Phase I Study of Amonafide Dosing Based on Acetylator Phenotype

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ABSTRACT

Amonafide is extensively metabolized, including N-acetylation to an active metabolite. Prior studies have demonstrated that patients who are fast acetylators of amonafide (and other drugs) have increased toxicity at standard doses of amonafide. The primary objective of this study was to define the recommended phase II dose of amonafide separately for slow and fast acetylators. Twenty-six patients with advanced cancer underwent acetylator phenotyping with caffeine and were assigned to a dose level. Slow acetylators were treated at 375 mg/m² (daily for 5 days) and had a median WBC nadir of 1600/µl. Fast acetylators were treated at both 200 and 250 mg/m², resulting in median WBC nadirs of 5300 and 2000/µl, respectively. Two patients were not typeable, and two patients appear to have been misphenotyped, one in each phenotype category. Pharmacodynamic analysis yielded a model for nadir WBC including acetylator phenotype. Pharmacodynamic analysis yielded a model for nadir WBC including acetylator phenotype.

INTRODUCTION

Amonafide (NSC 308847, benzoquinolinedione, nafidimide) is a new site-specific intercalating agent (1, 2) that completed initial phase I evaluation several years ago (3, 4). Its dose-limiting toxicity was found to be myelosuppression, which was not clearly related to the extent of prior therapy (4).

Among institutions that conducted phase I trials, there was variability in the dose recommended for phase II testing (4, 5). A 1-h infusion of 300 mg/m² for five consecutive doses (every 3–4 weeks) was selected for most phase II trials. At this dose and schedule amonafide has been shown to have activity in both breast (6) and prostate (7) cancer. In addition, amonafide is active in breast cancer when administered as a single dose of 800 mg/m² over 3 h every 4 weeks (8). A phase I study at higher doses suggests that amonafide has antileukemic activity as well (9).

Amonafide is extensively metabolized, including N-acetylation to an active metabolite, N-acetyl-amonafide (10). Preliminary analysis of a population pharmacodynamic study of amonafide had previously indicated that the myelosuppression observed at standard doses (300 mg/m² daily for 5 days) was highly correlated with the extent of acetylation of amonafide to N-acetyl-amonafide (11). On the basis of this finding, we hypothesized that phenotyping could eventually be used to individualize amonafide dosage.

In a recent study, we demonstrated that fast acetylators, defined by caffeine phenotyping, had significantly greater leukopenia than slow acetylators (12). The primary objective of the current study was to identify recommended phase II doses of amonafide for each of the genetically determined acetylator phenotypes.

MATERIALS AND METHODS

Patient Population. All patients had a solid tumor or lymphoma that was refractory to standard therapy or for which no standard therapy existed. Patients also had acceptable bone marrow and end-organ function including WBC ≥ 3,500/µl, platelets ≥ 100,000/µl, hemoglobin ≥ 9 g/dl, bilirubin ≤ 1.5 mg/dl (unless due to Gilbert’s syndrome), and creatinine ≤ 1.5 mg/dl. The minimum Karnofsky performance status permitted was 60%. Patients were required to have recovered from prior therapy (minimum of 4 weeks) and to be able to comply with the study of acetylator phenotype. Informed consent was obtained from all patients in accordance with institutional and federal guidelines.

Acetylator Phenotyping. Caffeine was used as a test drug to determine acetylator phenotype, using a modification of the method of Tang et al. (13). This previously published modification requires only a spot urine sample 4 to 6 h after administration of an adequate quantity of a caffeine-containing beverage (28–12 ounces) (12). The urinary concentrations of an acetylated (AAMU) and nonacetylated (1X) metabolite are determined by high-pressure liquid chromatography. The acetylator phenotype was based on the AAMU:1X molar ratio as follows: <1.8, slow; 1.8–2.4, indeterminate; >2.4, fast. After concentrations were obtained several patients’ phenotypes were reassigned based on dose received and NBI424 as noted in a population study of amonafide at standard dose (14).

Amonafide Dosing. The initial dose levels for slow and fast acetylators were 375 and 250 mg/m² (for 5 days), respectively. Patients who had an indeterminate acetylator phenotype were treated at the “standard” dose of 300 mg/m² (15).

The study was designed to enroll patients in cohorts, based on acetylator phenotype. For slow acetylators, subsequent cohorts were intended to be dose-escalated by 20%, but not beyond a level where the majority of patients developed grade 3–4 toxicity. For fast acetylators, subsequent cohorts were reduced by 50 mg/m², but not beyond a level where the majority of patients did not have grade 3–4 toxicity.

Dose modifications were used for subsequent cycles in individual patients as well. Patients with grade 0–1 toxicity during their first cycle of treatment were dose-escalated by one level, and patients with grade 4 toxicity were dose-reduced by one level. Subsequent cycles were delayed until all toxicity had resolved to grade 1 or less, but the interval between cycles was always at least 21 days.

Amonafide Administration. Amonafide was supplied by the National Cancer Institute. The drug was reconstituted in saline and administered over 1 h. Although not required, the vast majority of patients received the drug through a central venous catheter. Most patients received 10 mg prochlorperazine p.o. as a prophylactic antiemetic.

Laboratory Studies. Plasma samples for quantitation of amonafide and its active metabolite were obtained at 45 min and 24 h after the first dose (prior to the second dose). The area under the plasma concentration-time curve of amonafide was estimated using a previously published limited sampling model (12, 15). The high-pressure liquid chromatography method for amonafide and N-acetyl-amonafide has been described in detail previously (12).

Other laboratory studies included twice-weekly complete blood counts, as well as monitoring of serum chemistries before each cycle.

Statistical Analysis. The nonparametric Mann-Whitney test and Fisher exact test (two-sided) were used to compare characteristics of slow and fast acetylators. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received 11/5/92; accepted 3/11/93.

The abbreviations used are: AAMU, 5-acetylamino-6-amino-3-methyluracil; IX, 1-methylxanthine; RMSE, root mean square error; ANC, absolute neutrophil count; WBCP, pretreatment WBC; BIDA, amonafide; NBIDA, N-acetyl-amonafide; NBIDA24, 24-h NBIDA concentration; NBIDA24, NBIDA24 in slow acetylators (or zero); NBIDA24*, NBIDA24 in fast acetylators (or zero); BIDA24, 24-h BIDA concentration; NBIDA45, NBIDA concentration at 45 min; BIDA45, BIDA concentration at 45 min; SEX, variable defining gender in slow acetylators (or zero); SEXF, variable defining gender in fast acetylators (or zero).

1 The abbreviations used are: AAMU, 5-acetylamino-6-amino-3-methyluracil; IX, 1-methylxanthine; RMSE, root mean square error; ANC, absolute neutrophil count; WBCP, pretreatment WBC; BIDA, amonafide; NBIDA, N-acetyl-amonafide; NBIDA24, 24-h NBIDA concentration; NBIDA24, NBIDA24 in slow acetylators (or zero); NBIDA24*, NBIDA24 in fast acetylators (or zero); BIDA24, 24-h BIDA concentration; NBIDA45, NBIDA concentration at 45 min; BIDA45, BIDA concentration at 45 min; SEX, variable defining gender in slow acetylators (or zero); SEXF, variable defining gender in fast acetylators (or zero).
acetylators. All log transforms used the natural logarithm. To evaluate a prior pharmacodynamic model for amonafide (12), the predicted nadir WBC was compared to the observed nadir WBC. The mean predictive error ± SE and RMSE were calculated as measures of bias and precision, respectively (16). Stepwise multiple linear regression was used for constructing a new pharmacodynamic model (with log transformation of WBC), which was validated as described above.

Ideal body weight was defined based on height and gender: (a) males, 106 pounds plus 6 pounds for each inch over 5 feet; (b) females, 100 pounds plus 5 pounds for each inch over 5 feet. The ideal body weight ratio was defined as the ratio of actual body weight to ideal body weight.

**RESULTS**

Patient Characteristics. A total of 26 patients were entered in the study, including 13 males and 13 females. There were 21 Caucasians, 3 African-Americans, and 2 Hispanics. The median age was 61 years (range, 32–72 years), and the median performance status was 80%. Diagnoses included colorectal cancer (10 patients), non-small-cell lung cancer (5 patients), breast cancer (2 patients), thymoma (2 patients), soft tissue sarcoma (2 patients), and one patient each with hepatoma, gastric cancer, head and neck cancer, prostate cancer, and non-Hodgkin's lymphoma. Thirteen patients had previously received radiotherapy, and all but two had previously received chemotherapy, with a median of two prior regimens.

Acetylator Phenotyping. Of the 26 patients, there were 11 (42%) categorized as slow acetylators and 13 (50%) as fast acetylators (Fig. 1), with two (8%) untypeable patients. There was no significant difference between slow and fast acetylators in gender, age, performance status, race, or pretreatment blood counts (data not shown). We were particularly interested in comparing the ratio of actual to ideal body weight because of our prior finding that fast acetylators were significantly obese (median ratio, 1.12) compared with slow acetylators (median ratio, 0.92) (11). This prior finding was not confirmed in the current cohort of patients since the slow acetylators had a higher median ratio (1.21) than the fast acetylators (1.03) (P = 0.07, Mann-Whitney).

Hematological Toxicity by Dose Level. Ten patients, all of whom were phenotyped as slow acetylators, were treated at a dose of 375 mg/m² for their first cycle. One patient was not evaluable for myelosuppression, since he died on day 11 of the cycle of unknown causes with a WBC of 8200/µl. In the remaining nine slow acetylators, the median WBC nadir was 1600/µl, and the median ANC nadir was 740/µl. No additional cohorts were studied at higher dose levels since 6 of 9 evaluable patients had grade 3–4 neutropenia (including 3 patients with grade 4) at the 375 mg/m² dose level. However, 3 of the patients (who had only grade 0–1 toxicity) were subsequently dose-escalated in subsequent cycles, with one patient safely treated at 540 mg/m² (4 cycles, median ANC nadir, 1120/µl).

Seven patients determined to be fast acetylators were treated at the 250 mg/m² dose level for their first cycle. The first patient was erroneously considered a fast acetylator (molar ratio, 1.43) and had grade 0 myelosuppression (ANC nadir, 3710/µl). In the remaining 6 patients, the median WBC nadir was 2000/µl and the median ANC nadir was 1040/µl. Furthermore, there was grade 3–4 thrombocytopenia in 4 patients (2 patients with grade 4) with a median platelet nadir of 53,000/µl. Thus, the dose for fast acetylators was reduced to 200 mg/m².

Seven additional fast acetylators (by caffeine phenotype) were treated at 200 mg/m² with minimal toxicity (median WBC nadir, 5300/µl; median ANC nadir, 3500/µl). Four patients were subsequently dose-escalated to 250 mg/m² with acceptable toxicity (median nadirs for cycle 2: WBC, 3,000/µl; ANC, 2,190/µl; platelets, 160,000/µl). One of these patients appeared to be a slow acetylator of amonafide, as discussed below.

Both patients whose phenotype was indeterminate were treated at 300 mg/m². One patient each had grade 0 and grade 4 myelosuppression.

Nonhematological Toxicity. Amonafide was generally well tolerated; no patient suffered grade 3–4 nonhematological toxicity. The most common toxicities were nausea and vomiting, occurring in 62% and 35% of the patients, respectively. In all but one patient, this did not exceed grade 1 severity.

Other toxicities occurring in more than one patient included light-headedness (39%), diaphoresis (19%), blurred vision (12%), fatigue (12%), flushing (12%), and phlebitis (8%). This experience is similar to that reported in other studies of amonafide (3, 4, 8, 16–19).

Amonafide Plasma Levels and Pharmacokinetics. As previously reported (12), fast acetylators (by caffeine phenotyping) had greater acetylation of amonafide to N-acetyl-amonafide, which was particularly evident at 24 h following administration of the day 1 dose (Fig. 2). Within each dose level, however, significant interpatient pharmacokinetic variability was evident (Table 1). There was no significant difference in clearance between slow and fast acetylators (P = 0.8, Mann-Whitney), but there was a trend toward slower clearance of amonafide in the fast acetylators treated at 250 mg/m² (P = 0.062 versus 200 mg/m², Mann-Whitney). However, there was no evidence of dose-dependent clearance in analyzing patients treated at more than one dose level (data not shown).

Of the 2 patients who could not be phenotyped with caffeine (treated at 300 mg/m²), there was clearly one slow and one fast acetylator (Fig. 2), corresponding to grades 0 and 4 myelosuppression, respectively. As previously noted, one slow acetylator was erroneously treated at 250 mg/m² and had both low metabolite concentrations and mild toxicity. At least 2 patients appear to have been miscategorized with the caffeine assay. One patient, deemed a fast acetylator (AAMU:1X ratio of 8.16) and initially treated at 200 mg/m², had low N-acetyl-amonafide concentrations (and minimal toxicity), even with dose escalation to 300 mg/m². A misclassification occurred in only one of the 11 patients (9%) typed as slow acetylators (AAMU:1X ratio of 1.38), which resulted in overdosage and excessive toxicity. Thus, of the 26 patients, the caffeine phenotyping assay was not informative in at least 4 patients (15%).

Pharmacodynamic Modeling. In a prior report (12), we constructed a model for nadir WBC that included the NBIDA24, WBCP, and patient age:

\[
\text{LOG(WBCN)} = 0.0579 - 0.00887(\text{NBIDA24}) + 1.20\text{LOG(WBCP)} - 0.0197(\text{AGE})
\]
Fig. 2. Amonafide and N-acetyl-amonafide plasma concentrations (in ng/ml) at 45 min and 24 h after the first dose (first cycle only) by dose level. The height of the solid bar indicates the BIDA value. The NBIDA value is the difference between the top of the solid bar and the top of the striped bar. Total height is the sum of BIDA plus NBIDA. The suggested phenotype, based on both the caffeine assay and NBIDA24 (compared to historical controls; Ref. 14) is indicated as follows: S, slow; F, fast.

Table 1 Plasma concentrations (mean ± SD) of amonafide and N-acetyl-amonafide by acetylator phenotype and dose (for cycle I)

<table>
<thead>
<tr>
<th>Dose</th>
<th>Patients</th>
<th>45 min (ng/ml)</th>
<th>24 h (ng/ml)</th>
<th>BIDA AUC (μg min/ml)</th>
<th>BIDA clearance (ml/min/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>BIDA</td>
<td>NBIDA</td>
<td>BIDA</td>
<td>NBIDA</td>
</tr>
<tr>
<td>Fast</td>
<td>200</td>
<td>7</td>
<td>775 ± 273</td>
<td>510 ± 201</td>
<td>42.1 ± 18.6</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>6</td>
<td>1355 ± 660</td>
<td>555 ± 236</td>
<td>85.5 ± 51.3</td>
</tr>
<tr>
<td>Slow</td>
<td>375</td>
<td>10</td>
<td>2225 ± 1216</td>
<td>498 ± 465</td>
<td>65.7 ± 34.9</td>
</tr>
</tbody>
</table>

* P < 0.005 versus slow acetylators; all other P values > 0.05 (Mann-Whitney).

*The slow acetylator treated at this dose level was excluded, and one other patient's 24-h sample was lost.

We applied this model to our current data set but found it to be biased (mean predictive error ± SE, 1380 ± 485/μl), with a RMSE of 2700/μl, which indicated poor precision. Further analysis of this model demonstrated a significant relationship (for the current data set) between the predictive error and both amonafide dose (r = -0.56, P = 0.005) and caffeine phenotype (P = 0.01, t test).

Since there are significant pharmacokinetic/pharmacodynamic differences between slow and fast acetylators, we defined variables representing the NBIDA24 in slow (NBIDA24S) and fast (NBIDA24F) acetylators, respectively. These new variables defined the interaction between nadir WBC and NBIDA24 by acetylator phenotype. These indicator variables were set equal to either zero or NBIDA24, depending on the phenotype. Similar variables were created for BIDA24. Because exploratory data analysis revealed a possible difference in the relationship of gender to toxicity by phenotype group, we also defined variables for modeling the interaction between toxicity and gender by phenotype (SEXs, SEXf). Each variable was set to 1 for males and 2 for females for the patients with the corresponding acetylator phenotype, and 0 for the other patients. Seven variables, 2 variables each for phenotype interaction with NBIDA24, BIDA24, and gender, plus LOG(WBCP), were utilized for subsequent stepwise multiple regression. The patients from the current study were the training data set for model development, and the patients from our prior study (12) were the test data set. The phenotype was as determined prior to amonafide administration, with exclusion of the 2 indeterminate patients in the current study.

This analysis yielded the following model (F = 19.7; r² = 0.81):

\[
\text{LOG(WBCN)} = 0.360 - 0.0109(\text{NBIDA24}_s - 0.00687(\text{NBIDA24}_f) - 0.444(\text{SEX}_s) + 0.815\text{LOG(WBCP)}
\]
This model indicates that slow acetylators have greater toxicity at any given NBIDA24 than fast acetylators (although the NBIDA24 is much greater in fast acetylators) and that females who are slow acetylators have more toxicity than males who are slow acetylators. The model was validated on the test data set and was unbiased with minimal bias and reproducible precision when validated. The higher coefficient for NBIDA24 relative to NBIDA24 may reflect the greater exposure to parent drug at any particular NBIDA24 level, which is particularly evident in Fig. 2, due in part to the higher doses given to slow acetylators.

It is not entirely clear why measurements of the parent drug concentrations are not in the final model. BIDA24 was not found to be predictive, since it was strongly correlated to NBIDA24, although the BIDA24/NBIDA24 ratio was significantly greater in the slow acetylators (data not shown). It is possible that the sampling times were not optimal since they were derived from a heterogeneous group of fast and slow acetylators. Optimal sampling times may differ between slow and fast acetylators. The apparent importance of gender in the final model cannot be explained but has been reported to affect 6-mercaptopurine pharmacodynamics as well (24, 25). Alternative strategies not utilizing measured plasma concentrations are also feasible and are under development. We constructed a model based on all patients in both training and test data sets and found a similar model to be optimal [NBIDA24, NBIDA24S, LOG(WBCP)]. Gender was not predictive.

As noted, some patients may be misphenotyped with the caffeine assay. We do not have evidence that concurrent medications contributed to the misclassification in this group of patients. It is possible that this affects the pharmacodynamic modeling, although the model was validated on a separate test data set. It is unclear whether other test drugs available for acetylator phenotyping might be superior (26) or whether this represents a divergence between metabolism of caffeine and amonafide. An alternative method of great promise is direct genotyping (27–29), since the molecular mechanism of this polymorphism has recently been elucidated.

Pharmacogenetics is of increasing importance in regard to cancer chemotherapy. Deficiency of dihydropyrimidine dehydrogenase is a rare pharmacogenetic syndrome, resulting in life-threatening and novel toxicity at standard doses of 5-fluorouracil (30–32). There is also genetically determined variability in thiopurine methyltransferase (33), affecting both the efficacy and toxicity of 6-mercaptopurine (34–36). Of great importance are several identified polymorphisms within the cytochrome P450 system, associated with alterations in pharmacokinetics and/or pharmacodynamics of a number of drugs (37). Since the role of this system in metabolism of antineoplastic drugs available for acetylator phenotyping might be superior (26) or whether this represents a divergence between metabolism of caffeine and amonafide. An alternative method of great promise is direct genotyping (27–29), since the molecular mechanism of this polymorphism has recently been elucidated.

ACKNOWLEDGMENTS

We appreciate the data management support of Michael Kut and the preparation of the manuscript by Katherine Grefsheim. We also appreciate the assistance of Michaela Christian, M.D., and Lorraine Cazenave, M.D. (Developmental Chemotherapy Section, Investigational Drug Branch, Cancer Therapy Evaluation Program), in the design and implementation of this study.

REFERENCES


AMONAFIDE AND ACETYLATED PHENOTYPE


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