Abstract  Rationale: Monitoring plasma clozapine concentrations may play a useful role in the management of patients with schizophrenia, but information on the relationship between the plasma levels of the drug and response is still controversial.  Objective: The purpose of this study was to assess the relationship between plasma concentrations of clozapine and its weakly active metabolite norclozapine and clinical response in patients with schizophrenia resistant to conventional neuroleptics.  Methods: Forty-five patients, 35 males and ten females, aged 19–65 years, were given clozapine at a dosage up to 500 mg/day for 12 weeks. Steady-state plasma concentrations of clozapine and norclozapine were measured at week 12 by a specific HPLC assay. Psychopathological state was assessed at baseline and at week 12 by using the Brief Psychiatric Rating Scale, and patients were considered responders if they showed a greater than 20% reduction in total BPRS score compared with baseline and a final BPRS score of 35 or less.  Results: Mean plasma clozapine concentrations were higher in responders (n=18) than in non-responders (n=27) (472±220 versus 328±128 ng/ml, P<0.01), whereas plasma norclozapine levels did not differ between the two groups (201±104 versus 156±64 ng/ml, NS). A significant positive correlation between plasma levels and percent decrease in total BPRS score was found for clozapine (r_s=0.371, P<0.02), but not for norclozapine (r_s=0.162, NS). A cut-off value at a clozapine concentration of about 350 ng/ml differentiated responders from non-responders with a sensitivity of 72% and a specificity of 70%. At a cutoff of 400 ng/ml, sensitivity was 67% and specificity 78%. The incidence of side effects was twice as high at clozapine concentrations above 350 ng/ml compared with lower concentrations (38% versus 17%). Conclusions: These results suggest that plasma clozapine levels are correlated with clinical effects, although there is considerable variability in the response achieved at any given drug concentration. Because many patients respond well at plasma clozapine concentrations in a low range, aiming initially at plasma clozapine concentrations of 350 ng/ml or greater would require in some patients use of unrealistically high dosages and imply an excessive risk of side effects. Increasing dosage to achieve plasma levels above 350–400 ng/ml may be especially indicated in patients without side effects who failed to exhibit amelioration of psychopathology at standard dosages or at lower drug concentrations.

Key words  Clozapine · Norclozapine · Plasma concentration · Schizophrenia · Therapeutic drug monitoring

Introduction

Clozapine is an atypical antipsychotic with a low potential for inducing extrapyramidal side effects, used primarily in the management of patients with schizophrenia resistant to conventional neuroleptics (Baldessarini and Frakenburg 1998). Although clozapine is effective in up to 50–60% of these patients (Kane et al. 1988; Meltzer et al. 1990), its wider use has been limited by the risk of agranulocytosis, which makes frequent hematological monitoring necessary (Alvir et al. 1993).

Clozapine is metabolized in the liver by cytochrome P450 isoenzymes to several biotransformation products, the most important of which is the weakly active demethylated metabolite norclozapine (Jann et al. 1993). The disposition of clozapine is influenced by patient-
related factors such as age, gender and smoking habits (Haring et al. 1990), and by concomitant intake of other drugs (Edge et al. 1997). Due to wide pharmacokinetic variability, it has been suggested that monitoring plasma clozapine concentrations might play a useful role in clinical management (Freeman and Oyewumi 1997), but information on the relationship between plasma clozapine levels and response is still controversial. While in earlier investigations antipsychotic efficacy did not appear to be directly related to the drug concentration in plasma (Ackenheil et al. 1976; Thorup and Fog 1977; Brau et al. 1978), more recent data suggest that a positive correlation does exist (Perry et al. 1991; Hasegawa et al. 1993; Miller et al. 1994; Potkin et al. 1994; Kronig et al. 1995).

In some studies, a favourable response was found to be most likely above a cutoff value of 350–420 ng/ml (Perry et al. 1991; Hasegawa et al. 1993; Miller et al. 1994; Potkin et al. 1994; Kronig et al. 1995), but evidence has also been provided that these concentrations may be unnecessarily high and that a lower cutoff (around 250 ng/ml) may provide a more useful guide to adjustment of clozapine dosage (Van der Zwaag et al. 1996). The finding that some patients respond well at low plasma clozapine concentrations has also been taken as an argument against the use of a threshold value in therapeutic drug monitoring (Olesen 1998). The potential value of measuring the plasma concentration of the active metabolite norclozapine also remains to be established, and there is inadequate information about potential correlations between plasma levels of parent drug and metabolite and adverse effects.

In view of the evident implications of defining a correlation between plasma concentration and therapeutic and toxic effects, we conducted a study in which the plasma levels of clozapine and its active demethylated metabolite were measured in a sizeable group of patients with drug-resistant schizophrenia started on clozapine. Our results confirm that amelioration of psychotic symptoms correlates better with plasma clozapine levels than with the prescribed daily dosage and that norclozapine levels do not improve the predictive value of clozapine measurements.

Materials and methods

Patients

This study was part of an open follow-up evaluation of clozapine in patients with treatment-resistant schizophrenia. Patients under psychiatric care at the Centers of Mental Health, Azienda USL 5, Messina, Italy, were enrolled in the study according to the following eligibility criteria: 1) age above 18 years; 2) DSM-IV diagnosis of schizophrenia; 3) no response to at least two neuroleptics from two different chemical classes, used at dosages equivalent to at least 500 mg/day of chlorpromazine equivalents; 4) a total score of at least 45 on the 18-item Brief Psychiatric Rating Scale (BPRS) (Overall and Gorham 1962); 5) no exposure to depot antipsychotics during the previous 6 weeks. The protocol was approved by a local ethics committee and written informed consent was obtained from the patients or their relatives. All patients were hospitalized or attended the day hospital for at least 1–2 weeks after the initiation of treatment, and they were seen subsequently in the outpatient clinic at weekly intervals and treated by the same physicians and nursing personnel.

Study design

After a 1-week washout period, clozapine was initiated at a dosage of 25 mg/day and increased by 25–50 mg every few days up to a median dosage of 300 mg/day, given in two or three divided daily administrations. After completion of the titration period, which lasted for 2–3 weeks, dosage was in some cases adjusted by the treating physician on the basis of individual response as usually done in routine clinical practice and kept constant thereafter. No other psychotropic drugs were allowed, except for occasional benzodiazepines. Blood cell counts were performed weekly.

Clinical assessment

Antipsychotic response was assessed by determining BPRS scores at baseline (before initiation of clozapine) and after 4, 8 and 12 weeks. Ratings were done by trained research assistants who were not blind to the treatment. Because in some patients response to clozapine develops slowly after several weeks (Lieberman et al. 1994), the change in BPRS score at the final evaluation was used to differentiate responders from non-responders and to assess relationship between plasma drug levels and therapeutic response. For categorical analysis, patients were classified as responders if they showed a 20% or greater reduction in total BPRS score compared with baseline and their final BPRS score was 35 or less (Kane et al. 1988; Kronig et al. 1995). Adverse effects were evaluated at the same time points as psychopathology scores by means of medical examination and questioning of patients, relatives and nursing personnel.

Drug assays

To study the relationship between plasma drug levels and clinical outcome, blood samples for the determination of clozapine and norclozapine concentrations were obtained on 2 different days at the end of week 12, between 8 a.m. and 9 a.m., approximately 12 h after the bedtime dose. The mean of the two determinations was used for statistical evaluation. The plasma was separated within 3 h and stored at −20°C until assayed. Concentrations of clozapine and norclozapine were measured in duplicate by using the HPLC method described by Avenoso et al. (1998). The interday coefficient of variation was less than 3.4% for clozapine and less than 7.5% for norclozapine. The limit of quantitation was 1 ng/ml for both analytes.

Statistical analysis

Results are reported as means±SD. Demographic characteristics, plasma concentrations of clozapine, norclozapine or their sum in responders and non-responders were compared by using the Student's t-test for unpaired data. The Mann-Whitney U-test was used to compare total BPRS scores at baseline and at week 12 in responders and non-responders. Correlational analyses were also performed by model-independent non-parametric statistics. The Spearman rank correlation coefficient (r_S) was used to test correlations between plasma clozapine and norclozapine levels and dose, between percent improvement in BPRS scores and clozapine dose, and between percent improvement in BPRS scores (total and subscales for positive and negative symptoms) (Kane et al. 1988) and plasma concentrations of clozapine, norclozapine or their sum. Categorical analysis was carried out by means of receiver-operator curves (Perry et al. 1991; Kronig et al. 1995), in which percentages of true positives (responders at concentrations above a predetermined cut-off value as a fraction of the total number of respond-