Clinical pharmacology and pharmacogenetics of flavopiridol 1-h i.v. infusion in patients with refractory neoplasms

Suoping Zhai[a,b], Edward A. Sausville[c,d], Adrian M. Senderowicz[c], Yuichi Ando[a,b], Donna Headlee[c], Richard A. Messmann[c], Susan Arbuck[c,e], Anthony J. Murgo[c,e], Giovanni Melillo[c], Eiichi Fuse[a,b] and William D. Figg[a,b]

A phase I trial of flavopiridol administered as a 1-h i.v. infusion schedule was explored. Fifty-five patients were treated with flavopiridol at doses ranging from 12 to 78 mg/m^2 daily for 5, 3 and 1 day every 3 weeks. Pharmacokinetic and pharmacodynamic analysis was performed together with analysis of a promoter polymorphism of the *UGT1A1* gene. Peak concentrations and areas under the time–concentration curve of flavopiridol were linear within the doses studied. Estimated clearance was 13.8 $\pm$ 4.9 l/h/m^2 (mean $\pm$ SD), volume of distribution at steady-state was 64.9 $\pm$ 43.4 l/m^2 and elimination half-life was 5.2 $\pm$ 4.9 h. Forty-nine of the 55 patients were genotyped for the promoter polymorphism. We found five (10%) homozygous and 11 (22%) heterozygous patients for *UGT1A1*28*, which alters the reference sequence (TA)$_6$TAA to the variant (TA)$_7$TAA by an extra TA dinucleotide insertion within the TATA box. One patient was heterozygous for the sequence of five TA repeats, (TA)$_5$TAA. The remaining 32 patients did not have the *UGT1A1*28* allele (homozygous for the reference sequence). Associations of the *UGT1A1* promoter genotype with either the pharmacokinetic parameters or diarrhea (occurrence and severity) were not observed in this study. The pharmacogenetic analyses did not support that the *UGT1A1* promoter polymorphism could affect flavopiridol pharmacokinetics and alter the incidence and severity of diarrhea induced by the drug. *Anti-Cancer Drugs* 14:125–135 © 2003 Lippincott Williams & Wilkins.

Anti-Cancer Drugs 2003, **14**:125–135

Keywords: clinical trial, flavopiridol, pharmacogenetics, pharmacokinetics, polymorphism, UDP-glucuronosyltransferases, UGT1A1

[a]Clinical Pharmacology Research Core, Medical Oncology Clinical Research Unit, [b]Center for Cancer Research, [c]Developmental Therapeutics Program Clinical Trials Unit, [d]Developmental Therapeutics Program and [e]Cancer Therapy Evaluation Program, National Cancer Institute, Bethesda, MD, USA.

Correspondence to W.D. Figg, Molecular Pharmacology Section and Clinical Pharmacology Research Core, Center for Cancer Research, National Cancer Institute, Building 10, Room 5A01, MSC 1910, 9000 Rockville Pike, Bethesda, MD 20892, USA.
Tel: +1 301 402-3622; fax: +1 301 402-8606;
e-mail: wdfigg@helix.nih.gov

Received 20 October 2002 **Accepted** 26 November 2002

Introduction

Flavopiridol (NSC649890) [(–)*cis*-5,7-dihydroxy-2-(2-chlorophenyl)-8[4-(3-hydroxy-1-methyl)-piperidinyl]-4H-1-benzopyran-4-1] is a semisynthetic flavonoid derived from rohitukine, an alkaloid isolated from a plant indigenous in India. It is an inhibitor of several cyclin-dependent kinases (CDKs), key regulators of the cell cycle [1,2]. Flavopiridol can arrest cells in G_1 phase or at the G_2/M boundary, possibly by inhibiting CDK2 and CDK1 [3–5]. Another important feature of flavopiridol is the induction of apoptotic cell death in certain cell types. It causes the cell death in both cycling and non-cycling cell populations, although the mechanisms of induced apoptosis have not been fully elucidated [6]. In addition, flavopiridol has been shown to have other anticancer properties: reducing levels of cyclin D_1 (the product of an oncogene) [4,7], possessing antiangiogenic activity [8,9] and interacting with DNA [6].

Initial clinical trials were commenced with a schedule of 72-h continuous infusion, because a prolonged exposure to flavopiridol appeared to be necessary for its cytostatic effect according to the results of previous animal and *in vitro* models [10]. Using the 72-h schedule every 2 weeks in clinical trials, plasma concentrations of flavopiridol were achieved within the range of 200–500 nM, which were above a threshold necessary to inhibit cancer cell growth *in vitro* [11–14].

More recent preclinical animal studies have shown that an i.v. or i.p. of flavopiridol administered daily produced apoptosis in normal and malignant cells [7,15]. After daily treatments with 7.5 mg/kg flavopiridol by an i.v. or i.p. bolus for 5 consecutive days, 11 out of 12 advanced stage s.c. human HL-60 xenografts underwent complete regression of the tumor and animals remained disease-free for several months after one course of the treatment. Significant antitumor activity was also observed in animal models with head and neck squamous cell carcinoma using the i.p. bolus regimen. Conversely, flavopiridol administered as a 72-h infusion in similar leukemia models achieved only tumor growth delay [15]. These results suggested that repetitive high plasma concentrations of flavopiridol at the μmol range would be more effective to produce the maximum 'apoptosis'-related antitumor effects by the drug. Thus, a phase I trial of

0959-4973 © 2003 Lippincott Williams & Wilkins DOI: 10.1097/01.cad.0000054974.31252.f7

daily flavopiridol as a 1-h i.v. infusion was explored to achieve repetitive higher peak plasma concentrations.

Flavopiridol is conjugated and detoxified by UDP-glucuronosyltransferase (UGT, EC 2.4.1.17) to yield its β-glucuronides, which are mainly excreted into the bile and could be reabsorbed from the intestine into the enterohepatic circulation [16]. UGT1A1 is one of the UGT1 isozymes, which conjugates bilirubin, endogenous steroids, therapeutic drugs (e.g. ethinylestradiol) and xenobiotic compounds (e.g. phenols, anthraquinones and flavones) [17]. It has been reported that UGT1A9 and UGT1A1 are primarily involved in flavopiridol glucuronidating activity in humans [17,18]. Polymorphism in the promoter of the *UGT1A1* gene is closely related with Gilbert's syndrome, a benign form of unconjugated hyperbilirubinemia [19]. A promoter variant (*UGT1A1*28*) alters the reference sequence $(TA)_6TAA$ to the variant $(TA)_7TAA$ by insertion of an extra TA dinucleotide within the TATA box, resulting in reduced transcriptional activity of the *UGT1A1* gene [20,21]. According to an *in vitro* study, UGT1A9 appears to play a more important role in flavopiridol metabolism than UGT1A1 (18). However, the relative importance of the UGT1A9 compared with UGT1A1 for *in vivo* glucuronidation of flavopiridol remains to be determined because there may be a potential discrepancy between *in vitro* experiments and *in vivo* metabolism. Both UGT1A1 and UGT1A9 play major roles in glucuronidation of SN-38, an active metabolite of irinotecan, to a comparable extent in the *in vitro* study [22]. However, *UGT1A1* promoter genotype has known to have significant influence on the pharmacokinetics of SN-38 and clinical toxicity induced by its parent compound irinotecan [23,24]. We consider that the polymorphic activity of UGT1A1 enzyme may explain the variability of flavopiridol pharmacokinetics and, possibly, a likelihood of diarrhea, one of the dose-limiting toxicities of flavopiridol [14].

Our hypothesis is that variant genotypes of *UGT1A1* may increase the risk of toxicity by flavopiridol through the impaired glucuronidating (detoxifying) activity of the drug. If the clinical impact of the *UGT1A1* polymorphism on flavopiridol treatment were revealed, a patient carrying a variant genotype of *UGT1A1* might require lower doses of the drug to reduce the risks of adverse effects. This study reports the pharmacokinetic/pharmacodynamic analyses of flavopiridol administered as a 1-h i.v. infusion and pharmacogenetic analysis at the polymorphic promoter region.

Methods

Patients

Patients were eligible for entry onto the study if they had refractory tumors for which no standard therapy was available, were 18 years of age or older, had a serum creatinine $\leq$1.5 mg/dl. (or creatinine clearance $\geq$60 ml/min), had an absolute granulocyte count $\geq$1000/ml, platelets $>$75 000/ml, and had an AST and ALT $\leq$2.5 times ULN. Patients had a performance status (Eastern Cooperative Oncology Group) 0–2. Other eligibility criteria included no prior chemotherapy or radiotherapy within 4 weeks. Patients were excluded if they had metastatic disease to the brain, a myocardial infarction during the previous 6 months. This protocol was reviewed and approved by the National Cancer Center Institution's Institutional Review Board and all patients provided written informed consent prior to participation.

Design

At least three patients were enrolled at each dose level. If toxicity was noted at a particular dose, that dose level was expanded to include at least six patients. The starting dose of flavopiridol, 12 mg/m^2/day given as a 1-h infusion daily for 5 days every 3 weeks was one-half the toxic low dose in rats (24 mg/m^2/day). The dose was escalated at increments of 40% until grade 2 non-hematological or grade 3 hematological toxicity was observed in no more than two patients of a cohort during the first cycle. If three or more patients in a cohort experienced these toxicities, the rate of dose escalation was lowered to 25% of the preceding dose. When the maximum tolerated dose (MTD) was achieved, the protocol was amended in an effort to achieve higher peak plasma concentrations. Flavopiridol 1-h daily for 3 consecutive days and, then, for 1 day every 3 weeks were explored, in order to administer higher dose for higher peak plasma concentration.

Pharmacokinetic analysis

Blood samples were collected at the end of the 1-h infusion, 10 and 30 min, and at 1, 2, 4, 8, 12 and 24 h after the cessation of the infusion in cycle 1. To study flavopiridol pharmacokinetics over 5 days, blood samples from day 5 as well as day 1 were obtained at a dose of 37.5 mg/m^2/day for 5 days in six patients. Flavopiridol plasma concentrations were determined using a validated HPLC assay. In brief, 1 ml acetonitrile was added to 0.25 ml plasma and vortexed for 30 s. The samples were centrifuged at 10,000 r.p.m. for 5 min at 4°C. The supernatant was transferred to a glass tube and evaporated to dryness at 40°C under air. The sample was reconstituted with 200 μl mobile phase and vortexed. The 150 μl was injected onto a Water Nova-Pak C_{18} column. A gradient profile consisted of ammonium acetate (0.05 M, pH 4.15) and methanol (containing 0.025 M ammonium acetate), and a flow rate of 1 ml/min was utilized with a total run time of 21 min. Flavopiridol had an eluting time of 9.8 min and was detected with ultraviolet absorbance at 268 nm. The peak area was used for quantitation. The assay was linear over the concentration range of 0.025–3.0 μg/ml (57–6849 nM) [25].

The pharmacokinetic data were analyzed by compartmental analysis. ADAPT II version 4 (Biomedical Stimulation Resource, University of Southern California, Los Angeles, CA) was used [26]. A two-compartment open linear model fit the data best. The area under the plasma concentration–time curve (AUC) for each subject was calculated using the linear trapezoidal method from time zero to the last concentration-time point obtained and extrapolated to infinity by dividing the last concentration by the terminal elimination rate constant (C_{last}/k_e). Peak concentrations were the flavopiridol concentrations achieved at the end of 1-h infusion.

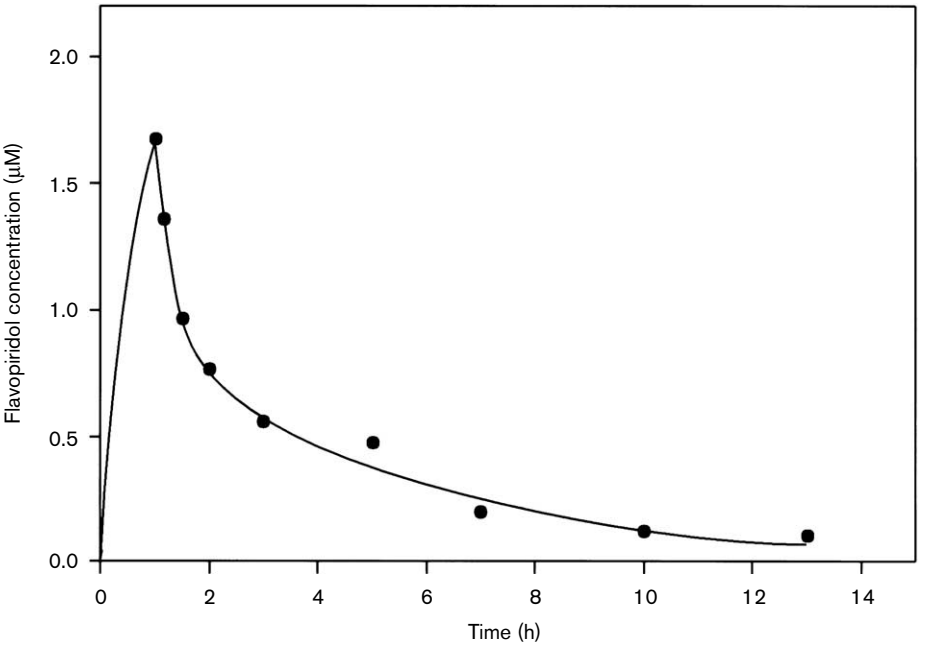
Pharmacogenetics

Genomic DNA from each patient was extracted from plasma [27]. All samples had been stored at −80°C prior to examination. A promoter variant (*UGT1A1*28*) which alters the reference sequence $(TA)_6TAA$ to the variant $(TA)_7TAA$ (nucleotide −39 to −53) by insertion of an extra TA dinucleotide within the TATA box was determined by direct sequencing [28]. This was accomplished by a PCR amplification of a 253-bp fragment (−147 to +106) using 0.25 μM forward primer 5′-AAGTGAACTCCCTGCTACCTT–3′ and 0.25 μM reverse primer 5′-CCACTGGGATCAACAGTATCT-3′ in a volume of 50 μl containing 0.2 mM of each deoxynucleoside triphosphate, 50 mM KCl, 20 mM Tris–HCl (pH 8.4), 2.0 mM $MgCl_2$ and 1.3 U of *Taq* polymerase (Platinum *Taq* DNA Polymerase; Invitrogen, Carlsbad, CA). PCR conditions were: 95°C for 5 min followed by 30 cycles of 95°C for 30 s, 58°C for 40 s and 72°C for 40 s (GeneAmp PCR System 9700; Applied Biosystems, Foster City, CA). A 1-μl product of the first PCR was subjected to the second round of PCR amplifications using another forward primer 5′-GTCACGTGACACAGTCAAAC-3′ and the same reverse primer as the first PCR. This was designed to amplify a 210-bp segment (−104 to +106). The second PCR conditions were identical to the first amplification reaction. Cycle sequencing was performed with a dye terminator sequence reaction (ABI Prism DNA Sequencing Kit; Perkin-Elmer, Foster City, CA) using the reverse PCR primer. The promoter polymorphism was observed on an ABI Prism 310 Genetic

Table 1 **Patient characteristics**

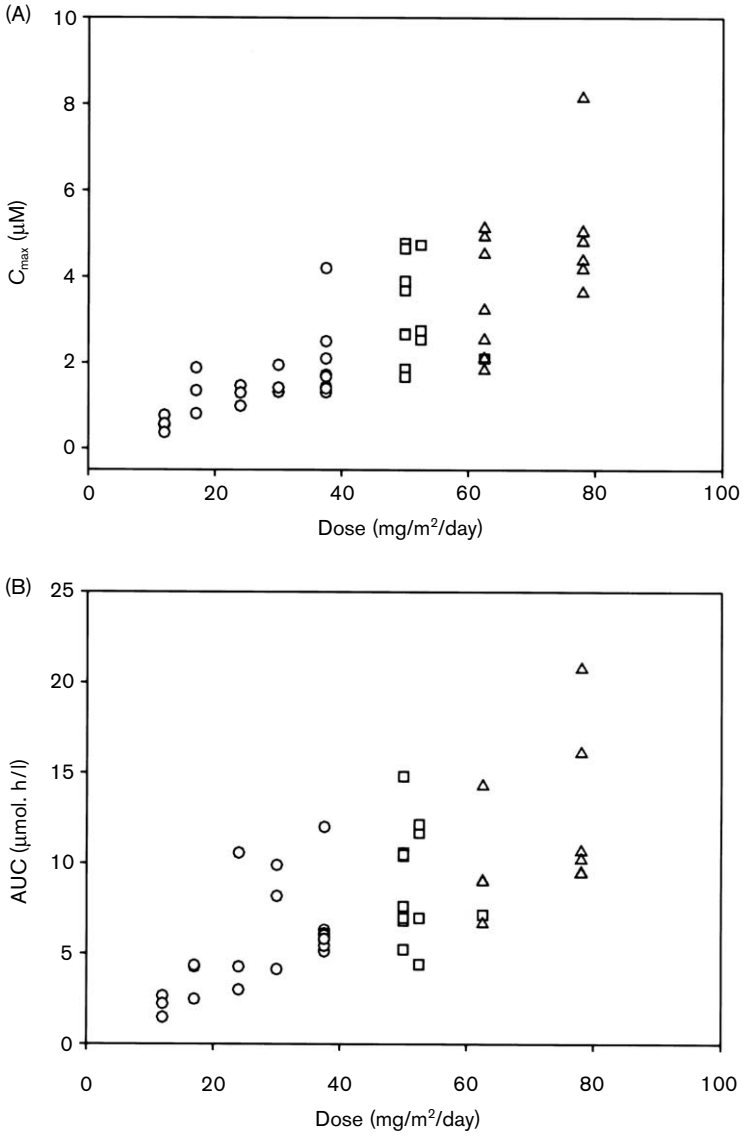
No. of patients	55
Male:female	36/19
Age [median (range) (years)]	56 (26–77)
Body surface area [median (range) (m^2)]	1.92 (1.33–2.4)
Tumor type	
colorectal	13
melanoma	10
renal	9
non-Hodgkin's lymphoma	5
mantle cell lymphoma	2
esophageal	2
breast	2
head and neck	2
Hodgkin's disease	2
others	8

Fig. 1



Flavopiridol plasma concentration–time profile. The data were from a representative patient who received flavopiridol at a dose of 37.5 mg/m^2/day for 5 consecutive days. A two-compartmental open model fits the data.

Fig. 2



(A) Flavopiridol plasma peak concentrations versus doses. Flavopiridol was administered as 1-h infusion. Peak concentrations were determined as the concentrations at the end of infusion. Data from 5- (circle), 3- (square) and 1-day (triangle) schedules. $N = 48$. (B) Flavopiridol plasma AUC versus doses. $N = 48$

Analyzer (Perkin-Elmer). The results were analyzed to see if the genotyping findings associate with clinical events, such as diarrhea [11] and the pharmacokinetic parameters.

Results

Clinical data

A total of 55 patients were treated with flavopiridol 1-h i.v. infusion on this phase I study. The clinical data for this trial of flavopiridol has been reported in detail elsewhere [29]. The MTD is 37.5 mg/m^2/day × 5 days, 50 mg/m^2/day × 3 days and 62.5 mg/m^2/day × 1 day. The dose-limiting toxicities (DLTs) were neutropenia, fatigue and diarrhea [29]. Table 1 shows the demographics of these patients and their sites of diseases.

Pharmacokinetics

Pharmacokinetic analysis was performed in 48 patients treated with flavopiridol at nine dose levels. A representative flavopiridol plasma concentration–time profile is presented in Figure 1. After the end of the 1-h infusion, flavopiridol plasma concentrations declined in a biexponential manner. The data fit a two-compartment model well in all patients with a mean coefficient of determination (r^2) of 0.98 ± 0.02. Flavopiridol peak concentration, C_{max}, which is the concentration at the end of the 1-h

Table 2 **Pharmacokinetic parameters of flavopiridol administered as a 1-h i.v. infusion to patients with refractory neoplasms**

Dose (mg/m^2/day)	*N*	C_{max} (μM)	AUC (μM h)	V_{ss} (l/m^2)	CL (l/h/m^2)	$t_{1/2(\alpha)}$(h)	$t_{1/2(\beta)}$(h)
12	4	0.56	2.22	75.5	13.7	0.39	4
		0.37–0.77	1.48–2.67	44–191.8	2.6–17.6	0.11–0.97	2.7–28.3
17	3	1.35	4.27	42.1	8	0.39	3.67
		0.81–1.88	2.49–4.34	28–91.5	6.2–16.1	0.16–0.51	2.95–9.01
24	3	1.29	4.27	122.5	10	0.81	10.31
		0.99–1.47	3.01–10.57	56.8–159.4	5.1–14.3	0.34–0.93	8.47–13.65
30	3	1.42	8.15	53.8	8.3	0.652	3.89
		1.32–1.95	4.13–9.89	46.7–81.5	6.3–16.5	0.2–0.86	2.6–11.1
37.5	9	1.68	5.80	58.2	15.4	0.55	3.50
		1.31–4.2	5.11–12	20.2–113.5	6.9–17.1	0.08–0.85	1.34–6.63
50	8	3.18	9	49.7	13.1	0.30	3.14
		1.67–4.77	5.23–14.79	19.5–207.2	8.4–20	0.08–1.54	1.4–19.5
52.5	4	2.71	9.3	39.3	15.5	0.31	2.85
		2.53–4.74	4.41–12.12	19.2–45.5	5.7–25.1	0.04–1.03	1.26–6.16
62.5	6	3.88	9	33.9	15.5	0.18	3.58
		1.83–5.13	6.64–15.17	23.2–162.8	8.7–21.5	0.06–0.89	2.26–7.47
78	6	4.6	10.4	49.8	16.7	0.33	3.34
		3.63–8.15	9.43–20.78	44–63.6	8.2–18.4	0.08–0.69	2.37–4.48
Median				52.3	15.1	0.39	3.46
Range				19.2–207.1	2.61–25.1	0.04–1.54	1.26–28.3
Mean				64.9	13.8	0.48	5.2
SD				43.4	4.9	0.37	4.9

infusion, was linear with the dose (Fig. 2A). Median C_{max} were 1.7 (1.3–4.2) ($n=9$), 3.2 (1.7–4.8) ($n=8$) and 3.9 (1.8–5.1) μM ($n=6$), respectively, at the recommended phase II doses of 37.5 mg/m^2/day × 5 days, 50 mg/m^2/day × 3 days and 62.5 mg/m^2/day × 1 day. The AUC of flavopiridol also appeared linear with all doses explored (Fig. 2B). The mean ± SD of the total clearance was 13.8 ± 4.9 l/h/m^2 and was independent of the dose. The terminal elimination half-life was 5.2 ± 4.9 h and the apparent volume of distribution at steady state was 64.9 ± 43.4 l/m^2. Pharmacokinetic parameters derived from the compartmental analysis are shown in Table 2.

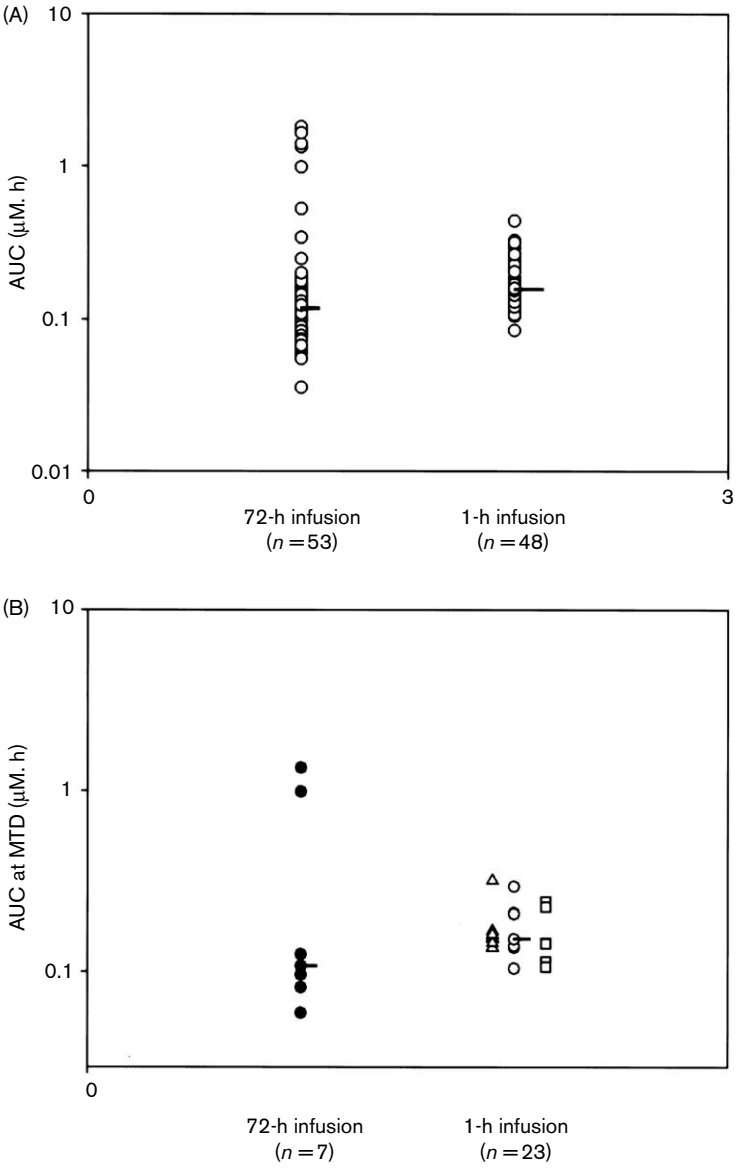
In a previous clinical trial, flavopiridol was administered as a 72-h continuous infusion [11]. The MTD was 50 mg/m^2/day and the DLT was secretory diarrhea. AUC results from this 72-h infusion study were compared to the present 1-h infusion study. The data from 53 patients with a 72-h infusion and 48 patients from 1-h i.v. infusion are shown in Figure 3(A). AUCs from MTDs were also shown in Figure 3(B). Since different doses were administered in these 2 trials, AUC values obtained were corrected for doses [i.e. AUC values were divided by dose (mg/m^2)]. Compared with the 72-h infusion, AUC from the 1-h infusion has less interpatient variability, but there is no statistical difference in AUC between the two schedules.

To study the consistence of flavopiridol pharmacokinetics during 5-day treatment, flavopiridol pharmacokinetics at day 1 and 5 was studied at a dose of 37.5 mg/m^2/day × 5 days in six patients. Although the mean C_{max} at day 5 appeared lower than that at day 1 (2.3 ± 1.1 versus 3.5 ± 1.7 μM, no statistically significant difference), there is no difference of flavopiridol clearance between day 1 and 5 (Fig. 4). Adjusted C_{max} and clearance obtained from day 5, 3 and 1 of the patients with different schedules were similar (Fig. 5), indicating that flavopiridol pharmacokinetic parameters such as C_{max} and clearance can be maintained during the treatment.

Pharmacogenetics

The genotypes were determined in 49 of the 55 patients (Table 3). Five (10%) homozygous (7/7) and 11 (22%) heterozygous (6/7) patients for *UGT1A1*28* were found. One patient was heterozygous for the sequence of five TA repeats, $(TA)_5TAA$. The remaining 32 patients did not have *UGT1A1*28* allele (homozygous for the reference sequence, 6/6). The allele frequency of *UGT1A1*28* was 0.214. Serum bilirubin levels for the 6/6, 6/7 and 7/7 patients were 0.40 ± 0.1, 0.6 ± 0.2 and 0.5 ± 0.1 (mg/dl, mean ± SD), respectively. There is a significant difference of bilirubin levels between 6/6 and 7/7 plus 6/7 group ($p<0.001$, Mann–Whitney rank-sum test). No apparent relationships between the genotype and flavopiridol pharmacokinetic parameters were observed (Table 3 and Fig. 6). For example, medians of the dose-adjusted AUC values (μM · h) are similar among the genotypes; 0.146 in 7/7 (potential low glucuronidating activity), 0.155 in 5/6 (potential high activity) and 0.152 in 6/6. Compared with the median of AUC values among the 6/6 patients, increased AUC was observed only in patient 5 with 7/7 (0.231 μM · h). The genotyping results were also compared with the occurrence and severity of diarrhea and neutropenia (Table 3 and Fig. 7). No obvious relationships between the genotype and the occurrence and severity of diarrhea and between genotype and neutropenia were observed for 1-, 3- (data not shown) and 5-day treatment.

Fig. 3



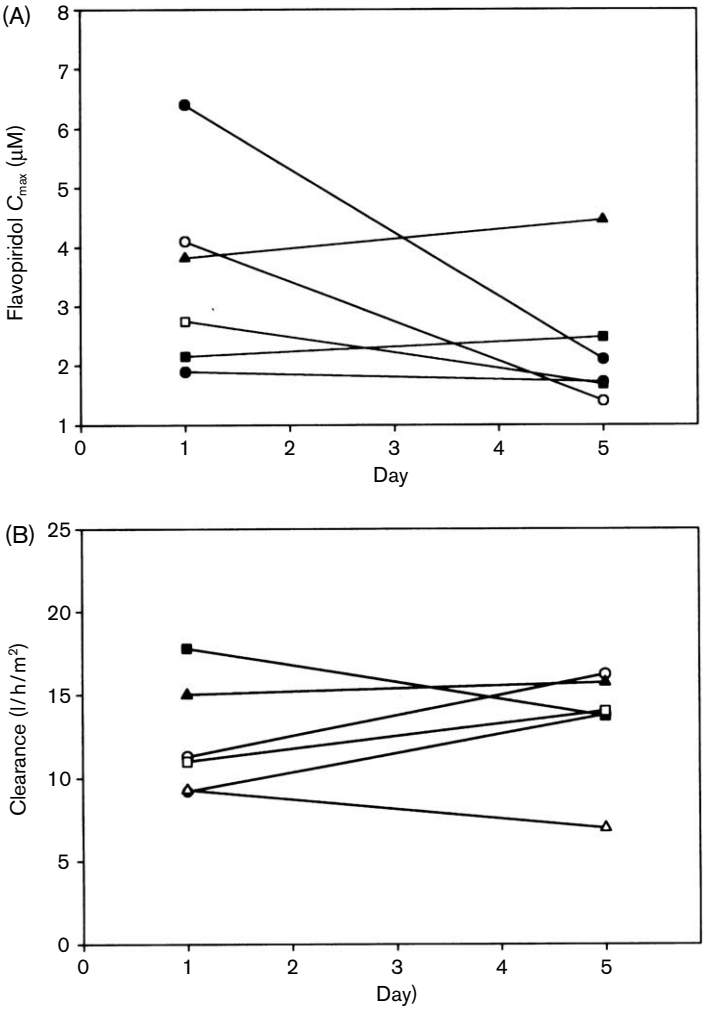
Flavopiridol AUC from 1-h infusion versus those from a 72-h infusion trial conducted at the NCI. Flavopiridol AUC values were adjusted based on dose administered [AUC were divided by dose (mg/m^2)]. (A) All AUC data obtained from two trials. (B) Only AUCs from MTDs were compared. For the 72-h trial, data obtained from 50 mg/m^2/day. For the 1-h trial, 37.5 (triangle, $n = 9$), 50 (circle, $n = 8$) and 62.5 mg/m^2/day (square, $n = 6$). The line is the median. Median of 1-h stands for all three MTDs.

Discussion

Flavopiridol is the first CDK inhibitor to enter clinical trials. Initial clinical trials have been intriguing but many questions remain. At this stage, it is still unclear what is the most effective regimen for the antitumor activity of this drug. This study is the first flavopiridol clinical trial using a 1-h schedule.

Rapid i.v. infusion dosing is necessary to produce sufficiently high concentrations. In more recent preclinical studies [7,15], better antitumor effects were observed using flavopiridol i.v./i.p. bolus. In these trials, flavopiridol peak concentrations of 4–9 μM were observed, followed by a bi-exponential decline to approximately 100 nM in 8 h. In this study, flavopiridol peak concentrations increased linearly with doses used. At the recommended phase II doses of 37.5 mg/m^2/day $\times$ 5 and 50 mg/m^2/day $\times$ 3, C_{max} values were between 1.9–4.2 and 1.7–4.8 μM, respectively. At a dose of 78 mg/m^2/day $\times$ 1, the highest dose in this trial, a peak concentration of 8.2 μM

Fig. 4



Flavopiridol C_{max} (A) and clearance (B) at day 1 versus those at day 5. Patients received flavopiridol at a dose of 37.5 mg/m^2/day for 5 consecutive days, and pharmacokinetic analysis was performed at day 1 and 5 ($n = 6$).

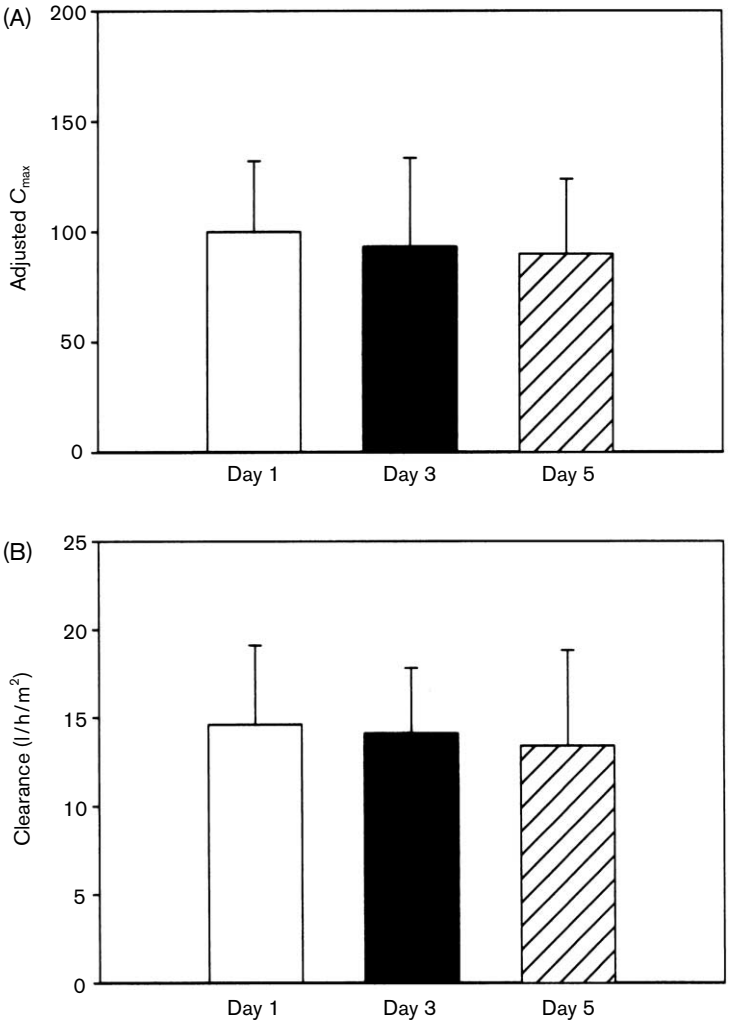
was observed, suggesting the high flavopiridol peak concentrations obtained in the preclinical animal models could be approached in human using a 1-h infusion.

Dose-proportional increase in peak plasma concentrations, AUC, and relative unchanged volume of distribution and clearance in this study support that flavopiridol follows linear pharmacokinetics at the doses used with the 1-h schedule. To assess antitumor activity and toxicity of flavopiridol, it is important to compare the AUCs between 1- and 72-h flavopiridol infusion. For example, the DLT for 72-h continuous infusion is secretary diarrhea, while the major DLT for this 1-h schedule is neutropenia as well as diarrhea. The results show that AUCs from1- and 72-h infusions are comparable, either at all dose levels or at MTD. Analysis of the clinical data suggested that neutropenia is related to flavopiridol peak concentrations [29]. The elimination half-life of flavopiridol from the 1-h infusion is shorter than the 72-h infusion (3.5 versus 11.6 h, median). It is possible that a different assay applied in the 72-h infusion study, which uses an electronic detector and has higher sensitivity, may explain or contribute to the difference in elimination half-life.

We also evaluated the consistence of flavopiridol pharmacokinetics over time. Our limited data showed that flavopiridol AUC and C_{max} appeared to be maintained during 5-day treatment. The C_{max} difference between day 1 and 5 could be due to intraindividual variation or sampling time errors.

Two glucuronidated metabolites of flavopiridol have been identified in humans: 5-*O*-*β*-glucopyranuronosyl-flavopir-

Fig. 5



C_{max} (A) and clearance (B) from day 1, 3 and 5 of flavopiridol administration at all dose levels. C_{max} values were adjusted based on the doses. C_{max} value at day 1 was set as 100%, data at day 3 and 5 were compared with that at day 1.

idol and 7-*O*-*β*-glucopyranuronosyl-flavopiridol [18]. Based on the *in vitro* studies using human liver microsomes, these metabolites are produced by UGT1 isozymes, the 5-*O*-*β*-glucuronide mainly by UGT1A1, and the 7-*O*-*β*-glucoronide by UGT1A9, UGT1A1 and UGT1A10, although several other UGT isozymes might be also involved [14,18,30]. Polymorphism in the promoter of the *UGT1A1* gene has been extensively evaluated in Gilbert's syndrome, as well as in relationships with pharmacokinetics/pharmacodynamics of substrate drugs of UGT1A1. For example, the genotype analysis reveals that aberrant *UGT1A1* promoter alleles alter the pharmacokinetic profile of SN-38, an active metabolite of irinotecan and a significant risk factor for severe toxicity of this drug [23,34]. Obviously, the knowledge of genetic polymorphisms in drug metabolizing enzymes of anticancer agents is particularly important in clinical oncology because of their cytotoxic effects and high doses used. Such pharmacogenetic and pharmacokinetic studies would identify patients with potential higher risk for severe toxicity and warrant pharmacogenetic-guided dosing of certain anticancer drugs.

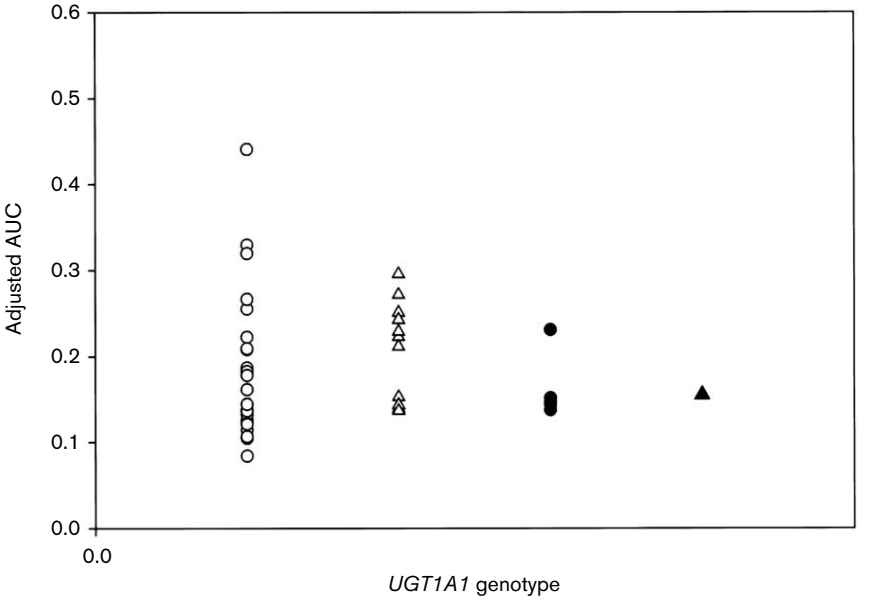
As an inverse relationship between the number of TA repeats and the activity using a luciferase reporter gene has been reported, the order of UGT1A1 enzyme activity was theoretically expected as 5/6, 6/6, 6/7 and 7/7 (from high to low) [21]. Although the elevated bilirubin concentrations observed in the patients with genotype 7/7 and 6/7 were consistent with low activity of UGT1A1 enzyme, the current study did not find the relationship between the *UGT1A1* genotype and flavopiridol pharma-

Table 3 **Patient's genotypes and their flavopiridol pharmacokinetics and pharmacodynamics**

Patient	Dose (mg/m^2)	Genotype		Bilirubin (mg/ml)	C_{max} (μM)[a]	AUC ($\mu M \cdot h$)[a]	$T_{1/2(\beta)}$ (h)	CL ($l/h/m^2$)	Diarrhea (grade)	Neutropenia (grade)
1	17	7/7		0.40	0.079	0.146	2.95	16.1	0	0
2	30	7/7		0.60	0.047	0.138	2.60	16.5	0	0
3	37.5	7/7		0.60	0.046	0.145	2.36	15.4	I	0
4	50	7/7		0.50	0.095	0.152	1.40	14.4	III	II
5	52.5	7/7		0.40	0.052	0.231	6.16	5.7	I	II
			mean	0.50	0.064	0.162	3.09	13.6		
			SD	0.10	0.022	0.039	1.81	4.5		
			median	0.50	0.052	0.146	2.60	15.4		
			range	0.4–0.6	0.046–0.095	0.138–0.231	1.4–6.16	5.7–16.5		
6	37.5	5/6		0.40	0.056	0.155	5.90	17.8	I	I
7–17		6/7	mean	0.59	0.057	0.209	6.3	11.0		
			SD	0.16	0.016	0.057	7.5	4.5		
			median	0.60	0.053	0.223	3.6	9.4		
			range	0.4–1.0	0.037–0.079	0.137–0.296	2.26–28.3	2.6–17.5		
18–48		6/6	mean	0.40	0.058	0.181	5.38	14.8		
			SD	0.10	0.025	0.083	4.25	5.1		
			median	0.40	0.051	0.152	3.54	15.2		
			range	0.2–0.6	0.029–0.112	0.084–0.440	1.26–19.5	5.1–25.1		

Normal range of bilirubin level is below 1 mg/ml.
[a]Dose-adjusted [C_{max} or AUC value were divided by dose (mg/m^2)].
Comparing the results between 6/6 and 7/7 plus 6/7 group, p value (unpaired t-test) is 0.0001 for bilirubin level, no statistical significance for flavopiridol pharmacokinetics.

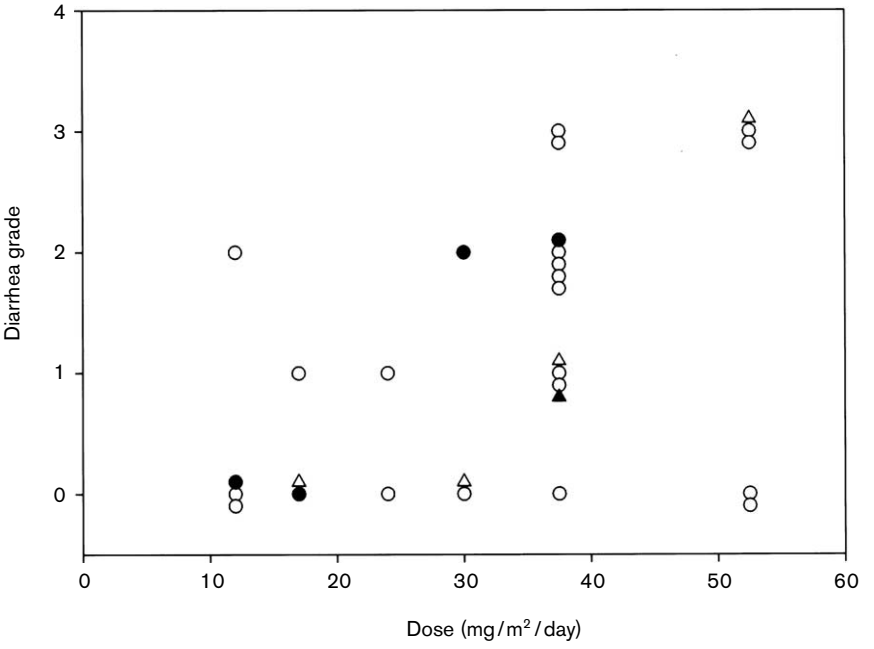
Fig. 6



UGT1A1 genotype versus the adjusted AUC. The genotype of patients was compared with their AUC values (dose-corrected). Homozygous for *UGT1A1*1* (6/6, open circle, $n = 32$), heterozygous for *UGT1A1*28* (6/7, triangle, $n = 11$), homozygous for *UGT1A1*28* (7/7, filled circle, $n = 5$) and heterozygous for $(TA)_5TAA$ (5/6, filled triangle, $n = 1$).

cokinetics. The reasons could be due to the fact that flavopiridol is metabolized by multiple isozymes of UGT1 isozymes, which might harbor the role of UGT1A1. Although genetic polymorphism of UGT1A9 gene was not identified in the previous research, we need to explore potential factors including other genetic variants that could regulate *in vivo* activity of UGT1A9 [18,31]. In addition, other enzymes such as the UGT2B family and UGT1A10 may also play some role in flavopiridol metabolism [14,18]. Secondly, despite the potentially significant role of UGT1A1 isozyme for the *in vivo* metabolism of flavopiridol, the effect of the promoter polymorphism of *UGT1A1* gene would be too weak to affect the pharmacokinetics and pharmacodynamics of the drug [20,32,33]. The promoter polymorphism analyzed in this study is originally related to Gilbert's

Fig. 7



The relationship between *UGT1A1* genotype and diarrhea. The genotypes of patients were compared with the occurrence and severity of diarrhea for 5-day flavopiridol treatment at doses from 12 to 37.5 mg/m^2/day. Homozygous for *UGT1A1*1* (6/6, open circle), heterozygous for *UGT1A1*28* (6/7, open triangle), homozygous for *UGT1A1*28* (7/7, filled circle) and heterozygous for $(TA)_5TAA$ (5/6, filled triangle).

syndrome, which clinically shows mild hyperbilirubinemia. Other variant *UGT1A1* alleles, which exist in the coding regions of the gene and remarkably reduce the enzyme activity, remain to be studied. Furthermore, genetic effect on UGT1A1 activity may be harbored by non-genetic factors such as hepatic disease, concomitant drugs, food and environmental exposure to other exogenous compounds (e.g. smoking). Indeed, the variant genotypes found in Gilbert's syndrome like *UGT1A1**28 are also noted in seemingly healthy individuals and do not always cause hyperbilirubinemia [33]. Based on the results of the current study, we suggest that the variations in UGT1A1 activity due to the promoter genotypes would be too small to explain such a wide variation of the pharmacokinetics and the pharmacodynamics of flavopiridol.

It has been suggested that flavopiridol-induced diarrhea is attributed to the lumenal exposure to flavopiridol, therefore flavopiridol glucuronidation may decrease lumenal exposure of flavopiridol. In a phase II trial administered as a 72-h continuous i.v. infusion, flavopiridol and its glucuronidation metabolite concentrations in plasma were measured in 22 cancer patients [14]. A bimodal distribution of metabolic ratios of flavopiridol glucuronide was found indicating that there might be a polymorphism in glucuronidation of flavopiridol. In addition, the relationship between the occurrence of diarrhea and the systemic glucuronidation of flavopiridol was evaluated. Eight of 11 extensive glucuronidators (flavopiridol glucuronide/flavopiridol ratio >1.2) did not develop diarrhea, whereas 10 of 11 poor glucuronidators (metabolic ratio <1.2) developed diarrhea ($p=0.008$). The risk of diarrhea induced by flavopiridol appeared to be inversely associated with flavopiridol glucuronidation. No information was available about relationships between these extensive/poor glucuronidators and their *UGT1A1* genotypes. In the current study, we evaluated whether there is a relationship between the patients' *UGT1A1* genotype and the occurrence and severity of diarrhea and the results did not support such relationship (Table 3 and Fig. 7).

In conclusion, the pharmacokinetic parameters of flavopiridol were determined in the 1-h i.v. schedule. The pharmacogenetic analyses did not support that the *UGT1A1* promoter polymorphism could affect flavopiridol pharmacokinetics, and alter the incidence and severity of diarrhea induced by the drug.

Acknowledgments

We would like to thank Dr Douglas K. Price and Dr Jurgen Venitz for helpful advice, and Dr Juliana Chan, Ms Siiri Forbes and Erin Lepper for technical assistance.

References

1 Sausville EA, Zaharevitz D, Gussio R, Meijer L, Louarn-Leost M, Kunick C, *et al.* Cyclin-dependent kinases: initial approaches to exploit a novel therapeutic target. *Pharmacol Ther* 1999; **82**:285–292.

2 Senderowicz AM, Sausville EA. Preclinical and clinical development of cyclin-dependent kinase modulators. *J Natl Cancer Inst* 2002; **92**:376–387.

3 Carlson BA, Dubay MM, Sausville EA, Brizuela L, Worland PJ. Flavopiridol induces G_1 arrest with inhibition of cyclin-dependent kinase (CDK) 2 and CDK4 in human breast carcinoma cells. *Cancer Res* 1996; **56**:2973–2978.

4 Carlson B, Lahusen T, Singh S, Loaiza-Perez A, Worland PJ, Pestell R, *et al.* Downregulation of cyclin D1 by transcriptional repression in MCF-7 human breast carcinoma cells induced by flavopiridol. *Cancer Res* 1999; **59**:4634–4641.

5 Losiewicz MD, Carlson BA, Kaur G, Sausville EA, Worland PJ. Potent inhibition of CDK2 kinase activity by the flavonoid L86-8275. *Biochem Biophys Res Commun* 1994; **201**:589–595.

6 Bible KC, Bible Jr RH, Kottke TJ, Svingen PA, Xu K, Pang YP, *et al.* Flavopiridol binds to duplex DNA. *Cancer Res* 2000; **60**:2419–2428.

7 Patel V, Senderowicz AM, Pinto D. Flavopiridol, a novel cyclin-dependent kinase inhibitor, suppresses the growth of head and neck squamous cell carcinomas by inducing apoptosis. *J Clin Invest* 1998; **102**:1674–1681.

8 Melillo G, Sausville EA, Cloud K, Lahusen T, Varresio L, Senderowizc A. Flavopiridol, a potent kinase inhibitor, down-regulates hypoxic induction of vascular endothelial growth factor expression in human monocytes. *Cancer Res* 1999; **59**:5433–5437.

9 Brusselbach S, Nettelbeck DM, Sedlacek HH, Muller R. Cell cycle-dependent induction of apoptosis by the antitumor drug flavopiridol in endothelial cells. *Int J Cancer* 1998; **77**:146–152.

10 Kaur G, Stetler-Stevenson M, Sebers S, Worland P, Sedlacek H, Myers C, *et al.* Growth inhibition with reversible cell cycle arrest of carcinoma cells by flavone L86–8275. *J Natl Cancer Inst* 1992; **84**:1736–1740.

11 Senderowicz AM, Headlee D, Stinson SF, Lush RM, Kalil N, Villalba L, *et al.* Phase I trial of continuous infusion flavopiridol, a novel cyclin-dependent kinase inhibitor, in patients with refractory neoplasmas. *J Clin Oncol* 1998; **16**:2986–2999.

12 Thomas J, Cleary J, Tutsch K, Arzoomanian R, Alberti D, Simon K. Phase I clinical and pharmacokinetic trial of the cyclin-dependent kinase (CDK) inhibitor flavopiridol. *Proc Am Soc Clin Oncol* 1998; **17**:209 (abstr).

13 Schwartz GK, Werner JL, Maslak P, Saltz L, O'Reilly E, Kelsen DP. Flavopiridol enhances the biological effects of paclitaxel: a phase I trial in patients with advanced solid tumors. *Proc Am Soc Clin Oncol* 1998; **17**:188 (abstr).

14 Innocenti F, Stadler WM, Iyer L, Ramirez J, Vokes EE, Ratain MJ. Flavopiridol metabolism in cancer patients is associated with occurrence of diarrhea. *Clin Cancer Res* 2000; **6**:3400–3405.

15 Arguello F, Alexander M, Sterry JA, Tudor G, Smith EM, Kalavar NT, *et al.* Flavopiridol induces apoptosis of normal lymphoid cells, causes immunosuppression, and has potent antitumor activity *in vivo* against human leukemia and lymphoma xenografts. *Blood* 1998; **91**:2482–2490.

16 Jager W, Zembsch B, Wolschann P, Pittenauer E, Senderowicz AM, Sausville EA, *et al.* Metabolism of the anticancer drug flavopiridol, a new inhibitor of cyclin dependent kinases, in rat liver. *Life Sci* 1998; **62**:1861–1873.

17 Senafi SB, Clarke DJ, Burchell B. Investigation of the substrate specificity of a cloned expressed human bilirubin UDP-glucuronosyltransferase: UDP-sugar specificity and involvement in steroid and xenobiotic glucuronidation. *Biochem J* 1994; **303**:233–240.

18 Hagenauer B, Salamon A, Thalhammer T, Kunert O, Haslinger E, Klingler P, *et al. In vitro* glucuronidation of the cyclin-dependent kinase inhibitor flavopiridol by rat and human liver microsomes: involvement of UDP-glucuronosyltransferases 1A1 and 1A9. *Drug Metab Disp* 2001; **29**:407–414.

19 Isselbacher KJ. Bilirubin metabolism and hyperbilirubinemia. In: Fauci AS, Braunwald E, Isselbacher KJ, Wilson JD, Martin JB, Kasper DL, et al. (editors): *Harrison's Principles of Internal Medicine*, 14th edn. New York: McGraw-Hill; 1997, p. 1672.

20 Monaghan G, Ryan M, Seddon R, Hume R, Burchell B. Genetic variation in bilirubin UDP-glucuronosyltransferase gene promoter and Gilbert's syndrome. *Lancet* 1996; **347**:578–581.

21 Beutler E, Gelbart T, Demina A. Racial variability in the UDP-glucuronosyltransferase 1 (*UGT1A1*) promoter: a balanced polymorphism for regulation of bilirubin metabolism?. *Proc Natl Acad Sci USA* 1998; **95**:8170–8174.

22 Gagné JF, Montminy V, Belanger P, Journault K, Gaucher G, Guillemette C. Common human *UGT1A* polymorphisms and the altered metabolism of irinotecan active metabolite 7-ethyl-10-hydroxycamptothecin (SN-38). *Mol Pharmacol* 2002; **62**:608–617.

23 Ando Y, Ueoka H, Sugiyama T, Ichiki M, Shimokata K, Hasegawa Y. Polymorphism of UGT-glucuronosyltransferase and pharmacokinetics of Irinotecan. *Ther Drug Monit* 2002; **24**:111–116.

24 Ando Y, Saka H, Asai G, Sugiura S, Shimokata K, Kamataki T. UGT1A1 genotypes and glucuronidation of SN-38, the active metabolite of irinotecan. *Ann Oncol* 1998; **9**:845–847.

25 Zhai S, Sausville ED, Figg WD. A high-performance liquid chromatography method using ultraviolet detection for the quantitation of flavopiridol from human plasma. *Biomed Chromatogr* 2002; **16**:379–382.

26 D'Argenio DZ, Schumitzky A. A program package for simulation and parameter estimation in pharmacokinetic system. *Comput Prog Biomed* 1979; **9**:115–134.

27 Dixon SC, Horti J, Guo Y, Reed E, Figg WD. Methods for extracting and amplifying genomic DNA isolated from frozen serum. *Nat Biotechnol* 1998; **16**:91–94.

28 Monaghan G, Ryan M, Seddon R, Hume R, Burchell B. Genetic variation in bilirubin UPD-glucuronosyltransferase gene promoter and Gilbert's syndrome. *Lancet* 1996; **347**:578–581.

29 Tan A, Headlee D, Messmann R, Sausville EA, Arbuck SG, Murgo AJ, *et al.* Phase I clinical and pharmacokinetic study of flavopiridol administered as a daily 1-h infusion in patients with advanced neoplasms. *J Clin Oncol* 2002; **20**:4074–4082.

30 Ramirez J, Iyer L, Lush RM, Mortell M, Feuer JA, Figg WD, *et al. In vitro* glucuronidation of flavopiridol (NSC649890) by human liver microsomes. *Clin Pharmacol Ther* 1998; **63**:149 (abstr).

31 Vogel A, Kneip S, Barut A, Ehmer U, Tukey RH, Manns MP, *et al.* Genetic link of hepatocellular carcinoma with polymorphisms of the UDP-glucuronosyltransferase UGT1A7 gene. *Gastroenterology* 2001; **121**:1136–1144.

32 Iyer L, Hall D, Das S, Mortell MA, Ramirez J, Kim S, *et al.* Phenotype–genotype correlation of *in vitro* SN-38 (active metabolite of irinotecan) and bilirubin glucuronidation in human liver tissue with UGT1A1 promoter polymorphism. *Clin Pharmacol Ther* 1999; **65**:576–582.

33 Bosma P, Chowdhury RJ, Bakker C, Gantla S, de Boer A, Oostra BA, *et al.* The genetic basis of the reduced expression of bilirubin UGD-glucuronyltransferase 1 in Gilbert's syndrome. *N Engl J Med* 1995; **333**:1171–1175.