The relationship between plasma clozapine concentration and clinical outcome: a cross-sectional study

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Abstract:

Objective: There is no report that statistically evaluates the therapeutic reference (350–600 ng/mL) and adverse drug reaction (ADR) range (>1000 ng/mL) of clozapine (CLZ) recommended by the *Arbeitsgemeinschaft für Neuropsychopharmakologie und Pharmakopsychiatrie (AGNP)* consensus guidelines in an isolated and large sampling study.

Methods: We administered CLZ to 131 Japanese patients with treatment-resistant schizophrenia in a multicenter cross-sectional study. Plasma CLZ concentrations were assayed by high-performance liquid chromatography using trough sampling. The Brief Psychiatric Rating Scale (BPRS) and severe dose-dependent ADR (sedation, myoclonus, and seizures) were analyzed statistically after adjusting for possible confounders.

Results: The daily CLZ dosage showed a moderately positive relationship with the plasma concentration ($r = 0.49$, $p < 0.001$). Every 100 ng/mL increase in plasma CLZ concentration improved the total BPRS score 1.95% (95% CI: 0.89–3.01, $p < 0.001$) and the odds ratio (OR) 1.38 (95% CI: 1.14–1.66, $p = 0.001$) for BPRS response. Compared with concentrations below 350 ng/mL CLZ, 350–600 ng/mL (11.12%; 95% CI: 2.52–19.72, $p = 0.012$) and 600–1000 ng/mL (11.05%; 95% CI: 2.40–19.71, $p = 0.013$) showed significant improvement in the total BPRS score. Dosages above 1000 ng/mL showed greater improvement (25.36%; 95% CI: 13.08–37.64, $p < 0.001$) of the total BPRS score but more severe ADRs than dosages below 1000 ng/mL (OR: 31.72; 95% CI: 1.04–968.81, $p = 0.048$).

Conclusion: The AGNP therapeutic reference range (350–600 ng/mL) is useful, and a dose above 1000 ng/mL is potentially more effective but carries the risk of severe ADRs in the central nervous system.

Keywords: Schizophrenia, Plasma clozapine concentration, Therapeutic drug monitoring, AGNP reference range, Adverse drug reaction

Significant outcomes

- The AGNP therapeutic reference range (350–600 ng/mL) is useful, and a dose above 1000 ng/mL is potentially more effective but carries the risk of severe ADRs in the central nervous system.
- The AGNP guidelines are potentially applicable to Japanese patients (and perhaps other East Asian patients) despite genetic differences from Caucasian patients.

Limitations

- The dose and concentrations of CLZ were not fixed or randomized.
- Discontinuous sampling may increase the possibility of selection bias (internal validity).
- Patients with longer durations of CLZ treatment may tend to be better responders and be tested by therapeutic drug monitoring (survivor and length bias).
- Because our study included only patients who agreed to participate in the study, the result may not be directly generalizable to clinical practice (external validity).

Introduction

About one-third of patients with schizophrenia have treatment-resistant schizophrenia (TRS) (1). Clozapine (CLZ) has the high efficacy of 30% to 60% in patients with TRS (2,3) but also carries the risk of adverse drug reactions (ADRs) (4). Many studies have assessed the relationship between plasma CLZ concentrations, clinical efficacy, and dose-dependent ADRs, but these results are controversial due to methodological limitations, such as small sample size, short duration of CLZ treatment, and unfixed sampling time. Therefore, the therapeutic reference range of CLZ in TRS is still debated (5).

According to the *Arbeitsgemeinschaft für Neuropsychopharmakologie und Pharmakopsychiatrie (AGNP)* consensus guidelines for therapeutic drug monitoring (TDM) (6), the proposed therapeutic reference range for CLZ is from 350 to 600 ng/mL, and the laboratory alert concentration for seizures is above 1000 ng/mL. However, there is no report that evaluates statistically the therapeutic reference and ADR range recommended by AGNP consensus guidelines in a large isolated sampling study after adjusting all the covariates.

A systematic review reported that the CLZ concentration-to-dose ratio (ng/mL per mg/day) was 1.5 times higher in East Asian patients (not including Japanese samples) than in Caucasian patients (7). Though there is one report on CLZ clearance in Japanese patients, the sample was small and did not include trough sampling (8). There is no report of a study that investigated the CLZ concentration and clinical efficacy in Japanese patients.

Aims of the study

The aim of this study was to estimate the validity of the AGNP therapeutic reference and ADR ranges. In addition, we evaluated the relationship between the daily CLZ dosage

and plasma CLZ concentrations, clinical efficacy, and severe dose-dependent ADRs in Japanese patients with TRS for the first time.

Method

Setting and Study Population

We employed a cross-sectional study to achieve our objectives. The diagnosis was based on the criteria of the International Statistical Classification of Diseases and Related Health Problems, 10th Revision, for schizophrenia. TRS was defined as a history of at least two failed trials of 600 mg of chlorpromazine equivalents for at least 4 weeks, and a Global Assessment of Functioning (GAF) (9) score <41 in each antipsychotic trial (by the criteria of the Clozaril Patient Monitoring Service in Japan). We conducted TDM of CLZ 404 times in 210 patients with TRS from March 15, 2015 to December 31, 2018 at four psychiatric hospitals in Japan. The physicians in charge decided to conduct TDM at the time of serious ADRs or as a barometer of effectiveness. In cases of multiple TDM sampling, we selected the last TDM in order to include late responders to CLZ. Patients were at least 16 years old. We excluded patients for a lack of trough sampling (n = 41), insufficient information on patients (n = 9), administration of a combination therapy of antipsychotics (n = 4), and less than 3 months CLZ administration (n = 25) because CLZ treatment requires a period of at least 6 weeks to 3 months to be effective (10,11). Finally, the data of 131 patients treated with CLZ at Okayama Psychiatric Medical Center (n = 65; 49.6%), Kanagawa Psychiatric Center (n = 29; 22.1%), Shizuoka Psychiatric Medical Center (n = 23; 17.6%), and the National Center of Neurology and Psychiatry (n = 14; 10.7%) were available for analysis. The Institutional Review Board of the Okayama Psychiatric Medical Center and the

Okayama University ethics committee approved this study. All patients gave written consent to take part in the study.

CLZ was initiated at a dosage of 12.5 mg/day and increased by 25–50 mg every few days up to 200 mg/day over 3 weeks according to tolerability, given in two or three divided daily administrations. After completion of the titration period, the dosage was adjusted up to 600 mg/day by the treating physician based on clinical response and tolerance, as in routine clinical practice. In Japan, 18 weeks of hospitalization are required for the initiation of CLZ because the risk of agranulocytosis is higher in the first 18 weeks (12), and augmentation of CLZ with antipsychotics is not allowed to avoid the risk of side effects associated with a drug-induced high CLZ concentration (13).

Clinical Assessment

As a primary outcome, clinical symptoms at the initiation of CLZ and the time of CLZ TDM were assessed using the 18-item Brief Psychiatric Rating Scale (BPRS) (14), in which items were rated from 1 (not present) to 7 (extremely severe). Subscores of BPRS were constructed from positive symptoms (6 items), negative symptoms (2 items), and general psychopathology symptoms (10 items). As secondary outcomes, the Clinical Global Impression Scale (CGI), subscales for severity of illness (CGI-S) and improvement (CGI-I) (15) and the GAF score were rated to assess the severity of the illness and the therapeutic outcome as changes in psychopathology. Severe dose-dependent ADRs of the central nervous system (sedation (16), myoclonus (17), and seizures (18)) were assessed. Two psychiatrists, the physician in charge of each patient and a senior trained psychiatrist (n = 27), assessed the rating of BPRS, CGI, and GAF. The assessments were blind to the TDM

result and made independently, and the two scores were averaged. The two raters were blind to each other's assessments.

Data Collection

On the day of blood sampling, the clinical data (sex, age, duration of illness, weight, height, caffeine and smoking history, diagnoses, concomitant medications, time and date of last CLZ dose, daily CLZ dosage, duration of CLZ treatment, clinical assessments (BPRS, CGI, and GAF), and severe dose-dependent ADRs) were recorded on the questionnaire by the physician.

Clozapine Assay

Blood samples (10 ml) were collected from patients in tubes containing EDTA-2Na during a steady state (more than 7 days on a stable dosage (19)) and trough (blood sampling 10–14 h after the last dose (20)). Samples were then centrifuged at 3000 rpm for 10 min, and plasma collected and stored at −20℃ until assay, which was performed within a week (21,22). CLZ and norclozapine (NCLZ) plasma concentrations were evaluated by high-performance liquid chromatography (HPLC) with ultraviolet detection at 254 nm, modified from the analytical method of Novartis, with loxapine as the internal standard. A fluorinated silica-gel based column (Wakopak Fluofix) was used as the solid phase. The mobile phase was composed of acetate buffer (50:50). The extraction recoveries were >85%. The inter- and intra-assay variance was <5%. Intra- and inter-day coefficients of variations were <11%. The standard curves were linear within the range of 100–2000 ng/mL. Routine psychotropic drugs or other drugs did not interfere with this assay. All reagents used were of HPLC grade.

Statistical Analyses

The Pearson correlation coefficient (r) was estimated to analyze the relationship between daily CLZ dosage and plasma concentrations of CLZ and NCLZ. Patients were classified as responders if they showed a 20% or greater reduction from baseline in the total BPRS score following previous papers (23,24). We first examined the relationship between the plasma CLZ concentration and the percent BPRS score improvements by using a linear regression model (Crude Model). Subsequently, we adjusted for age and sex as covariates (Adjusted Model, AM), and finally, we additionally adjusted for duration of illness, CLZ dosage, duration of CLZ treatment (categorized as less than 6 months, 6 to 12 months, and more than 12 months), smoking, caffeine intake, number of CLZ TDM (single or multiple), concomitant medications, such as lithium carbonate, antiepileptics, or antidepressants supposed to be related to plasma CLZ concentration and clinical assessments, as covariates (Fully Adjusted Model, FAM). In a similar way, the association between the plasma CLZ concentration and BPRS response was examined using a logistic regression model.

To evaluate the usefulness of the AGNP therapeutic reference ranges, we compared the clinical efficacy of four plasma CLZ concentration ranges as categorical variables (< 350, 350–600, 600–1000, and >1000 ng/mL), adopting a multiple linear regression and a logistic regression model only after adjusting all the covariates (FAM; age, sex, duration of illness, CLZ dosage, duration of CLZ treatment, smoking, caffeine intake, number of CLZ TDM (single or multiple), and concomitant medications (lithium carbonate, antiepileptics, or antidepressants). To estimate the risk of severe dose-dependent ADRs in patients above 1000 ng/mL, we adopted a logistic regression model, adjusting for age, sex, weight, smoking,

caffeine intake, number of CLZ TDM (single or multiple), concomitant medications (lithium carbonate, antiepileptics, or antidepressants) as covariates (AM). All statistical analyses were performed using IBM SPSS Statistics 24 (Chicago, IL, USA).

Receiver operating characteristic (ROC) curve analysis was performed predict a threshold value of the plasma CLZ concentration for distinguishing responders and non-responders (25).

Results

Relationship Between Dosage and Plasma CLZ Concentration

The daily CLZ dosage was moderately positively related to the plasma concentrations of CLZ and NCLZ ($r = 0.49$, $p < 0.001$, $r = 0.55$, $p < 0.001$) (Figure 1). The mean CLZ concentration-to-dose ratio (C/D) was 1.80 ± 0.79 (range 0.20–4.57) (ng/mL per mg/day). No patient was prescribed any antipsychotic drug other than clozapine. Several concomitant medications were prescribed, including lithium carbonate (n = 57; 43.6%), antiepileptics [n = 47; 35.9%; valproic acid (32), lamotrigine (13), topiramate (6), clonazepam (12), carbamazepine (1), levetiracetam (1)], antidepressants [n = 11; 8.4%; escitalopram (6), trazodone (3), sertraline (1), fluvoxamine (1)], famotidine (n = 4; 3.1%), and atomoxetine hydrochloride (n = 3: 2.3%). Fifty-eight female patients were included [smoking (n = 6), lithium carbonate (n = 24), antiepileptics (n = 28), and antidepressants (n = 6)]. No patient was pregnant or taking hormonal contraceptives.

Clinical Characteristics of Responders and Non-Responders

The clinical characteristics of responders and non-responders are shown in Table 1.

The mean BPRS scores at baseline did not differ between responders and non-responders. Similarly, the two groups did not differ by sex, age, duration of illness, smoking, caffeine intake, or CLZ dosage. The mean plasma CLZ concentrations were higher in responders than in non-responders (678.8 ± 300.6 vs. 555.7 ± 258.0 ng/mL), whereas NCLZ concentrations did not differ between the two groups (335.4 ± 160.5 vs. 291.5 ± 147.2 ng/mL). The responders also showed better therapeutic outcomes in GAF improvement, CGI-S improvement, and CGI-I. The duration of CLZ treatment was longer in responders than in non-responders (916.7 ± 687.3 vs. 526.8 ± 478.9 days). A histogram of responder and non-responder values at each 50 ng/mL concentration is shown in Supplementary Figure 1.

Relationship Between Clinical Efficacy and Plasma CLZ Concentration

The percent BPRS score improvements per 100 ng/mL plasma CLZ concentration increment are shown in the upper part of Table 2. In the crude model, we observed statistically significant improvement (1.49%; 95% CI: 0.52–2.45) of the total BPRS score and in two subscores (positive and general psychopathology, but not negative). In the adjusted model as well, they were statistically significant and more prominent. In the fully adjusted model, the percent improvements of total BPRS and three subscores were all statistically significant. Every 100 ng/mL increase in plasma CLZ concentration improved the total BPRS score 1.95% (95% CI: 0.89–3.01), positive score 2.15% (95% CI: 0.87–3.43), negative score 1.40% (95% CI: 0.07–2.72), and general psychopathology score 1.72% (95% CI: 0.51–2.93), respectively.

The odds ratios (ORs) for BPRS response associated with CLZ concentration are shown in the lower part of Table 2. In crude, adjusted, and fully adjusted models, the ORs

were statistically significant (ORs: 1.17–1.38) for every 100 ng/mL increase in plasma CLZ concentration. In the covariates, the duration of CLZ treatment was significantly related to the total BPRS response. The OR for duration of CLZ treatment over 12 months was 5.08 (95% CI: 1.65–15.65) compared with the OR at 3–6 months.

Similarly, the plasma NCLZ concentration also showed a significant association with BPRS improvement. Every 100 ng/mL increase in plasma NCLZ concentration improved the total BPRS score 4.15% (95% CI: 2.10–6.10), positive score 4.02% (95% CI: 1.50–6.54), negative score 3.65% (95% CI: 1.10–6.20), and general psychopathology score 3.98% (95% CI: 1.65–6.31), respectively. The OR for BPRS response associated with NCLZ concentration was 1.48 (95% CI: 1.05–2.07).

The ROC curve showed that a threshold value of the plasma CLZ concentration for distinguishing between responders and non-responders was 550 ng/mL with sensitivity of 63.3% and specificity of 50% (area under the curve: 0.603, $p < 0.041$, 95% CI: 0.504–0.701) (Supplementary Figure 2).

Validity of AGNP Therapeutic Reference Range

The percent BPRS score improvements and ORs for BPRS response are shown according to CLZ plasma concentrations in Table 3. Compared with the <350 ng/mL concentration, 350–600 and 600–1000 ng/mL concentrations showed significant improvements of 11.12% (95% CI: 2.52–19.72) and 11.05% (95% CI: 2.40–19.71), and concentrations >1000 ng/mL showed much improvement at 25.36% (95% CI: 13.08–37.64). Compared with the <350 ng/mL concentration, the ORs of 350–600 and 600–1000 ng/mL were significantly higher at 7.09 (95% CI: 1.85–27.24) and 8.11 (95% CI: 2.09–31.53), and

that of >1000 ng/mL was 57.28 (95% CI: 4.36–753.32), the highest.

Relationship Between Severe Dose-Dependent ADRs and Plasma CLZ Concentration

Clinical characteristics of severe dose-dependent ADRs and non-ADRs are shown in Table 4. Eight (6.1%) patients had ADRs, sedation (n = 2; 1.5%), myoclonus (n = 4; 3.1%), and seizures (n = 2; 1.5%). They were able to continue CLZ after a reduction of CLZ dose or addition of antiepileptic medication. ADR patients were younger than non-ADR patients. The mean plasma concentration of CLZ and NCLZ in ADR patients was not different from that of non-ADR patients. Compared with plasma CLZ concentrations below 1000 ng/mL, more severe dose-dependent ADRs were observed in patients with concentrations above 1000 ng/mL (OR: 31.72; 95% CI: 1.04–968.81, p = 0.048) (bottom of Table 4).

Discussion

This is a first report about the relationship between plasma CLZ concentration, clinical response, and severe dose-dependent adverse drug reactions in Japanese patients with treatment-resistant schizophrenia. In addition, we first confirmed statistically the validity of the AGNP therapeutic reference (350–600 ng/mL) and ADR ranges (>1000 ng/mL) in an isolated study after adjusting by possible confounders.

Supplementary Table 1 summarizes the previous studies of the relationship between plasma CLZ concentration and clinical efficacy. Our findings are consistent with many studies that showed positive relationships between CLZ concentrations and clinical efficacy. On the other hand, there are many studies that do not show positive relationships. There are

several reasons involving methodological factors that explain the discrepant findings. First, sample sizes in almost all studies were less than 100 patients and thus too small for detection of a true difference (type II error). Second, though TDM of CLZ is recommended 10–14 h after the last CLZ administration (trough sampling) (20), there are several studies which did not sample in the trough and did not determine a steady state of CLZ concentration (19). Third, a fixed dose is not suitable for optimal CLZ prescription. Fourth, the duration of CLZ treatment was too short to detect late responders who improved after over three months of CLZ treatment (23,24,26). Finally, almost all studies did not adjust for possible confounders (27). Our study avoids these previous methodological deficiencies and assessed the largest sample size (131 patients), used trough sampling (10–14 h), adjustment of background covariates, flexible doses fitting clinical practice, and long-term treatment durations of CLZ (mean 761.9 days and more than 3 months).

In the validity of the commonly used AGNP therapeutic reference range and alert concentration for seizures (6), improvement with 350–600 and 600–1000 ng/mL CLZ concentrations is better than that at <350 ng/mL, and >1000 ng/mL is best. On the other hand, more severe dose-dependent ADRs occur above 1000 ng/mL. Our findings confirm the validity of the AGNP therapeutic reference and ADR range. Though many previous studies have focused separately on estimating lower response thresholds or high safety-related thresholds (5), our study statistically validated efficacy and safety together. Remington et al. (28) reported that increasing CLZ concentrations over 600–838 ng/ml didn't improve clinical response, though there is not enough evidence of efficacy at high concentrations of CLZ. One retrospective study (29) showed that high (1077±457 ng/mL) and intermediate (838±699 ng/mL) doses of CLZ have the same effectiveness, though the

comparison range of CLZ concentration was possibly too narrow to clarify the difference in clinical efficacies. Because a wide range of CLZ concentrations was compared in this study, CLZ concentrations above 1000 ng/mL may be potentially useful for patients with poor response to CLZ, if without severe ADRs. In these cases, augmentation of CLZ with electroconvulsive therapy (30) or switching to long-acting injectable antipsychotics (31) may be an option due to the risk of dose-dependent side effects (4). Augmentation of CLZ with another antipsychotic may be more useful (32), but is not approved in Japan.

In our study, the mean CLZ concentration-to-dose (C/D) ratio was 1.80 (ng/mL per mg/day), relatively higher than in Caucasian samples. Our findings are consistent with the above-mentioned East Asian review (7). The CLZ C/D ratio depends on CLZ clearance, which is primarily metabolized by cytochrome P450 (CYP) 1A2 (33). Variant CYP1A2 allele frequencies associated with poor metabolization are more common in Japanese (23.4%) and Asians (21.0–26.7%) than in Caucasians (0.8–4.0%) (34-36). Thus, differences in variant CYP1A2 allele frequencies between ethnic groups may explain differences in CLZ clearance. Due to the large individual variation in CLZ clearance, Japanese ethnic groups especially may require stricter TDM of CLZ.

A 17-country survey of CLZ utilization shows that it is least frequently administered in Japan (37). TDM of CLZ is rarely used in Japan, unlike Sweden (30%) and China (44%) (38,39), because it is not covered by insurance and is limited to use by a few research institutions in Japan. We hope that our findings will promote the optimal use of TDM of CLZ and induce more effective and safer treatment of TRS patients with CLZ.

Limitations

The main limitation of this study is that the dose and concentrations of CLZ were not fixed or randomized; rather the dose and concentrations were determined by the clinical state of the patient (response and side-effects). To find the therapeutic response thresholds for CLZ concentrations, Schulte (19) reported that the best methodological studies of titration with TRS patients use a predefined fixed dose or randomize patients to different CLZ concentrations ranges. Our flexible dose study may have a bias; for example, non-responders were subjected to high doses and CLZ concentrations. On the other hand, CLZ doses were adjusted to the best clinical response and tolerance expected by patients, their families, and physicians under real-world clinical conditions. This different approach supports the validity of the AGNP therapeutic reference range (350–600 ng/mL) and may show the potential effectiveness of a dose above 1000 ng/ml. Because this was a cross-sectional study, these results must be interpreted carefully.

Next, eight weeks are reported to be the minimum for an adequate evaluation of the effectiveness of CLZ because patients may be given unnecessarily high doses of CLZ and late responders to CLZ may be overlooked (19). In this study, we stabilized the CLZ dose for more than seven days before measuring its concentration and performed the clinical assessments on the same day as blood sampling because reaching the steady state of CLZ is reported to require 5–7 days (19) and several previous studies administered CLZ for 5–7 days before measuring its concentration and performed the clinical assessments on the same day as blood sampling (40,41). Thus, stabilizing the CLZ dose for more than seven days, as was done in this study, may not be adequate for measuring CLZ concentration and clinical assessments.

There are several other limitations. First, the nature of our study design (i.e.,

intermittent sampling) may increase the possibility of selection bias (internal validity). Patients with longer durations of CLZ treatment (over 12 months) may tend to be better responders and be tested by TDM (survivor and length bias). Therefore, we considered the possibility by adjusting CLZ treatment durations in the fully adjusted model. Second, because our study included only patients who agreed to participate in the study, the result may not be directly generalizable to clinical practice (external validity). Third, clinical assessment by physicians in several different institutions may suffer from potential inter-rater unreliability. The Positive and Negative Syndrome Scale (PANSS) may be better than BPRS. However, this reliability could skew our results toward the null and thus did not threaten the significance of our results. Fourth, outpatients' TDM may include poor adherence, though the attending physicians checked the possibility of a lack of adherence at the time of TDM. Fifth, even in trough steady-state collections, there is substantial variability due to medication adherence, smoking, caffeine intake, and concomitant medications by using a single sample. Sixth and last, our study evaluated only severe dose-dependent ADRs and did not evaluate non-severe dose-dependent ADRs, such as constipation and hypersalivation. Further studies are warranted to include the information on concomitant medications and non-severe dose-dependent ADRs.

Conclusion

Our findings suggest that the AGNP therapeutic reference range (350–600 ng/mL) is useful and potentially more effective above 1000 ng/mL, but clinicians need to be aware of severe adverse effects on the central nervous system (sedation, myoclonus, and seizures). The AGNP guidelines are potentially applicable to Japanese patients (and perhaps other East

Asian patients) despite genetic differences from Caucasian patients. Our study confirmed the relationship between plasma CLZ concentrations, clinical efficacy, and severe dose-dependent ADRs in Japanese patients with TRS.

Declaration of interest

Y.Y. has received honoraria for his participation as a speaker at educational events sponsored by Novartis and Dainippon Sumitomo. N.Y. has received unrestricted research funding from Daiichi Sankyo, Eisai, Pfizer, Otsuka, Astellas, and Merck Sharp & Dohme, which was deposited into research accounts at Okayama University. N.Y. has received honoraria for his participation as a speaker at educational events from UCB Japan, Tsumura, Pfizer, Dainippon-Sumitomo, Daiichi-Sankyo, Merck Sharp & Dohme, Pfizer, Eisai, Meiji-Seika, and Mochida. M.T. has received honoraria for his participation as a speaker at educational events sponsored by Daiichi Sankyo, Takeda, Tsumura, Otsuka and Dainippon Sumitomo. S.S. has received unrestricted research funding from Eli Lilly, which was deposited into research accounts at Okayama University Hospital. S.S. has received honoraria for his participation as a speaker at an educational event sponsored by Otsuka and Meiji-Seika. Y.K. has received honoraria for his participation as a speaker at educational events sponsored by Novartis and Dainippon Sumitomo. K.K., Y.O., A.O., A.N., H.K. and S.T. report no additional financial or other relationship relevant to this article.

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Author Contribution

Yuji Yada, M. Takaki, S. Sakamoto, Y. Okahisa and N. Yamada participated in the design of the study, supervised the project, and contributed intellectually to the interpretation of the data. K. Kitagawa assayed the laboratory data. Y. Y, K. K, Y. Kishi, A. Ozawa, A. Nakada and H. Kashiwagi investigated patient clinical records. S. Takao interpreted the statistical analyses. M. T., S. S. and S. T. revised critically. All authors contributed to and have approved the final manuscript.

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Figure 1 legend

Relationship between dosage and plasma concentrations of clozapine and norclozapine for 131 patients. Therapeutic reference range of 350–600 ng/mL is highlighted. Seizure-alert concentration of 1000 ng/mL is (- - -). The daily clozapine dosage was moderately positively related to the plasma concentrations of clozapine and norclozapine (Pearson's $r = 0.49$, $p < 0.001$, $r = 0.55$, $p < 0.001$).

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Supplementary Fig. 1 Histogram of responders and non-responders at each 50 ng/mL increment of concentration

Supplementary Table 1 CLZ concentrations and clinical efficacy studies

Supplementary Fig. 2: Receiver operating characteristic (ROC) curve for the relationship between responder status and plasma concentration of CLZ (50 ng/mL increments)

Table 1. Clinical characteristics of responders and non-responders

Characteristics	All patients (n = 131)		Responders (n = 79)		Non-responders (n = 52)	
	Mean ± SD	range	Mean ± SD	range	Mean ± SD	range
SEX (male /female)	73 / 58		45 / 34		28 / 24	
Age (years)	40.1 ± 12.0	16 - 72	40.1 ± 11.5	16 - 72	39.9± 12.5	18 - 66
Duration of illness (years)	18.5 ± 11.1	0.08 - 47	18.7 ± 10.3	1.2 - 44	18.4 ± 12.3	0.08 - 47
Weight (kg)	63.7 ± 12.6	37.4 - 104	65.7 ± 11.9	42.5 - 104	60.7 ± 13.3	37.0 - 88.2
Smoking, n (%)	28 (21.4)		16 (20.3)		12 (23.1)	
Habitual caffeine intake, n (%)	37 (28.2)		15 (19.0)		22 (42.3)	
Number of TDM (single/multiple)	63 / 68		39 / 40		24 / 28	
Concomitant medications						
Lithium carbonate, n (%)	57 (43.5)		34 (43.0)		23 (44.2)	
Antiepileptics, n (%)	47 (35.9)		24 (30.4)		23 (44.2)	
Antidepressants, n (%)	11 (8.4)		6 (7.6)		5 (9.6)	
Daily CLZ dosage (mg/day)	372.1 ± 140.1	75 - 600	369.3 ± 130.6	125 - 600	376.4 ± 154.7	75 - 600
Duration of CLZ treatment (day)	761.9 ± 640.4	90 - 2736	916.7 ± 687.3	111 - 2736	526.8 ± 478.9	90 - 2115
CLZ concentration (ng/mL)	630.0 ± 289.8	118.2 - 1465.5	678.8 ± 300.6	131 - 1466	555.7 ± 258.0	118 - 1092
NCLZ concentration (ng/mL)	318.0 ± 156.3	60.7 - 1102.2	335.4 ± 160.5	85 - 1102	291.5 ± 147.2	61 - 648
CLZ concentration-to-dose ratio (ng/mL per mg/day)	1.80 ± 0.79	0.20 - 4.57	1.94 ± 0.84	0.52 - 4.57	1.59 ± 0.68	0.20 - 3.64
BPRS score at baseline						
Total	66.0 ± 15.4	31 - 113	68.2 ± 16.0	35 - 113	62.8 ± 13.9	31 - 102
Positive	25.1 ± 6.8	9 - 42	26.1 ± 7.3	9 - 42	23.7 ± 5.8	11 - 36
Negative	8.2 ± 2.8	2 - 14	7.9 ± 3.0	2 - 14	8.5 ± 2.3	3 - 14
General psychopathology	32.8 ± 9.3	15 - 60	34.2 ± 9.3	20 - 60	30.6 ± 8.9	15 - 57
BPRS score at last observation						
Total	49.4 ± 15.0	20 - 88	44.4 ± 14.2	20 - 88	57.0 ± 13.0	31 - 83
Positive	17.1 ± 6.0	7 - 30	14.9 ± 5.5	7 - 30	20.4 ± 5.1	8 - 30
Negative	6.7 ± 2.5	2 - 12	5.9 ± 2.3	2 - 11	7.9 ± 2.3	4 - 12
General psychopathology	25.7 ± 8.3	11 - 49	23.7 ± 8.2	11 - 49	28.6 ± 7.8	15 - 48
CGI - S						
Baseline	5.9 ± 0.7	3 - 7	6.0 ± 0.8	3 - 7	5.9 ± 0.6	4 - 7
Last observation	4.5 ± 1.1	1 - 6	4.0 ± 1.1	1 - 6	5.1 ± 0.8	3 - 6
Improvement (baseline - last)	1.5 ± 1.1	0 - 5	2.0 ± 1.0	0 - 5	0.8 ± 0.7	0 - 3
CGI - I	2.5 ± 0.9	1 - 6	2.1 ± 0.8	1 - 5	3.0 ± 0.8	2 - 5
GAF score						
Baseline	24.3 ± 7.7	5 - 50	23.1 ± 7.5	10 - 50	26.1 ± 7.7	5 - 40
Last observation	37.7 ± 11.1	10 - 65	40.9 ± 10.9	20 - 65	32.7 ± 9.7	10 - 58
Improvement (%) (baseline - last / baseline)	67.4 ± 67.4	- 25 - 310	91.8 ± 72.3	0 - 310	30.3 ± 35.7	− 25 - 167

SD, standard deviation; TDM, therapeutic drug monitoring; CLZ, clozapine; NCLZ, norclozapine; BPRS, Brief Psychiatric Rating Scale; CGI, Clinical Global Impression; GAF, Global Assessment of Functioning

Table 2. Percent BPRS score improvement and ORs for BPRS response associated with plasma CLZ concentration (n = 131)

BPRS score improvements, %	Crude model			Adjusted model			Fully adjusted model		
	B	(95% CI)	p	B	(95% CI)	p	B	(95% CI)	p
Total									
for CLZ concentration (100 ng/mL increments)	1.49	(0.52-2.45)	0.003	1.52	(0.55-2.50)	0.003	1.95	(0.89-3.01)	<0.001
Positive									
for CLZ concentration (100 ng/mL increments)	1.62	(0.43-2.82)	0.008	1.69	(0.48-2.89)	0.006	2.15	(0.87-3.43)	0.001
Negative									
for CLZ concentration (100 ng/mL increments)	1.14	(-0.03-2.30)	0.057	1.18	(-0.03-2.33)	0.056	1.40	(0.07-2.72)	0.039
General psychopathology									
for CLZ concentration (100 ng/mL increments)	1.30	(0.25-2.36)	0.016	1.32	(0.25-2.39)	0.016	1.72	(0.51-2.93)	0.006
ORs for BPRS response	**Crude model**			**Adjusted model**			**Fully adjusted model**		
	OR	(95% CI)	p	OR	(95% CI)	p	OR	(95% CI)	p
for CLZ concentration (100 ng/mL increments)	1.17	(1.03-1.17)	0.019	1.18	(1.03-1.35)	0.016	1.38	(1.14-1.66)	0.001

BPRS, Brief Psychiatric Rating Scale; OR, odds ratio; CLZ, clozapine; BPRS score improvement, (baseline - last observation/ baseline *100); B, parameter estimate; CI, confidence interval; Adjusted model: Adjusted for age and sex. Fully adjusted model: Adjusted for age, sex, duration of illness, clozapine dosage, duration of CLZ treatment (categorized into less than 6 months, 6 to 12 months and more than 12 months), smoking, caffeine intake, number of CLZ therapeutic drug monitoring (single or multiple), concomitant medications (lithium carbonate, antiepileptics or antidepressants)

The percent BPRS score improvements per 100 ng/mL plasma CLZ concentration increment are shown in the upper part. The odds ratios (ORs) for BPRS respon

Table 3. Comparison of percent BPRS score improvements and ORs for BPRS response at four CLZ concentration ranges

CLZ concentration range		n	Responders / Non-responders	BPRS score improvements, % (Fully adjusted model)			ORs for BPRS response (Fully adjusted model)		
				B	(95% CI)	p	OR	(95% CI)	p
< 350	(ng/mL)	23	(10 / 13)	0	reference	-	1	reference	-
350 - 600	(ng/mL)	42	(26 / 16)	11.12	(2.52 - 19.72)	0.012	7.09	(1.85 - 27.24)	0.004
600 - 1000	(ng/mL)	55	(33 / 22)	11.05	(2.40 - 19.71)	0.013	8.11	(2.09 - 31.53)	0.003
> 1000	(ng/mL)	11	(10 / 1)	25.36	(13.08 - 37.64)	< 0.001	57.28	(4.36 - 753.32)	0.002

BPRS, Brief Psychiatric Rating Scale; OR, odds ratio; CLZ, clozapine; B, parameter estimate; CI, confidence interval;
Fully adjusted model: Adjusted for age, sex, duration of illness, clozapine dosage, duration of CLZ treatment (categorized as less than 6 months, 6 to 12 months, and more than 12 months), smoking, caffeine intake, number of CLZ therapeutic drug monitoring (single or multiple), concomitant medications (lithium carbonate, antiepileptics or antidepressants)

Table 4. Clinical characteristics of severe dose-dependent ADRs and non-severe ADRs

Characteristics				
	Severe ADRs (n = 8)		Non - severe ADRs (n = 123)	
	Mean ± SD	Range	Mean ± SD	Range
Sex (male/female)	2 / 6		71 / 52	
Age (years)	30.3 ± 10.0	19 - 46	40.7 ± 11.7	16 - 72
Duration of CLZ treatment (days)	815.9 ± 691.7	161 - 2112	758.4 ± 639.8	90 - 2736
Weight (kg)	56.5 ± 11.3	39.5 - 68.9	64.2 ± 12.6	37.4 - 104
Smoking, n (%)	1 (13.0)		27 (22.0)	
Habitual caffeine intake, n (%)	2 (25.0)		35 (28.5)	
Number of TDM (single/multiple)	3 / 5		60 / 63	
Concomitant medications, n (%)				
Lithium carbonate, n (%)	0		57 (46.3)	
Antiepileptics, n (%)	6 (75.0)		41 (33.3)	
Antidepressants, n (%)	2 (25.0)		9 (7.3)	
Daily CLZ dosage (mg/day)	453.1 ± 107.3	300 - 600	366.9 ± 140.7	75 - 600
BPRS total improvement (%)	23.5 ± 15.1	- 2.3 - 44	24.7 ± 16.8	- 25.8 - 73.3
BPRS responder, n (%)	4 (50.0)		75 (61.0)	
CLZ concentration (ng/mL)	784.4 ± 318.3	526.3 - 1337.6	619.9 ± 286.4	118.2 - 1465.5
NCLZ concentration (ng/mL)	350.0 ± 162.0	209.5 - 660.4	315.9 ± 156.3	60.7 - 1102.2
ORs for severe dose-dependent ADRs categorized by CLZ concentration ranges (Adjusted model)				
	ADRs / Non-ADRs	OR	(95% CI)	p
CLZ concentration <1000 ng/mL (n)	6 / 114	1	reference	-
CLZ concentration >1000 ng/mL (n)	2 / 9	31.72	(1.04 - 968.81)	0.048

ADRs, adverse drug reactions; SD, standard deviation; CLZ, clozapine; TDM, therapeutic drug monitoring; NCLZ, norclozapine; BPRS, Brief Psychiatric Rating Scale; improvement, (baseline last observation/baseline *100); OR, odds ratio; CI, confidence interval; Adjusted Model: Adjusted for age, sex, weight, smoking, caffeine intake, number of CLZ therapeutic drug monitoring (single or multiple), concomitant medications (lithium carbonate, antiepileptics or antidepressants)

Fig. 1. Relationship between dosage and plasma concentrations