available at www.sciencedirect.com

ScienceDirect

journal homepage: www.ejconline.com

Phase I study of continuous weekly dosing of dimethylamino benzoylphenylurea (BPU) in patients with solid tumours

Wells A. Messersmith[a,*,c], *Michelle A. Rudek*[a,c], *Sharyn D. Baker*[a], *Ming Zhao*[a], *Connie Collins*[a], *A. Dimitrios Colevas*[b], *Ross C. Donehower*[a], *Michael A. Carducci*[a], *Antonio C. Wolff*[a]

[a]*The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Bunting-Blaustein Cancer Research Building, CRB 1M88, 1650 Orleans Street, Baltimore, MD 21231, United States*

[b]*Cancer Therapeutics Evaluation Program, National Cancer Institute, Bethesda, MD, United States*

ARTICLE INFO

Article history:
Received 22 August 2006
Received in revised form
8 September 2006
Accepted 14 September 2006
Available online 3 November 2006

Keywords:
Tubulin inhibitor
Pharmacokinetics
Accelerated titration introduction

ABSTRACT

A phase I study of dimethylamino benzoylphenylurea (BPU), a tubulin inhibitor, was performed using a weekly continuous schedule. Patients with refractory solid tumours received oral BPU once weekly without interruption at doses ranging from 5 to 320 mg using an accelerated titration design. Nineteen subjects received 54 cycles of BPU. Early pharmacokinetic findings of decreased clearance with increasing dose and plasma accumulation led to the expansion of the 320 mg dose level. Two subjects then developed late haematologic dose-limiting toxicities (DLTs) that were associated with the highest plasma exposure to BPU and metabolites. Study enrollment resumed at dose 150 mg with real-time pharmacokinetic monitoring. Seven additional subjects (6 evaluable) were treated for a median of 2 cycles (range 1.5–4) without further myelotoxicity. A long half-life and accumulation of BPU and active metabolites were observed, recommending against a continuous administration. Weekly oral BPU therapy should be further tested using an interrupted schedule.

1. Introduction

Dimethylamino Benzoylphenylurea (BPU, NSC #639829) is a novel, small-molecule, oral tubulin-interactive agent with potent anti-tumour activity.[1–3] Benzolyphenylureas were originally developed as insecticides, and after their anti-tumour properties were discovered a derivative, BPU (N-[4-(5-bromo-2-pyrimidinyloxy)-3-methylphenyl]-N′-(2-dimethylaminobenzoyl)urea) with improved physicochemical properties was synthesised.[4,5] The mechanism of action of BPU is not completely elucidated. BPU inhibits tubulin polymerisation and microtubule depolymerisation by weakly binding to the colchicine site of tubulin.[2,4,6] It also inhibits DNA polymerase-α, and HL-60 leukemia cells treated with BPU accumulate in the G_1-S phase of the cell cycle.[7]

BPU demonstrated *in vivo* activity against a wide range of human xenografts.[8] It was more effective than paclitaxel in the PC-3 prostate cancer model, and produced a sustained anti-tumour effect following discontinuation in several animal models.[9] Animal toxicity studies showed GI toxicity and dose-limiting myelosuppression. Human hematopoietic progenitor cells were found *in vitro* to be less sensitive than canine or murine progenitors. Animal pharmacokinetic (PK) studies showed the drug to be poorly bioavailable.[10] BPU

* *Corresponding author:* Tel.: +1 410 502 6873; fax: +1 410 614 9006.
E-mail address: wmesser1@jhmi.edu (W.A. Messersmith).
[c] These authors contribute equally to the work presented.
0959-8049/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.ejca.2006.09.006

was found to be extensively metabolised to two metabolites with anti-tumour activity, desmethyl-BPU (mmBPU) and didesmethyl-BPU (aminoBPU), which showed variable but higher exposure than did parent compound.[11] AminoBPU is the most potent metabolite, with BPU and mmBPU demonstrating intermediary potency, and four metabolites (G280, G308, G322 and G373) demonstrated no cytoxicity (for chemical structures see Ref. 11).[11,12]

The present trial was designed to evaluate BPU administered on a continuous weekly schedule in patients with refractory solid tumours using an accelerated titration design.[13] The study objectives were to define the maximum tolerated dose (MTD) of BPU and to characterise the toxicities of BPU on this schedule as well as its PK profile and any preliminary evidence of anti-tumour activity. A separate study is evaluating a weekly, interrupted schedule.[14]

2. Patients and methods

2.1. Eligibility

Patients with histologically confirmed advanced solid malignancies without conventional treatment options were eligible. Inclusion criteria included age $\geqslant$18; ECOG performance status (PS) of 0–2; at least 4 weeks elapsed since prior chemotherapy or radiation therapy (6 weeks if the regimen included nitrosources or mitomycin C); and adequate haematologic, hepatic and renal function. Patients were excluded if they had known brain metastases, active infections, chronic diarrhoea, malabsorption, peripheral neuropathy >grade 1 [National Cancer Institute Common Toxicity Criteria version 2 (NCI CTCv2)], pregnancy, HIV infection, or serious concurrent medical conditions.

The clinical protocol was approved by the Johns Hopkins Institutional Review Board and NCI Cancer Therapeutics Evaluation Program (CTEP). All subjects provided written informed consent prior to study drug administration.

2.2. Drug dosage and administration

Oral BPU was administered continuously as a fixed, non BSA-adjusted, dose once weekly. One cycle was defined as 28 days. An accelerated titration design was used starting at 1/10th the LD_{10} in dogs. Subsequent dose levels were double the previous dose. One patient was enrolled at each dose level, with plans of cohort expansion to three patients if grade 2 or greater toxicity (any cycle) or DLT (first cycle) occurred. Intra-patient dose-escalation was permitted only for patients enrolled to the initial dose levels. The highest dose level evaluated, 320 mg, was expanded to six patients.

BPU was supplied by the CTEP in conjunction with the Division of Cancer Treatment and Diagnosis (DCTD) and Ishihara Sangyo Kaisha, Ltd. Initially 5 mg capsules were used, but due to the large number of capsules required, a 25 mg capsule was subsequently made available for the 150 mg dose level. The 5 mg and 25 mg capsule had similar bioavailability in dogs.[10] Subjects were instructed to fast for 1 h before and 2 h after each dose, and were not routinely premedicated. At the final dose level evaluated, 150 mg, concomitant use of CYP3A4 inhibitor/inducers and CYP2D6 inhibitors was prohibited after in vitro metabolism data became available.[15] Doses were administered under direct supervision in the outpatient clinic for the first 8 weekly doses, after which patients were given a treatment diary and evaluated every 4 weeks. Treatment with growth factors was not permitted during the first 2 cycles of study drug administration. Study drug was not administered on scheduled days if there were dose-limiting toxicities (DLT), platelets < 100,000/mm^3, or ANC < 1500/mm^3. Missed doses were not substituted. Treatment was continued until unacceptable toxicity, disease progression, or withdrawal of consent.

2.3. Toxicity assessment

Toxicity was assessed weekly during the first 2 cycles, and monthly thereafter, using the NCI CTCv2. DLT was defined as dose delays >2 weeks, grade 4 haematologic toxicity (except grade 4 neutropenia lasting <5 days), or grade 3 non-haematologic toxicity. The MTD was considered to be the dose level where $\leqslant$1 of 6 subjects experienced DLT.

2.4. Pretreatment and follow-up studies

Baseline evaluations were conducted within two weeks before study entry and included: history and physical exam, review of concurrent medications, complete blood counts (CBC), serum chemistries, liver function tests and urinalysis. Radiologic evaluations (including chest X-ray) and baseline ECG were performed within 4 weeks of study entry. Serum chemistries and liver function tests were performed weekly during the first 2 cycles before treatment and before each cycle thereafter. A CBC with differential was performed twice weekly during cycle 1, and weekly thereafter. Physical exams were repeated at the beginning of each cycle. The response of measurable lesions was assessed using RECIST criteria every 2 cycles.[16] A 30-day off-study follow-up evaluation of toxicities and blood tests was performed.

2.5. Drug assay and pharmacokinetic analysis

2.5.1. BPU

PK studies were performed during the first 2 cycles of study drug administration for all subjects, and throughout treatment for the subjects enrolled at the last dose level. The serial sampling of venous blood was performed pre-treatment and post-treatment to 168 h during weeks 1 and 4. Weekly pre-treatment samples were obtained during the first 8 weeks on treatment and for the duration of study and for 4 weeks following discontinuation at the final dose level. Blood samples were collected in heparinised tubes and were processed by centrifugation at 1000*g* at 4 °C for 10 min. Plasma was divided into multiple aliquots and was stored at −70 °C until analysis. Urine was collected, and stored in the refrigerator, during weeks 1 and 4 from 0 to 72 h. Urine was divided into multiple aliquots and was stored at −70 °C until analysis.

BPU concentrations in plasma were measured in plasma using validated analytical methods: LC–MS–MS[17] and LC/UV.[18] A third validated method, LC–MS–MS, was used for the final two dose levels to quantitate BPU, mmBPU and aminoBPU in plasma and urine, and three non-cytotoxic metabo-

lites of BPU (G280, G308 and G322) in urine (for chemical structures see Ref. 11).[19] A fourth non-cytotoxic metabolite, G373, was qualitatively assessed in the urine.[19] The LC/UV method was used as a rapid technique in order to perform real-time PK monitoring of plasma concentrations every 2–4 weeks in the final dose level after a grade 5 event and characterisation of long half-life of cytotoxic metabolites. Subjects would be removed from the trial for safety reasons if the concentrations of BPU or mmBPU exceeded pre-determined thresholds based on PK and toxicity data from previous patients treated on the trial (see Table 1). All results reported below utilise the LC–MS–MS method.

Individual PK parameters were estimated by standard non-compartmental analysis, which was performed using WinNonlin version 5.0 (Pharsight Corporation, Mountain View, CA).[20]

2.6. Midazolam

The oral midazolam test was performed during the first 2 cycles of study drug administration for the subjects enrolled at the final dose level. Within 72 h prior to BPU treatment, patients were given a single 3 mg dose of oral midazolam (Versed® Syrup, Roche). The serial sampling of venous blood was performed pre-treatment and post-treatment to 7 h during weeks 1 and 8. Blood samples were processed to plasma and quantitated using a validated LC–MS–MS assay as described previously.[21] Individual PK parameters were estimated by standard non-compartmental analysis using WinNonlin.[20]

2.7. Statistical considerations

All study subjects who received at least one dose of study drug are included in the toxicity and efficacy analysis. For PK analysis, parameters were summarised using descriptive statistics. The differences between PK parameters were compared by a paired Student's *t*-test or repeated measures ANOVA. The associations between CYP3A phenotype as determined by the oral midazolam test at baseline and at week 8 and BPU PKs obtained during weeks 1 and 8 were evaluated by the use of Pearson's correlation coefficient. Statistical calculations were performed with the software package JMP version 3.1 (SAS Institute, Carey, NC). The a priori level of significance was set at $P < 0.05$.

Table 1 – Threshold concentrations of BPU and metabolites for continuation of therapy on 150 mg dose level

Patients would have study drug held for >3 weeks, and then restarted at 50% (75 mg) if any of the following were exceeded[a]

Week	BPU		mmBPU	
	C_{min} (nM)	AUC (μg*h/ml)	C_{min} (nM)	AUC (μg*h/ml)
2–4	>60		>400	
4		>15		>100
5–12	>60		>600	
>12[b]	–	–	–	–

a IRB-approved thresholds were empirically chosen based on visual inspection of Figs. 2 and 3 for the protocol amendment subsequent to the grade 5 event at the 320 mg dose level.
b Patients would not be discontinued from the study for PK thresholds after 12 weeks since by definition some type of clinical benefit (even stable disease) would have been achieved.

3. Results

3.1. Patient characteristics

Nineteen patients were enrolled between August 2001 and October 2004. The subjects had a variety of solid tumour types, including head and neck, non-small cell lung, neuroendocrine and renal cancer (Table 2). In all, 54 four-week cycles of BPU were administered over a dose range of 5–320 mg (Table 3). The median number of cycles was 2 (range 1.5–15). One patient at the 150 mg dose level did not complete cycle two due to rapid disease progression. One patient at the 20 mg dose level was escalated to 40 mg for cycles 6–8, but subsequently returned to the original dose level.

Although few toxicities >grade 1 were seen at the first 6 dose levels, the 320 mg dose level was expanded to six subjects to obtain more PK data due to (1) an ≈8-fold decrease in clearance seen between 40 mg and 80 mg, and (2) the excessive number of 5 mg capsules to be swallowed at the subsequent dose level (128 capsules at 640 mg). Two among six subjects at the 320 mg dose level experienced delayed haematologic toxicities (see below). The study was subsequently re-opened and completed at a reduced dose of 150 mg using 25 mg capsules. Further dose levels were not explored due to safety concerns.

Table 2 – Patient characteristics

Characteristic	No. of patients (*N* = 19)
Sex	
Male	11
Female	8
Age, years	
Median	60
Range	41–77
ECOG performance status	
0	6
1	12
2	1
Diagnosis	
Head and neck	3
Lung (NSC)	2
Renal	2
Neuroendocrine	2
Other[a]	10
Prior treatment	
Chemotherapy	17
Radiotherapy	14

Abbreviations: ECOG, Eastern Cooperative Oncology Group.
a Includes one case each of cancer of the gallbladder, rectum, pancreas, oesophagus, stomach, breast, liver and thyroid; adenocarcinoma of unknown primary; and one sarcoma.

Table 3 – Dose escalation

Dose level	BPU dose (mg)	No. of patients	No. of cycles[a]	No. of DLT
1	5	1	2	0
2	10	1	2	0
3	20[b]	1	15	0
4	40	1	2	0
5	80	1	2	0
6	160	1	2	0
7	320[c]	6	14	2
8	150[d]	7[e]	15	0

a Each cycle consisted of four weekly doses of oral BPU (one month).
b This patient received three cycles (cycles 6–8) of drug administration at 40 mg, and was subsequently returned to 20 mg due to grade 3 syncope. This was the only subject who was dose-escalated.
c Dose level expanded due to decline in apparent clearance.
d For this dose level, a 25 mg capsule was used. At all previous dose levels, a 5 mg capsule was administered.
e One patient not evaluable for toxicity due to early progression.

3.2. *Toxicity*

Most subjects reported little or no toxicities (Table 4). The most common adverse events were fatigue (42%), nausea (21%), and cytopenias (11–16%). The patient at 20 mg experienced a grade 3 syncopal event after escalation to 40 mg that was felt to be possibly related to study drug. He was also on antihypertensives and was orthostatic at the time, and no further episodes occurred after returning to the 20 mg dose level. Mild (grade 1) diarrhoea and headache were also each reported in 2 subjects. Even the most serious adverse event, neutropenia, was seen in only 2 subjects.

DLT was observed in two patients at the 320 mg dose level. One subject, a 67-year-old female with adenocarcinoma of unknown primary (metastatic to liver and lung, previously treated with two lines of cytotoxic chemotherapy over 15 months) developed sudden, prolonged neutropenia beginning at week 8, when her absolute neutrophil count (ANC) dropped within one week from 5460 to 21 cells/mm^3. She was admitted and died after 24 days from complications of *Pseudomonas aeruginosa* bacteremia and *Candida albicans* fungemia. She also experienced grade 3 anaemia and thrombocytopenia and her bone marrow never recovered despite growth factor support (bone marrow biopsy was aplastic). A second subject, a 53-year-old male with hepatocellular carcinoma, experienced an ANC nadir of 320 cells/mm^3 after 12 weeks of study drug administration. This was a more gradual decline which began on week 13 (ANC = 1140) with a nadir on week 16 and a full recovery by week 18. He also experienced grade 1 thrombocytopenia. He was considered a DLT because study drug was held >2 weeks.

After a full review of the clinical and PK data and assessment of drug exposure–toxicity relationships (see below), the study was subsequently amended to include real-time PK monitoring in which the concentrations of BPU and mmBPU were measured every 2 weeks (twice) then every 4 weeks (Table 1). The study was reopened and 7 patients (six evaluable) received a dose of 150 mg (<50% of the previous dose) using a 25 mg capsule. None of these patients exceeded the PK thresholds during the first 2 cycles and only one grade > 1 toxicity episode (grade 2 anaemia) was observed. The dose of 150 mg PO weekly was defined as the MTD for safety reasons despite the lack of DLT's.

Table 4 – Treatment-related side effects per dose level

Toxicity	Dose level (mg)							
	5 (n = 1)	10 (n = 1)	20 (n = 1)[a]	40 (n = 1)	80 (n = 1)	160 (n = 1)	320 (n = 6)	150 (n = 7)
Neutropenia							**G4:1**	
Neutropenic infection							**G5:1**	
Anaemia							G1:1 G3:1	G2:1
Thrombocytopenia							G1:1 G3:1	
Fatigue			G2:1				G1:4	G1:3
Headache			G1:1					G1:1
Diarrhoea	G1:1						G1:1	
Nausea		G1:1	G1:1				G1:1	G1:1
Vomiting								G1:1
Alopecia							G1:2	
Rhinitis								G1:1
Cough								G1:1
Myalgia								G1:1
Arthralgia			G1:1					
Syncope			G3:1					

Note: Number of worst grade adverse events possibly, probably, or definitely attributed to BPU during study drug administration. Toxicities are graded per the NCI CTC version 2 criteria. 'G1:2' denotes 2 patients had grade 1 toxicity. Dose-limiting toxicities are indicated in bold.
a This subject was the only patient dose-escalated; after receiving five cycles (cycle 1–5) at 20 mg and three cycles (cycles 6–8) at 40 mg, the patient was returned to 20 mg (cycles 9–15) due to grade 3 syncope during cycle 8. No further syncopal events occurred after the subject's antihypertensive medication was discontinued.

3.3. *Response*

No responses were seen in this pre-treated patient population. One patient with adenoid cystic carcinoma experienced stable disease for 13 months and came off study because of progression. Two patients with disease stabilisation after two cycles subsequently progressed.

3.4. *Pharmacokinetic studies*

3.4.1. *BPU in plasma*

PK data were obtained and evaluable for 19 patients. Representative BPU, mmBPU and aminoBPU plasma concentration–time profiles are illustrated in Fig. 1 and plasma PK parameters are listed in Tables 5 and 6.

For BPU, C_{max} values were reached on average at 2.3 h, 1.7 h and 3.3 h during weeks 1, 4 and 8, respectively. BPU concentrations were measurable at 1 week post-treatment in all patients treated at 80 mg or higher. As the BPU dose was increased, a disproportionate increase in BPU exposure (C_{max} and AUC) was observed (see Fig. 2 and Table 5). This prompted, in part, an expansion of the patient cohort at 320 mg. The mean terminal half-life of BPU ranged from 27 to 198 h during weeks 1, 4 and 8 over all dose levels. The inspection of weekly pre-treatment concentrations indicates that BPU reached steady-state at ≈8 weeks (Fig. 3).

On average, mmBPU C_{max} values were reached between 1 and 8 h and always occurred at the same time or after the T_{max} for BPU (Table 6). Mean terminal half-life values ranged from 103 to 1526 h, which were longer than BPU. mmBPU reached steady-state at ≈10–14 weeks (Fig. 3). Average mmBPU:BPU AUC ratios were 3.9, 3.7 and 4.7 during weeks 1, 4 and 8, respectively.

Mean aminoBPU T_{max} values were 3.1 h with the 5 mg capsule and 29.1 h with the 25 mg formulation and always occurred after the T_{max} for BPU or mmBPU (Table 6). Because concentrations declined slowly during the 168-h sampling period, the terminal half-life for aminoBPU could not be estimated for most patients. The mean terminal half-life was 607 h (67% CV), when it could be estimated. AminoBPU reached steady-state at ≈10–14 weeks (Fig. 3). Average aminoBPU:BPU AUC ratios were 0.5, 1.4 and 1.8 during weeks 1, 4 and 8, respectively, and were consistently lower for aminoBPU than for mmBPU.

The presence of the BPU metabolites G280, G308, G322 and G373 in plasma were monitored and quantitated on the BPU calibration curve; all concentrations were BLQ.

3.5. *BPU in urine*

The amount of BPU and metabolites excreted in urine was collected and analysed in 7 patients. On average, less than

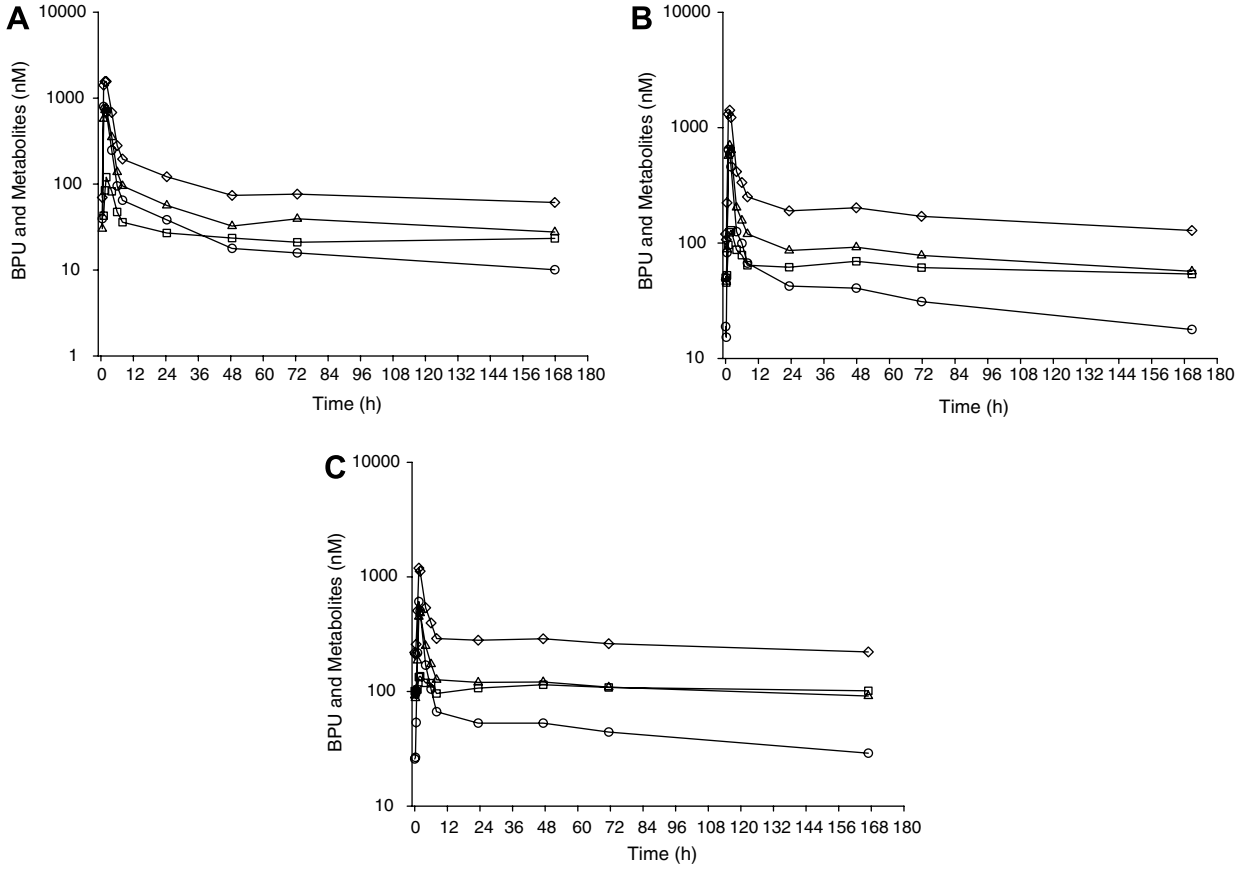

Fig. 1 – Plasma concentration time curve in a single patient for BPU administered orally at a dose of 150 mg during week 1 (A), 4 (B) and 8 (C). The open circle (○), open triangle (△), open square (□) and open diamond (◇) represent BPU, mmBPU, aminoBPU, and cumulative exposure to BPU and the cytotoxic metabolites concentrations, respectively.

Table 5 – Pharmacokinetic parameters for BPU in plasma

Dose (mg)	Week	No. of patients	Pharmacokinetic parameters[a]					
			T_{max} (h)	C_{max} (nM)	V_z/F (l)	Cl_s/F (l/h)	$t_{1/2,z}$ (h)	AUC[b] (μM*h)
5	1	1	1.0	13.8	9667	121	56	0.09
5	4	1	1.0	9.6	NR	142	NR	0.08
10	1	1	4.2	9.1	16282	134	84	0.16
10	4	1	1.0	14.3	NR	101	27	0.21
20	1	1	1.5	115	4165	97	30	0.44
20	4	1	2.0	36.2	NR	160	26	0.27
40	1	1	1.0	192	11992	158	52	0.54
40	4	1	1.5	130	NR	114	27	0.75
80	1	1	6.0	579	1657	14	81	12.0
80	4	1	2.0	994	NR	15	111	11.3
160	1	1	1.0	2040	5802	22	182	15.4
160	4	1	1.5	838	NR	53	138	6.4
320	1	6	1.8 ± 0.4	3484 ± 799	3305 ± 2018	17 ± 6	161 ± 133	46.2 ± 22.6
320	4	6	1.7 ± 0.4	3129 ± 1366	NR	21 ± 9	123 ± 65	40.4 ± 24.1
150	1	7	2.7 ± 2.0	630 ± 349	6056 ± 2592	50 ± 19	95 ± 48	7.4 ± 3.0
150	4	7	2.0 ± 2.0	774 ± 278	NR	47 ± 28	186 ± 161	9.0 ± 5.1
150	8	6	3.3 ± 2.2	349 ± 173	NR	51 ± 35	198 ± 79	9.0 ± 6.1

Abbreviations: AUC, area under the concentration–time curve; C_{max}, maximal plasma concentration; NR, not reportable; T_{max}, time of the maximal plasma concentration; $T_{1/2,z}$, terminal half-life; V_z/F, apparent volume of distribution.
a Values are reported as the mean ± standard deviation for the 150 and 320 mg dose level.
b AUC_{inf} is reported for all dose levels for week 1; AUC_{last} is reported for all dose levels for week 4; $AUC_{0-168\,h}$ is reported for all dose levels for week 8.

Table 6 – Pharmacokinetic parameters for mmBPU and amino-BPU in plasma

	Dose (mg)	Week	No. of patients	Pharmacokinetic parameters[a]				
				T_{max} (h)	C_{max} (nM)	$t_{1/2,z}$ (h)	AUC[b] (μM*h)	Metabolite: BPU AUC ratio
mmBPU	320	1	6	1.93 ± 0.21	2858 ± 742	279 ± 170	130 ± 73.0	3.5 ± 1.4
mmBPU	320	4	6	2.26 ± 0.88	3279 ± 996	483 ± 301	122 ± 56.2	3.2 ± 0.9
mmBPU	150	1	7	3.51 ± 2.63	517 ± 306	317 ± 89	29.2 ± 13.9	4.3 ± 2.2
mmBPU	150	4	7	2.51 ± 2.42	732 ± 338	283 ± 122	28.0 ± 16.0	4.1 ± 3.2
mmBPU	150	8	6	4.05 ± 1.81	349 ± 94	736 ± 420	29.8 ± 9.5	4.7 ± 3.9
Amino-BPU	320	1	6	2.59 ± 1.10	251 ± 131	734 ± 101	15.7 ± 8.9	0.5 ± 0.2
Amino-BPU	320	4	6	3.67 ± 1.50	582 ± 228	415 ± 158	54.3 ± 33.7	1.4 ± 0.6
Amino-BPU	150	1	7	31.20 ± 67.88	46 ± 40	255 ± 276	2.6 ± 1.7	0.4 ± 0.4
Amino-BPU	150	4	7	32.27 ± 62.41	87 ± 56	623 ± 283	9.0 ± 6.5	1.4 ± 1.4
Amino-BPU	150	8	6	23.33 ± 23.40	71 ± 37	986 ± 504	10.4 ± 4.7	1.8 ± 1.8

Abbreviations: AUC, area under the concentration–time curve; C_{max}, maximal plasma concentration; T_{max}, time of the maximal plasma concentration; $T_{1/2,z}$, terminal half-life.
a Values are reported as the mean ± standard deviation for the 150 and 320 mg dose level.
b AUC_{inf} is reported for all dose levels for week 1; AUC_{last} is reported for all dose levels for week 4; $AUC_{0-168\,h}$ is reported for all dose levels for week 8.

1.5% of the BPU dose was excreted in urine as parent compound or metabolites, with a maximum of 3.6% observed in one patient during week 4. The most predominant metabolite in urine was G280, which is inactive.[11]

3.6. *Midazolam*

The addition of the oral midazolam test as a phenotypic probe for CYP3A was added during weeks 1 and 8 at the 150 mg dose level. CYP3A activity varied about 11-fold (midazolam apparent oral clearance mean, 40 l/h; range = 10–111 l/h), which is consistent with previously published studies.[21] CYP3A activity at baseline was not correlated with BPU PK parameters during week 1 ($P > 0.05$). However, CYP3A activity during week 8 was correlated with BPU clearance ($P = 0.007$) and the relative extent of conversion to mmBPU ($P = 0.003$) and aminoBPU ($P < 0.001$) during the week 8 pharmacokinetic assessment.

3.7. *Pharmacokinetic–toxicity relationship*

Data from 19 patients receiving BPU are available to characterise drug exposure–toxicity relationships. The two patients who experienced a DLT had the highest BPU C_{max}, BPU AUC,

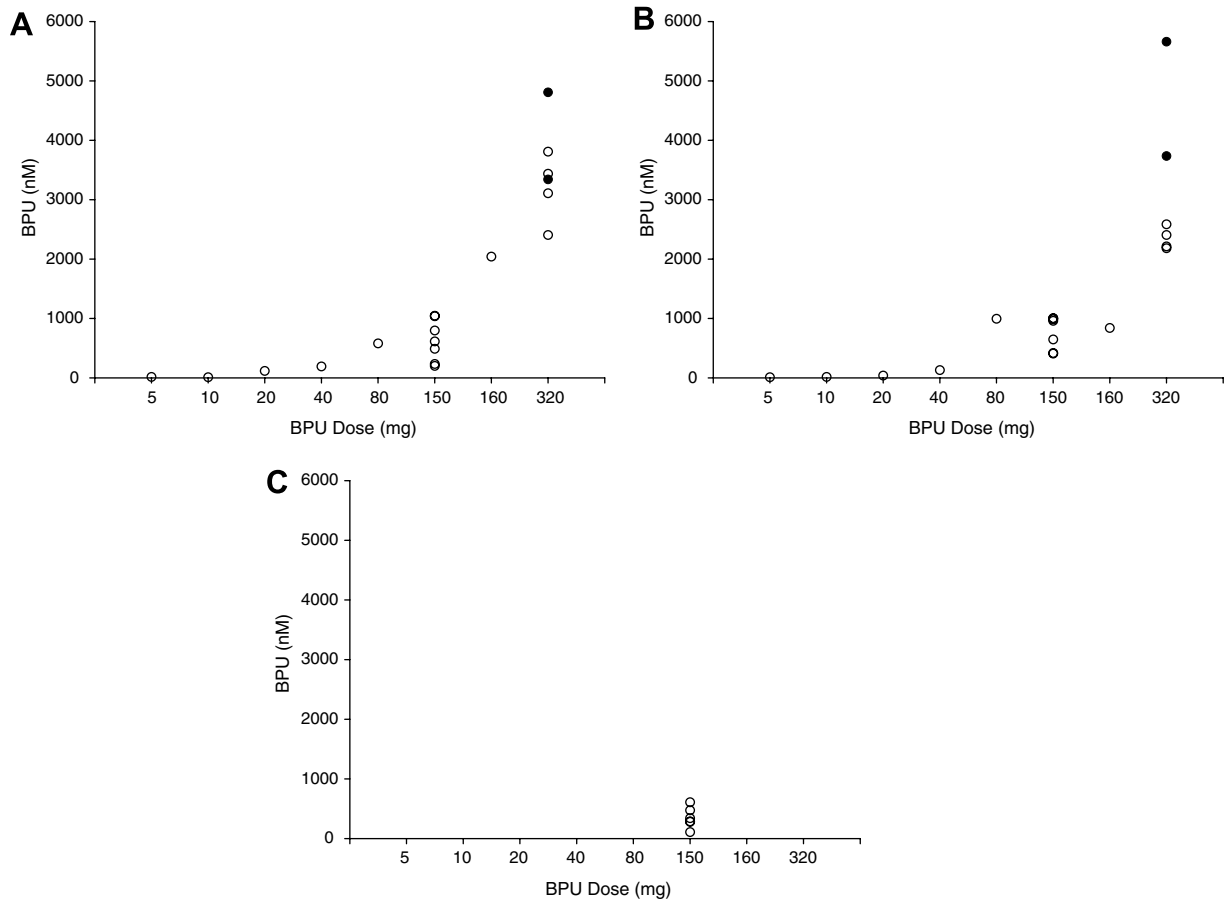

Fig. 2 – BPU maximum plasma concentrations (C_{max}) as a function of dose level and week 1 (A), 4 (B) and 8 (C). The closed circles (●) represent the two patients that experienced DLTs at the 320 mg dose level.

mmBPU C_{max}, mmBPU AUC, aminoBPU C_{max} (first and third highest), aminoBPU AUC values during week 4 and the highest pre-treatment concentrations (Fig. 2 for BPU C_{max}; Fig. 3 for pre-treatment concentrations; AUC values not shown). BPU and metabolite exposure in the 7 subjects treated at 150 mg were much lower than that observed at the 320 mg dose level, potentially explaining the observed lack of neutropenia at 150 mg (Figs. 2 and 3).

4. Discussion

This first-in-human phase I study of BPU in patients with refractory, advanced solid tumours has defined 150 mg PO weekly as the MTD for its administration in an uninterrupted weekly schedule. A sudden and life-threatening myelosuppression was observed at the 320 mg dose level, and is likely related to the long half-life of both parent compound and cytotoxic metabolites. Although no DLTs and only a single grade 2 event were observed among the six patients treated at 150 mg, the safety and PK schedule of this administration schedule discouraged us from exploring intermediate doses between 150 and 320 mg. Of note, it is possible that the toxicities at the 150 mg dose level are underestimated since only one subject out of seven received study drug for more than 8 weeks, when steady-state levels are reached.

Alternative, interrupted schedules of BPU should be considered. A contemporary trial using a six-week on, two-week off schedule has successfully enrolled patients above the 320 mg dose schedule without excessive myelotoxicity.[14] As with this study, the 5 mg capsule formulation was changed to a 25 mg capsule due to the need to swallow an excessive number of pills.

This trial shows several limitations of accelerated titration designs, which were developed as a method to reduce the number of patients treated at low doses of phase I agents and speed the completion of trials.[13] Although the average number of patients is reduced, accelerated titration does not necessarily decrease the average number of patients who experience grade 3/4 toxicities, and the built-in delays between dose levels do not speed up the completion of phase I trials as hoped. In this study, the toxicities observed in the fourth and fifth patients in dose level 320 mg were delayed, sudden and severe. The predetermined safety parameters to discontinue single-patient cohorts (grade 2 toxicity in any cycle or DLT in cycle 1) would have allowed dose escalation to 640 mg after the one patient treated at 320 mg, but the 320 mg dose level was expanded due to the decreased clearance observed and number of pills required. This study also illustrates the utility of real-time access to clinical pharmacology resources in the early stages of human testing.

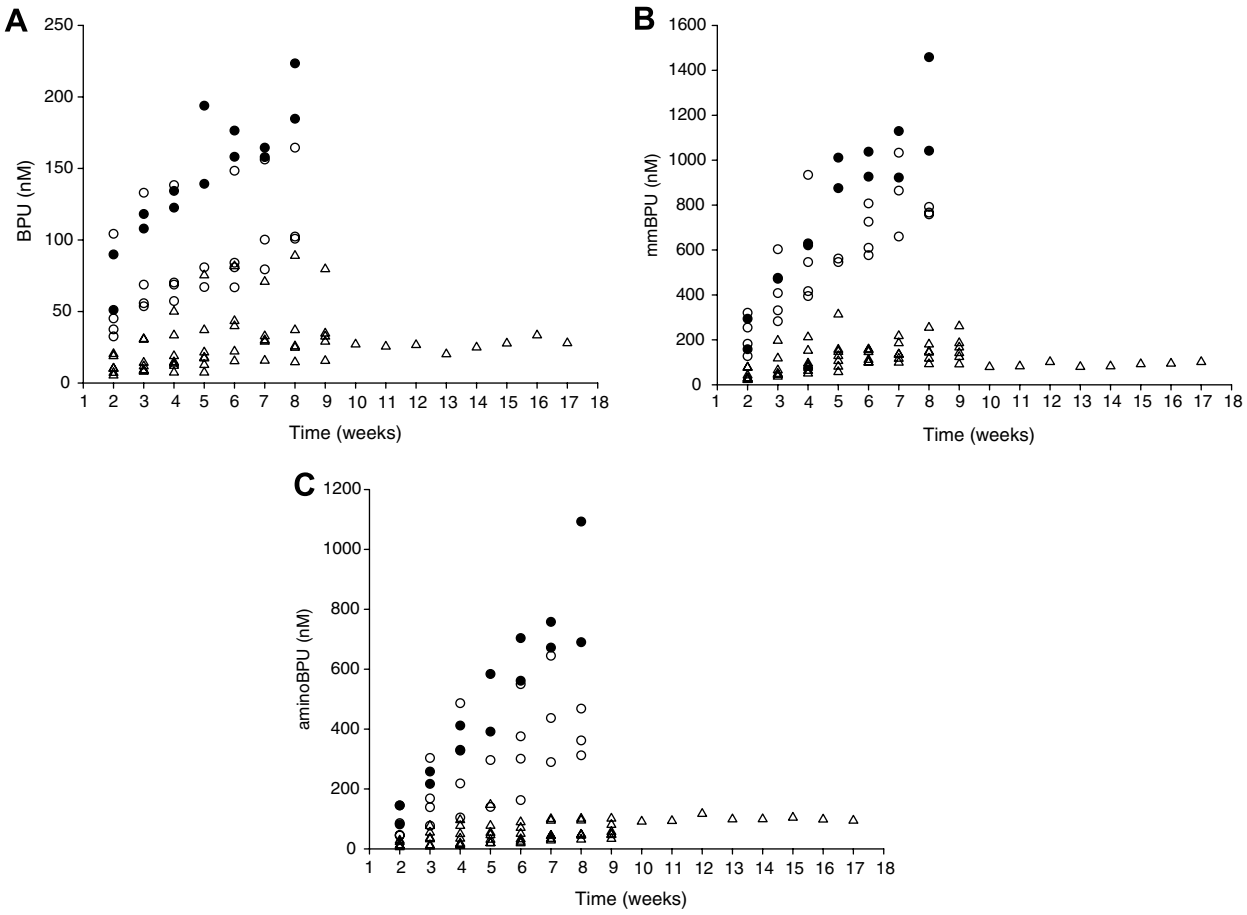

Fig. 3 – BPU (A), mmBPU (B) and aminoBPU (C) minimal plasma concentrations (C_{min}) over time. The open triangle (△) and open circle (○) represent the 150 mg dose level and 320 mg dose level, respectively. The closed circles (●) represent the two patients that experienced DLTs at the 320 mg dose level.

BPU has several interesting PK properties, including a long terminal half-life, which allows once weekly dosing, and extensive metabolism to cytotoxic metabolites, which have longer half-lives and represent up to 1250% of the exposure to the parent compound. The reason for the marked decrease in clearance between low and higher dose levels is not clear. A possible explanation is saturation of the metabolic pathways. We have determined that BPU is metabolised predominantly by CYP3A4 and CYP1A1 and to a lesser extent by CYP2C8, CYP2D6, CYP3A5 and CYP3A7.[11,19] These studies will further guide the future development of this drug using alternative dosing schedules and possible combination trials with CYP3A inhibitors. An examination of the concomitant medications taken by our subjects for CYP3A4 inhibitors or inducers did not show any clear relationships.

Clinical investigators involved in early-phase testing of new drugs are constantly reminded of the need to minimise exposing human subjects to potentially subtherapeutic levels while obtaining critical safety and PK data. Several methods for early phase studies of cytotoxic agents in humans have been suggested as alternatives to the modified Fibonacci series.[22] Although accelerated titration schedules have been successfully employed in many studies, our study should serve as a reminder of its potential limitations.[23–25]

Conflict of interest statement

None declared.

Funding

Funded by U01 CA70095. Correlative studies supported by National Institutes of Health grant P30CA069773, National Cancer Institute Cancer Therapy and Evaluation Program's Translational Research Fund, and the Commonwealth Foundation for Cancer Research.

Acknowledgements

We thank Ping He and Yelena Zabelina for their technical support; Susan Davidson for her quality assurance of the data; Manuel Hidalgo and Saeed Khan for their scientific input.

REFERENCES

1. Okada H, Koyanagi T, Yamada N, Haga T. Synthesis and antitumor activities of novel benzoylphenylurea derivatives. *Chem Pharm Bull (Tokyo)* 1991;**39**(9):2308–15.

2. Hollingshead MG, Sackett DL, Alley MC, et al. The anticancer activity of six benzoylphenylurea compounds and their interaction with tubulin. *Proc Am Assoc Cancer Res* 1998;**39**:164 [abstract 1126].
3. Okada H, Koyanagi T, Yamada N. Synthesis and antitumor activities of prodrugs of benzoylphenylureas. *Chem Pharm Bull (Tokyo)* 1994;**42**(1):57–61.
4. Okada H, Kato M, Koyanagi T, Mizuno K. Synthesis and antitumor activities of water-soluble benzoylphenylureas. *Chem Pharm Bull (Tokyo)* 1999;**47**(3):430–3.
5. Jain N, Yang G, Tabibi SE, Yalkowsky SH. Solubilization of NSC-639829. *Int J Pharm*. 2001;**225**(1–2):41–7.
6. Ando N, Nakajima T, Masuda H, et al. Antimicrotubule effects of the novel antitumor benzoylphenylurea derivative HO-221. *Cancer Chemother Pharmacol* 1995;**37**(1–2):63–9.
7. Abdel-Aziz W, Hickey R, Edelman M, Malkas L. Effect of novel benzoylphenylurea derivatives on DNA polymerase alpha activity using the synthesome-based in vitro model system. *Invest New Drugs* 2003;**21**(4):421–8.
8. Alley MC, Covey JM, Pacula-Cox CM, et al. In vitro and in vivo pharmacologic evaluations of N,N-dimethyl-amino-benzoylphenylurea and a principal metabolite which exhibit multi-spectrum anticancer activities. *Proc Am Assoc Cancer Res* 2001;**42**:378 [abstract 2035].
9. Nakajima T, Masuda H, Okamoto T, et al. Antitumor activity on murine tumors of a novel antitumor benzoylphenylurea derivative, HO-221. *Cancer Chemother Pharmacol* 1991;**28**(5):351–6.
10. Noker PE, Weinberg DS, Page JG, Schweikart KM, Tomaszawski JE. Oral bioavailability of dimethyl amino benzoylohenyl urea (BPU) in dogs. *Proc Am Assoc Cancer Res* 2003;**44**:394 [abstract 1737].
11. Rudek MA, Zhao M, Smith NF, et al. In vitro and in vivo clinical pharmacology of dimethyl benzoylphenylurea, a novel oral tubulin-interactive agent. *Clin Cancer Res* 2005;**11**(23):8503–11.
12. Gurulingappa H, Amador ML, Zhao M, Rudek MA, Hidalgo M, Khan SR. Synthesis and antitumor evaluation of benzoylphenylurea analogs. *Bioorg Med Chem Lett* 2004;**14**(9):2213–6.
13. Simon R, Freidlin B, Rubinstein L, Arbuck SG, Collins J, Christian MC. Accelerated titration designs for phase I clinical trials in oncology. *J Natl Cancer Inst* 1997;**89**(15):1138–47.
14. Edelman MJ, Bauer KS, Meiller T, et al. Phase I, pharmacokinetic (PK) and pharmacodynamic study of benzoylphenylurea (BPU, NSC 639829), a novel antitubulin agent. *Proc Am Soc Clin Oncol* 2003;**22**:137 [abstract 550].
15. Garimella TS, Edelman MJ, Horn JJ, Colevas AD, Bauer KS. In vitro metabolism of the novel antimicrotubule agent benzoylphenylurea (bpu) currently under phase I investigation in patients with advanced malignancy. *AAPS Pharm Sci*. 2003;**5**(4) [abstract T3328].
16. Therasse P, Arbuck SG, Eisenhauer EA, et al. New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. *J Natl Cancer Inst* 2000;**92**(3):205–16.
17. Zhao M, Zabelina Y, Rudek MA, Wolff AC, Baker SD. A rapid and sensitive method for determination of dimethyl benzoylphenyl urea in human plasma by using LC/MS/MS. *J Pharm Biomed Anal* 2003;**33**(4):725–33.
18. Rudek MA, Zabelina Y, Zhao M, Wolff AC, Baker SD. A method for determination of dimethyl benzoylphenyl urea (BPU) in human plasma by using LC/UV. *Biomed Chromatogr* 2004; **18**(5):282–7.
19. Rudek MA, Zhao M, He P, et al. Validation and implementation of a liquid chromatography/tandem mass spectrometry assay to quantitate dimethyl benzoylphenylurea (BPU) and its five metabolites in human plasma and urine for clinical pharmacology studies. *J Chromatogr B Analyt Technol Biomed Life Sci*. 2005;**828**(1–2): 41–54.
20. Gibaldi MPD. *Noncompartmental analysis based on statistical moment theory. Pharmacokinetics*. New York (NY): Marcel Dekker; 1982. p. 409–17.
21. Li J, Karlsson MO, Brahmer J, et al. CYP3A4-phenotyping approach to predict systemic exposure to EGFR tyrosine kinase inhibitors. *J Natl Cancer Inst* [in press].
22. Bolognese JA. A Monte Carlo comparison of three up-and-down designs for dose ranging. *Control Clin Trials* 1983;**4**:187–96.
23. Grem JL, Morrison G, Guo XD, et al. Phase I and pharmacologic study of 17-(allylamino)-17-demethoxygeldanamycin in adult patients with solid tumors. *J Clin Oncol* 2005;**23**(9):1885–93.
24. Syed S, Takimoto C, Hidalgo M, et al. A phase I and pharmacokinetic study of Col-3 (Metastat), an oral tetracycline derivative with potent matrix metalloproteinase and antitumor properties. *Clin Cancer Res* 2004;**10**(19): 6512–21.
25. Goetz MP, Toft D, Reid J, et al. Phase I trial of 17-allylamino-17-demethoxygeldanamycin in patients with advanced cancer. *J Clin Oncol* 2005;**23**(6):1078–87.