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/ul. Fast acetylators were treated at both 200 and 250 mg/m², resulting in median WBC nadirs of 5300 and 2000/ul, 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, sex, gender, and pretreatment WBC. We recommend doses of 250 and 375 mg/m² (for 5 days) for further phase II testing of amonafide in fast and slow acetylators, respectively.

INTRODUCTION

Amonafide (NSC 308847, benzisquinoxilinedione, nafidime) 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 both in 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.
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 to 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/ul. In the remaining nine slow acetylators, the median WBC nadir was 1600/ul, and the median ANC nadir was 740/ul. 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/ul).

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/ul). In the remaining 6 patients, the median WBC nadir was 2000/ul and the median ANC nadir was 1040/ul. Furthermore, there was grade 3–4 thrombocytopenia in 4 patients (2 patients with grade 4) with a median platelet nadir of 53,000/ul. 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/ul; median ANC nadir, 3500/ul). Four patients were subsequently dose-escalated to 250 mg/m² with acceptable toxicity (median nadirs for cycle 2: WBC, 3000/ul; ANC, 2190/ul; platelets, 160,000/ul). 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 misclassified 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:

\[
\text{LOG(WBCN)} = 0.0579 - 0.00887(\text{NBIDA24}) + 1.20 \text{LOG(WBCP)} - 0.0197(\text{AGE})
\]
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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>Fast</td>
<td>7</td>
<td>510 ± 201</td>
<td>382 ± 122d</td>
<td>564 ± 149</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>6</td>
<td>1555 ± 660</td>
<td>132.3 ± 91.0</td>
<td>375 ± 137</td>
</tr>
<tr>
<td></td>
<td>375</td>
<td>10</td>
<td>2225 ± 1216</td>
<td>51.4 ± 42.7</td>
<td>492 ± 199</td>
</tr>
<tr>
<td>Slow</td>
<td>200</td>
<td>7</td>
<td>775 ± 273a</td>
<td>42.1 ± 18.6</td>
<td>85.5 ± 51.3</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>6</td>
<td>1355 ± 660</td>
<td>56.6 ± 18.6</td>
<td>731 ± 309</td>
</tr>
<tr>
<td></td>
<td>375</td>
<td>10</td>
<td>2225 ± 1216</td>
<td>375 ± 137</td>
<td>375 ± 137</td>
</tr>
</tbody>
</table>

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

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):

\[
\text{LOG}(\text{WBCN}) = 0.360 - 0.0109(\text{NBIDA24}_s - \text{NBIDA24}_f) - 0.444(\text{SEX}_s) + 0.815\text{LOG}(\text{WBCP})
\]
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 comparable accuracy to the training data set (Fig. 3). The model displayed comparable precision (based on RMSE) when applied to either dataset (training, 1640/μl; test, 1800/μl). The variation, although sizable, is still an improvement over the variation in toxicity observed when a standard dose (300 mg/m²) was administered (12, 14).

A stepwise regression on 7 main effects only [phenotype, NBIDA24, BIDA24, NBIDA45, BIDA45, sex, and LOG(WBCP)] produced a similar model that included phenotype, sex, LOG(WBCP), and NBIDA24.

**DISCUSSION**

This study demonstrates that acetylator phenotyping based on urinary concentrations of caffeine metabolites (13) can be used to guide dosing of amonafide. Although 300 mg/m² administered daily for 5 days was used for the vast majority of phase II trials of this agent, this appears to be the wrong dose for all patients, too high for fast acetylators and too low for slow acetylators. Thus, the conclusions of prior phase II studies (17–20) may be questioned on the basis of inadequate dosing.

At this time, we would recommend doses of 250 and 375 mg/m² for fast and slow acetylators, respectively. These dose recommendations are subject to all the concerns of any phase I study based on relatively few patients per cohort. Patients who cannot be phenotyped, due to either indeterminate status or lack of access to an assay, should be treated initially at 250 mg/m² with dose escalation to 375 mg/m² in the absence of hematological toxicity. Asian subjects, in particular, should be treated at 250 mg/m² or less, since fast acetylators comprise approximately 90% of this population (21). Other non-Caucasian subjects may also be at greater risk for amonafide toxicity, as based on both a preliminary analysis of a Cancer and Leukemia Group B trial in metastatic breast cancer (14) and the noted higher proportion of fast acetylators among African-American children (22). Since there is still significant interpatient variability in toxicity at the recommended phase II doses, future studies might incorporate pharmacodynamic modeling to guide dosing, as previously utilized for etoposide (23, 24).

The model defined in this report is potentially useful in guiding dosing since it incorporates only the acetylator phenotype, pretreatment WBC, gender, and one measured plasma concentration (N-acetyl-amonafide) prior to the second dose. The importance of both acetylator phenotype and pretreatment WBC was expected based on our previous study (12), although the prior model appears to have been biased. By using separate covariates for the NBIDA24 in each phenotype group, there was minimal bias and reproducible precision when validated. The higher coefficient for NBIDA24S relative to NBIDA24F 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 [NBIDA24s, 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 agents has not been well studied, this may be a particularly fruitful area for future investigation.

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**REFERENCES**


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