Population pharmacokinetics of clozapine evaluated with the nonparametric maximum likelihood method

Markus Jerling,¹ Yann Merlé,² France Mentré² & Alain Mallet²

¹Department of Clinical Pharmacology at the Karolinska Institute, Huddinge Hospital, 141 86 Huddinge, Sweden and ²INSERM U 436, SIM, CHU Pitié-Salpêtrière, 91, Bd de l'Hôpital, 750 13 Paris, France

Aims To evaluate the distribution of population kinetic parameters for clozapine and their relationship to age and gender in patients on continuous treatment with the drug.

Methods Retrospective therapeutic drug monitoring data (391 samples from 241 patients) were evaluated using the nonparametric maximum likelihood method. Patients treated concomitantly with drugs known to interact with clozapine were not included. The distribution of clozapine clearance was compared with the distribution of the activity of the drug metabolic enzyme CYP1A2 found in other populations, as recent studies indicate that CYP1A2 is a major determinant for clozapine elimination. The kinetic linearity for clozapine was studied in 41 patients who each provided data from more than one dose level.

Results Clozapine clearance was highly variable in the population and skewed towards high values. Men had higher clearances CL/F (median with 25% and 75% quartiles 38.2 (22.0, 60.0) *vs* 28.3 (15.2, 48.6) $l\ h^{-1}$) and a larger volume of distribution V/F (694 (224, 970) *vs* 401 (189, 932) l) than women. Clearance did not decrease with age in any gender. Clozapine clearance was similarly distributed as the indices of CYP1A2-activity found in other populations by other authors. Evidence of nonlinear kinetics was not found.

Conclusion The large kinetic variability for clozapine found in this study implies that the dose of clozapine needs to be individualised over a wide dose range. The similarity of the distribution of clozapine clearance in this study and the CYP1A2-activity in other populations support the assumption that CYP1A2 is a major determinant for clozapine elimination.

Keywords: clozapine

Introduction

The use of the neuroleptic drug clozapine has in recent years become widespread, despite the risk of causing agranulocytosis [1]. One reason is that some schizophrenic patients resistant to other drugs may respond to clozapine [2]. Another reason is that clozapine rarely induces extrapyramidal adverse effects, which is a major problem with other neuroleptics.

Clozapine is eliminated almost entirely by hepatic metabolism [3]. Several metabolites are formed by demethylation and hydroxylation reactions [4, 5], but the pathways and enzymes involved have not been mapped out in detail. One recent study of drug interactions with clozapine showed that the antidepressant fluvoxamine effectively inhibits the elimination of clozapine, thereby increasing the dose-adjusted clozapine concentration by a factor of 5–10 [6]. Fluvoxamine is also a potent inhibitor of the enzyme CYP1A2 [7]. Together, this led to the hypothesis that clozapine to a significant degree is metabolized by CYP1A2 and that the activity of this enzyme is rate-limiting for the

elimination of clozapine. When subjects who previously participated in a panel study of clozapine kinetics [8] on a later occasion were examined with regard to their CYP1A2-activity, a high correlation ($r = -0.84$, $P < 0.005$) between the AUC of clozapine and the N3-demethylation of caffeine was found [9]. This strongly supports the notion that clozapine is metabolized chiefly by CYP1A2. Another indication of a connection between clozapine elimination and CYP1A2 is that clozapine inhibits the degradation of theophylline *in vitro* [10], where it is known that theophylline is metabolized by CYP1A2 [11].

A study of 13 patients showed linear kinetics of clozapine under steady-state conditions at trough concentrations in the range 60–250 $mg\ ml^{-1}$ [12]. The peak concentration when adjusted for dose and the clearance at the dose levels 37.5, 75 and 150 $mg\ day^{-1}$ were compared intraindividually and were found to be similar. Given that a relationship between the kinetics of clozapine and the activity of CYP1A2 exists, a comparison with the properties of caffeine is relevant. The kinetics of caffeine may in certain subjects be dose-dependent with lower clearances at high doses, as shown in a study where single doses of 70 mg, 200 mg and 300 mg were given to subjects on different occasions [13]. The nonlinearity was seen mainly in those with a high

initial clearance with the lowest dose. If clozapine clearance is closely related to the activity of CYP1A2 and this enzyme has a limited capacity, dose-dependent kinetics might be expected for clozapine.

The aims of this study were to evaluate the distribution of kinetic variables for clozapine from therapeutic drug monitoring data using a population kinetic approach, to compare the clearance distribution in this population with the distribution of the CYP1A2-indices in other populations, and to investigate the kinetic linearity in patients providing concentration measurements from more than one dose rate.

Methods

Study population

A therapeutic drug monitoring service for clozapine was set up in 1989 at the research laboratory at Ulleråkers Psychiatric Hospital, Uppsala, Sweden. All analyses performed up to the end of 1992 were considered for evaluation, in total 978 analyses from 467 patients. The material has been used in a previous study [6]. All information stated on request forms was screened and collected in a computerized database. Some of the information was not available in all patients. Body weight was stated in only about 16% of the cases and this covariate was therefore not considered in the analysis. Smoking habits were not described. Lack of essential information such as the time of sampling or the current dosing schedule for clozapine necessitated exclusion of several observations. We also excluded analyses from patients treated simultaneously with carbamazepine, cimetidine, fluvoxamine or phenytoin, which all may alter the kinetics of clozapine. The reason for this exclusion was that one objective of the present study was to compare the clozapine clearance distribution with the distribution of CYP1A2-activity in other non drug-treated populations. Drugs allowed along with clozapine were benzodiazepines and drugs that mainly are metabolised by CYP2D6, as a previous study has shown that neither of them affect the kinetics of clozapine [6]. The data for this study were selected in a stepwise procedure. From the original 978 analyses, 339 were excluded due to lack of information on the time interval between last dose intake and sampling, 51 analyses due to unknown dosage schedules, 140 analyses due to unknown treatment duration and 57 analyses from patients treated with drugs known to interact with clozapine. The remaining data for 391 analyses from 241 patients were used in the evaluation where almost all samples came from inpatients (360 of the 391 samples). The material was split according to gender with separate evaluations for men and women. Table 1 shows the descriptive statistics of the groups. All samples were taken after a dosing period of at least 7 days.

Pharmacokinetic model

A two-compartment, open model has been applied in several kinetic studies of clozapine [12, 14, 15]. However, only about 7% of the samples in our material were drawn within 8 h after the previous drug intake which excluded the possibility to assess intercompartmental constants. A

one-compartment model with oral absorption was therefore chosen for this study, as described by Equation (1):

$$C(t, \theta) = \frac{D ka}{V/F(ka - k)} (e^{-kt} - e^{-ka t}) \quad (1)$$

where ka is the absorption rate constant, V/F is the volume of distribution directed by bioavailability and k is the elimination rate constant. D is the dose and $C(t, \theta)$ is the model-predicted plasma drug concentration at time t after a single drug intake. ka , k and V/F constitute the parameter vector θ . Oral clearance was calculated from $k \times V/F$.

The samples were not obtained at steady state for every patient. The expected concentration at the time of sampling after multiple-dose regimens was calculated according to the superposition principle [16]. We considered the contribution of dose intakes more than 30 days before the day of sampling as negligible. Therefore, we included only doses given within 30 days preceding the sampling.

Error model

For the NPML-method that was used in this study, as well as for several other methods, a model describing the relationship between the measured and the predicted concentrations must be specified beforehand. In addition, with the NPML-method, the variance parameters have to be given. Since almost all patients in this study were treated in specific psychiatric wards where the drug intake was carefully supervised and where deviations between the true and stated timing of drug intake and sampling was known to be low due to controlled procedures, we chose to include only the error of the laboratory method. This does not exclude the possibility of errors from other sources, but the influence of errors in e.g. timing of drug intake and sampling was considered to have equal importance over the whole concentration range.

Plasma levels of clozapine were determined by gas chromatography—mass spectrometry [17]. The performance of the laboratory method over the entire range of concentrations included in this study was assessed and described by a quadratic polynomial regression model of the coefficient of variation against the concentration. The model was derived from replicated assays at several known concentration values denoted as C [18]. The regression model is given by Equation (2):

$$CV(C) = 0.125 - 0.0294 \ln(C) + 0.00247 [\ln(C)]^2 \quad (2)$$

which gives a $CV(C)$ between 4% and 6% within the concentration range 20–5000 ng ml⁻¹.

A log-normal description of the errors was found to be appropriate [18].

Methodology of the population analysis

The kinetic parameter distribution in the population was evaluated with the previously described nonparametric maximum likelihood (NPML-) method [18, 19]. The reason for using a nonparametric method in this study was to achieve maximum resolution in the description of the kinetic parameter distributions. The NPML approach relies

Table 1 Descriptive statistics of two patient groups (men and women) used for evaluation of the population kinetic parameters of clozapine. (Mean $\pm$ s.d. (range minimum—maximum))

	Women	Men
Number of patients	82	159
Number of observations	141	250
Age (years)	39 $\pm$ 11 (20–74)	37 $\pm$ 9 (20–86)
Total daily dose (mg)	398 $\pm$ 160 (50–800)	378 $\pm$ 158 (12.5–800)
Dose events per day	2.1 $\pm$ 0.8 (1–4)	2.1 $\pm$ 0.7 (1–4)
Interval last dose—sampling (h)	12.0 $\pm$ 3.0 (2–24)	12.3 $\pm$ 3.0 (0.5–24)
Plasma concentration (ng ml ⁻¹)	515 $\pm$ 628 (18–5363)	333 $\pm$ 354 (19–3772)

upon the maximisation of the likelihood of the available data. The estimated distributions of the included kinetic variables are not restricted to belong to a parametric family and no *a priori* assumptions on their distribution are required. Multimodal distributions are, therefore, described with a higher resolution than when using parametric methods. No second stage model (a regression model between the variables and the covariates) is needed and nonlinear relationships between kinetic variables and covariates can be described. The resulting distribution is a discrete set of vectors of variables and covariates, so-called locations, with associated probabilities. Each location is composed of information from all raw data points, but the contribution of the different data points to a specific location varies extensively.

We analysed the materials from men and women separately. For the covariate age, a standard error of 5 years was used. The underlying assumption is that the chronological age is a noisy measurement of the real covariate which is the biological age. Hence, there is an error when using the chronological age which is accounted for by the assignment of a standard deviation to the value of the chronological age.

We had in our material different number of data from different patients, from one up to five samples taken on different occasions from the single patient. This may be modelled by referring multiple samples from one subject to the specific subject, or to treat all samples independently regardless of the number of subjects from which they were drawn. The first approach may be used when the intraindividual relationship between the kinetic parameters and the covariates is relatively stable and the within- and between subject kinetic variability can then be determined. The second method will give a more true estimate of the kinetic variability in the population if the relationship between kinetic parameters and covariates is changing over time and the samples are taken wide apart. By using the latter approach, the result will include both interindividual and intraindividual variability. However, knowing the extent of intraindividual variability is of limited use for individual kinetic predictions unless the temporal pattern of this variation is known. The CYP1A2-activity varies considerably over time in the same subject [20]. We therefore treated all data independently in the analysis.

The relation of the kinetic parameters to the covariate age was evaluated by computing the mean and variance of all the kinetic parameters conditional on the chosen covariates. The smoothed distributions of the parameters according to a gaussian kernel function are also obtained [21].

Comparison with the index of CYP1A2-activity

Two studies relevant for the this purpose were used [20, 22]. Raw data for both phenotyped materials were made available by courtesy of Professor Werner Kalow and Dr Mary Relling. A great variety of metabolites are formed in the metabolism of caffeine and different ratios between metabolites in urine have been proposed as markers of the activity of CYP1A2. The ratio (AAMU + 1X + 1U)/17U was used in the first study [20] and the ratio (AFMU + 1U + 1X)/17X was used in the second [22]. The first ratio has been claimed to be more reliable as a measure of CYP1A2-activity as the excretion of the metabolite 17X is dependent on urine flow, which is not the case for 17U [23]. However, no reference method has been established so far. We therefore included both studies in the comparison. We did not split the materials according to gender as no major differences between their CYP1A2-activities have been found, and as we wanted to keep the materials as large as possible. Accordingly, we merged the NPML-results for clozapine clearance from both genders for this comparison. The index distributions [20, 22] were compared with the clozapine clearance found in this study by using a quantile-quantile plot. A linear relationship in such a plot indicates that the two distributions have a similar shape.

Investigation of the kinetic linearity

We found in the total material 41 patients each having concentration data from more than one daily dose rate. Very few had data from more than two dose rates, and we used in those cases data from the lowest and the highest daily dose. Bayesian estimation of clearances for these individuals at high and low doses was performed and the ratio (clearance at the high dose/clearance at the low dose) was plotted against the corresponding ratio of the two doses. If the metabolizing enzyme(s) becomes saturated at therapeutic doses, the first ratio will be below unity and it will be negatively correlated to the ratio of the two doses.

Results

The numerical results of the population kinetic analysis of clozapine are presented in Table 2. The distributions of k_a should be interpreted cautiously due to the low amount of data early in the dose interval. Men had higher oral clearances of clozapine associated with a higher V/F , leaving the elimination rate constant k at a similar magnitude as in women. The variability in clearance was pronounced,

Table 2 Population kinetic parameters for clozapine.

	Women			Men		
	Median with 25% and 75% quartiles	Mean	Coefficient of variation	Median with 25% and 75% quartiles	Mean	Coefficient of variation
k_a (h^{-1})	1.24 (0.80, 1.50)	1.24	0.42	1.30 (0.87, 1.72)	1.37	0.49
V/F (l)	401 (189, 932)	564	0.8	694 (224, 970)	719	0.79
k (h^{-1})	0.086 (0.037, 0.131)	0.1	0.72	0.083 (0.054, 0.127)	0.095	0.62
CL/F ($l h^{-1}$)	28.3 (15.2, 48.6)	39.9	1.22	38.2 (22.0, 60.0)	47.9	0.72

especially in women. The higher values of the mean than of the median for the clearance indicate a skewed distribution in both genders.

This is evident also in Figure 1 where the discrete clearance distribution is shown together with the smoothed distribution. Each spike shows the probability of the corresponding unique clearance value and the sum of all probabilities equals 100%. No antimodes dividing the distributions into subpopulations were found apart from a small, separate location of very high clearances in women. The clearance locations covered a 40-fold range in both genders. There were separate locations at a very low clearance of about $5 l h^{-1}$ with a probability close to 1% in both genders.

Figure 2 shows the cumulative probability distribution of the clearance where the curve for males is located to the right of the curve for females, except at very low and high clearance rates. The cumulative distribution of V/F for each gender is presented in Figure 3. We found a difference between the genders also for this variable.

The mean value of the clearance conditional on age is shown in Figure 4. The value was similar at the two endpoints of the ranges for which enough data were available (20–70 years). Men had a convex curve whereas women had a biphasic curve with lowest clearances at ages between 35 and 45 years.

Figure 5 shows the oral clearance of clozapine in this study compared with the CYP1A2-indices in two other

Figure 2 Cumulative distribution of the probability of clozapine clearance (CL/F) in women (A) and men (B).

populations. With logarithmic axes, the distributions of the two indices differ from each other at high values and the relationship to clozapine clearance remains linear only for the index $(AAMU + 1X + 1U)/17U$ [20].

Among the patients who had their clozapine concentration measured on two different occasions at different daily doses, the average clearance at the low dose was 49.4 (s.d. 24.2) $l h^{-1}$ and at the high dose 43.7 (s.d. 28.0) $l h^{-1}$. This difference is not statistically significant. The corresponding

Figure 1 Discrete distribution of clozapine clearance in women (a) and men (b). The dashed line shows the smoothed distribution according to a gaussian kernel function. The y-axis represents the probability associated with each location of the NPML-estimated discrete distribution. For the smoothed distribution it represents a probability density function (pdf) which has been rescaled.

Figure 3 Cumulative distribution of the probability of volume of distribution (V/F) for clozapine in women (A) and men (B).

doses were 306 ± 120 mg and 446 ± 126 mg. A plot of the ratio (clearance at the high dose/clearance at the low dose) against the corresponding ratio of the two doses revealed no relationship (plot not shown).

Discussion

Most studies of clozapine kinetics have used panels of patients [12, 14, 15, 24] or therapeutic drug monitoring data [25, 26], as the toxicity of the drug limits the possibilities to administer the drug to healthy volunteers [27]. A very large interindividual variability in the clearance has been verified. Factors proposed to be associated with a low clearance are advanced age [26], female gender [26], and concomitant intake of cimetidine [28]. By contrast, smoking [26], and concomitant intake of carbamazepine [6, 29] or phenytoin [30] may increase the clearance of clozapine. We could in this study confirm a very large interindividual variability in the clearance of clozapine in the absence of interacting drugs, and a difference between genders. A pronounced skew towards high clearances was found and a small probability of a very low clearance in both genders.

Figure 5 Quantile-quantile plot of the clozapine clearance distribution estimated in this study and two CYP1A2-index distributions collected from separate studies in other populations. The continuous line refers to Kalow & Tang [20] and the dashed line to Relling *et al.* [22].

The mean values of the kinetic parameters are in accord with previous results [12, 14, 15, 24] but we found a larger variability in clearance. The relationship between age and clearance was nonlinear with no clear tendency to lower capacities in elderly patients up to 70 years old. We have no information on why women around 40 years of age had the lowest clearance values. Intake of oral contraceptives is one possible factor but is probably as frequent at ages between 20 and 30 years. Differences in smoking habits is another possible explanation.

The caffeine test has been used to discern the CYP1A2-activity in the general population [20, 22]. The distribution is skewed towards high activities and contains a high degree of variability between subjects. The existence of several modes in the distribution has not been verified. In analogy with the clearance of clozapine, smoking increases the activity [31], cimetidine decreases the activity [32], and

Figure 4 Influence of age on the clearance of clozapine in women (a) and men (b). The continuous line shows the mean value of the clearance and the dashed line the standard deviation, both conditional on age.

females have lower activities than males [20, 22]. The latter may not be a true gender difference but rather due to the fact that oral contraceptives cause a significant decrease of the CYP1A2-activity [20]. However, significantly lower CYP1A2-ratios in women than in men were found in a population where no subject was taking oral contraceptives [22]. The age factor is more unclear. One study found a higher activity in children 3 to 11 years old than in adults 18 to 30 years old [33]. Another study found no decrease in the CYP1A2-index with increasing age in subjects from 2 to 46 years of age [22]. The shape of the cumulative probability distribution of the clozapine clearance found in this study and the CYP1A2 indices in two other populations were relatively similar, as shown by a linear relationship in the major part of the quantile-quantile plot. Theoretically, a closer relationship should be expected at high enzyme activities at which a larger fraction of the drug metabolism is catalysed by the specific enzyme. If the CYP1A2-activity is low, other enzymes will be quantitatively more important and a low correlation to the clozapine clearance is seen. The two indices of CYP1A2-activity gave similar results except at the highest activities. The ratio $(AAMU + 1X + 1U)/17U$ may indicate a falsely low enzyme activity as the formation of 17U from 17X is partly catalysed by CYP1A2 [34]. This error is most prominent at high enzyme activities and will diminish the variability at high values.

We did not find evidence of nonlinear kinetics in intraindividual comparisons of the clearance at different dose rates. Some patients had a lower clearance at the high dose and some patients a higher clearance. Neither the initial clearance with the low dose nor the difference between the doses were related to the display of nonlinear kinetics. Another finding was a large intraindividual kinetic variability where the coefficient of variation of the ratio of the two clearance rates was 48%. This variability can theoretically be explained by changes in any of the factors that determine the disposition of clozapine and that may vary with time.

A few studies have investigated the concentration-effect relationship for clozapine [35–37]. No strong correlations were found, but one study defined a cut-off value at 350 ng ml^{-1} above which therapeutic response was more common [36]. The incidence of EEG abnormalities and seizures seems to increase with dose [38]. As clozapine has a large kinetic variability and a narrow therapeutic index, the dose individualization is a difficult but important task in order to optimise the treatment [39]. It is therefore important to identify the major sources of kinetic variability to enable prediction of individual kinetic parameters. The factors age and gender account for a minor part of the kinetic variability. Several data support that CYP1A2 is rate limiting for the clearance of clozapine in most subjects. To perform a caffeine test in patients before initiating clozapine therapy is one possibility to study the predictive power of the CYP1A2-activity for the kinetics of clozapine.

We acknowledge Professor Leif Lindström and Professor Ulf Bondesson at the Psychiatric Research Centre, University of Uppsala, for giving us access to the therapeutic drug monitoring data used in this study. We also wish to express our gratitude to Professor Werner Kalow and Dr Mary

Relling for sending us raw data for the CYP1A2-indices from their materials.

This study was supported by the Swedish Medical Research Council (3902) and the Bank of Sweden Tercentenary Foundation.

References

- 1 Lieberman JA, Alvir JM. A report of clozapine-induced agranulocytosis in the United States. *Drug Safety* 1992; **7**(suppl 1): 1–2.
- 2 Kane J, Honigfeld G, Singer J, Meltzer H. Clozapine for the treatment-resistant schizophrenic: A double blind comparison versus chlorpromazine. *Arch Gen Psychiatry* 1988; **45**: 789–796.
- 3 Gauch R, Michaelis W. The metabolism of 8-chloro-11-(4-methyl-1-piperazinyl)-5H-dibenzo-[b,c][1, 4] diazepine(clozapine) in mice, dogs and human subjects. *Farmaco* 1971; **26**: 667–681.
- 4 Ackenheil M. Clozapine-pharmacokinetic investigations and biochemical effects in man. *Psychopharmacology (Berl)* 1989; **99** Suppl: S32–S37.
- 5 Rosenthaler J, Nimmerfall F, Sigrist R, Munzer H. Non-equilibrium method for the radioimmunoassay of clozapine in the presence of metabolites. *Eur J Biochem* 1977; **80**: 603–609.
- 6 Jerling M, Lindström L, Bondesson U, Bertilsson L. Fluvoxamine inhibition and carbamazepine induction of the metabolism of clozapine: evidence from a therapeutic drug monitoring service. *Ther Drug Monit* 1994; **16**: 368–374.
- 7 Brösen K, Skjelbo E, Rasmussen BB, Poulsen HE, Loft S. Fluvoxamine is a potent inhibitor of cytochrome P4501A2. *Biochem Pharmacol* 1993; **45**: 1211–1214.
- 8 Dahl ML, Llerena A, Bondesson U, Lindström L, Bertilsson L. Disposition of clozapine in man: lack of association with debrisoquine and S-mephenytoin hydroxylation polymorphisms. *Br J Clin Pharmacol* 1994; **37**: 71–74.
- 9 Bertilsson L, Carrillo JA, Dahl ML, et al. Clozapine disposition covaries with the CYP1A2 activity determined by the caffeine test. *Br J Clin Pharmacol* 1994; **38**: 471–473.
- 10 Rasmussen BB, Mäenpää J, Pelkonen O, et al. Selective serotonin reuptake inhibitors and theophylline metabolism in human liver microsomes: Potent inhibition by fluvoxamine. *Br J Clin Pharmacol* 1995; **39**: 151–159.
- 11 Gu L, Gonzalez FJ, Kalow W, Tang BK. Biotransformation of caffeine, paraxanthine, theobromine and theophylline by cDNA-expressed human CYP1A2 and CYP2E1. *Pharmacogenetics* 1992; **2**: 73–77.
- 12 Choc MG, Lehr RG, Hsuan F, et al. Multiple-dose pharmacokinetics of clozapine in patients. *Pharm Res* 1987; **4**: 402–405.
- 13 Cheng WS, Murphy TL, Smith MT, Cooksley WG, Halliday JW, Powell LW. Dose-dependent pharmacokinetics of caffeine in humans: relevance as a test of quantitative liver function. *Clin Pharmacol Ther* 1990; **47**: 516–524.
- 14 Cheng YF, Lundberg T, Bondesson U, Lindström L, Gabrielsson J. Clinical pharmacokinetics of clozapine in chronic schizophrenic patients. *Eur J Clin Pharmacol* 1988; **34**: 445–449.
- 15 Choc MG, Hsuan F, Honigfeld G, et al. Single- vs multiple-dose pharmacokinetics of clozapine in psychiatric patients. *Pharm Res* 1990; **7**: 347–351.
- 16 Rowland M, Tozer TN. Dose and time dependencies. In *Clinical Pharmacokinetics (concepts and applications)* 2nd ed, London: Lea and Febiger, 1989: 376.
- 17 Bondesson U, Lindström LH. Determination of clozapine and

- its N-demethylated metabolite in plasma by use of gas chromatography-mass spectrometry with single ion detection. *Psychopharmacology* 1988; **95**: 472–475.
- 18 Jerling M, Merlé Y, Mentré F, Mallet A. Population pharmacokinetics of nortriptyline during monotherapy and during concomitant therapy with drugs that inhibit CYP2D6—an evaluation with the nonparametric maximum likelihood method. *Br J Clin Pharmacol* 1994; **38**: 453–462.
 - 19 Mallet A. A maximum likelihood estimation method for random coefficient regression models. *Biometrika* 1986; **3**: 645–656.
 - 20 Kalow W, Tang BK. Use of caffeine metabolite ratios to explore CYP1A2 and xanthine oxidase activities. *Clin Pharmacol Ther* 1991; **50**: 508–519.
 - 21 Parzen E. On estimation of probability density function and mode. *O.N.R. Technical Report* 1961; 40.
 - 22 Relling MV, Lin JS, Ayers GD, Evans WE. Racial and gender differences in N-acetyltransferase, xanthine oxidase, and CYP1A2 activities. *Clin Pharmacol Ther* 1992; **52**: 643–658.
 - 23 Grant DM, Tang BK, Kalow W. Variability in caffeine metabolism. *Clin Pharmacol Ther* 1983; **33**: 591–602.
 - 24 Lin SK, Chang WH, Chung MC, Lam YW, Jann MW. Disposition of clozapine and desmethylclozapine in schizophrenic patients. *J Clin Pharmacol* 1994; **34**: 318–324.
 - 25 Haring C, Barnas C, Saria A, Humpel C, Fleischhacker WW. Dose-related plasma levels of clozapine, letter. *J Clin Psychopharmacol* 1989; **9**: 71–72.
 - 26 Haring C, Meise U, Humpel C, Saria A, Fleischhacker WW, Hinterhuber H. Dose-related plasma levels of clozapine: influence of smoking behaviour, sex and age. *Psychopharmacology (Berl)* 1989; **99** Suppl: S38–S40.
 - 27 Pokorny R, Finkel MJ, Robinson WT. Normal volunteers should not be used for bioavailability or bioequivalence studies of clozapine :letter:. *Pharm Res* 1994; **11**: 1221.
 - 28 Szymanski S, Lieberman JA, Picou D, Masiar S, Cooper T. A case report of cimetidine-induced clozapine toxicity. *J Clin Psychiatry* 1991; **52**: 21–22.
 - 29 Raitasuo V, Lehtovaara R, Nuttunen MO. Carbamazepine and plasma levels of clozapine :letter:. *Am J Psychiatry* 1993; **150**: 169.
 - 30 Miller DD. Effect of phenytoin on plasma clozapine concentrations in two patients. *J Clin Psychiatry* 1991; **52**: 23–25.
 - 31 Kalow W. Variability of caffeine metabolism in humans. *Arzneimittelforschung* 1985; **35**: 319–324.
 - 32 Shaw-Stiffel TA, Tang BK, Spielberg SP, Shear NH. Caffeine—a novel probe to assess drug effects on hepatic cytochrome P-450 activity [Abstract]. *Hepatology* 1988; **8**: 1385.
 - 33 Campbell ME, Spielberg SP, Kalow W. A urinary metabolite ratio that reflects systemic caffeine clearance. *Clin Pharmacol Ther* 1987; **42**: 157–165.
 - 34 Kalow W, Tang BK. The use of caffeine for enzyme assays: a critical appraisal. *Clin Pharmacol Ther* 1993; **53**: 503–514.
 - 35 Ackenheil M. Clozapine—pharmacokinetic investigations and biochemical effects in man. *Psychopharmacology (Berl)* 1989; **99** Suppl: S32–S37.
 - 36 Perry PJ, Miller DD, Arndt SV, Cadoret RJ. Clozapine and norclozapine plasma concentrations and clinical response of treatment-refractory schizophrenic patients. *Am J Psychiatry* 1991; **148**: 231–235.
 - 37 Hasegawa M, Gutierrez-Esteinou R, Way L, Meltzer HY. Relationship between clinical efficacy and clozapine concentrations in plasma in schizophrenia: effect of smoking. *J Clin Psychopharmacol* 1993; **13**: 383–390.
 - 38 Gunther W, Baghai T, Naber D, *et al.* EEG alterations and seizures during treatment with clozapine. A retrospective study of 2843 patients. *Pharmacopsychiatry* 1993; **26**: 69–74.
 - 39 Freeman DJ, Oyewumi K. Will routine therapeutic drug monitoring have a place in clozapine therapy? *Clin Pharmacokinet* 1997; **32**: 93–100.

(Received 8 August 1996,
accepted 26 June 1997)