Pharmaceutical Research, Vol. 7, No. 4, 1990

Report

Single- vs Multiple-Dose Pharmacokinetics of Clozapine in Psychiatric Patients

Miles G. Choc,[1,2] Francis Hsuan,[3] Gilbert Honigfeld,[1] William T. Robinson,[1] Larry Ereshefsky,[4,5,8] Miles L. Crismon,[5] Stephen R. Saklad,[4,5,8] Jack Hirschowitz,[6] and Richard Wagner[7]

Received May 18, 1989; accepted September 25, 1989

Clozapine plasma levels were monitored in 16 patients during a series of three consecutive treatments (single dose–multiple dose–single dose). Each patient received a single 75-mg dose (3 × 25 mg) with clozapine tablets, and serial plasma samples were collected over 48 hr after the dose. At 48 hr, a multiple-dose regimen was started, consisting of an initial dose escalation period followed by dosing at a constant regimen for at least 6 days. After the last dose, serial plasma samples were again obtained over 72 hr. Drug was then withheld for at least 7 days, a final single 75-mg dose was given, and plasma sampling was repeated. A subset of the patient population ($N = 7$) was used to test for a food effect during the single-dose treatments. The pharmacokinetic parameters between the initial and the final single dose periods were not significantly different. Similarly, there were no differences within patients when given the dose after fasting (fed 1 hr after dose) or with a meal. In contrast, the terminal elimination rate differed between the single-dose and the multiple-dose treatments ($t_{1/2}^{m3} = 7.9$ hr single dose and 14.2 hr multiple dose) ($P < 0.05$) and the dose-normalized area under the plasma concentration/time curves increased 27% with multiple dosing. Since a previous study in patients (Choc *et al.*, *Pharm. Res.* 4:402–405, 1987) showed dose proportionality of clozapine plasma concentrations during multiple-dose regimens, the present results cannot be described by Michaelis–Menten kinetics.

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

INTRODUCTION

Clozapine (Clozaril) is a tricyclic dibenzodiazepine neuroleptic. In a previous report on steady-state pharmacokinetics in patients (1), we have shown that plasma levels of clozapine measured during three ascending dose regimens could be adequately described using a linear pharmacokinetic (two-compartment) model with a terminal elimination half-life of 15.8 hr. In a separate single-dose study conducted in normal volunteers, elimination from blood occurred with a much shorter half-life of 5.1 hr, and hence, single-dose data were not predictive of the levels observed in the multiple-dose study (2).

The present study addresses the pharmacokinetic differences between the two studies and tests the hypothesis whether the differences are caused by different dosing regimens (single vs multiple doses) or by the different study populations, i.e., normal healthy volunteers vs psychiatric patients. Accordingly, patients were exposed to sequential regimens, single dose–multiple dose–single dose, and the pharmacokinetics were assessed at each stage through the measurement of clozapine plasma concentrations. The effect of food was also examined in a subpopulation; these patients were randomized with respect to food administration in the two single-dose periods.

MATERIALS AND METHODS

Patients were entered into the study at three separate sites. Four patients completed the study at center 1 (Northport, New York), one at center 2 (Cranston, Rhode Island), and eleven at center 3 (San Antonio, Texas). For statistical analysis, data from all centers were pooled. Twenty-three patients entered into the study: males between 21 and 53 years of age, diagnosed as schizophrenic. Their average age was 33.3 years (±8.8 years), average weight 74.3 kg (±11.0 kg), and average height 175 cm (±6.35 cm). Patients were not entered into the study unless they were physically healthy as judged by medical history, physical examination,

[1] Sandoz Research Institute, East Hanover, New Jersey 07936.
[2] To whom correspondence should be addressed.
[3] Department of Statistics, School of Business Administration, Temple University, Philadelphia, Pennsylvania 19122.
[4] Departments of Pharmacology and Psychiatry, University of Texas, San Antonio, Texas 78284.
[5] College of Pharmacy, University of Texas at Austin, Austin, Texas 78712.
[6] VA Medical Center, Northport, New York 11768.
[7] Rhode Island Psychiatric Research and Training Center, Cranston, Rhode Island 02920.
[8] San Antonio State Hospital, San Antonio, Texas 78284.

0724-8741/90/0400-0347$06.00/0

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 as shown in Table I. Every dose was administered with four oz of water, with oral inspections made to ensure that the medication was ingested. In general, the timing of breakfast in relation to the morning dose was such that drug administration was 30 min or more prior to the meal. However, some of the patients were randomized in Periods 1 and 3 with respect to administration of the drug: in a fasted state (overnight prior to the dose and 1 hr after the dose) and in a fed state (dose given after start of the standard institutionalized meal). A total of seven patients (from two of the study centers) completed this aspect of the study.

In each period serial blood samples were collected according to the schedule shown in Table II. At each time point, a 10-ml blood sample was collected by venous puncture into a heparinized tube (green-top Vacutainer BD6480). After sampling, the plasma was separated by centrifugation and frozen in polypropylene screw-cap vials (Sarstedt) until the time of analysis.

Clozapine plasma concentrations were determined using a specific high-performance liquid chromatography method described previously (1). The limit of detection was 3 ng/ml, with a linear concentration–response observed up to 10,000 ng/ml. The parameters for statistical analysis were calculated directly from the clozapine plasma concentration raw data and from the kinetic constants obtained from the fitting of a three-exponential equation (1) to the raw data:

$$C(t) = Ae^{-m_1 t} + Be^{-m_2 t} + Ce^{-m_3 t} \quad (1)$$

The apparent terminal elimination rate constant (m_3) and its associated half-life for clozapine were the primary pharmacokinetic parameters of interest. This parameter (m_3) was determined from the curve-fit analysis (NONLIN; Ref. 3) and also by least-squares regression analysis of the logarithmic transformed plasma concentrations beyond 9 hr.

For the comparison of Periods 1 and 3 (pharmacokinetics and food effect) AUC was calculated both from the raw data (addition of trapezoids formed by the individual concentration/time points) and from the curve-fit estimates. For comparisons with Period 2 (single vs multiple dose), in which the dosing regimens were not constant across all patients, AUC was calculated exclusively from parameters obtained from curve-fitting. The model for curve-fitting was based upon the triexponential equation above, applied using the principle of superposition. Using the curve-fit parameters, AUC was normalized to a 75-mg dose for direct comparison with results from Periods 1 and 3.

Table I. Sequential Design of Three-Period Open-Label Study

Study period	Study day	Conditions
Baseline	1–7	Drug-free
1	8	3 × 25-mg tablets at 7 AM
	9	Drug-free
2	10–16[a]	Dose escalation to 150–300 mg/day
	17–22[b]	Maintenance dosing (150–300 mg/day)
	23	Dosing in AM only
3	24–29	Drug-free
	30	3 × 25-mg tablets at 7 AM
	31–32	Drug-free

[a] The dose escalation period could be less than but was not to exceed 7 days.

[b] The multiple-dosing period at the fixed regimen could be extended beyond 7 days.

Table II. Schedule for Serial Blood Samples

Study period	Study day	Hours after dose
1	8	0, 1, 3, 5, 7, 9, 12, 15, 24, 30, 36, 48
2	23	0, 1, 3, 5, 7, 9, 12, 15, 24, 30, 36, 48, 72
3	30	0, 1, 3, 5, 7, 9, 12, 15, 24, 30, 36, 48

The volume of distribution (V_{dss}) was determined by the use of the equation described by Benet and Galeazzi (3):

$$V_{dss} = \frac{f \times F \times \text{dose} \times \text{AUMC}}{\text{AUC}^2}$$

where AUMC is the area under the first moment of the plasma concentration/time curve, f represents the fraction of the absorbed dose reaching the systemic circulation as unchanged drug, and F is the fraction of the administered dose which is absorbed (0.95 based upon Ref. 2).

The fraction bioavailable, f, was determined by the use of the equation proposed by Gibaldi *et al.* (4):

$$f = \frac{F\ (\text{flow rate})}{\text{flow rate} + [(F\ \text{dose})/\text{AUC}]}$$

where the flow rate is the hepatic plasma flow, estimated to be 940 ml/min.

Plasma clearance (CL_p) after an oral dose was estimated by the following equation:

$$CL_p = \frac{f \times F \times \text{dose}}{\text{AUC}}$$

Statistical analyses were performed with two objectives: (i) comparison of the results from the two single-dose administrations (Period 1 and Period 3) and (ii) comparison of the single-dose results to those observed after multiple dosing. Prior to examining data from Periods 1 and 3 for changes in pharmacokinetic parameters, the effect of food was assessed in the subpopulation participating in this aspect of the study. For the comparison of single- and multiple-dose pharmacokinetic characteristics, parameters from Periods 1 and 3 were averaged and then compared with Period 2 parameters. For both comparisons (Period 1 vs Period 3 and single vs multiple dose), the analysis of variance method appropriate for a randomized block design was used to check for statistical significance. Statistical significance was declared if $P < 0.05$; if $0.05 < P < 0.10$, the differences were considered "borderline" statistically significant.

RESULTS

Seven of the patients participated in an assessment of the effect of food on clozapine bioavailability. These patients

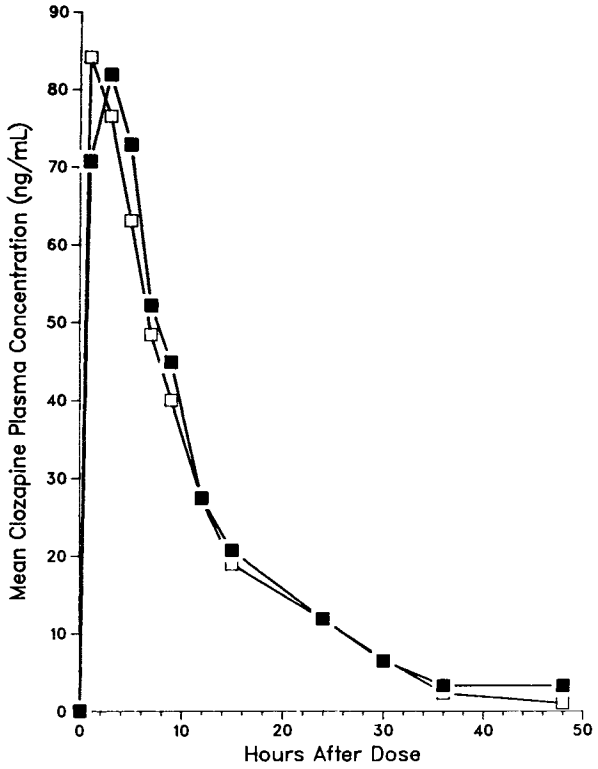

Fig. 1. Mean plasma concentrations of clozapine observed in 16 patients receiving single 75-mg (3 × 25-mg Clozaril tablets) doses prior to receiving multiple-dose therapy with clozapine (Period 1, filled squares) or after receiving multiple-dose clozapine therapy (Period 3, open squares) (see Materials and Methods).

were randomized, in Periods 1 and 3, with respect to dose administration with and without food (dosing either with the meal or 1 hr before the meal). Qualitatively and quantitatively, there were no differences in the mean plasma levels or plasma level variability when the dose was administered with food [AUC = 1132 (48% CV), C_{max} = 116 (40% CV)] when food was delayed until 1 hr after dosing [AUC = 1102 (52% CV), C_{max} = 118 (49% CV)]. In both treatments, the time to peak plasma concentration was between 2 and 3 hr.

Based upon the lack of any significant food effect, an analysis to detect differences between the two single-dose periods (1 and 3) was conducted. Figure 1 shows the mean plasma concentrations observed in the initial and final single-dose treatment periods, and Table III summarizes the relative bioavailability and pharmacokinetic parameter comparisons. No significant differences were noted between these two single-dose treatments. For the parameter AUC, a 30% difference could be detected with adequate power (≥0.80). Since the two single-dose periods were shown to produce equivalent plasma level responses, parameters from each individual were averaged for subsequent comparisons with multiple-dose parameters.

Figure 2 displays the mean plasma concentrations of the combined Periods 1 and 3 (single dose) and compares them to the mean plasma concentrations observed at the end of the multiple-dose Period 2 (shown as the mean of the individual raw data time points). Since the multiple doses were administered using dosage regimens that were individualized for each patient, the resulting single- and multiple-dose levels were not directly comparable. However, Fig. 2 does offer a visual comparison of the relative rates of elimination under the two types of dosing conditions. It shows that the apparent rate of elimination after multiple doses was slower than

Table III. Single-Dose Pharmacokinetic Parameters

Parameter	Arithmetic mean ± SD Geometric mean (Range) Period 1	Period 3	Percentage difference	Significance (P)
Raw data				
AUC (ng/hr/ml)	1021 ± 605	1958 ± 422	−6.2	NS
	876	881	0.6	NS
	(394–2318)	(385–2187)		
C_{max} (ng/ml)	104 ± 58	100 ± 37	−3.9	NS
	92	94	2.2	NS
	(44–238)	(42–190)		
Curve-fit data				
AUC (ng hr/ml)	1044 ± 871	983 ± 404	−5.8	NS
	797	920	15.4	NS
	(330–3036)	(473–2257)		
m_1 (hr^{-1})	—	—		
	2.90	4.28	47.6	NS
	(0.49–15.9)	(0.49–21.9)		
m_2 (hr^{-1})	—	—		
	0.34	0.47	38.2	NS
	(0.20–1.00)	(0.21–1.29)		
m_3 (hr^{-1})	—	—		
	0.086	0.083	−3.5	NS
	(0.047–0.24)	(0.027–0.12)		

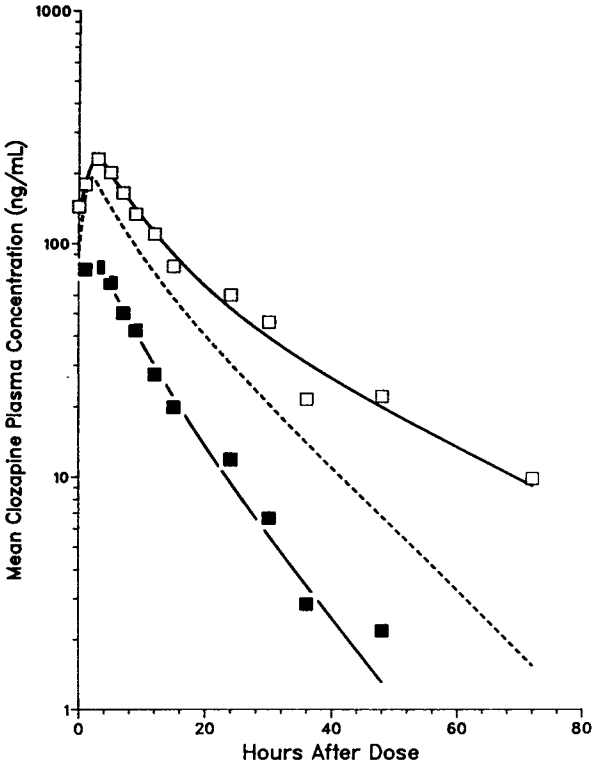

Fig. 2. Mean of the individual raw data clozapine plasma concentrations observed after multiple dosing (open squares) compared to the combined single-dose data (filled squares). The dashed line represents the average of individual multiple-dose projections which were based upon Period 1 curve-fit parameters applied to the individual multiple-dosing regimens.

that observed after single-dose administration. Accordingly, a projection of multiple-dose plasma concentrations, based upon the single-dose parameters and the multiple-dose regimens actually employed (dashed line in Fig. 2), fails to describe adequately the observed concentrations. Conversely, the multiple-dose pharmacokinetic parameters can be used to calculate single-dose plasma concentrations [using Eq. (1), above] for comparison to those actually observed. These calculations indicate that the slower elimination phase observed after multiple dosing, if it were present after a single dose, would have resulted in the observation of measurable plasma concentrations during most of the terminal elimination phase (i.e., failure to observe this phase after a single dose is not due to any assay sensitivity limitation). A comparison of the pharmacokinetic parameters for a single- vs multiple-dose conditions is provided in Table IV. Statistically significant differences ($P < 0.05$) were observed for the parameters m_3 and AUC (multiple-dose AUC normalized to 75-mg dose for comparison purposes). Differences in the relative rates of absorption (m_1) were borderline significant ($P < 0.10$).

A summary of results for additional derived pharmacokinetic parameters is given in Table V. While the fraction of drug bioavailable (f) and the plasma clearance (CL_p) remained essentially constant throughout the study, the steady-state volume of distribution (V_{dss}) and the apparent elimination half-life ($t_{1/2}^{m3}$) increased after multiple dosing. However, statistical testing using a paired t-test (two-tailed, $P = 0.05$) showed that the only parameter in Table V which displayed statistically significant differences between treatments (Periods 1 → 2, 2 → 3) was the apparent elimination half-life, $t_{1/2}^{m3}$. The increase in V_{dss} (ca. 72%) was skewed by one individual having a large value in the multiple-dosing

Table V. Summary of Pharmacokinetic Parameters

Parameter	Period 1	Period 2	Period 3
V_{dss} (L)	342	587	397
f (%)	41.8	47.4	41.1
CL_p (L/hr)	32.8	29.6	33.2
$t_{1/2}^{m3}$ [a]	8.1	14.1*	8.3
	(2.9–14.9)	(5.2–59.7)	(5.8–25.3)

[a] Half-life = ln 2/geometric mean; (range) represents the 5 and 95% quantiles.

* Significantly different ($P < 0.05$) from the Period 1 and 3 parameters using a two-tailed paired t test.

Table IV. Comparison of Single- and Multiple-Dose Pharmacokinetic Parameters

Curve-fit parameter	Arithmetic mean ± SD Geometric mean (Range) Single dose	Multiple dose	Percentage difference	Significance (P)
m_1 (hr^{-1})	—	—		
	4.62	2.06	−55.4	0.06
	(0.95–14.9)	(0.50–10.1)		
m_2 (hr^{-1})	—	—		
	0.42	0.40	−4.8	NS
	(0.23–0.753)	(0.081–1.90)		
m_3 (hr^{-1})	—	—		
	0.088	0.049	−44.3	<0.01
	(0.052–0.17)	(0.012–0.13)		
AUC[a] (ng hr/ml)	1040 ± 493	1420 ± 972	36.5	0.04
	948	1197	26.3	0.08
	(447–2293)	(443–4420)		

[a] Multiple-dose AUC was calculated using individual parameters from curve-fit analysis but normalized to a 75-mg dose.

period. With that individual removed, the average increase was less than 20%.

DISCUSSION

The results of this study demonstrate a clear effect of dosage regimen upon the apparent rate of elimination of clozapine from plasma, as indicated by the parameters $t_{1/2}{}^{m3}$ and AUC. The effect was reversible, in that there was a lack of significant differences between the two single-dose treatments which bracketed the multiple-dose period. The study design did not allow an examination of how the kinetics of clozapine elimination changed during the start of the multiple dose period. However, it was shown in a previous study (1) that under steady-state dosing conditions there was no dose-dependent change in elimination rate (i.e., dose-proportional plasma concentrations were observed at three different multiple-dose levels: 37.5, 75, and 150 mg bid).

Because of the dose proportionality previously reported at steady state, it is clear that the differences observed here cannot be explained by simple Michaelis–Menten kinetics. Multiple dosing may simply expose an additional pharmacokinetic compartment not visualized in the single dose data, as has been reported for other antipsychotics (5). Alternatively, metabolites present in the systemic circulation may inhibit the biotransformation of the parent drug, giving rise to slower elimination at steady-state (6). This would explain the reversibility of the effect after a suitable washout period.

The clinical implications of this study for clozapine are minimal. Because of the orthostatic hypotension often observed in patients being dosed with clozapine for the first time, the typical protocol is upward dose adjustment by titration to an effective dose level. The study does, however, explain the apparent discrepancies in kinetic behavior we have observed in previous single (2)- and multiple (1)-dose studies.

ACKNOWLEDGMENTS

The authors would like to gratefully acknowledge the technical assistance of Ms. J. Hendler, C. Might, G. Kalafsky, and N. Wong in the preparation of this report.

REFERENCES

1. M. G. Choc, R. G. Lehr, F. Hsuan, G. Honigfeld, H. T. Smith, R. Borison, and J. Volavka. *Pharm. Res.* 4:402–405 (1987).
2. F. Ballard and G. Honigfeld. Unpublished data.
3. L. Z. Benet and R. L. Galeazzi. *J. Pharm. Sci.* 68:1071–1974 (1979).
4. M. Gibaldi, R. N. Boyes, and S. Feldman. *J. Pharm. Sci.* 60:1338–1340 (1971).
5. L. Ereshefsky, M. W. Jann, S. R. Saklad, and C. M. Davis. *J. Clin. Psych.* 47:6–15 (1986).
6. D. Perrier, J. J. Ashley, and G. Levy. *J. Pharmacokin. Biopharm.* 1:231–242 (1973).