Flavopiridol Metabolism in Cancer Patients Is Associated with the Occurrence of Diarrhea

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Abstract

Flavopiridol, a cyclin-dependent kinase inhibitor currently undergoing clinical evaluation, has a dose-limiting toxicity of diarrhea. Preclinical data on flavopiridol metabolism indicate that flavopiridol undergoes hepatic glucuronidation. The purpose of this study is to evaluate whether the occurrence of diarrhea is related to the systemic glucuronidation of flavopiridol. Parent drug and metabolite concentrations in plasma were measured by high-pressure liquid chromatography in 22 metastatic renal cancer patients treated on a Phase II trial of 50 mg/m²/day of flavopiridol administered every 2 weeks as a 72-h continuous infusion. Pharmacokinetics of flavopiridol and its glucuronide were assessed during the first cycle at 23, 47, and 71 h during the infusion. Flavopiridol concentrations at 23, 47, and 71 h were 389 nM (296–567 nM), 412 nM (297–566 nM), and 397 nM (303–597 nM) [median (interquartile range), respectively. Flavopiridol glucuronide reached a plateau of 358 nM (196–553 nM) at 47 h. Metabolic ratios of flavopiridol glucuronide:flavopiridol at 71 h showed an apparent bimodal distribution with an antimode of 1.2. Thirteen patients experienced diarrhea and had lower metabolic ratios [0.72 (0.53–0.86)] than patients without diarrhea [2.24 (1.76–2.3); P = 0.002]. Eight of 11 extensive glucuronidators (ratio > 1.2) did not develop diarrhea, whereas 10 of 11 poor glucuronidators (ratio < 1.2) developed diarrhea (P = 0.008). The glucuronidation of flavopiridol is apparently polymorphic, suggesting a genetic etiology. The systemic glucuronidation of flavopiridol is inversely associated with the risk of developing diarrhea.

Introduction

The National Cancer Institute drug screening selected flavopiridol (L86 8275; NSC 649890) as a potential candidate for clinical development (for review, see Ref. 1). Flavopiridol exhibited a unique pattern of differential growth-inhibitory activity at concentrations below 200 nM, as well as cytostatic properties in xenografted human tumors (2–4). Investigations into its mechanism of action revealed that flavopiridol is an inhibitor of several cyclin-dependent kinases (5–8). These mediators of cell cycle progression are often deregulated in transformed cells (9) and have been suggested to be excellent targets for antineoplastic drug development (10). Flavopiridol can be cytostatic or cytotoxic to neoplastic cells, depending on the concentration and the duration of exposure (4, 11–15). The preclinical studies suggested that prolonged exposure was important for maximizing flavopiridol activity (16), and thus continuous infusion schedules have been most extensively explored in human trials (17, 18).

The metabolism of flavopiridol in humans has not been characterized in detail. An in vivo animal study indicated that the glucuronidation of flavopiridol is the major mechanism of flavopiridol transformation (19). We demonstrated the ability of human liver microsomes to convert flavopiridol to its corresponding glucuronide (20), providing evidence for existence of this metabolic pathway in humans as well. Pharmacokinetic studies during Phase I trials focused only on the disposition of the parent drug and showed that plasma concentrations necessary to inhibit in vitro cyclin-dependent kinase activity and cell proliferation can be easily achieved (17, 18). The dose-limiting toxicity for flavopiridol administered as a 72-h continuous infusion is secretory diarrhea (17, 18). The occurrence of intestinal toxicity may limit the utility of flavopiridol, especially because in vitro induction of apoptosis in xenograft models seems to require concentrations above those achievable with 72-h continuous infusions of flavopiridol at the maximum tolerated dose (4). Interestingly, the maximum tolerated dose of flavopiridol can be increased from 50 to 78 mg/m²/day when patients are treated with a prophylactic anti-diarrheal treatment with cholestyramine and loperamide (18). The amelioration of diarrhea by the use of cholestyramine is consistent with the finding that cholestyramine binds flavopiridol, thus preventing its toxic action on the gut mucosa (18). Moreover, preliminary results in vitro showed that flavopiridol is a modulator of the intestinal epithelial chloride secretion (21).

Because results to date suggest that flavopiridol-induced diarrhea is attributable to the lumenal exposure to flavopiridol and glucuronidation may decrease lumenal exposure, this study aimed to explore the glucuronidation of flavopiridol in cancer patients and its relationship to the dose-limiting toxicity of diarrhea.
Materials and Methods

Patients and Treatment. The clinical results of this study have been reported separately (22). All patients had metastatic renal cancer. Laboratory criteria for adequate liver and kidney function were total bilirubin ≤ 1.5 mg/dL, transaminases ≤ 2.5× the upper limit of normal, and creatinine ≤ 2 mg/dL (or estimated creatinine clearance ≥ 60 mL/min). Written informed consent was obtained from each patient in accordance with institutional and federal guidelines.

The drug was supplied by the National Cancer Institute and administered every 2 weeks as a 72-h continuous infusion at the dose of 50 mg/m²/day, the recommended Phase II dose without diarrhea prophylaxis. Flavopiridol was reconstituted in benzyl alcohol-preserved saline at a final concentration of 0.25–2.25 mg/mL in polyvinyl chloride bags and administered via an ambulatory pump. Toxicity assessment was done according to the Cancer and Leukemia Group B expanded toxicity criteria. For diarrhea episodes of grade 3 or greater, patients were given loperamide or diphenoxylate hydrochloride plus atropine sulfate (Lomotil). If diarrhea continued, treatment with octreotide acetate was begun. Any grade 3 and 4 toxicity led to a 25% and 50% dose reduction in subsequent cycles, respectively.

Assay Method and Pharmacokinetics. Heparinized blood samples were drawn at 23, 47, and 71 h during the infusion. Plasma was separated immediately by centrifugation and stored at −70°C until analysis. Chemicals were obtained from Fisher Scientific (Itasca, IL) and Sigma Chemical Co. (St. Louis, MO). Quantitation of flavopiridol and its glucuronide in plasma was performed by HPLC. One mL of plasma was combined with 20 μL of internal standard (flavone, 33.8 μM) and 5 mL of acetonitrile. After shaking at low speed for 10 min, the samples were centrifuged at 2500 rpm for 15 min (4°C). The supernatant was evaporated to dryness using nitrogen gas (37°C). After adding 500 μL of 0.1 M sodium phosphate buffer (pH 6.4) and 5 mL of acetonitrile, the samples were shaken (10 min) and centrifuged (15 min, 2500 rpm, 4°C). The samples were then dried down and reconstituted in 300 μL of mobile phase, and aliquots of 150 μL were injected into the HPLC system. Because flavopiridol glucuronide for calibration standards was not available in our laboratory, the estimation of flavopiridol systemic metabolism with the development of diarrhea was determined using a reversed-phase μBondapak Phenyl guardpak (Waters Corp.). The mobile phase consisted of 35% acetonitrile and 65% 50 mM ammonium acetate containing 1.46 g/L triethyamine (pH was adjusted to 4.15 with 17.4 mM acetic acid). At a flow rate of 1 mL/min, the retention times of flavopiridol and flavone were 9 and 21 min, respectively. Standard curves for flavopiridol in plasma were linear within the range of 37.3–3953.5 nM. Human plasma for the preparation of calibration standards was purchased from the blood bank at the University of Chicago Hospitals. The interday and intraday variability in the accuracy of plasma concentrations investigated ranged from 1.0–7.9% and 1.6–7.6%, respectively. The interday and intraday variability in the precision (expressed as the coefficient of variation) was between 2.6% and 8.4% and 0.7% and 8.4%, respectively. The extraction recovery at three different concentrations within the linear range of the calibration curve was 99.0 ± 1.5% (mean ± SD).

Blood samples for pharmacokinetics were collected during the first cycle of treatment (at 23, 47, and 71 h), and plasma was immediately separated by centrifugation. Metabolic ratio refers to the ratio of the concentrations of flavopiridol glucuronide to flavopiridol. Metabolic ratios were calculated at 23, 47, and 71 h during the infusion. Total clearance of flavopiridol at 23, 47, and 71 h was calculated by dividing the dosing rate by the drug concentration at each time point. The median clearance value of each patient was used to calculate the median clearance of flavopiridol among patients.

Statistical Analysis. Data are expressed as median values and interquartile range (the range of values between the 25th and 75th percentiles of the distribution). Significance has been tested by two-sided statistical tests. The Wilcoxon signed-rank test was used to compare the concentrations of flavopiridol and flavopiridol glucuronide and the metabolic ratios at 23, 47, and 71 h during the infusion. For this analysis, the adjusted P < 0.017 (0.05/3, Bonferroni correction) was considered significant. Otherwise, a P of <0.05 was considered the cutoff of significance. Plasma concentrations of flavopiridol and flavopiridol glucuronide and metabolic ratios were compared in patients with and without diarrhea by means of a Mann-Whitney test. Moreover, Fisher’s exact test was used to assess the association of flavopiridol systemic metabolism with the development of diarrhea. The distribution of the metabolic ratios at 71 h was investigated by constructing a frequency distribution histogram.

Results

Patient Characteristics and Demographics. Twenty-two patients (10 men and 12 women) with metastatic or unresectable renal carcinoma were evaluated for pharmacokinetics. The median age of patients was 58 years (56–63 years). Two of 22 patients were minorities (one Hispanic patient and one black patient). Thirteen patients underwent prior nephrectomy. All but one patient had a Cancer and Leukemia Group B performance status of 0–1, and all patients met the laboratory criteria for adequate liver and kidney function.

Pharmacokinetics and Systemic Metabolism of Flavopiridol. A steady-state level was apparently attained after 23 h of infusion, with flavopiridol median concentrations of 389 nM (296–567 nM), 412 nM (297–566 nM), and 397 nM (303–597 nM) at 23, 47, and 71 h, respectively (Fig. 1A). No significant
difference was observed between the three collection times. Median total clearance of flavopiridol was 12.6 L/h/m² (9.7–17.0 L/h/m²).

The pharmacokinetics of flavopiridol glucuronide show that the metabolite accumulated from 23–47 h during the infusion, reaching a plateau from 47–71 h. Plasma levels increased significantly from 194 nM (118–321 nM) at 23 h to 358 nM (196–533 nM) at 47 h (P < 0.0001). At 71 h, the median flavopiridol glucuronide was 308 nM (218–990 nM). This value was lower than the median concentration at 47 h (P = 0.04; Fig. 1B). No clear correlation was observed between flavopiridol and flavopiridol glucuronide concentrations (data not shown).

Metabolic ratio levels during the infusion reflected the increasing concentrations of flavopiridol glucuronide from 23–47 h and the constant levels of the parent drug during the infusion. A median value of 0.49 (0.27–0.9) at 23 h was significantly lower than values of 0.96 (0.44–1.87; P = 0.0002) and 1.18 (0.65–1.96; P = 0.0003) at 47 and 71 h, respectively (Fig. 1C). Metabolic ratios at 47 and 71 h were not significantly different (P = 0.35).

Maximal interpatient variability for flavopiridol was observed at 23 h (coefficient of variation, 81%). Flavopiridol glucuronide levels were more variable, with coefficients of variation of 83%, 102%, and 107% at 23, 47, and 71 h, respectively. The interpatient variability in metabolic ratios was 93%, 99%, and 72% at the corresponding sampling times.

The frequency distribution histogram of the metabolic ratios at 71 h seemed to be bimodal, suggesting the presence of two different groups of patients (Fig. 2). By adopting an antimode value of 1.2, they were tentatively categorized as “poor” and “extensive” glucuronidators of flavopiridol. Poor and extensive glucuronidators had metabolic ratios lower and higher than 1.2, respectively.

**Association between Flavopiridol Metabolism and Diarrhea.** Thirteen of 22 patients experienced grade 1–3 diarrhea during the first cycle. Five patients had grade 1 diarrhea, six patients grade 2 diarrhea, and two patients had grade 3 diarrhea. The typical onset of this toxicity was during the last day of the infusion, becoming less intense in the following 2–3 days. Grade 2 diarrhea was controlled by loperamide. To treat grade 3 diarrhea in two patients, octreotide was administered, and flavopiridol dosage was reduced by 20% in subsequent cycles.

Patients who developed diarrhea had lower metabolic ratios at 71 h than patients without intestinal toxicity, with median metabolic ratios of 0.72 (0.53–0.86) and 2.24 (1.76–2.3), respectively (P = 0.002). A less significant correlation was observed between metabolic ratios at 47 h (P = 0.006). Diarrhea was not correlated with other pharmacokinetic parameters, such as flavopiridol and flavopiridol glucuronide levels during the infusion and metabolic ratios at 23 h. The presence or absence
flavopiridol glucuronide and enable flavopiridol to be absorbed and enter an enterohepatic cycle. Because considerable urinary recoveries of flavonoids were found after a soy meal in humans (33), the role of renal elimination of flavopiridol and its metabolite should be evaluated.

The superfamily of human UGT enzymes is divided into UGT1A and UGT2B families, each of which has individual isoforms (34). Preliminary results suggested that although flavopiridol is a substrate of the UGT1A1 isoform, an enzyme isoform belonging to the UGT2B family seems to play a major role in this reaction. We found intact glucuronidation activity in microsomes from UGT1A1-deficient species and a moderate correlation between the glucuronidation rates of flavopiridol and UGT1A1 substrates (20). In vitro studies are ongoing in our laboratory to identify the human UGT2B isoform responsible for the glucuronidation of flavopiridol. This could be of particular value because combination trials with flavopiridol have been planned, and drug interactions might arise from the induction/inhibition of specific UGTs when flavopiridol is administered with agents undergoing glucuronidation.

In theory, the closest measure of drug-metabolizing enzyme activity in vivo is the intrinsic clearance down that route. Practical reasons dictate the use of indirect indexes of in vivo enzyme activity, such as metabolic ratios between the metabolite and the parent drug (35). In this study, plasma metabolic ratios of flavopiridol glucuronide:flavopiridol are both surrogate markers of individual glucuronidating activity and a measure of the metabolic clearance of flavopiridol.

The distribution of the glucuronidating phenotypes among patients is apparently bimodal, indicating the presence of a genetic polymorphism of flavopiridol glucuronidation. Drug-metabolizing enzyme genes are clearly among the modifying factors that affect enhanced risk of drug toxicity (36). Anticancer agents are detoxified by metabolizing enzymes that usually show genetic variations (37). The knowledge of a pharmacokinetic polymorphism is particularly important in oncology because cytotoxic agents have a narrow therapeutic index and are administered at the maximum tolerated doses (38). Such genetic polymorphisms have played a crucial role in drug-related toxicity in cancer chemotherapy, leading to either life-threatening toxicities or unpredictable variability in response and toxicity among patients (39–41). UGT2B variants might account for the apparent polymorphic metabolism of flavopiridol. Mutated UGT2B4, UGT2B7, and UGT2B15 enzymes have been described recently (42–44), but the functional role of these enzymes in vivo is still unknown.

The importance of a possible polymorphic metabolism of flavopiridol stems from the clinical relevance of flavopiridol glucuronidation in patients. The glucuronidating phenotype is inversely reflective of the extent of biliary excretion of flavopiridol and hence of the risk of developing diarrhea. Diarrhea was present in 91% of poor glucuronidators of flavopiridol, whereas 73% of extensive glucuronidators did not develop diarrhea (Fig. 3).

**Discussion**

This is the first report of in vivo flavopiridol metabolism in humans demonstrating the presence of a major glucuronidated metabolite. Glucuronidation is generally considered an inactivating metabolic pathway and provides an important means of elimination for many clinically used drugs (23). Conjugation with glucuronic acid by UGT usually renders the parent drug less active and more easily excreted from the body (24). For extensively glucuronidated drugs, altered glucuronidation activity in the liver is likely to have important consequences for clinical use (23).

Two different flavopiridol glucuronides containing only one glucuronic acid in position 5 or 7 of the flavonoid core were found in rat bile (19). The number and the chemical structure of flavopiridol glucuronides produced in humans are still unknown. If more than one flavopiridol glucuronide are present in rat bile, the concentration of each metabolite could not be measured by means of our analytical method. The concentrations of flavopiridol glucuronide were determined after enzymatic hydrolysis. This analytical approach has been used previously for measuring the glucuronidation of SN-38, the active metabolite of irinotecan (25–29). Our assay method is able to quantify all of the existing O-glucuronidated metabolites of flavopiridol in patient plasma, providing a measure of overall systemic glucuronidation of flavopiridol.

The current data suggest that flavopiridol is transformed to flavopiridol glucuronide in the liver and excreted in the bile. The liver is largely responsible for the metabolism of absorbed dietary flavonoids, which undergo biliary excretion after conjugation with glucuronic acid or sulfate (30). Enterohepatic circulation has been proposed for flavopiridol and related flavonoids (31, 32). Microorganisms in the colon can hydrolyze...
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Flavopiridol induces apoptosis of normal lymphoid cells, causes immune glucuronidation and response rates in our study. Future studies should evaluate the relationship of flavopiridol glucuronidation to activity or response.

This report provides experimental evidence for future studies on the clinical pharmacogenetics of flavopiridol glucuronidation. To identify the genetic variants most likely to be responsible for this polymorphic phenotype, flavopiridol glucuronidation rates in vitro will be related to the UGT genotype in human livers and in cell systems expressing mutated UGT2B variants. A safer administration of flavopiridol might be obtained by the prediction of the risk of toxicity on the basis of the glucuronidation phenotype and/or genotype of patient candidates to flavopiridol. Finally, to lower the incidence of intestinal toxicity, pharmacological modulation of flavopiridol glucuronidation might be explored by administration of inducers of metabolism as well as inhibitors of drug biliary excretion, as has been proposed for irinotecan (26, 45, 46).

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References


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