A Collaborative, Double-Blind Randomized Study of Cetiedil Citrate in Sickle Cell Crisis

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We have recently completed a double-blind, placebo-controlled, noncrossover study, the goal of which was to determine whether cetiedil citrate (cetiedil) could affect the course of vaso-occlusive crises in sickle cell disease. Patients, who presented to the emergency room at least 4 but no more than 24 hours after the onset of a painful vasoocclusive crisis severe enough to require hospitalization, were considered candidates for the study. Each patient received either placebo or cetiedil at one of the following three dosages: 0.2, 0.3, or 0.4 mg/kg body weight. The assigned drug dosage was given as a 30 minute intravenous infusion every 8 hours for 4 consecutive days. A total of 67 patients was enrolled in the study. Cetiedil, at its highest dosage (0.4 mg/kg body weight), was found to be significantly superior to placebo both in reducing the number of painful sites present on all 4 treatment days and in shortening the total time in crisis. No serious adverse reactions were observed during the course of the study. We conclude that cetiedil, given at a dosage of 0.4 mg/kg body weight, is therapeutically advantageous for sickle cell crisis.

AMONG the most troublesome sequelae of the substitution of valine for glutamic acid at the sixth position of the beta chain of the hemoglobin molecule are recurrent vaso-occlusive crises.1,2 To date, no specific treatment that will terminate or prevent these episodes is available.3,4 Urea and sodium cyanate showed promise during initial in vitro studies but were found to be either ineffective or toxic in subsequent clinical testing.5-11 A wide variety of other agents has also been proposed, but none has as yet been proven effective in controlled clinical trials.12-14

Cetiedil citrate (cetiedil), an iminoester developed from 3-thienyl acetic acid, was originally used as a vasodilator for peripheral vascular diseases such as Raynaud's phenomenon and intermittent claudication.15-17 Cabannes reported that cetiedil, when administered intravenously on an open basis, shortened the duration of acute sickle cell crisis.18 Further interest in cetiedil as a potential therapeutic agent in sickle cell anemia came from the observation by several groups in the United States that the drug exhibited in vitro antisickling activity at micromolar concentrations.19,22 This laboratory evidence, along with the Cabannes experience and a favorable safety profile, served as the impetus to perform a controlled, double-blind, randomized trial to test the safety and efficacy of cetiedil in the treatment of acute painful crisis in sickle cell anemia.

MATERIALS AND METHODS

Patients. Patients with a known history of sickle cell disease, either sickle cell anemia (SS), sickle cell-beta Thalassemia (SB\textsuperscript{Thal}, SB\textsuperscript{Thal}), or sickle cell-hemoglobin C disease (SC), entered into the study. Hemoglobinopathies were confirmed by electrophoresis at a central laboratory. In this study, sickle cell crisis was defined as the sudden onset of pain involving one or more sites (extremities, back, abdomen, chest) typical of the patient's usual crisis for which there was no other explanation. Atypical isolated chest or abdominal pain was not considered to be crisis pain. Each patient was 19 years of age or older, capable of giving informed consent, and in sickle cell crisis for at least four hours, but not longer than 24 hours. Admission to the study was limited to one crisis per patient.

Criteria for exclusion were pregnancy, women of childbearing potential, incarcerated persons, patients with a history of drug abuse or drug dependency, transfusion within 90 days preceding the trial, acute cerebrovascular accident, overt infection, renal failure with a serum creatinine concentration greater than 2 mg percent (0.18 mmol/L), clinical or roentgenographic evidence of pulmonary edema, glaucoma, urinary retention, or a history of high sensitivity to anticholinergic or atropine-like drugs.

All studies were approved by the appropriate institutional review process and informed written consent was obtained from each patient. Whenever possible, this consent was obtained prior to the onset of the studied crisis.

Study design. The study was a double-blind, multi-investigator trial conducted at five clinical centers. Patients were randomly assigned at a central data center to treatment with either placebo (0.9 mg/mL, normal saline) or cetiedil (0.2 mg/kg, 0.3 mg/kg, or 0.4 mg/kg body weight) in normal saline. Drug, active or placebo, was presented in identically appearing ampules. The randomization plan specified a coded ampule and a volume (dosage) for each patient. The investigators were aware of the volume of medication but were not aware of the ampule's contents. Each patient received an IV infusion of study medication every eight hours over a four-day treatment period for a total of 12 doses. Fluid replacement and analgesic therapy were standardized. Choice of analgesics was limited to parenteral meperidine hydrochloride (1.2 mg/kg) or the equivalent of morphine sulfate for severe pain, and oral acetaminophen (300 mg) with codeine phosphate (30 mg) for moderate pain. Analgesia was not given within two hours before or after the beginning of medication infusion.

Determinants of efficacy and safety. Effectiveness of the study medication was examined by assessing pain intensity, number of
painful sites, duration of crisis, the need for analgesic medication, and the investigator's overall evaluation of the patient's response to study medication.

For each patient, pain location and intensity were assessed and recorded on a modification of the standardized form utilized in earlier studies. The scale for assessment of intensity was 0 = no pain; 0.5 = residual tenderness or soreness; 1 = mild, movement does not increase pain; 2 = moderate, movement increases pain; 3 = severe, cannot move portions of body. Pain was evaluated by investigators and research nurses trained in assessing pain, just prior to therapy and at 1, 2, 4, and 6 hours after dosing. For pain intensity and number of painful sites, an average value was computed for each day of the study. Changes from baseline were analyzed for each treatment group, and each active drug group was compared to placebo.

Duration of crisis in this study refers to the time from the initiation of drug administration to the end of crisis. The sickle cell crisis was considered terminated when three of the following four criteria were satisfied: (1) absence of fever for at least 8 hours; (2) no progression of painful symptoms with absence of need for parenteral analgesics for at least 8 hours; (3) ability to walk without apparent difficulty; and (4) disappearance of pain symptoms and reduction of pain rating to ≤1 as evaluated on the scale as described above.

The average time in crisis for each of the three active drug groups was compared with that of the placebo group. Between-group differences in the proportion of patients requiring analgesic medication and the number of analgesic administrations were also examined.

To evaluate safety, clinically observed adverse reactions were recorded with regard to severity, onset, date, duration, frequency, drug relationship, and action taken. At the same time intervals as for pain assessment, vital signs (pulse, blood pressure, temperature, and respiration) were recorded. On days 1, 2, 3, 4, and 5 of the study and at the time of discharge, hemoglobin, hematocrit, complete blood count with differential, red cell indices, platelets, reticulocyte counts, prothrombin time, and fibrinogen levels were done. On days 1, 5, and day at discharge, electrocardiogram and additional laboratory tests, including fasting blood chemistries (urea nitrogen, glucose, SGOT, SGPT, alkaline phosphatase, uric acid, albumin, creatinine, total and direct bilirubin, cholesterol, triglycerides) and urinalysis, were performed. Finally, in each case an overall evaluation of the patient's response to study medication, taking into account both efficacy and toxicity, was performed by the investigators and rated as excellent, good, fair, or poor. A summary of the patient's course was also recorded. Patients were retained in the study for a minimum of 7 days.

Statistical methods. Continuous variables for patient characteristics and details of therapy were analyzed using a two-way analysis of variance. Discrete variables in these categories were analyzed using the Chi-square test or, for ranked data, the Kruskal-Wallis analysis of variance on ranks. For crisis evaluation parameters, a two-way analysis of covariance was performed, using baseline as a covariate. Pairwise comparisons using t tests on adjusted treatment means were also made. For the variables, number of painful sites and pain intensity, a slope was calculated for each patient's collection of data points. Using the least squares regression technique, a two-way analysis of variance was performed on the slopes of the summary statistics. Using PIR multiple linear regression analysis program of the BMD-P statistical software (1983 edition), analysis of variance was performed on the slopes that were determined for all patients at each time point of the raw data. All within-treatment comparisons were accomplished by a paired t test on change from baseline. Global or overall evaluation and use of analgesic medication were analyzed by a two-way analysis of variance on ranks. The Mantel-Haenszel procedure was used for comparisons of active drug to placebo for overall evaluations. For analgesic medication, comparisons were made using t tests on adjusted mean ranks.

Treatment by investigator interaction was evaluated for all parameters using a two-way analysis of variance. Since investigators did not know whether active drug or placebo was being administered to a given patient, but were aware of the volume of study medication given, study drug volume was included as a covariate in the statistical analysis of efficacy. It was subsequently determined that drug volume was not a significant source of variation in any of the analyses of key efficacy determinants, including number of painful sites, crisis duration, and global evaluation.

RESULTS

Sixty-seven patients were entered in the trial. Sixty-three patients were included in the analysis of efficacy. Four patients were found retrospectively to have high hemoglobin A concentration at baseline due to recent transfusions and were excluded. All 67 patients were included in the safety analysis. Baseline data for the 63 patients evaluated for efficacy are shown in Table 1. Fifty-seven per cent were male, 67% were between the ages of 20 and 29, and 87% had sickle cell anemia (SS). Statistical analysis revealed no significant differences among treatment groups for any of the baseline variables including pain intensity and number of painful sites.

Summary data for the change in average number of
painful sites during the study are presented in Fig 1. The paired t within-group analyses of change from baseline documented significant improvement from baseline on all four days for both the middle dose (0.3 mg/kg) and high dose (0.4 mg/kg) of cetiedil (P ≤ 0.02), while improvement from baseline in the low dose (0.2 mg/kg) and placebo groups was significant only on day 4. When treatment groups were compared to placebo (utilizing the baseline score as a covariate), only the high dose of active drug, cetiedil 0.4 mg/kg, was significantly superior (P < 0.05) on all four treatment days. These results for the 0.4 mg/kg dose compared with placebo were corroborated by analysis of the slopes calculated by using either the summary statistics for each patient (P < 0.05) or the raw data for each patient at each time point (P < 0.001).

In Fig 2, the influence of cetiedil on average pain intensity is seen. For within-group analysis of average pain, only the placebo group was not significantly improved on day 1: all treatments showed significant improvement on each day thereafter (P < 0.02). Comparisons of active drug to placebo (using baseline score as a covariate) indicated improvement in average pain intensity of statistical significance for the 0.3 mg/kg dose group on days 1 and 2 (P = 0.02). Cetiedil was not significantly superior to placebo at any doses upon
analysis of the slopes of summary statistics for each patient. Analysis of the slopes calculated by multiple linear regression analysis of raw data for each patient at each time point indicated improvement in pain intensity by the 0.4 mg/kg dose when compared with placebo ($P < 0.01$).

Figure 3 shows results of statistical comparisons between cetiedil and placebo for duration of crisis (time from first administration of study medication to end of crisis). Cetiedil dosages of 0.3 mg/kg and 0.4 mg/kg were significantly superior to placebo ($P < 0.05$). There was no significant correlation between the degree of shortening of crisis after administration of study medication and the length of time in crisis within the 4 to 24 hour time period prior to treatment.

No significant differences between any of the cetiedil groups and placebo could be detected in either the proportion of patients requiring parenteral analgesic medication or the total number of doses given. Meperidine was the agent used greater than 95% of the time.

No serious adverse reactions were observed. The incidence of adverse effects (Table 2) was similar for the cetiedil 0.3 mg/kg, cetiedil 0.4 mg/kg, and placebo groups, and significantly lower ($P < 0.04$) for the cetiedil 0.2 mg/kg group. The most commonly reported adverse effects were headache and nausea/vomiting, for which no treatment or dose relationship was apparent, and dry mouth, which was noted only in the middle- and high-dose cetiedil groups.

Few laboratory variables were significantly different with cetiedil treatment when compared with placebo. The red blood cell count and hematocrit decreased significantly from baseline ($P < 0.05$) with all treatment regimens except the 0.4 mg/kg cetiedil dose. Analyses of electrocardiograms revealed a statistically but not clinically significant difference in the P-R interval between the middle-dose cetiedil group and placebo on day 5 but the effect was not dose-related since it was not apparent in the higher dose group.

Taking into account both efficacy and safety considerations, investigators rated the patient’s overall response to treatment as excellent, good, fair, or poor (Table 3). The difference in the mean scores between the high dose of cetiedil and placebo was significant ($P < 0.01$). Excellent or good responses were recorded for 85% of patients who received cetiedil 0.4 mg/kg, 56% of patients who received cetiedil 0.3 mg/kg, 56% of patients who received cetiedil 0.2 mg/kg, and 25% of patients who received placebo. No

### Table 2. Adverse Reactions by Treatment Group

<table>
<thead>
<tr>
<th>Adverse Reactions</th>
<th>Placebo</th>
<th>Cetiedil 0.2 mg/kg</th>
<th>Cetiedil 0.3 mg/kg</th>
<th>Cetiedil 0.4 mg/kg</th>
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<tr>
<td>Dry mouth</td>
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<td>Nausea</td>
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<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Vomiting</td>
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<td>2</td>
<td>2</td>
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<td>Constipation</td>
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<td>1</td>
<td>1</td>
<td>1</td>
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### Table 3. Overall Evaluation Distribution by Treatment

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<th>Study Drug</th>
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<th>Fair</th>
<th>Poor</th>
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<th>Mean*</th>
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<tbody>
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<td>Placebo</td>
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<td>8</td>
<td>4</td>
<td>16</td>
<td>2.13</td>
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<tr>
<td>Cetiedil 0.2 mg/kg</td>
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<td>3</td>
<td>4</td>
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<td>2.56</td>
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<tr>
<td>Cetiedil 0.3 mg/kg</td>
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<td>4</td>
<td>5</td>
<td>3</td>
<td>18</td>
<td>2.72</td>
</tr>
<tr>
<td>Cetiedil 0.4 mg/kg</td>
<td>7</td>
<td>4</td>
<td>2</td>
<td>0</td>
<td>13</td>
<td>3.38†</td>
</tr>
</tbody>
</table>

*Scale for means: 4, Excellent; 3, Good; 2, Fair; 1, Poor.
†Cetiedil 0.4 mg/kg was significantly superior to placebo ($P < 0.01$).
significant treatment by investigator interactions was noted in the efficacy and safety analyses.

**DISCUSSION**

This investigation demonstrates a measurable improvement in the course of the vaso-occlusive crisis in patients treated with cetiedil. Compared to placebo, the highest dose of cetiedil tested (0.4 mg/kg total body weight) was associated with significant reduction in the number of painful sites on all treatment days and a shortening of the time in crisis after receiving the study medication. The investigators’ global evaluations were excellent or good in 85% of the patients receiving the highest dosage of cetiedil, compared with 25% of the patients receiving placebo.

The safety of the intravenous administration of cetiedil was also demonstrated in this study, as only minimal side effects were observed. The only side effect that appeared to have a dose-dependent relationship was dry mouth, an expected result because of cetiedil’s known anticholinergic activity. In order to determine whether dry mouth might have unblinded the study, the data were reanalyzed. It appears that this side effect had no such influence with respect to reduction in painful sites and duration of crisis. The overall or global evaluation in the high-dose cetiedil groups was better for those with, than without, dry mouth. However, the overall evaluations for the patients who received active drug but did not have dry mouth remained significantly superior to those of placebo patients.

The results of this study and in vitro studies with cetiedil are noteworthy for two principal reasons. This study demonstrates the drug’s potential usefulness as an effective and safe therapy for the painful crisis of sickle cell disease. A beneficial effect, occurring after the initiation of vaso-occlusion, has not been noted in controlled double-blind antisickling trials of other agents.

Second, in vitro studies with cetiedil suggest that factors involved in the pathogenesis of painful crisis can be influenced by approaches independent of modification of the abnormal hemoglobin molecule. Unlike cyanate or urea, cetiedil does not increase the solubility or oxygen affinity of hemoglobin. Recent studies have suggested that cetiedil’s antisickling activity might be due to the ability of the drug to alter erythrocyte membrane permeability. Sickling is influenced by the permeability of the red cell membrane primarily because the rate of hemoglobin S gelation is enhanced by cell dehydration and slowed by an increase in cell water content. Calcium-induced potassium loss and cell dehydration, which have been observed in very dense sickle cells, are inhibited by cetiedil. Alternatively, the drug has been shown to enhance passive sodium permeability, thus causing cell swelling. Other possible contributing factors to cetiedil’s in vivo activity include such pharmacologic actions as peripheral vasodilation, calmodulin inhibition, reduction in plasma fibrinogen concentration and blood viscosity, and inhibition of platelet aggregation.

At present, the use of cetiedil citrate for treatment of vaso-occlusive crises in sickle cell anemia is restricted to investigations like the one described in this paper. Data from this study, coupled with the ability of cetiedil to inhibit sickling in vitro, serve as a rationale to continue investigations of cetiedil as a treatment in sickle cell disease. The importance of incorporating the rigorous methodology of this study with objective criteria for evaluation in future trials cannot be overemphasized. Only if subsequent trials confirm the findings in this report, should cetiedil be made available to practicing physicians for management of vaso-occlusive crises in sickle cell anemia.

**ACKNOWLEDGMENT**

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