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Original article

The usefulness of monitored therapy using Clozapine concentration in the blood serum for determining drug dose in Polish schizophrenic patients

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ABSTRACT

Background: The aim of the study is to evaluate the advisability of systematic monitoring of clozapine (CLO) concentration in serum during treatment of schizophrenia in Polish psychiatric patients.
Method: The concentration of CLO and its metabolites: norclozapine (NCLO) and clozapine N-oxide (CLO-NO) in serum obtained from 107 patients suffering from schizophrenia was determined by high performance liquid chromatography (HPLC) method. There were two groups of patients. In the first group of patients (n = 95) the concentration of drug and its metabolites was determined by one-time testing. Correlations were tested using the test statistics. In the second group of patients (n = 12), 51 samples of serum were provided by the same patient in different time spans (from 6 days to 14 months after the beginning of the treatment).
Results: Concentrations of CLO and its metabolites in blood serum do not always show a linear dependence on the applied dose for individual patients.
Conclusion: The high volatility of CLO concentrations in blood serum of patients treated with identical doses of the drug confirmed the validity of the monitored therapy.

Introduction

Schizophrenia is a disease occurring in all regions of the world, and the number of people suffering from it is now estimated at 0.8–1% of the population. The age between 15 and 45 years old is considered the period of risk (highest incidence). This risk is the same in both sexes, but the incidence differs in terms of the onset – earlier in men (the age 15–24 years), and later in women (the age of 25–34 years) [1].

Despite the development of novel drugs, which have a greater efficacy and fewer side effects, some patients still do not respond to treatment. The number of patients who are refractory to treatment is estimated at between 30–60%, depending on applicable criteria [2]. In this case clozapine (CLO) gives good results. Despite the significantly increasing over the last 20 years amount of atypical neuroleptics, CLO is still a unique drug. It is regarded to be superior to other antipsychotics, both typical and atypical, in the treatment of refractory schizophrenia. It is recommended that each of these patients should undergo the test of CLO therapy [3].

CLO is a drug of choice in the treatment of patients resistant to other neuroleptics, who have "drug-resistant schizophrenia." The proportion of drug-resistant patients in whom CLO ameliorates reaches 40–70% [4]. Although in patients known as drug-resistant CLO works most effectively, due to the risk of many adverse effects, including serious (agranulocytosis, myocarditis, other inflammatory conditions, convulsions, obesity, diabetes, metabolic syndrome), treatment with CLO must be systematically and carefully monitored. In addition, there is a risk of drug-resistant exacerbation after its withdrawal. Therefore, some propose the creation of specialized CLO treatment centers, closely monitoring and having the ability to introduce a quick appropriate medical intervention [5]. Drug monitoring is aimed to individualize dosage for individual patients, leading to the achievement of the maximum effectiveness of the substance while minimizing adverse side effects of the drug. Both the effectiveness and the induction of

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unwanted response to a drug are dependent on the dose. During treatment with a particular drug, measurements are made of its concentration, which allows for determining the dose, whose concentration at the site of action is appropriate to achieve optimal therapeutic effects [6].

A preferred result of CLO treatment correlates with serum concentrations of 350–420 ng/ml, which corresponds to 169–825 mg daily doses of the drug [7].

Increasing the daily dose above 600 mg is associated with a high risk of complications (convulsions, salivation, fainting, pressure drop) [8]. According to Vogel et al. [9] a therapeutic dose oscillates between 300–600 mg, and even 900 mg in the maintenance treatment. It is recommended that the concentration was maintained above 400 ng/ml. There are studies showing that 40–70% of patients treated with CLO do not manifest optimal response to treatment, and a dose increase as well as alternative treatments performed to get a better response are not supported by consistent evidence [3].

CLO is metabolized in the liver by the cytochrome P450 system to multiple metabolites, of which the most important are NCLO and CLO-NO. There are wide individual differences in the formation of the two metabolites. It was found that NCLO may contribute to the therapeutic effect of CLO with a new mechanism involving modulation of muscarinic receptor and 5-HT2 [10,11]. CLO is metabolized by the isoforms CYP1A2, CYP3A4, CYP2D6. All substances (including drugs) that increase activity of these isoforms (inductors) or decrease activity of these isoforms (inhibitors) influence also significantly the concentration of CLO in serum.

Terapeutic drug monitoring (TDM) serum concentrations of CLO is uncommon outside of specialist clinics [10], in spite of numerous works showing the effects of smoking cigarettes and caffeine levels in the blood serum concentrations of CLO [12–14], only in Japan CLO is mandatorily (routine) monitored in clinical practice [15] and not only prescribed for monitoring (according to the guidelines AGNP-TDM).

The main objective of this study was to evaluate the utility of monitoring therapy in patients taking CLO. We are trying to assess concentrations of CLO and its metabolites in the blood serum of patients treated with different doses of the drug and whether it could be sufficient evidence of the need for routine monitoring of CLO in clinical practice.

Materials and methods

Patients – clinical materials

The concentration of CLO, NCLO and CLO-NO in serum obtained from 107 patients suffering from schizophrenia. There were two groups of patients. In the first group the concentration of drug and its metabolites was determined by one-time testing. In this group there were 95 patients (34 women and 61 men) treated for schizophrenia with different doses of CLO, ranging from 25 to 900 mg/day. In the second group there were 12 patients (6 women and 6 men, aged from 24 to 63), treated for schizophrenia with different doses of CLO, ranging from 200 to 900 mg/day, from whom 51 samples were obtained. In this group several samples of serum were provided by the same patient in different time spans (the period between first and last sampling for different patient ranged from 6 days to 14 months).

The reason for testing the concentration of CLO in the serum of patients were the following clinical problems:

1. No response or not full clinical response to the applied treatment;
2. The undesirable symptoms of treatment;
3. The need of the control of the appropriateness of the drug concentration in serum;
4. The application of the drug in the politherapy.

The information card of the patient contained the information about the number of cigarettes a day by the patient, the weight of the patient, height, the amount of caffeine daily (in mg), the treatment duration and about the other drugs taken by the patient. Some patients were subjected to monotherapy, while a part of them received additional treatment associated with a risk of other diseases, or aimed to mitigate adverse effects following CLO. The serum was collected from the patients after they reached the steady state when receiving the stable dose of the drug. The serum was collected in the morning before the next dose of the drug was applied, so the dosing range was measured. Patients were recruited from the Departments of Psychiatry at Medical University in Warsaw and were hospitalized during treatment. Serum was prepared by centrifugation of blood samples at $-20\,^{\circ}C$ until assayed. Before the treatment was started, an informed consent was obtained from all patients. Local Bioethical Committee approved the trial.

Methodology

We have developed the HPLC method based on the work of Liu et al. [16], with many modifications concerning the composition of the stationary phase, the kind of column and extraction method. The HPLC method for simultaneous determination of CLO, NCLO, CLO-NO and doxepine (DOX) as internal standard (IS) was described. This method was developed, optimized and validated. Analyses were performed on Shimadzu Chromatograph with UV detector, at lambda = 215 nm. After extraction on SPE (Solid Phase Extraction) column, the sample was injected on Symmetry Waters C18 (5 μm x 4.6 × 150 mm) column (stationary phase). Eluating mixture contained acetonitryle:H_2O (3:7) with 146 μl triethylamine and 200 μl 85% H_3PO_4, pH = 3.3, flow rate: 0.8 ml/min.

Assay Validation

Validation of HPLC method of determining CLO and its metabolites in standard serum containing known amounts of drug were spiked with standard solutions to obtain final concentrations of 50–1600 ng/ml CLO, NCLO and 20–400 ng/ml CLO-NO. The precision and accuracy of the method were determined by replicate analyses of serum samples containing different concentrations (low, medium and high) of CLO, NCLO and CLO-NO.

The limit of quantification (LOQ) and the limit of detection (LOD) were calculated according to Lunn et al. [17].

Statistical analysis

Descriptive analysis of clinical features and dose-concentration relationship was carried out for two groups of study patients (n = 95 and n = 12). We compared the mean concentrations of CLO and its metabolites in the appropriate dosing intervals. Results are presented as mean +/− standard deviation (SD). Statistical analysis were performed for the group of patients using the Statistica v. 10.0 software by StatSoft Inc., USA. Area under the curve was calculated by the trapezoid method. The plasma concentrations of CLO and its metabolites scores were analyzed using a repeated measures MANOVA (multivariate analysis of variance). Chi-square test was used to assess the variables. Student's t-test was applied in the assessment of differences in interval variables. The threshold of significance for all tests was set at 5% (<0.05).