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Short communication

Retrospective evaluation of therapeutic drug monitoring of clozapine and norclozapine in Belgium using a multidrug UHPLC-MS/MS method

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Abstract

Objective: Clozapine is an atypical antipsychotic with a narrow therapeutic range and serious toxic side effects. According to AGNP-TDM consensus guidelines, therapeutic drug monitoring (TDM) of clozapine and its metabolite norclozapine is strongly recommended. 330 serum samples, sent to the toxicological laboratory of Ziekenhuis Netwerk Antwerpen for monitoring of clozapine, were tested with a new ultra-high performance liquid chromatography-tandem mass spectrometric method (UHPLC-MS/MS). The aim of this research was to evaluate this method for TDM of clozapine and norclozapine, but also to determine other antipsychotics present in these serum samples.

Design and methods: Serum samples were taken just prior to the morning dose of the antipsychotic (trough concentration). All samples were, after a simple liquid-liquid extraction with methyl *t*-butylether, analyzed using a fully validated UHPLC-MS/MS method which is able to quantitate 16 different antipsychotics and 8 of their major metabolites. Serum concentrations were compared with the therapeutic ranges as defined by the AGNP-TDM guidelines.

Results: For clozapine, only 22.3 % of the serum concentrations were within the therapeutic range of 350-600 ng/mL, while 67.9 % of the concentrations were below 350 ng/ml. For norclozapine, 68.2 % of the serum samples were within the therapeutic range of 100-600 ng/ml. The mean clozapine:norclozapine ratio was 1.7 (SD 0.8). 218 of the 330 serum samples contained other antipsychotics than clozapine. Only 52.5 % of these concentrations were within the proposed range.

Conclusion: This retrospective study highlights the importance of TDM for clozapine and other APs, since many patients show suboptimal serum concentrations.

Keywords

Clozapine, antipsychotic drugs, therapeutic drug monitoring, UHPLC-MS/MS

Abbreviations

Antipsychotic, AP; Clozapine, CLO; norclozapine, NORCLO; Therapeutic drug monitoring, TDM; ultra-high performance liquid chromatography-tandem mass spectrometry, UHPLC-MS/MS;

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Introduction

Therapeutic drug monitoring (TDM) is of great importance for drugs with a high interindividual variability in serum concentration, a narrow therapeutic range or serious adverse effects. Clozapine (CLO), a tricyclic dibenzodiazepine belonging to the atypical antipsychotics (APs), meets these requirements. According to the AGNP Consensus Guidelines for TDM in Psychiatry (Arbeitsgemeinschaft für Neuropsychopharmakologie und Pharmakopsychiatrie), TDM of CLO is strongly recommended and TDM can help in dose optimization and in monitoring adherence.[1] Clozapine's major metabolite, norclozapine (NORCLO) is mainly formed by the oxidative metabolism of CLO by CYP1A2, while CYP2C9, CYP2C19, CYP2D6 and CYP3A4 contribute moderately. NORCLO levels usually range between 50-90 % of the total CLO concentrations. [2-5]

CLO is recommended for treatment-resistant schizophrenia and for psychosis in Parkinson disease, not responding to standard therapy.[2] Although not FDA approved, CLO is also used for severe mania in bipolar disorder.[6] Serious side effects, like weight gain, sedation, postural hypotension, metabolic disturbances, cardiomyopathy, seizures and considerable agranulocytosis, impede dose optimization and is often a reason for suboptimal dosage.[3] Determination of steady-state serum concentration of CLO is important in the clinical management of psychiatric patients.[7]

Of all APs, only CLO is monitored routinely in Belgium. Recently, we developed a multi-analyte ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) method for quantification of 16 different APs including CLO and NORCLO.[8] Serum samples for CLO monitoring were collected in order to evaluate this analytical method, to evaluate the therapeutic range of the CLO and NORCLO results and the value of the

CLO:NORCLO ratio. Additionally, presence of other APs in these serum samples was also established.

Materials & methods

Patients samples

A total of 330 serum samples were collected from 171 patients (62.6 % male, 37.4 % female), aged 19 to 74 years. More than one serum sample was received from 39.1% of the patients. These samples were send for routine TDM of CLO and NORCLO and collected between November 2012 and September 2013 at the laboratory for toxicology and TDM of Ziekenhuis Netwerk Antwerpen (Antwerp, Belgium). This laboratory receives about 500 samples a year for CLO monitoring. In Belgium, approximately 120 000 patients outside the hospital setting are treated each day with an antipsychotic. According to the National Institute for Health and Disability Insurance (NIHDI), at least 2000 patient are prescribed CLO each day. However, the number of hospitalized patients treated with CLO were not taken into account since these data were not available.

As recommended, serum samples were taken just prior to the morning dose of the AP (trough concentration). After routine analysis using a gas chromatography-nitrogen phosphorus detection method, based on the assay described by Caldwell and Challenger [9], the remainder of the serum samples were sent to the Toxicological Centre of the University of Antwerp.

Clozapine assay

Samples were analyzed by a fully validated UHPLC-MS/MS method for quantification of 16 APs and 8 metabolites in serum, as described before.[8]

Briefly, sample preparation involved liquid-liquid extraction with methyl *tert*-butylether at pH 9.5 using 200 µl of patient serum. After transfer and evaporation of the upper organic layer, the extract was reconstituted in acetonitrile, and injected into the UHPLC-MS/MS system, which was operated in dynamic multiple reaction monitoring mode.

For CLO and NORCLO, calibration curves were linear between 50 and 1500 ng/mL and 10 and 1500 ng/mL, respectively. The LOD was 0.5 ng/ml for CLO and 1 ng/ml for NORCLO. Proficiency testing (Arvecon, Walldorf, Germany), performed 3 times a year, resulted in a limited bias (calculated as % bias against target value) between -8.4 and 7.5 % for CLO and -8.3 and 15.8 % for NORCLO in the period between February 2013 and March 2014.

Results and discussion

323 of the 330 serum samples contained CLO and 324 of the 330 contained NORCLO concentrations above LOD. For CLO, the therapeutic range defined by the AGNP Guidelines is 350 to 600 ng/ml.[1] Concentrations of the order of 250 ng/ml can be adequate once symptoms are controlled. Concentrations above 600-1000 ng/ml are associated with serious side effects.[3, 7] Only 22.3 % (72/323) of the serum concentrations were within the therapeutic range (Figure 1). On the other hand, 21.4 % (69/323) of the serum concentrations were between 250-350 ng/ml, which may be sufficient to obtain clinical effect. 46.5 % (150/323) of the serum concentrations were lower than 250 ng/ml. Overall, 67.9 % (219/323) of the results were lower than 350 ng/ml, which was suggested as a possible reason for poor response. In comparison, *Couchman et al.* found 42.5 % of serum samples lower than 350 ng/ml (n=104 127).[3] As can be seen in the histogram on Figure 1, a

wide distribution of serum concentrations was found and most of them were between 150-350 ng/ml. In this retrospective study, knowledge about the administered dose, duration of clozapine treatment and smoking status was lacking, which makes it difficult to address these subtherapeutic concentrations to underdosing. It is possible that CLO was monitored during start-up of the therapy or when non-compliance was suspected. In Belgium, clinicians are not used to give additional information when requesting TDM. However, laboratories should ask to report this information, since it can aid in interpretation of the results.

For NORCLO, the therapeutic range defined by AGNP is 100-600 ng/ml.[1] Since these range is broader, 68.2 % (221/324) of the NORCLO concentrations were found within the proposed range (Figure 1). Only 29.9 % (97/324) of the serum concentrations were found below 100 ng/ml and 0.3 % (1/324) of the concentrations were higher than 600 ng/ml. Despite its doubtful activity, monitoring of NORCLO has some advantages. NORCLO shows a longer plasma half-life and less day-to-day variability than CLO.[1, 3] So far, no studies have looked to NORCLO alone in relation to clinical response [3]. However, the CLO:NORCLO ratio has been suggested as an interesting parameter for TDM. A CLO:NORCLO ratio greater than 3 can be caused by saturation of the CLO metabolism at high dose, inhibition of the CLO metabolism (CYP1A2 inhibitor e.g. fluvoxamine) or a poor metabolizer phenotype. A CLO:NORCLO ratio below 0.5 can suggest poor adherence in the last 24 h before blood withdrawal, induction of the CLO metabolism (CYP1A2 inductor e.g. smoking) or a rapid metabolizer phenotype.[1, 3] In treatment-refractory patients with schizophrenia, co-administration of fluvoxamine results in higher CLO:NORCLO ratios and potentially reduces the CLO dosage needed to obtain clinical effect.[4, 10, 11] For most of the 330 serum samples, the calculated ratio was between 0.5-3, which was in correlation with the findings of *Couchman et al.*[3] The mean ratio was 1.7 (SD 0.8; range 0.1-5.9). Twenty-four samples had a ratio above 3, only 2 samples had a ratio

lower than 0.5. In Figure 2 the evolution of the CLO and NORCLO serum concentrations of 2 patients is presented, together with the calculated CLO:NORCLO ratio. As can be seen, even when the serum concentrations of CLO and NORCLO increase, the ratio remains stable. Only when the metabolic state of the patient changes (for example, when co-administered drugs influence CYP1A2 enzymes) or when problems of compliance are present, the ratio will change.[1] As was proven before, the ratio is a valuable, stable parameter which can be helpful in the interpretation of TDM data.[1, 3]

A lot of patients are treated with more than one AP at the same time. Analysis of the serum samples for presence of other APs revealed that 218 of the 230 samples (66.1 %) contained at least one other AP than CLO or its metabolite. Ten different APs were found: amisulpride (n=29), aripiprazole (n=38), bromperidol (n=6), haloperidol (n=37), olanzapine (n=19), paliperidone (n=86), pipamperone (n=8), risperidone (n=16), quetiapine (n=55) and zuclopenthixol (n=21). For correct interpretation, co-medication with APs should be mentioned on the TDM-application, since it can be important for interpretation. According to the AGNP guidelines, TDM is strongly recommended for amisulpride, CLO, haloperidol and olanzapine. TDM is recommended for aripiprazole, bromperidol, paliperidone, quetiapine and risperidone. For pipamperone and zuclopenthixol TDM can be useful to control whether plasma or serum concentrations are plausible for a given dose or clinical improvement may be attained by dose increase in non-responders.[1] The serum concentrations of the other APs were compared with the recommended therapeutic ranges and only about half of the concentrations (mean 52.5%) were within the proposed range. Data about co-medication and dosage were unknown, which makes interpretations difficult.

Conclusion

The importance of monitoring both CLO and NORCLO is highlighted by this retrospective study, especially since many patients show suboptimal serum concentrations. Calculating the CLO:NORCLO ratio can give additional information about the metabolic state and adherence of the patient. An important advantage of the analytical method used here, is the possibility of monitoring other co-administered APs concurrently. Clinicians should be notified that monitoring of all APs can aid in optimization of the therapy. They should be aware of the importance of subtherapeutic concentrations and correlate the results with the clinical response of the patient. However, this study has some drawbacks, since clinical data, dosage and co-medication are lacking. Nevertheless, the results were comparable with the limited number of studies published so far and contributes to the growing importance of TDM and individualized therapy [1, 3, 7].

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Figure captions

Figure 1: Histogram of the serum concentrations of clozapine (A) en norclozapine (B) found in 330 serum samples.

Figure 2: Evolution of the serum concentrations of clozapine and norclozapine in comparison with the clozapine:norclozapine ratio of 2 patients (patient A & B). For patient A, 17 different serum samples were send to the laboratory in a period of 14 months. For patient B, 17 different serum samples send to the laboratory in a period of 5 months.