August 1992 and closed to accrual in June 1996. The protocol and consent form were reviewed and approved by the human investigation committees of participating institutions prior to patient enrollment.

Eligibility Criteria. Patients were required to meet all of the following eligibility criteria prior to entry onto study: (a) histological confirmation of malignancy; (b) age 18 years or greater; (c) Eastern Cooperative Oncology Group performance status of 2 or better; (d) an expected survival of at least 2 months; (e) full recovery from the effects of any previous cancer treatment and/or infection; (f) adequate hematological function (granulocyte count of at least 1500/mm³ and platelet count of at least 100,000/mm³); (g) adequate renal function (serum creatinine less than 2 mg/dl or creatinine clearance greater than 60 ml/min); and (h) adequate hepatic function (total bilirubin less than or equal to 2 mg/dl, aspartate aminotransferase less than or equal to 100 units/liter). In addition, patients had to be competent and had to give their signed, informed consent to comply with the procedures of the protocol. Females of child-bearing age were required to be nonpregnant, nonlactating, and using effective contraceptive measures for inclusion. Patients with concurrent serious medical problems or major mental disability were excluded from this protocol. No chemotherapy or radiation therapy was permitted for a minimum of 4 weeks prior to entering on the clinical trial.

Dose Escalation. At least three patients were entered in each treatment level of BAU. Specifically, the first patient
Phase I Studies of BAU

Table 2  Tissue distribution of BAU in dogs and pigs, 4 h after drug administration\(^{a}\)

<table>
<thead>
<tr>
<th>Dose</th>
<th>Liver</th>
<th>Kidney</th>
<th>Spleen</th>
<th>Gut</th>
<th>Muscle</th>
<th>Lung</th>
<th>Heart</th>
<th>Pancreas</th>
<th>Adipose tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dog, 30 mg/kg (p.o.)</td>
<td>68 (62–74)</td>
<td>66 (63–69)</td>
<td>51 (43–59)</td>
<td>58 (52–64)</td>
<td>49 (45–53)</td>
<td>61 (59–63)</td>
<td>61 (54–68)</td>
<td>55 (51–59)</td>
<td>17 (14–20)</td>
</tr>
<tr>
<td>Dog, 120 mg/kg (p.o.)</td>
<td>67 (60–74)</td>
<td>74 (71–77)</td>
<td>49 (46–52)</td>
<td>68 (61–75)</td>
<td>60 (52–68)</td>
<td>64 (58–70)</td>
<td>ND(^{b})</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Pig, 120 mg/kg (p.o.)</td>
<td>63 (58–68)</td>
<td>88 (85–91)</td>
<td>51 (47–55)</td>
<td>67 (61–73)</td>
<td>55 (50–60)</td>
<td>73 (68–78)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

\(^{a}\) Values represent means obtained from two different animals. Values in parentheses are the two single determinations. The data are expressed as % of plasma concentration measured 4 h after BAU administration.

\(^{b}\) ND, not determined.

entered at each dose level was observed for 1 week. If no significant toxicity was observed, the next two patients were enrolled in the study the following week. Additional patients could be enrolled if the first three patients at the current dose level did not experience significant toxicity within three weeks of receiving BAU. Intratropic dose escalation was permitted.

Definition of MTD and DLT. DLT was defined as any nonhematological toxicity grade 2 or higher or any hematological toxicity grade 3 or higher, using the National Cancer Institute Common Toxicity Criteria. If DLT occurred in any of the first three patients enrolled at a particular dose level, a total of 6 patients were to be enrolled at least 1 week apart and followed for 3 weeks prior to entering any additional patients at the next dose level. The MTD of BAU was defined as the dose level producing DLT in a majority (>50%) of all patients treated at that level, and this dose was a stopping point for this trial. The planned dose escalation of BAU was 200, 400, 800, 1600, and 3200 mg/m².

Drug. BAU powder and a liquid diluent were provided in separate vials by Farmitalia Carlo Erba (Pharmacia Upjohn) and were only reconstituted within 1 h of administration. Patients were given a single oral dose of BAU. They were instructed not to take any medications or food within 1 h of BAU administration and encouraged to drink at least 1 liter of fluid on the day of BAU administration.

Clinical and Laboratory Monitoring for Toxicity. Patients were required to have weekly history and physical examinations (including performance status), blood analysis (complete blood count with differential, glucose, blood urea nitrogen, creatinine, uric acid, electrolytes, calcium and phosphorus, alkaline phosphatase, LDH, aspartate aminotransferase, and total bilirubin), and urinalysis while enrolled in the study.

BAU and Uridine Sampling Points. The MED of BAU was defined as the dose that elevated plasma uridine levels 4-fold above pretreatment uridine levels. All patients enrolled in the study underwent serial peripheral venous blood sampling from an indwelling i.v. catheter at the following times after receiving BAU: pretreatment, 10 min, 30 min, and 1, 2, 3, 6, 8, and 24 h. The starting time for all patients was between 6 and 8 a.m. The samples were immediately placed in ice-cold Vacutainer tubes containing EDTA and centrifuged at 1000 rpm for 10 min at 4°C. The plasma from each sample was separated and stored at −20°C until further analysis. Urine samples were also taken at the following times after patients had received BAU: 3, 6, 12, and 24 h. All urine samples were stored at −20°C until further analysis.

Urinary excretion of BAU during the first 24 h from the administration was calculated by adding the amounts of drug present in the fraction samples collected during that time period.

RESULTS

BAU was administered p.o. and parenterally by bolus i.v. injection at 30 and 120 mg/kg to dogs. The lower dose of BAU (30 mg/kg) exhibited first-order elimination, with a \( t_{1/2} \) of 1.75 and 1.84 h, respectively for the i.v. and p.o. dosing (Fig. 1 and Table 1). Oral drug achieved a peak concentration after 1 h and a bioavailability of approximately 86%. At 120 mg/kg (Fig. 1), oral absorption was less effective and was delayed, with a bioavailability of 42%, and the peak concentration was reached 2.5–3 h after the initial administration. The \( t_{1/2} \) of this higher dose of BAU was increased to 3.2 h for parental and 3.6 h for oral administration (Table 1) as a consequence of a marked decrease in plasma clearance from 284 ml/h/kg for the 30 mg/kg dose to 121 ml/h/kg for the higher dose of BAU. The volumes of distribution remained constant, at 284 and 255 ml/kg for the low and high doses, respectively. Distribution into tissues was evaluated 4 h after oral administration of BAU at 30 and 120 mg/kg. The data indicate a similar level of distribution in all tissues, regardless of the dose, with a tissue to plasma ratio of 0.6–0.7 (Table 2). Only adipose tissue showed a minimal drug distribution, with an average ratio below 0.2 compared to plasma level.

The effect of BAU on UrdPase was evaluated by measuring the plasma uridine concentration. Dogs have very low plasma uridine concentrations (0.2–0.3 \( \mu \)M) compared to most other animal species and humans (2–5 \( \mu \)M). Nevertheless, BAU at 30 mg/kg administered i.v. caused a rapid increase in uridine concentration up to 2.8 \( \mu \)M, a 14-fold elevation over baseline level (data not shown). Oral administration delayed by 30 min the elevation of plasma uridine, but with a similar 13-fold increase in plasma uridine at 2.6 \( \mu \)M (Fig. 2). An oral dose of 120 mg/kg (Fig. 2) achieved a 28-fold elevation in plasma level (5.7 \( \mu \)M). Seven h after drug administration, the concentration of uridine was still above 4 \( \mu \)M.

A tracer dose of \[^{3}H\]uridine (1 mCi) was administered i.v. to a dog in the absence and presence of 30 mg/kg of BAU (i.v. bolus). \[^{3}H\]Uridine was cleared from plasma within 3–4 min in the control group. However, the administration of BAU (120 mg/kg) 1 h before the injection of the radiolabeled nucleoside greatly reduced the rate of uridine clearance (Fig. 3A).

Unlike most of the other species, including humans, dogs present significantly lower uridine plasma concentration and rapid uridine clearance from plasma. The pharmacokinetic and
pharmacodynamic properties of BAU were also investigated in pigs to more carefully evaluate the properties of this UrdPase inhibitor before clinical introduction. BAU was administered i.v. and p.o. at 120 mg/kg and p.o. at 300 mg/kg. At 120 mg/kg, the $t_{1/2}$ was very similar for p.o. and i.v. dosage (Table 3) and bioavailability (39%) had a value similar to that seen in dogs (42%). Plasma clearance (122 ml/h/kg) and volume of distribution (183 ml/kg) were similar to those seen at the same dose in dogs (Table 1). Oral administration of 300 mg/kg of BAU resulted in a $t_{1/2}$ of 1.97 h and an AUC that was 2.15-fold higher than the value obtained with the lower oral dose of BAU (Fig. 4). The drug was readily distributed in all tissue with tissue to plasma ratio of approximately 0.6, except for kidneys, which had a 0.9 ratio 4 h after initial administration (Table 2).

BAU (120 mg/kg) also elevated plasma uridine in the pig within 30 min, and the increased level was sustained up to 6 h with at least a 2-fold level over baseline (Fig. 5). A higher oral dose (300 mg/kg) caused a greater and more sustained elevation of plasma uridine (Fig. 5).

Also in this animal model, we injected a tracer dose of [$^3$H]uridine (2 mCi) in the absence or presence of 120 mg/kg BAU given i.v. (Fig. 3B). The UrdPase inhibitor also caused, in this animal model, a reduction in the catabolic rate of uridine. In the absence of BAU, we observed a rapid clearance of uridine from plasma, with 0.9 and 10.0 min for the $t_{1/2}$ and $t_{3/4}$, respectively; in the presence of the drug, the catabolism slowed down, with values of 1.7 and 22.9 min, respectively.

To extend these studies to humans, a Phase I clinical trial of BAU was initiated at Yale and at Brown universities to determine the dose that would cause a 4-fold increase in the concentration of plasma uridine and to measure the pharmacokinetics of BAU in humans. Our starting dose was 200 mg/m², 10–20-fold lower than our target dose.

A total of nine patients, six male and three female, were entered into this study from August 1992 until June 1996, at which time the trial was closed to further accrual (Table 4). The age of the patients ranged from 48 to 74 years old, with a median age for the study population of 58.5 years. Two patients (J. P. and R. M.) received multiple dose levels of BAU, as outlined below. All patients had advanced cancer with adequate hematological, renal, hepatic, and performance status and had given signed informed consent. All patients had received at least one cycle of chemotherapy and/or radiation with progression of disease prior to entry onto study. Baseline Eastern Cooperative Oncology Group performance status ranged from 0 to 2; the median patient performance status was 1.
In general, BAU was extremely well tolerated, and even with doses up to 1600 mg/m², it was essentially nontoxic. No significant (grade 3 or higher) hematological toxicities were observed. One patient had an episode of bleeding (melena) prior to entry that required periodic blood transfusions; this patient required a transfusion during the 3 weeks after receiving BAU.

Table 3  Pharmacokinetic parameters of BAU in pigsα

<table>
<thead>
<tr>
<th></th>
<th>120 mg/kg i.v.</th>
<th>120 mg/kg p.o.</th>
<th>300 mg/kg p.o.</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC₀→₅₀ (mg·h/liter)</td>
<td>982.7 (900.3–1045)</td>
<td>385.4 (333.9–436.9)</td>
<td>825.8</td>
</tr>
<tr>
<td>t₁/₂ (min)</td>
<td>3.54 (2.76–4.32)</td>
<td>135 (117–153)</td>
<td>97 (89–105)</td>
</tr>
<tr>
<td>Cmax (mg/liter)</td>
<td>655.6 (589.4–721.8)</td>
<td>93.8 (72.3–115.3)</td>
<td>137.7</td>
</tr>
<tr>
<td>CL (ml/h/kg)</td>
<td>122.1 (103.4–140.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vd (ml/kg)</td>
<td>183.1 (165.4–200.8)</td>
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</tbody>
</table>

α The data represent the mean value obtained from two different animals for the 120 mg/kg dosage. Values in parentheses are the two single determinations. At 300 mg/kg, only one animal was evaluated.

β The value in parentheses indicates the % of oral bioavailability.

γ Values in parentheses indicate t₁/₂.
The anemia was thought not to be related to BAU. No significant (grade 2 or higher) nonhematological toxicities were observed in this trial. One patient had documented fevers prior to entry that were thought to be cancer-related. This patient continued to have fevers after receiving BAU, but the fevers were thought not to be related to BAU. One patient had a persistent rise in uric acid during BAU therapy that was asymptomatic and was felt to be related to BAU therapy. One patient had a high baseline alkaline phosphatase (397 mg/dl) that was felt to be secondary to underlying liver disease, and with BAU treatment, this value subsequently decreased over the next 3 weeks to 207 mg/dl. One patient was not eligible for toxicity evaluation after it was discovered that the dose of BAU given was 10-fold lower (40 mg/m² rather than 400 mg/m²).

The median follow-up was 1 month after initiation of therapy (range, 3–9 weeks). No responses to treatment were observed, and no deaths occurred during the follow-up period. Survival data were not collected for this study.

Peak concentrations and AUC of BAU were related in an essentially linear fashion to the dose of the administered drug (Table 5 and Fig. 6), with a correlation coefficient for linear regression of $r = 0.986$ for the AUC and $r = 0.972$ for the $C_{\text{max}}$. The volume of distribution was essentially consistent with dose escalation, and the consistent clearance rate suggests a first-order nonsaturated process. Twenty-four-h urinary excretion of unchanged BAU was variable with each dose and ranged from 21.7 to 47.5% of the oral dose, suggesting that metabolism and/or elimination pathways other than renal excretion were present. No major BAU metabolites were detected in this study.

The peak plasma uridine level within the first 4 h after BAU treatment was always higher than the pretreatment (baseline) plasma uridine concentration (Fig. 7). The ratio of the peak plasma uridine level after treatment to the pretreatment uridine level increased with each dose of BAU up to the 1600 mg/m² BAU dose level: 200 mg/m², 120% of the pretreatment level; 400 mg/m², 150%; 800 mg/m², 250%; and 1600 mg/m², 188%.

**DISCUSSION**

This series of preclinical and clinical studies was conducted to evaluate the pharmacokinetics, biochemical effects, and safety of BAU. Several groups have proposed this UrdPase inhibitor as a rescue agent when administered after 5-FU in cancer patients (7, 10) or to enhance the antitumor effect of fluoropyrimidines through simultaneous administration (20–22) and to reverse AZT-induced hematological toxicity in HIV-positive patients (23, 24).

The i.v. administration of BAU in dogs and pigs resulted in a two-compartment first-order elimination with a rapid $t_{1/2}$ of 2–3 min, followed by a $t_{1/2}$ of 100–130 min for the 30 mg/kg dose in dogs and the 120 mg/kg in pigs. In dogs at 120 mg/kg, $t_{1/2}$ increased to 3 h, probably indicating saturation in
the elimination process. In patients throughout the range of BAU doses, the $t_{1/2}$ varied from 3.0 to 3.9 h.

In contrast to the dog studies, in which a marked decrease in bioavailability was seen at the higher dose of 120 mg/kg, possibly indicating a saturation effect in the absorption mechanism, in patients, we observed a linear increase of the AUC with the dose, suggesting a constant absorption. As shown in Table 5, patients had a constant plasma clearance of BAU not accompanied by an increase in urinary excretion that was quite variable among individuals but did not show an overall increase at higher doses. This paradox could be attributable to the existence of other, parallel metabolic pathways that do not involve urinary excretion of the parent compound or the presence of metabolites that we were not able to identify because we did not coadminister any radiolabeled tracer.

Dogs presented a very low basal plasma uridine concentr-

\[ \text{Table 4 Patient characteristics} \]

<table>
<thead>
<tr>
<th>Study</th>
<th>Patient</th>
<th>Age</th>
<th>Sex</th>
<th>Race</th>
<th>Cancer</th>
<th>BAU dose level (mg/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>001</td>
<td>N. W.</td>
<td>62</td>
<td>M</td>
<td>W</td>
<td>NSCLC</td>
<td>200</td>
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<td>48</td>
<td>M</td>
<td>W</td>
<td>Thyroid</td>
<td>200</td>
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<td>F</td>
<td>W</td>
<td>Brain</td>
<td>200</td>
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<td>Breast</td>
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<tr>
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<td>W</td>
<td>Thyroid</td>
<td>400</td>
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<tr>
<td>006</td>
<td>J. P.</td>
<td>59</td>
<td>M</td>
<td>W</td>
<td>Carcinoid</td>
<td>400</td>
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<tr>
<td>007</td>
<td>H. S.</td>
<td>83</td>
<td>F</td>
<td>W</td>
<td>Parotid</td>
<td>400$^a$</td>
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<tr>
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<td>48</td>
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<td>F</td>
<td>W</td>
<td>Colon</td>
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<tr>
<td>013</td>
<td>L. P.</td>
<td>62</td>
<td>M</td>
<td>B</td>
<td>Appendix</td>
<td>1600</td>
</tr>
</tbody>
</table>

$^a$ W, white; B, black; NSCLC, non-small cell lung cancer.

$^b$ Patient 007 received a dose of BAU of 40 mg/m².
Table 5  BAU pharmacokinetic parameters by dose level

<table>
<thead>
<tr>
<th>BAU dose (mg/m²)</th>
<th>AUC₅₀→₉₀ (µg · h/ml)</th>
<th>t½,µ (h)</th>
<th>Cₘₐₓ (µg/ml)</th>
<th>tₘₐₓ (h)</th>
<th>CI (liters/h/m²)</th>
<th>Vₐₛₜ (liters/h/m²)</th>
<th>Urinary excretion (% of total dose during first 24 h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>200</td>
<td>35.0 ± 9.8</td>
<td>3.3 ± 1.2</td>
<td>5.3 ± 1.6</td>
<td>1.3 ± 0.5</td>
<td>7.6 ± 2.8</td>
<td>27.6 ± 9.0</td>
<td>21.7 ± 5.5</td>
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<tr>
<td>400</td>
<td>48.5 (37.2–59.9)</td>
<td>3.0 (2.5–3.4)</td>
<td>8.8 (7.7–9.8)</td>
<td>1.7 (1.6–1.8)</td>
<td>8.7 (6.7–10.7)</td>
<td>28.5 (24.1–32.7)</td>
<td>47.5 (45–50)</td>
</tr>
<tr>
<td>800</td>
<td>134.6 ± 38.6</td>
<td>3.3 ± 0.7</td>
<td>19.8 ± 6.4</td>
<td>1.7 ± 0.3</td>
<td>8.2 ± 2.8</td>
<td>33.9 ± 15.9</td>
<td>31.7 ± 12.7</td>
</tr>
<tr>
<td>1600</td>
<td>215.3 ± 27.8</td>
<td>3.0 ± 1.1</td>
<td>27.7 ± 4.1</td>
<td>2.3 ± 0.2</td>
<td>8.4 ± 1.3</td>
<td>30.5 ± 12.2</td>
<td>29.3 ± 4.5</td>
</tr>
</tbody>
</table>

* The data represent the mean ± SD of the pharmacokinetic values obtained from three patients at each dose level.
* For the 400 mg/m² dose, data from only two patients were available; the value represents the average (both data points are in parentheses).

Fig. 6  Plasma concentration-time curves of BAU in patients at different dose levels. BAU was administered at the indicated doses in patients as a liquid suspension. Blood was serially collected through an indwelling i.v. catheter, and plasma was separated and analyzed for BAU by HPLC.

tration (0.2–0.3 µM), an order of magnitude lower than that in most of the other species investigated. The low uridine plasma concentration (0.8 µM) and high hepatic UrdPase activity seen in rats strongly suggests that rapid clearance in dogs may be associated with high UrdPase activity (25, 26). The biochemical effect of BAU in dogs was as extensive as that seen in mice, with a 28-fold elevation in plasma uridine concentration with a dose of 120 mg/kg. However, in both humans and pigs, the increase in plasma uridine was less remarkable. What is not known is whether the uridine concentration in tissues is greatly increased, as was seen in mice.

The primary objective of the clinical trial was to determine the safety profile of BAU in cancer patients. DLT was not reached at the highest dose level (1600 mg/m² BAU given as a single oral dose), suggesting that even higher concentrations of BAU are needed to produce DLT. Given the high safety profile seen in preclinical animal studies, this finding was not unexpected. Theoretical concerns included the possibility of hyperthermia, seen with exogenous uridine infusions (27) but not observed at even the highest dose levels of BAU. Because of concern about crystallization of BAU in the bladder due to its limited solubility, hydration of 1 liter was given to all patients on the day of BAU administration, but no decrease in renal function or bladder toxicity was observed. On the basis of all preclinical *in vitro* and *in vivo* work, BAU was not expected to have any antitumor effect on its own, and no objective responses were noted in this trial.

The secondary objective of this clinical trial was to deter-
mine the MED of BAU required to elevate plasma uridine levels. Elevations in plasma uridine were seen at BAU doses of 200 and 400 mg/m². Because plasma uridine levels do not completely correlate with tissue pools of uridine, it is possible that the 20% increase in plasma uridine levels seen with the 200 mg/m² starting dose of BAU was associated with more extensive expansion of tissue uridine pools. The greatest change detected was a 2.5-fold increase in plasma uridine levels following a dose of 800 mg/m² BAU. The magnitude of this elevation suggests that UrdPase enzyme activity was inhibited at this dose level of BAU, although hepatic phosphorylase activity, the primary site of uridine clearance, was not directly measured in this trial. Increasing the BAU dose to 1600 mg/m² did not lead to a proportionate increase in plasma uridine levels. In two of three patients at this dose level, only a minimal biochemical effect was observed. The other patient (R. M.), who received BAU at each dose level, showed a progressive increase in uridine peak concentration, with a maximum 3.5-fold elevation over the baseline level with BAU at 1600 mg/m².

The variability in the capacity of BAU to effectively elevate the plasma concentration of BAU raises the possibility that UrdPase activity is not the sole mechanism for uridine homeostasis and catabolism, and it is possible that other factors contribute to the more or less successful effect of BAU. The clinical study also indicated the relatively short-term effect of BAU on elevating uridine concentration. This effect succeeded 4 h after the drug administration, and the concentration of the nucleoside was back to baseline level. This is quite similar to what has been observed in the preclinical studies reported here and in the initial data in a murine model. The plateau effect on the plasma uridine level seen in patients between 800 and 1600 mg/m² and the relatively short-lasting elevation observed induced us to terminate the clinical trial at the 1600 mg/m² dose level. This suggests that especially for the “rescue” use of BAU for 5-FU containing regimens, a frequent administration of the inhibitor would be necessary to achieve a significant host protection.

The plateau in the response of plasma uridine levels to a single dose of BAU in humans is similar to changes observed in the plasma uridine levels of rhesus monkeys treated with increasing doses of BAU-succinate. A maximal 3.2-fold increase in plasma uridine levels was achieved even with exogenous uridine administration, a finding thought to be due to compensatory increases in renal excretion of uridine (26). Dilazep administration increased plasma uridine levels in this species, presumably by inhibiting transport of uridine in renal tubular cells and decreasing urinary excretion (28); if renal elimination of uridine were a major mechanism in humans, combining BAU with dilazep might lead to sustained elevations in uridine.

UrdPase enzyme activity undergoes circadian variation in mice (29), which can result in alterations in serum uridine levels of nearly 2-fold (5–10 μM; Ref. 30). Because all patients who entered this trial were studied at the same time of day (starting between 6 and 10 a.m.) to allow the 6-h time point to be performed at an appropriate time of day, circadian variation is
less likely to explain the uridine levels noted over the first 6 h of blood sampling and would not explain the lack of sustained high uridine levels.

On the basis of this initial clinical study, subsequent Phase II trials of BAU in combination with 5-FU have be initiated with a starting BAU dose of 800 mg/m². An important consideration for these trials will be to incorporate appropriate biochemical and/or molecular end points in tumor and/or surrogate tumor tissue. For example, actual determination of UrdPase activity, as well as quantitation of uridine and uracil pools in tumor and normal tissues biopsies, would be less likely to be confounded by nucleoside transport inhibition or non-UrdPase-mediated elimination of uridine. Moreover, evaluation of key fluoropyrimidine cytotoxic metabolites (such as FdUMP and FUTP), along with determination of TS enzyme inhibition and incorporation of 5-FU metabolites into tumor RNA and/or DNA would significantly enhance our understanding of the mechanism(s) by which BAU modulates 5-FU antitumor activity.

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