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ORIGINAL ARTICLE

**Apurva A. Desai · Federico Innocenti · Linda Janisch
Mark DeMario · Dale Shepard · Jacqueline Ramirez
Gini F. Fleming · Mark J. Ratain**

A phase I trial of pharmacokinetic modulation of carboxyamidotriazole (CAI) with ketoconazole in patients with advanced cancer

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Abstract *Purpose*: Carboxyamidotriazole (CAI) is a novel antineoplastic agent in clinical development with limited oral bioavailability. In vitro, ketoconazole has been demonstrated to inhibit CYP3A4-mediated metabolism of CAI. We performed this phase I trial to determine if ketoconazole-mediated CYP3A4 inhibition would lead to favorable alteration of CAI pharmacokinetics, and to evaluate the safety, toxicity and tolerability of the proposed combination. *Design*: Forty-seven patients were treated using a standard three patients per cohort CAI dose-escalation scheme. In cycle 1, CAI was administered alone on day −6 followed by a single dose of ketoconazole (200 mg) on day 0. CAI and ketoconazole (200 mg/day) were subsequently coadministered on days 1 and 3–28. Plasma samples for pharmacokinetic analysis were obtained following the doses on days −6 and 1. All subsequent cycles were of 28-day duration, and consisted of daily CAI and ketoconazole coadministration. *Results*: Pharmacokinetic analysis was performed on samples from 44 patients. In most patients administration of ketoconazole produced an increase in CAI AUC and C_{max} with a decrease in CAI clearance. Seven patients experienced stable disease for up to 12 months. Gastrointestinal and constitutional toxicities were the most common toxicities. *Conclusions*: Coadministration of CAI with ketoconazole increased CAI exposure in most of the patients without altering the toxicity profile of CAI. The highest CAI dose administered on the trial was 300 mg/day. The clinical utility of such a modulation strategy might be explored in future clinical trials of CAI.

Keywords Cytochrome P450 · P-gp inhibition · Oral chemotherapy

A. A. Desai · F. Innocenti · L. Janisch · M. DeMario
J. Ramirez · G. F. Fleming · M. J. Ratain (✉)
Section of Hematology/Oncology, Department of Medicine,
University of Chicago, 5841 S. Maryland Ave., MC 2115,
Chicago, IL 60637-1470, USA
E-mail: mratain@medicine.bsd.uchicago.edu
Tel.: +1-773-7024400
Fax: +1-773-7023969

F. Innocenti · D. Shepard · M. J. Ratain
Committee on Clinical Pharmacology and Pharmacogenomics,
Chicago, USA

F. Innocenti · G. F. Fleming · M. J. Ratain
Cancer Research Center, The University of Chicago,
Chicago, IL, USA

Introduction

Carboxyamidotriazole (CAI) is a synthetic oral inhibitor of calcium influx that has demonstrated antiangiogenic, antiinvasive and antiproliferative properties [1–5]. CAI has antitumor activity both in vitro and in xenograft models [3]. CAI has also displayed cytostatic properties against various malignancies in previous clinical trials [6–10]. Based on these preliminary results, CAI-based treatment regimens are being explored for the treatment of various solid tumors [11–13]. Ludden and colleagues [14] have analyzed the in vitro and in vivo metabolism of CAI, and demonstrated that the two major metabolites of CAI are products of the cytochrome P450 (CYP450) system. They also established that hepatic microsome coincubation with CAI and ketoconazole significantly inhibits CYP450-mediated metabolism of CAI at ketoconazole concentrations as low as 3.0 μM.

Ketoconazole is an orally administered imidazole that is routinely used as an antifungal agent. Ketoconazole is hepatically metabolized, and is a well-studied inhibitor of CYP3A4, an important drug-metabolizing enzyme expressed in the liver and the small bowel. [15–18]. CYP3A4 inhibition has been demonstrated to increase the bioavailability of various orally administered medications that are CYP3A4 substrates [15–17]. For example, ketoconazole coadministration with cyclosporine has been shown to substantially reduce the required dose and cost of chronic cyclosporine

administration [16]. P-glycoprotein (P-gp), a member of the ATP-binding cassette transporter family, has also been demonstrated to play a role in decreasing the bioavailability of several oral medications and P-gp shares many substrates with CYP3A4 [19–21]. Wang and colleagues [22] have recently demonstrated that ketoconazole also inhibits P-gp function, and thus ketoconazole may play an important role in improving oral bioavailability of drugs such as CAI by inhibiting both P-gp and CYP3A4.

Previous CAI clinical trials have shown significant interpatient variability in CAI kinetics [7–10], which might partially explain the discrepancies in the observed toxicities amongst those trials. The micronized formulation has been the best tolerated, albeit the least bioavailable formulation [9, 10]. Therefore, increasing the exposure to the parent drug by inhibiting its metabolism could potentially enhance the efficacy. Based on these observations, we hypothesized that the potent CYP3A4/P-gp inhibitor ketoconazole, when coadministered with CAI would substantially reduce CAI metabolism, increase CAI bioavailability and potentially diminish the interindividual variability in CAI pharmacokinetics. Increasing the bioavailability of CAI while decreasing its kinetic variability could theoretically improve the therapeutic index of CAI. The purpose of this phase I trial was to determine the toxicity profile of CAI administered on a daily administration schedule with ketoconazole, and to describe the resultant pharmacokinetic effects of the proposed drug modulation.

Patients and methods

Patient eligibility

All patients participating in the trial provided written informed consent before study enrollment in accordance with institutional and Federal guidelines. Patients with a histologically confirmed diagnosis of nonhematologic malignancy that was refractory to standard therapy or for which no known effective therapy was available were eligible to participate in this study. Other eligibility criteria included measurable or assessable disease by radiographic studies or physical examination, age at least 18 years, Karnofsky performance status of at least 70%, and adequate organ function defined as serum creatinine <1.5 mg/dl, serum total bilirubin <1.5 mg/dl, AST and ALT levels less than 2.5 times the upper limit of normal, absolute neutrophil count of at least 2000/μl and platelet count of at least 100,000/μl. Eligible patients must have been off previous anticancer therapy including chemotherapy, radiotherapy, biological therapy, or investigational therapy for at least 4 weeks before study entry (6 weeks if prior therapy included nitrosoureas or mitomycin C). All patients of childbearing capability were required to use contraception during therapy and for 2 months after completion of therapy, and pregnant or lactating patients were excluded. Patients with malabsorption disorders and peripheral neuropathy exceeding grade 1 were also excluded. The oral bioavailability of ketoconazole is reduced by concurrent antacid administration; hence, patients requiring antacid therapy were excluded from the study. Furthermore, patients needing concomitant administration of CYP3A4 substrates, inhibitors, and inducers were also excluded.

Drug administration and study design

The National Cancer Institute (NCI), Division of Cancer Treatment and Diagnosis supplied CAI. Due to the lipophilic nature of CAI, it was prepared by dissolution in polyethylene glycol-400 (PEG-400) and was available in a gelatin capsule (Gelcap) formulation containing CAI dissolved in PEG-400, or a micronized capsule formulation containing 50 mg CAI and microcrystalline cellulose. Ketoconazole was obtained commercially as 200-mg scored tablets (Janssen Pharmaceuticals, Titusville, N.J.). A single 200-mg oral dose of ketoconazole produces mean peak plasma concentrations of 3.5 μM 1–2 h after administration, and such levels have been demonstrated to be adequate for inhibition of CYP3A4-mediated CAI metabolism [14]. Hence, a fixed 200-mg/day oral dose of ketoconazole was chosen for the study. Given the high degree of interindividual variability in the pharmacokinetic parameters of CAI, and limited number of dose sizes available for CAI capsules that generally require "rounding off" doses calculated based on body surface area (BSA), a more practical flat dosing schema was selected for this study. Furthermore, apparent oral clearance would not be expected to correlate with BSA. The study participants were instructed to administer the study drugs in the morning, under fasting conditions, and at least 1 h prior to any food intake.

The initial three dose cohorts were treated with the Gelcap formulation of CAI. The first cohort of study participants received 50 mg/day of CAI (Gelcap formulation) concomitantly with a fixed dose of 200 mg/day ketoconazole. Additionally, 100 and 150 mg/day CAI dose levels were also studied for the Gelcap formulation. The protocol was amended at that point to study dose escalation utilizing the better-tolerated micronized formulation of CAI [9, 10]. The initial patient cohort treated with the micronized CAI was started at 100 mg/day dose level, and subsequent dose escalation consisted of 50 mg/day dose increments per dose cohort until a maximum tolerated dose (MTD) was reached or up to a maximum daily dose of 300 mg/day (Table 1).

In order to determine if ketoconazole had any effect on CAI pharmacokinetics, the following treatment scheme was designed for cycle 1 (Fig. 1). A single CAI test dose was administered on day −6 followed 7 days later by a single dose of ketoconazole (200 mg/day) on day 0. Subsequently, CAI and ketoconazole were

Table 1 Dose levels

Formulation	Dose level (mg/day)	No. of patients treated
Gelatin capsule	50	5
	100	4
	150	6
Micronized capsule	100	6
	150	3
	200	4
	250	4
	300	15

coadministered on day 1; no study medications were administered on day 2. CAI and ketoconazole were then coadministered on days 3–28. Patients received the same CAI dose on day −6 (test dose) as the dose they were given on day 1 and on days 3–28. Plasma samples were drawn as described below on days −6 and day 1 of the first cycle. Starting with cycle 2, each treatment cycle consisted of daily CAI plus ketoconazole coadministered for 28 days. There was no interruption of therapy between treatment cycles, and treatment was continued until disease progression or dose limiting toxicity (DLT). In the event of a DLT, therapy was held pending resolution of toxicity and therapy could subsequently be resumed at the next lower dose level, at the discretion of the patient and treating physician.

For the purposes of determining the MTD, only DLT occurring during the first cycle of therapy was considered. Patients not completing cycle 1 for reasons other than DLT were replaced. DLT was defined as: (a) any grade 2 or greater nonhematologic toxicity excluding hepatic transaminase elevation, nausea, emesis, and alopecia; (b) grade 3–4 nausea/emesis; (c) grade 3–4 hepatic transaminase elevations with an increase of at least 50% from the prestudy value; (d) any toxicity resulting in greater than a 5-day delay of drug administration; or (e) grade 4 hematologic toxicity or febrile neutropenia. Treatment delays for toxicities more than grade 1 but less than that constituting a DLT were allowed for 5 days, and if the toxicity failed to resolve to less than grade 1 within 5 days of dose delay, then it was considered a DLT. CAI doses that were missed were not replaced. Toxicity was assessed using NCI Common Toxicity Criteria (version 2.0). It was required that three patients complete at least one cycle of therapy prior to entry of patients at the next higher dose level. Dose escalation was permitted if none of the initial three patients incurred DLT. If one of the initial three patients experienced DLT in cycle 1, the dose cohort was expanded to six evaluable patients. The MTD was defined as the highest dose level tested at which no more than one of six patients experienced a DLT.

Pretreatment and follow-up studies

Before starting therapy, all patients were assessed with a complete history and physical examination. Karnofsky performance status was also assessed, and tumor burden evaluated radiographically, along with a chemistry panel, complete blood count, and beta HCG level (when applicable). Hematologic parameters were assessed weekly, and complete serum chemistries were assessed every 2 weeks while on therapy. Physical examination and performance status evaluations were performed every 2 weeks during treatment. All of the above parameters were monitored more frequently if deemed necessary by the treating physicians. Tumor response assessments were performed after the first two cycles of therapy and every two cycles thereafter. Two-dimensional measurement criteria were used to classify response using World Health Organization response criteria. Patients with partial and complete response or stable disease were allowed to continue therapy.

CAI pharmacokinetics

Blood samples were obtained using sodium heparinized vacuum tubes for pharmacokinetic analysis at 0, 1, 2, 3, 4, 8, 24 and 48 h after CAI doses on days −6 and 1. At the time of collection, plasma was separated by centrifugation and stored in polypropylene tubes at −80°C

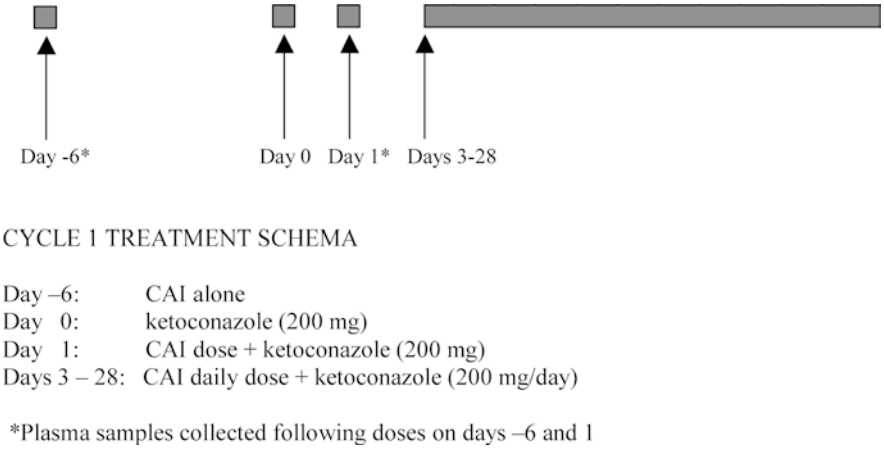

Fig. 1 Cycle 1 treatment schedule for CAI and ketoconazole administration

until analysis. The assay methodology described below was modified slightly from previously published methods [23]. Briefly, plasma samples (0.5 ml) were combined with 30 µl internal standard (33 µg/ml harmine in methanol) and 3 ml chloroform. After shaking (10 min, high speed) and centrifuging (15 min, 2500 rpm, 25°C), the organic layer was evaporated under nitrogen gas (37°C). The samples were reconstituted in 200 µl mobile phase and centrifuged (10 min, 14,000 rpm, 25°C). An aliquot of 60 µl was injected into the HPLC (Hitachi Instruments, San Jose, Calif.). The mobile phase consisted of 63/37 methanol/81.7 m*M* ammonium acetate (pH 6) at a flow rate of 1 ml/min. A µBondapak (10 µ*M*, 3.9×300 mm ID) column and µBondapak C18 guard-pak (Waters Corporation, Milford, Mass.) were used. The retention times of the internal standard and CAI were 8.5 and 11 min, respectively. In some patients, CAI was detected in plasma at time 0 of day 1. Hence, these "residual" CAI levels were subtracted from the levels obtained after giving CAI on day 1, and the principle of superposition was applied as previously described [24]. Thus, pharmacokinetic parameters of CAI were determined by noncompartmental analysis and the principle of superposition. The area under the concentration vs time curve (AUC) was determined using the log-linear trapezoidal rule (WinNonlin software; Pharsight Corporation, Mountain View, Calif.). Analysis of the peak plasma concentration (C_{max}) was determined by inspection. Clearance was calculated for each patient using the formula dose/AUC.

Statistical analysis

The modulation of CAI pharmacokinetics by ketoconazole was assessed by comparing the pharmacokinetic parameters obtained after the CAI test dose (hereafter referred to as baseline CAI pharmacokinetics) with those obtained after CAI administered with ketoconazole. For this purpose, paired *t*-test analysis was used. We also compared the differences in pharmacokinetic parameters amongst two patient groups according to severity of reported toxicity (more than grade 3 toxicity vs less than grade 3 toxicity) using unpaired *t*-test analysis. All the *P* values reported are two-tailed and all tests were performed at the 0.05 significance level. The statistical analyses of the data were conducted using GraphPad software (San Diego, Calif.).

Results

Patient characteristics

Between March 1998 and July 2000, 47 patients were enrolled on the trial. The characteristics of the patients enrolled are listed in Table 2. Most patients were heavily pretreated. Several different tumor types were represented.

Table 2 Patient characteristics. Values are number of patients, except age in years

Male	25
Female	22
Age (years)	
Median	62
Range	27–76
Karnofsky performance status (%)	
100	21
90	12
80	12
70	2
Disease	
Colorectal	11
Ovarian	10
Renal	5
Lung	5
Sarcoma	5
Uterine	3
Breast	2
Head and neck	2
Other	4
Prior treatment regimens	
1	5
2	15
3	10
4	12
5 or more	5

Table 3 Toxicity for all dose levels

Toxicity	Grade 1	Grade 2	Grade 3
Gastrointestinal			
Nausea/emesis	16	6	3
Constipation	6	0	0
Diarrhea	1	0	0
Constitutional			
Anorexia	8	5	0
Fatigue	14	9	1
Weight loss	4	3	0
CNS			
Peripheral neuropathy	9	1	0
Light headedness	1	0	1
Headache	3	0	1
Hematologic			
Neutropenia	2	0	0
Anemia	5	1	1
Miscellaneous			
Metabolic	2	1	1
Infection	0	0	2

Toxicity

Table 3 provides a summary of toxicity reported by 44/47 patients on-study (three patients who committed errors in medication administration were excluded). In addition to these three patients, ten other patients were not assessable for MTD determination, as they did not complete the first cycle of therapy due to disease progression (four patients), study withdrawal due to subjective intolerance of toxicity (two patients), and hospitalization due to intercurrent illness (four patients).

Gastrointestinal toxicity was the most common toxicity, particularly nausea/emesis, which was observed in

57% of the patients (25/44). Most of the patients did not report a significant benefit from previously reported pharmacologic and non-pharmacologic measures used to alleviate nausea [7–10]. Two of three patients who experienced grade 3 nausea/emesis during the second treatment cycle withdrew from the study; one patient experienced the toxicity during the first cycle and accounted for a DLT observed at the 150 mg/day dose level of the Gelcap formulation. Two patients withdrew from the study due to intolerance of grade 2 nausea/emesis.

Constitutional symptoms of fatigue and anorexia were also common. Fatigue was reported by 54% of the patients (24/44). One patient experienced grade 3 fatigue that accounted for a DLT at the 300 mg/day dose level of the micronized formulation. The toxicity resolved with discontinuation of CAI, and the patient was rechallenged with CAI at the next lower dose without recurrence of the toxicity. Of the 44 patients, 13 (29%) experienced grade 1 or 2 anorexia.

Neurologic symptoms were reported by 34% of patients (15/44), and mild (grade 1) sensory neuropathy accounted for most (67%, 10/15) of the neurologic toxicities. One patient experienced dose limiting "light headedness" (150 mg/day Gelcap dose level) that resolved upon discontinuation of CAI. The patient otherwise had a negative neurologic examination, and was subsequently treated at the next lower dose of CAI without recurrence of neurologic symptoms. Another patient developed new-onset grade 3 headache and vertigo on therapy; however, the diagnostic evaluation demonstrated new brain metastases.

Four additional patients experienced grade 3 toxicities that were deemed to be unrelated to CAI therapy: two patients were diagnosed with non-neutropenic chronic indwelling catheter infections requiring hospitalization; one patient was noted to have an elevated bilirubin level resulting from progressive liver metastases; and one patient developed grade 3 anemia due to disease progression that resulted in continuous gastrointestinal blood loss (confirmed by a colonoscopy).

Response data

Although assessment of tumor response was not a primary objective of this study, patients were evaluated for tumor response after every two cycles of treatment. A total of 36 patients completed at least two cycles of therapy, and thus were eligible for response evaluation. No partial or complete responses were observed. Seven patients (19%) were noted to have stable disease for periods up to 12 months (range 4–12 months). Two heavily pretreated patients with leiomyosarcoma had disease stabilization of 6 and 12 months duration. Additionally, two patients with renal cell carcinoma who had been previously treated with cytotoxic therapy had disease stabilization for 4 months each. One patient each with non-small-cell lung cancer and ovarian cancer experienced disease stabilization for 6 months, and one patient with colorectal cancer experienced disease stabilization for 5 months.

Pharmacokinetics of CAI

Pharmacokinetic data were available for 44 of the 47 treated patients (two patients erroneously self-administered the medications at incorrect times and one patient missed blood sampling times). Table 4 provides a summary of the CAI pharmacokinetic data. In all 44 patients, CAI AUC and C_{max} values were significantly higher after CAI and ketoconazole coadministration compared to the baseline CAI AUC and C_{max} mean ± SD baseline CAI AUC in all 44 patients was 15.6 ± 9.4 μg/ml h, which increased to 21.2 ± 12.3 μg/ml h ($P = 0.009$, paired t-test) after CAI and ketoconazole coadministration. Although ketoconazole treatment led to a 136% increase in the mean CAI AUC for all 44 patients, we noted that many patients demonstrated minimal or no increase in the CAI AUC. Previous phase I trials have shown approximately 20% intrapatient variability in CAI pharmacokinetics [7–10]; hence, we retrospectively adopted a >20% increase in AUC as the cut-off for effective modulation by ketoconazole. Thus, 62% of patients ($n = 27$) demonstrated a >20% change in CAI AUC after CAI and ketoconazole coadministration. The mean ± SD baseline CAI AUC was 11.3 ± 1.4 μg/ml h and 22.1 ± 2.2 μg/ml h for patients with >20% and <20% modulation, respectively. In the 27 patients with >20% modulation of CAI AUC, a 206% increase was noted in the CAI AUC after CAI and ketoconazole coadministration (the mean ± SD CAI AUC after CAI and ketoconazole coadministration was 25.0 ± 13.3 μg/ml h vs a baseline value of 11.3 ± 1.4 μg/ml h). A substantial decline in clearance of CAI was observed across all dose levels after coadministration of ketoconazole as shown in Table 4. No significant decrease in the interpatient or intrapatient variability of CAI kinetics was noted after CAI was administered with ketoconazole (Table 4). In order to quantify the change in the various pharmacokinetic parameters before and after ketoconazole administration, we calculated the ratio of the mean values for the parameters of interest before and after modulation with ketoconazole (i.e., day 1/day −6). The mean of the ratios and the SD are represented as "modulation ratios" for each parameter in Table 5.

Discussion

There are various examples in the literature of deliberate inhibition of CYP3A substrate metabolism to potentially enhance the clinical benefit of certain oral medications [15, 16]. This phase I study demonstrated that coadministration of CAI and ketoconazole increased the AUC and C_{max} while decreasing the clearance of CAI in

Table 4 CAI pharmacokinetics

Formulation	Dose level (mg/day)	Patients	AUC (μg/ml h)		C_{max} (μg/ml)		Apparent oral clearance (l/h)	
			CAI alone	CAI + ketoconazole	CAI alone	CAI + ketoconazole	CAI alone	CAI + ketoconazole
Gelcap	50	4	9.1 ± 1.4	21.8 ± 10.5	0.4 ± 0.1	0.6 ± 0.3	5.6 ± 0.8	2.8 ± 1.5
	100	3	16.9 ± 7.5	26.3 ± 15.6	0.8 ± 0.5	0.6 ± 0.6	7.1 ± 3.9	4.7 ± 2.4
	150	6	17.7 ± 7.9	28.6 ± 11.4	0.8 ± 0.3	1.1 ± 0.4	10.7 ± 6.6	6.1 ± 2.6
	Total	13	14.8 ± 7.2	25.9 ± 11.5	0.7 ± 0.3	0.9 ± 0.4	8.3 ± 5.2	4.8 ± 2.6
Micronized	100	5	7.4 ± 4.2	9.9 ± 4.7	0.2 ± 0.2	0.3 ± 0.2	18.3 ± 11.2	12.5 ± 7.1
	150	3	11.3 ± 6.8	13.9 ± 2.1	0.4 ± 0.3	0.4 ± 0.1	16.1 ± 7.3	10.9 ± 1.5
	200	4	2.9 ± 14.2	24.8 ± 7.5	0.7 ± 0.3	0.7 ± 0.3	9.7 ± 5.1	8.8 ± 3.2
	250	4	17.3 ± 7.7	32.8 ± 23.5	0.5 ± 0.2	0.9 ± 0.7	17.1 ± 8.4	12.8 ± 10.8
	300	15	16.3 ± 10.1	20.9 ± 9.7	0.5 ± 0.3	0.6 ± 0.3	40.4 ± 60.1	17.1 ± 7.7
	Total	31	15.7 ± 10.3	20.5 ± 12.4*	0.5 ± 0.3	0.6 ± 0.3*	27.5 ± 43.4	14.1 ± 7.5

*$P < 0.05$, baseline values compared to post ketoconazole modulation, two-tailed paired *t*-test.

Table 5 CAI pharmacokinetics: modulation ratios

Formulation	Dose level (mg/day)	Patients	AUC	C_{max}	Apparent oral clearance
Gelcap	50	4	2.3 ± 0.8	1.6 ± 0.9	0.5 ± 0.2
	100	3	1.8 ± 1.0	0.9 ± 0.6	0.8 ± 0.7
	150	6	2.2 ± 2.2	1.4 ± 0.4	0.7 ± 0.4
	Total	13	2.2 ± 1.6	1.4 ± 0.6	0.7 ± 0.4
Micronized	100	5	2.1 ± 1.8	2.7 ± 2.6	1.1 ± 1.2
	150	3	1.4 ± 0.5	1.4 ± 0.7	0.7 ± 0.3
	200	4	1.2 ± 0.9	1.5 ± 1.4	1.1 ± 0.6
	250	4	1.8 ± 1.0	2.1 ± 1.3	0.7 ± 0.4
	300	15	2.8 ± 5.0	1.5 ± 1.0	0.8 ± 0.6
	Total	31	2.2 ± 3.6	1.8 ± 1.4	0.9 ± 0.7

most patients. However, almost 40% of patients studied did not have an appreciable increase in CAI exposure after coadministration of ketoconazole. These patients were distributed across the different dose levels for both formulations, and hence the formulation or CAI dose might not have been contributing factors in the observed differences in modulation. Patient error in reporting or compliance with concomitant medications is a possibility that cannot be excluded as a factor that might have contributed to the observed variability in the modulation as well.

Data from other studies with similar pharmacokinetic modulation strategies also suggest that such strategies are not effective uniformly in all of the study subjects [17, 25]. The interindividual variability in CYP3A4 activity [26, 27] probably plays an important role in the diminished modulation of CAI kinetics observed in some patients. The significantly higher baseline CAI AUC observed in the patients with minimal modulation of CAI pharmacokinetics suggests that they might have had functionally or quantitatively reduced CYP3A4 at baseline, thus, diminishing the impact of further inhibition of CYP3A4 by ketoconazole. In agreement with this hypothesis, Brophy and colleagues [28] conducted a trial to evaluate the potential impact of coadministration of two CYP3A4 substrates (amprenavir and clarithromycin), and they also observed that subjects with low baseline amprenavir AUC had a higher likelihood of a larger pharmacokinetic interaction with clarithromycin. Review of data from other trials [17, 25] in which interactions between azoles and CYP3A substrates were investigated also suggests similar nonuniform pharmacokinetic interactions. Similarly, interindividual differences in P-gp levels [29, 30] could also play an independent and/or synergistic role in altering the modulatory effect of ketoconazole.

One of the objectives of the study besides increasing patients' systemic exposure to CAI was to decrease the previously described variation in CAI kinetics among patients [7–10]. We were not able to demonstrate a significant reduction in the interpatient variability in the pharmacokinetic parameters measured in the study. The fact that CAI exposure increased significantly in some patients while exhibiting limited change in others contributed to the observed lack of reduction in the interpatient variability of CAI kinetics.

The highest micronized CAI dose administered in the current trial was 300 mg/day, which was similar to that recommended by Berlin et al. [10] for a "fixed-dose" schedule of single-agent micronized CAI. Mild to moderately severe constitutional, gastrointestinal and neurologic toxicities were the most common toxicities observed in the current trial. The overall toxicity profile of CAI when coadministered with ketoconazole was

similar to that observed previously with single-agent CAI by Kohn and colleagues [9]. A negligible increase in grade 3 gastrointestinal toxicity was observed on this trial compared to the single-agent micronized CAI study reported by Kohn and colleagues [9]. Ketoconazole is known to cause gastrointestinal toxicity at high doses, but is well tolerated at the dose utilized in the present study [31, 32]. One possible explanation for the mildly increased incidence of severe gastrointestinal toxicity observed in the current trial is potential synergistic impact of a relatively innocuous dose of ketoconazole when combined with CAI. In the current trial, the incidence of serious neurotoxicity was negligible compared to previous reports by Berlin and colleagues [8, 10]. Berlin et al. [10] have reported a much higher rate of grade 3 neurotoxicity compared to that observed in the present trial. Although comparing data from different trials conducted with dissimilar trial designs is difficult, analysis of available data from the various CAI clinical trials using the micronized formulation does not provide specific insight regarding the observed discrepancies in the toxicity. Small sample sizes of the individual studies and the inherent selection bias associated with such small studies might have contributed to some of the observed discrepancies.

To conclude, this study demonstrates that rationally designed drug modulation strategies for oral medications can be implemented successfully and safely. In this trial, modulation of CAI pharmacokinetics using ketoconazole led to increased CAI exposure in most patients, and the modulation did not lead to any appreciable increase in toxicity. However, the modulation was not useful in decreasing the interpatient variability in CAI pharmacokinetics. As several new oral anticancer therapies are now becoming available, such drug modulation strategies might be explored early in therapeutic development to investigate their potential role in improving the therapeutic index of such agents.

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References

1. Kohn EC, Liotta LA (1990) L651582: a novel antiproliferative and antimetastasis agent. J Natl Cancer Inst 82:54–60
2. Hupe DJ, Boltz R, Cohen CJ, Felix J, Ham E, Miller D, Soderman D, Van Skiver D (1991) The inhibition of receptor-mediated and voltage-dependent calcium entry by the antiproliferative L-651582. J Biol Chem 266:10136–10142
3. Kohn EC, Sandeen MA, Liotta LA (1992) In vivo efficacy of a novel inhibitor of selected signal transduction pathways including calcium, arachidonate, and inositol phosphates. Cancer Res 52:3208–3212
4. Jacobs W, Mikkelsen T, Smith R, Nelson K, Rosenblum ML, Kohn EC (1997) Inhibitory effects of CAI in glioblastoma growth and invasion. J Neurooncol 32:93–101
5. Lambert PA, Somers KD, Kohn EC, Perry RR (1997) Antiproliferative and antiinvasive effects of carboxyamido-triazole on breast cancer cell lines. Surgery 122:372–378
6. Figg WD, Cole KA, Reed E, Steinberg SM, Piscitelli SC, Davis PA, Soltis MJ, Jacob J, Boudoulas S, Goldspiel B et al (1995) Pharmacokinetics of orally administered carboxyamido-triazole, an inhibitor of calcium-mediated signal transduction. Clin Cancer Res 1:797–803
7. Kohn EC, Reed E, Sarosy G, Christian M, Link CJ, Cole K, Figg WD, Davis PA, Jacob J, Goldspiel B, Liotta LA (1996) Clinical investigation of a cytostatic calcium influx inhibitor in patients with refractory cancers. Cancer Res 56:569–573
8. Berlin J, Tutsch KD, Hutson P, Cleary J, Rago RP, Arzoomanian RZ, Alberti D, Feierabend C, Wilding G (1997) Phase I clinical and pharmacokinetic study of oral carboxyamido-triazole, a signal transduction inhibitor. J Clin Oncol 15:781–789
9. Kohn EC, Figg WD, Sarosy GA, Bauer KS, Davis PA, Soltis MJ, Thompkins A, Liotta LA, Reed E (1997) Phase I trial of micronized formulation carboxyamidotriazole in patients with refractory solid tumors: pharmacokinetics, clinical outcome, and comparison of formulations. J Clin Oncol 15:1985–1993
10. Berlin J, Tutsch KD, Arzoomanian RZ, Alberti D, Binger K, Feierabend C, Dresen A, Marnocha R, Pluda J, Wilding G (2002) Phase I and pharmacokinetic study of a micronized formulation of carboxyamidotriazole, a calcium signal transduction inhibitor: toxicity, bioavailability and the effect of food. Clin Cancer Res 8:86–94
11. Kohn EC, Reed E, Sarosy GA, Minasian L, Bauer KS, Bostick-Bruton F, Kulpa V, Fuse E, Tompkins A, Noone M, Goldspiel B, Pluda J, Figg WD, Liotta LA (2001) A phase I trial of carboxyamido-triazole and paclitaxel for relapsed solid tumors: potential efficacy of the combination and demonstration of pharmacokinetic interaction. Clin Cancer Res 7:1600–1609
12. Bauer KS, Figg WD, Hamilton JM, Jones EC, Premkumar A, Steinberg SM, Dyer V, Linehan WM, Pluda JM, Reed E (1999) A pharmacokinetically guided phase II study of carboxyamido-triazole in androgen-independent prostate cancer. Clin Cancer Res 5:2324–2329
13. Stadler WM, Rosner G, Small E, Ratain MJ (2001) Feasibility of the randomized discontinuation clinical trial design for assessing cytostatic drug activity (abstract 377). Proceedings of AACR-NCI-EORTC International Conference: Molecular Targets and Cancer Therapeutics: Discovery, Biology, and Clinical Applications 7:77
14. Ludden LK, Strong JM, Kohn EC, Collins JM (1995) Similarity of metabolism for CAI (NSC 609974) in human liver tissue in vitro and in humans in vivo. Clin Cancer Res 1:399–405
15. Dresser GK, Spence JD, Bailey DG (2000) Pharmacokinetic-pharmacodynamic consequences and clinical relevance of cytochrome P450 3A4 inhibition. Clin Pharmacokinet 38:41–57
16. Keogh A, Spratt P, McCosker C, Macdonald P, Mundy J, Kaan A (1995) Ketoconazole to reduce the need for cyclosporine after cardiac transplantation. N Engl J Med 333:628–633
17. Gomez DY, Wacher VJ, Tomlanovich SJ, Hebert MF, Benet LZ (1995) The effects of ketoconazole on the intestinal metabolism and bioavailability of cyclosporine. Clin Pharmacol Ther 58:15–19
18. McKinnon RA, Burgess WM, Hall PM, Roberts-Thomson SJ, Gonzalez FJ, McManus ME (1995) Characterisation of CYP3A gene subfamily expression in human gastrointestinal tissues. Gut 36:259–267
19. Watkins PB (1997) The barrier function of CYP3A4 and P-glycoprotein in the small bowel. Adv Drug Deliv Rev 27:161–170
20. Ito K, Kusuhara H, Sugiyama Y (1999) Effects of intestinal CYP3A4 and P-glycoprotein on oral drug absorption—theoretical approach. Pharm Res 16:225–231

21. Wacher VJ, Silverman JA, Zhang Y, Benet LZ (1998) Role of P-glycoprotein and cytochrome P450 3A in limiting oral absorption of peptides and peptidomimetics. J Pharm Sci 87:1322–1330
22. Wang EJ, Lew K, Casciano CN, Clement RP, Johnson WW (2002) Interaction of common azole antifungals with P glycoprotein. Antimicrob Agents Chemother 46:160–165
23. Holmes KA, Chaffins S, Osborn BL, Liotta LA, Kohn EC (1993) Quantification of human plasma levels of the anticancer agent carboxyamidotriazole by high-performance liquid chromotography. J Chromatogr 613:317–325
24. Gibaldi M, Perrier D (1982) Pharmacokinetics. Dekker, New York 15:451–457
25. Niemi M, Neuvonen PJ, Kivisto KT (2001) The cytochrome P4503A4 inhibitor clarithromycin increases the plasma concentrations and effects of repaglinide. Clin Pharmacol Ther 70:58–65
26. Westlind A, Lofberg L, Tindberg N, Andersson TB, Ingelman-Sundberg M (1999) Interindividual differences in hepatic expression of CYP3A4: relationship to genetic polymorphism in the 5′-upstream regulatory region. Biochem Biophys Res Commun 259:201–205
27. Xie HG, Kim RB, Wood AJ, Stein CM (2001) Molecular basis of ethnic differences in drug disposition and response. Annu Rev Pharmacol Toxicol 41:815–850
28. Brophy DF, Israel DS, Pastor A, Gillotin C, Chittick GE, Symonds WT, Lou Y, Sadler BM, Polk RE (2000) Pharmacokinetic interaction between amprenavir and clarithromycin in healthy male volunteers. Antimicrob Agents Chemother 44:978–984
29. Ameyaw MM, Regateiro F, Li T, Liu X, Tariq M, Mobarek A, Thornton N, Folayan GO, Githang'a J, Indalo A, Ofori-Adjei D, Price-Evans DA, McLeod HL (2001) MDR1 pharmacogenetics: frequency of the C3435T mutation in exon 26 is significantly influenced by ethnicity. Pharmacogenetics 11:217–221
30. Tanabe M, Ieiri I, Nagata N, Inoue K, Ito S, Kanamori Y, Takahashi M, Kurata Y, Kigawa J, Higuchi S, Terakawa N, Otsubo K (2001) Expression of P-glycoprotein in human placenta: relation to genetic polymorphism of the multidrug resistance (MDR)-1 gene. J Pharmacol Exp Ther 297:1137–1143
31. Trump DL, Havlin KH, Messing EM, Cummings KB, Lange PH, Jordan VC (1989) High-dose ketoconazole in advanced hormone-refractory prostate cancer: endocrinologic and clinical effects. J Clin Oncol 7:1093–1098
32. Isik AZ, Gokmen O, Zeyneloglu HB, Senoz S, Zorlu CG (1996) Low dose ketoconazole is an effective and a relatively safe alternative in the treatment of hirsutism. Aust N Z J Obstet Gynaecol 36:487–489