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Report

Multiple-Dose Pharmacokinetics of Clozapine in Patients

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After a 2-day buildup, patients were dosed continuously with clozapine solution at three ascending dose levels (37.5, 75, and 150 mg bid for 7 days at each dose level). Following the morning administration on the twenty-third day of dosing a drug holiday was instituted which lasted for a minimum of 48 hr. Serial plasma samples were obtained during each of the periods and during the drug holiday for the calculation of the steady-state parameters AUC^{SS}, C^{SS}_{max}, and C^{SS}_{min} at each dose level as well as for the assessment of the terminal elimination rate. Mean parameter values for AUC^{SS}, C^{SS}_{max}, and C^{SS}_{min} showed a linearly increasing response with the dose, well described by a straight line passing through the origin. The terminal elimination appeared to follow linear kinetics and had a mean half-life of 15.8 hr (range, 5.8–33 hr).

KEY WORDS: clozapine; pharmacokinetics; multiple-dose regimen.

INTRODUCTION

Clozapine (Clozaril) is a tricyclic dibenzodiazepine neuroleptic. It has been shown to be extensively metabolized (1,2) prior to excretion, but the pharmacokinetics of the unchanged drug and metabolites have not received much attention. Two papers have reported a positive correlation between the total daily dose and the daily minimum plasma concentrations (3,4). However, these studies made comparisons across patient groups and no kinetic analyses were attempted.

The present study was designed to assess the pharmacokinetics at steady state and the area–dose relationship in a patient population. Patients were dosed at three different dose levels using a multiple-dose (bid q 12 hr) regimen. Each patient served as his own control in this sequential (nonrandom) study design.

MATERIALS AND METHODS

Patients were entered into the study at two separate study sites. Seven patients completed the study at center 1 (New York) and six completed it at center 2 (Augusta). For statistical analysis, data from both centers were pooled. Patients completing the study were males between 18 and 55 years of age and diagnosed as schizophrenics. Their average age was 34.2 years (SD, 6.8 years), their average height was 176 cm (SD, 8.6 cm), and their average weight was 78 kg (SD, 11.7 kg). Patients were not entered into the study unless they were physically healthy as judged by medical history, physical examination, and clinical laboratory tests. All patients gave written consent after being advised of the nature and risks of the study.

This three-period open-label study employed a sequential design which called for each patient to be stabilized on clozapine therapy prior to testing three dose regimens (37.5, 75, and 150 mg bid q 12 hr) in a sequential dose-rising manner. Dosage buildup and stabilization consisted of a single-37.5 mg dose on day 6 and 37.5 mg bid q 12 hr (7 AM, 7 PM) on day 7. Thereafter dosing was bid q 12 hr (7 AM, 7 PM), throughout until day 7 of the final (third) period. In period 1 the dose regimen was 37.5 mg bid; in period 2, 75 mg bid; and in period 3, 150 mg bid. Dosing was discontinued after the first dose on day 7 of period 3 (two subjects were accidentally dosed also at 7 PM on this final study day). Serial plasma samples were collected on day 7 in each period to assess the area under the concentration–time curve at each dose level, and samples were collected during the drug holiday at the end of period 3 to measure the elimination rate of unchanged drug from plasma. Sequential minimum (predose, trough) plasma samples were collected on days 6 and 7 in each study period as a means to evaluate the steady-state condition.

Clozapine plasma concentrations were determined using a specific high-performance liquid chromatographic (HPLC) method. The method is a modification of a previously developed proprietary procedure (H. Holz and J. Meier, Internal Communication, Sandoz LTD, Basel). The method was validated in terms of linearity, precision (±6.7%), and reproducibility (±7.6%) over the range 0–10,000 ng/ml. The minimum limit of detection was set at 3 ng/ml based upon the precision and accuracy in determina-

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tions of prepared plasma samples in the range 0–10 ng/ml. The assay procedure was as follows: 1 ml of plasma in a glass centrifuge tube (45 ml) was alkalinized with 0.5 ml of 2 *N* sodium hydroxide. To this solution was added 6 ml of methyl-t-butyl ether. The tube was stoppered (glass) and shaken for 15 min in a platform shaker (Eberbach, 120 cycles/sec, with all tubes sitting at a 20° incline to the plane of shaking). The tube was then centrifuged for 5 min at ~2000 rpm (IEC 253 with swinging bucket head). After centrifugation, 5 ml of the upper phase was transferred by pipette to another centrifuge tube and evaporated to dryness under vacuum (water aspirator) in a vortex evaporator (Buchler, heating block at 50°C). The sample was reconstituted with 500 μl of chromatographic mobile phase (methylene chloride–methanol–triethylamine; 97.5:2.5:0.003, v/v/v) and separated from endogenous material on a silica column (150 μl on column injection, Spherisorb 5 μm, 250 × 4.6 mm) thermostated (Perkin–Elmer LC-100) at 50°C. Clozapine was detected in the column effluent by monitoring uv absorption at 260 nm (Perkin–Elmer LC-75).

The parameters for analysis were calculated from the raw clozapine plasma concentration data and from the fit of a two-compartment pharmacokinetic model (see below) to the raw data.

In each 12-hr dosing interval serial plasma concentrations obtained just prior to and at 0.5, 1, 2, 4, 6, 9, and 12 hr after the dose were used to calculate the $AUC^{SS}_{0-12\ hr}$ (addition of the trapezoids formed by the individual concentration/time points), C^{SS}_{max} (highest plasma concentration within the dosing interval), C^{SS}_{min} (average of the 0- and 12-hr concentrations), and t_{max} (the time within the dosing interval at which the maximum plasma concentration was observed).

The clozapine plasma concentration data were fit with a triexponential (two-compartment, first-order absorption) linear model:

$$C(t) = Ae^{-m_1 t} + Be^{-m_2 t} + Ce^{-m_3 t}$$

where

$$A = \frac{Fm_1X_0\{k_{21} - m_1\}}{V\{m_2 - m_1\}\{m_3 - m_1\}}$$

$$B = \frac{Fm_1X_0\{k_{21} - m_2\}}{V\{m_1 - m_2\}\{m_3 - m_2\}}$$

$$C = -(A - B)$$

Curve fitting was performed with the nonlinear regression program (NONLIN) of Metzler (5). The plasma concentrations in each study period were fitted simultaneously with a function that represented a summation of the individual doses (superposition) up to and including the dose administered just prior to the taking of the respective serial plasma samples. The appropriate dose level (X_0, as μg) was input for each study period. The macroconstants m_2 and m_3, the rate constant for transfer from the peripheral to the central compartment (k_{21}), and the volume of distribution (V) were assumed constant across study periods. Within each period the apparent absorption rate (m_1) and the relative bioavailability, as defined by a variable F, were allowed to vary (in reference period 3 it was assumed for simplicity that $F = 1$).

Analysis of variance was performed for purposes of comparison of the three dose levels. The data were analyzed statistically as a two-factor experiment having repeated measurements on the same subject (6). Statistical significance was declared if $P < 0.05$, with all tests being two tailed. A linear regression line was fitted to the available C^{SS}_{min} values for each subject in each period (−24-, 0-, 12-, and 24-hr values in periods 1 and 2; −24-, 0-, and 12-hr values in period 3). The resulting slopes were tested to see if they differed significantly from zero (steady-state condition). Linear regression was also used to establish the relationship of dose for the bioavailability parameters AUC^{SS}, C^{SS}_{max}, and C^{SS}_{min}. The appropriateness of the linear model was assessed by a test for "lack of fit" (7) in conjunction with the test of a slope different from zero.

RESULTS

The mean steady-state plasma data, displayed in Fig. 1, showed a regular increase in systemic plasma concentrations of clozapine with each increase in daily dose level. The parameters $AUC^{SS}_{0-12\ hr}$, C^{SS}_{max}, and C^{SS}_{min} (Table I) demonstrate an ordered response to the dose. Their dose relationships are well described by a straight line which passes through

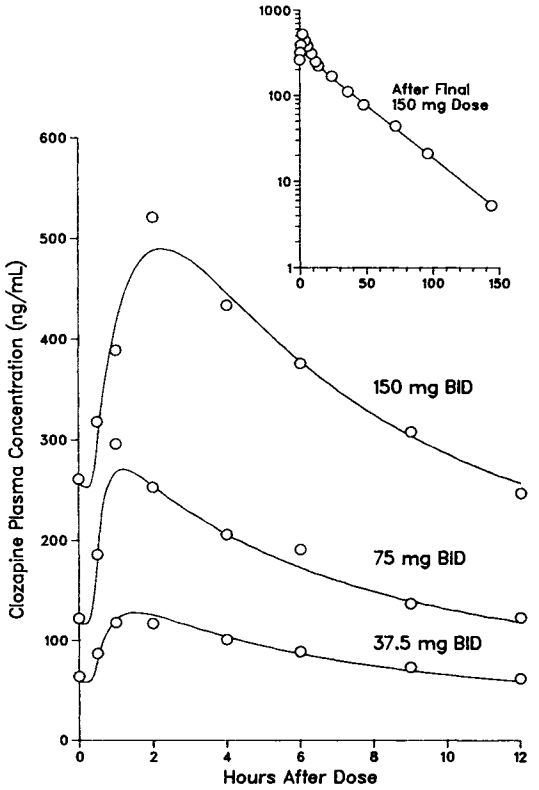

Fig. 1. Steady-state plasma concentrations of clozapine after continuous dosing with clozapine solution at (bottom) 37.5 mg bid, (middle) 75 mg bid, and (top) 150 mg bid. The open circles represent the mean of individual plasma concentrations at each time point; the solid lines represent a fit of the multiple-dosing function of the mean values. Inset: Mean clozapine plasma concentrations at the end of the study, showing the results after the final 150-mg dose through the drug holiday. The open circles represent the mean of the individual data ($N = 11$) and the solid line represents theoretical levels from the curve-fit analysis.

Table I. Pharmacokinetic Parameters

Parameter	Arithmetic mean ± SD (range)		
	37.5 mg bid	75 mg bid	150 mg bid
$AUC^{SS}_{0-12\ hr}$ (ng *hr/ml)	1048 ± 887 (214–2944)	2185 ± 1789 (770–7180)	4429 ± 3163 (1784–12712)
C^{SS}_{max} (ng/ml)	138 ± 82.4 (49.8–304)	314 ± 190 (119–770)	564 ± 307 (277–1300)
C^{SS}_{min} (ng/ml)	60.4 ± 64.8 (10.6–229)	117 ± 127 (25.6–484)	249 ± 246 (66.6–959)
t_{max} (hr)	1.69 ± 1.42 (0.50–6.00)	1.08 ± 0.45 (0.50–2.00)	1.88 ± 0.82 (0.50–4.00)

the origin, based upon highly significant results for tests of slope greater than zero and nonsignificant results for lack-of-fit tests. The individual results showed a positive ordered dose response in nearly every case (13 of 13 for AUC^{SS}, 13 of 13 for C^{SS}_{min}, and 12 of 13 for C^{SS}_{max}). For the parameter t_{max} there was no clear relationship to the dose.

Descriptive statistics for the different parameters are summarized in Table I. Intersubject variability for each parameter was large. For AUC^{SS} the coefficient of variation (%CV) about the means ranged from 71 to 85% across dosing levels, for C^{SS}_{max} the %CV ranged from 54 to 60%, and for C^{SS}_{min} the %CV ranged from 99 to 109%.

The differences between means for the parameters AUC^{SS}, C^{SS}_{max}, and C^{SS}_{min} in the three pairwise comparisons (37.5 vs 75, 37.5 vs 150, and 75 vs 150 mg) were statistically significant ($P < 0.05$) as determined by analysis of variance. The inset in Fig. 1, a plot of the mean plasma concentration data from the 11 patients who entered the drug holiday on the morning of day 7 in period 3, shows that elimination of clozapine after multiple dosing occurs with apparent linear kinetics (as noted above under Materials and Methods, two patients were accidently dosed at 12 hr into the drug holiday and therefore their data have not been included for Fig. 1). Elimination plots for each individual (not shown) were consistent with this hypothesis [for the individual curve-fit analyses the goodness of fit (r^2) ranged from 0.93 to 0.99, with an average of 0.98]. Individual terminal elimination half-lives (taken from the curve-fitting procedure) ranged from 5.8 to 33 hr, with a geometric mean of 15.8 hr. Individual distribution phase half-lives ranged from 0.78 to 4.9 hr, with a geometric mean of 2.0 hr.

Table II summarizes the calculated pharmacokinetic parameters. Both the bioavailability relative to the 150-mg dose level (F) and the plasma clearance (CL) were constant throughout the study. There were no consistent trends in the individual data which would suggest that either parameter was changing through the study (i.e., as the plasma concentrations increased). The apparent first-order rate constant (m_1) decreased as the dose level increased, but the limited number of data points (typically 0.5, 1, and 2 hr) defining the absorptive phase does not allow much confidence in the "best fit" for this parameter.

The measurement of consecutive predose (C^{SS}_{min}) plasma concentrations is presented as the mean of individual data in Table III. Based upon the measured plasma elimination half-life (above), each patient should have attained steady-state plasma concentrations after 6 days of dosing at each dose level. This is supported by the consecutive C^{SS}_{min} measurements, which indicate no apparent trends in the means. Analysis of the individual results showed that in no case were the slopes of the regression lines (based upon consecutive C^{SS}_{min} values) significantly different from zero (steady-state condition).

DISCUSSION

The results from this study establish the linear dose–proportionality relationship at steady state between clozapine plasma concentrations and the administered dose. These data are consistent with results from previous studies in which the daily minimum plasma concentrations were re-

Table II. Pharmacokinetic Parameters

Parameter	Arithmetic mean ± SD, geometric mean (range)		
m_2 (hr^{-1})	0.40 ± 0.23 0.34 (0.14–0.89)		
m_3 (hr^{-1})	0.050 ± 0.027 0.044 (0.021–0.119)		
$t_{1/2}$ (m_3) (hr)	17.4 ± 7.7 15.8 (5.84–33.2)		
V (liters/kg)	5.14 ± 2.33 4.65 (2.20–10.2)		
	37.5 mg bid	75 mg bid	150 mg bid
m_1 (hr^{-1})	15.0 ± 22.6 4.91 (0.39–81.5)	10.5 ± 15.0 5.64 (1.34–54.5)	3.10 ± 3.96 1.55 (0.52–11.1)
F	0.97 ± 0.34 0.91 (0.48–1.65)	0.98 ± 0.23 0.96 (0.63–1.44)	—[a]
CL (liters/hr)	57.5 ± 31.7 47.0 (12.4–113)	53.3 ± 29.3 44.7 (11.0–105)	50.0 ± 26.3 42.7 (12.5–84.3)

[a] By definition $F = 1.0$ at the 150-mg bid dose level.

Table III. Steady-State Minimum Plasma Concentrations

Dose level (mg)	Average C_{min}^{SS} value (ng/ml)[a]			
	−24 hr	0 hr	12 hr	24 hr
37.5	67.5	61.1	59.7	68.6
75	127	118	117	127
150	269	255	244	—

[a] Sampling times referenced to predose (0 hr) on day 7 of each study period.

lated to the dose, with ng/ml/mg dosed/kg body wt calculated to be in the range 55–90 (3,4). In the present study this relationship is estimated to be ~80 ng/ml/mg dosed/kg body wt. The broad range in concentration–dose response observed in earlier studies reflects the intersubject variability inherent in the steady-state clozapine plasma concentrations (above and Ref. 3).

Within each subject the response to dose was predictable (see Fig. 1). Using the final period as a standard it was calculated that greater than 60% of the individuals at the low-dose level (37.5 mg) and greater than 75% of the individuals at the mid-dose level (75 mg) had a relative bioavailability within 25% of this reference.

The dose–proportionality of clozapine plasma concentrations observed here strongly suggests linear steady-state pharmacokinetics over the range of plasma concentrations studied (10.6–1300 ng/ml). The determinations made during the drug holiday at the conclusion of the study, when levels dropped to or below the detection limit of the analytical method (3 ng/ml), provide evidence that the elimination kinetics remain linear over an approximately thousandfold range of plasma concentrations (see inset, Fig. 1). The plasma level data from this patient population were well fitted using a triexponential model, resulting in individual terminal elimination half-lives which ranged from 5.8 to 33 hr (geometric mean, 15.8 hr). Calculating on the mean data projects the attainment of ~97% of the theoretical steady-state plasma concentrations [$F^{ss} = (1 - e^{-nm_3\tau})$] after approximately 7 days or less.

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