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Therapeutic drug monitoring of targeted anticancer therapy with use of imatinib in CML patients

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Summary

Background. Imatinib at 400 mg once daily is a standard treatment of chronic myeloid leukemia (CML). Many analysis showed that effective steady-state trough concentration (C_{trough}) of imatinib in patients with CML was >1002 ng/mL. This tyrosine kinase inhibitor characterizes by big intersubject variability. Therefore, therapeutic drug monitoring (TDM) for imatinib is recommended. ***Aim of study.*** The purpose of the study was to analyse the steady-state trough concentrations of imatinib in patients with CML. ***Material and methods***. Plasma concentrations were measured in 44 CML patients (mean [SD]; age, 50.0 [12.4] years; weight 78.3 [15.3] kg; and creatinine clearance, 93.3 [34.2] mL/min). One blood sample was drawn just before the next dose to measure C_{trough} of imatinib. Imatinib concentrations were determined by high-performance liquid chromatography. ***Results.*** The mean plasma trough level of imatinib at steady state in our patients with CML was 838,9±672,3 ng/mL. The recommended steady-state trough concentration of imatinib was reached only in 27% of analysed patients. ***Conclusions.*** Considerable inter-subject variability for C_{trough} in CML patients requires systematic monitoring. *(Farm Współ 2012; 5: 163-169)*

Keywords: therapeutic drug monitoring, tyrosine kinase inhibitor, imatinib, CML

Introduction

Therapeutic drug monitoring (TDM) applied to patients undergoing a therapy is a costly process. Therefore it should be applied in the following situations when it is difficult to measure the pharmacological effect, when there is good correlation between the drug concentration in the blood and its effect and when the drug has a narrow therapeutic index. The implementation of TDM is also recommended when the symptoms of drug poisoning are difficult to be clinically diagnosed at an early stage, the pharmacokinetics of the drug is complex (e.g. non-linear), the drug causes numerous interactions or when a critically ill patient is treated. However, when there is simple and direct measurement of the pharmacological effect (e.g. the concentration of glucose or glycated haemoglobin for hypoglycemic drugs), there are no indications to apply TDM. Most often the concentrations of the following drugs are monitored: digoxin, antibiotics (aminoglycosides, vancomycin), theophylline, antiepileptic drugs (phenytoin, phenobarbital, carbamazepine, valproic acid), antiarrhythmic drugs (disopyramide, lidocaine, procainamide, propafenone), analgesic drugs (paracetamol, acetylsalicylic acid), immunosuppressive drugs (cyclosporin A), antineoplastic drugs (methotrexate), tricyclic antidepressants (amitriptyline, nortriptyline) and other drugs with effect on the central nervous system (lithium, pentobarbital). TDM is a tool rarely used in oncology but it has been unquestionably tested in imatinib therapy. Imanitib

is a competitive inhibitor of tyrosine kinases: ABL kinase (BCR-ABL gene product), c-Kit receptor kinase for the stem cell growth factor (SCF) and receptor kinase for the platelet-derived growth factor α and β (PDGFRα and PDGFRβ). By competitive blocking of the ATP binding sites of the aforementioned kinases the drug inhibits transmission of signals leading to the development, growth and division of neoplastic cells, inhibiting the phosphorylation of proteins participating in the transmission of cell signals [1-3]. TDM for imatinib involves specific implications. According to numerous publications, the minimum concentration at steady state in patients with CML (chronic myeloid leukaemia) should be about 1000 ng/mL [4-6], and when this concentration is achieved, but there is no clinical response, it is recommended to change the treatment to another tyrosine kinase inhibitor [6]. Usually the dose of 400mg/24h is applied. However, if the body surface area is smaller and the risk of adverse reactions is higher, the dose of 300mg/24h is suggested [7,8]. Dose diversification may also result from the genotype, because the dose of 400mg/24h is recommended to patients with ABCG2 421C/C genotype, whereas the dose of 300mg/24h is recommended to patients with 421C/A or 421A/A genotype [6]. In order to increase treatment efficacy in patients with acquired cytogenic resistance to the standard dose of imatinib larger doses of the drug are applied (600-800 mg/24h) [1,9,10]. Increased drug clearance and, in consequence, reduced imatinib concentrations may occur in patients with higher body mass. Reduced drug clearance and increased concentrations are expected in patients with impaired renal function and those applying CYP 3A4 inhibitors [11].

The purpose of the study was to analyse the steady-state trough concentrations of imatinib in patients with CML.

Materials and methods

Patients

The research was conducted at the Department of Hematology and the Department of Clinical Pharmacy and Biopharmacy, University of Medical Sciences, Poznań, Poland with the approval from the Bioethics Committee of the Poznań University of Medical Sciences. The research was explained to the patients, and those who consented to imatinib administration and blood collection were enrolled as subjects. The subjects of the research were patients who were on single-agent imatinib treatment at least one month and received a daily dose of 400 mg between June 2009 and April 2012. The patients were treated with imatinib (Glivec®, Novartis SA; tablets 400 mg) in the oral dose of 400 mg, once a day. All the patients were treated with one dose of imatinib irrespective of their bodyweight, sex and age. Creatinine clearance for each patient was calculated with Cockroft-Gault formula [12]. One blood sample was drawn just before the next dose to determine plasma concentrations of imatinib.

Assays

The concentration of imatinib was carried out by means of a high pressure liquid chromatographic (HPLC) method with UV detection, which was a modified version of the method developed by N. Widmer et al. [13]. The high-performance liquid chromatographic (HPLC) system (Alliance, model 2695; Waters Association, Milford, MA, USA) was used with a DAD (Diode Array Detector) (model 2487; Waters Association) and a data acquisition and processing module (Empower Pro Software; Waters Association). Imatinib (batch number 4012571) was provided by Novartis; clozapine (batch number 1204), used as the internal standard, was purchased from Sigma-Aldrich. Separation was achieved by gradient elution of the mobile phase, solvent A consisted of water containing 0.05% (w/v) of NH_4Ac and solvent B was methanol containing also 0.05% (w/v) of NH_4Ac, at a flow rate of 1.0 ml/min through a XBridge®C 18 column (150 mm × 4.6 mm, 5 μm particle size) (Waters). The column temperature was maintained at 25°C, the UV–Vis detection wavelength was set at 261 nm, and the injection volume was 50 μL. The total analysis time for each run was 46 min. Examples of chromatograms are shown in Figure 1.

The lower limits of quantification for imatnib was 0.1 μg/mL, with calibration curve 0.1 to 10 μg/mL (Figure 2). Intra- and inter-day accuracies were 98.62% to 102.68% and 94.60% to 105.95% for imatinib, respectively. Precision variations of imatinib was lower than 8.38%.

Statistical analysis

The statistical calculations were made with SAS software package (SAS Institute Inc. 2002-2003. The SAS System for Windows version 9.1. Cary, NC 27513-2414 USA). The distribution of all of the measured parameters was checked for agreement with the nor-

mal distribution by the Shapiro-Wilk test (p = 0.05). Student's t-test was used to estimate statistical significance of differences between the results obtained in groups of men and women. Trough plasma concentrations of imatinib at steady state (n = 44) were correlated with age, body weight, and body surface area (BSA) at baseline using linear regression analysis (Pearson correlation coefficient provided).

Results

Fourty four patients (23 males and 21 females) with a diagnosis of chronic myeloid leukemia were enrolled on the study. Median age was 51 years (range 23-73 years). The background of all patients enrolled in the research is shown in Table 1.

Table 1. The characteristics of patients included in the research (n = 44)

parameter	median	range
Duration of treatment [months]	18.5	1-99
Age [years]	51	23-73
Body mass [kg]	79	50-127
Height [cm]	170	155-185
BSA [m2]	1.92	1.50-2.43
CCR [mg/dL]	1.0	0.5-1.46
CLCR [mL/min]	85.5	48.5-176.0
ALT [U/L]	23	9-137
AST [U/L]	25	10-104
Males/females	23/21	

BSA, body surface area; C_{CR}, creatinine concentration; CL_{CR}, creatinine clearance estimated by the Cockroft-Gault formula [12]; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

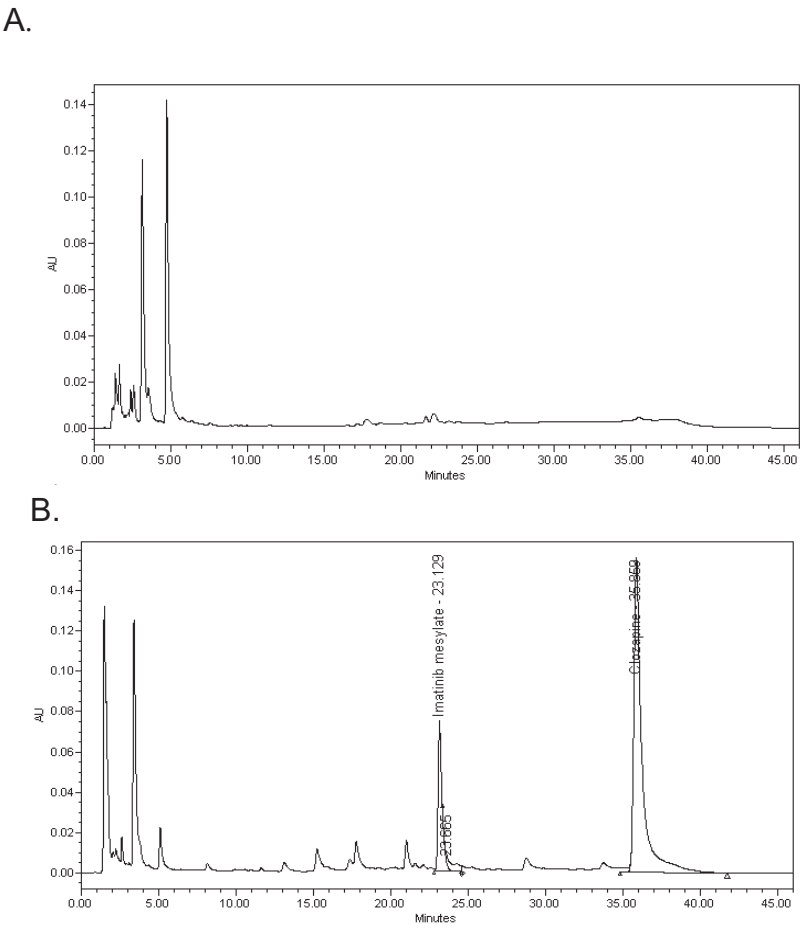

Figure 1. The chromatogram of imatinib: a drug-free plasma (A), patient's plasma (B)

The mean plasma trough level of imatinib at steady state was slightly higher in males than females (854 ng/mL *vs* 804 ng/mL, respectively), but there is no significant difference (p = 0.7480). All measurements are presented in Table 2. There was large interpatient variability in plasma trough concentrations (80%). A weak correlation was identified between steady-state trough levels of imatinib and body weight for all patients (r^2 = 0.0108), for females (r^2 = 0.0281) and males (r^2 = 0.1216), but for males < 100kg was good (r^2 = 0.3009). A weak correlation was identified between steady-state trough levels of imatinib and BSA (r^2 = 0,0206).

Table 2. The steady-state trough concentrations (C_{trough}) of imatinib in male and female patients with CML (n = 44)

No	Ctrough [ng/mL]	
	males	females
1.	461	359
2.	2482	1016
3.	684	612
4.	947	715
5.	597	1306
6.	619	653
7.	419	1189
8.	665	755
9.	327	2312
10.	594	294
11.	601	1532
12.	403	699
13.	751	435
14.	233	258
15.	893	190
16.	505	564
17.	3885	484
18.	1216	888
19.	1037	228
20.	688	1113
21.	438	1287
22.	549	
23.	1030	
24.	461	
S±SD	854±784	804±521

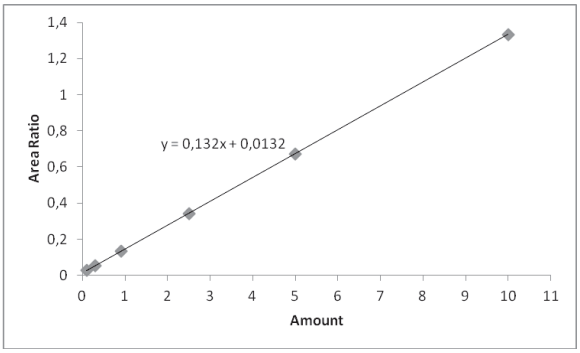

Figure 2. Standard calibration curve for imatinib (n = 3)
Each curve is based on 6 calibration standards with triplicate injections

Discussion

Independent studies point to the correlation between the concentration of imatinib in the plasma and the response in patients with CML. French studies suggest that the minimum drug concentration in the plasma at steady state necessary to achieve the MMR is 1002 ng/mL [5]. On the other hand, the IRIS studies by Larson et al. indicate that the concentration which enables achievement of the CCR is 1009 ng/mL [14].

In the group of patients under analysis the minimum concentration values at steady state had high variability, which confirms the intersubject variability of the pharmacokinetic parameters of imatinib proved by many authors. Only in 12 out of 44 patients (27%) the concentration of the drug in the blood exceeded the recommended value. The group of patients under analysis was diversified in terms of their renal and hepatic function, but it is known that these factors do not have significant influence on imatinib levels [15]. Similarly to other studies, the obtained results confirm the low correlation between the minimum concentration of imatinib and the age, sex and body weight [16,17].

Imatinib is metabolised by means of cytochrome P450 isoenzymes, chiefly by CYP3A4 and CYP3A5, and to a lesser extent by the CYP1A2, CYP2D6, CYP2C9 and CYP2C19 isoenzymes [14,16,18]. For this reason there is high likelihood of interaction with the other drugs that patients simultaneously receive, especially with CYP3A4 inductors or inhibitors, and in consequence, there is a risk of subtherapeutic concentrations or the occurrence of adverse reactions [19-23]. On the other hand, some drug combinations do not have any clinical consequences [24,25]. In 3 patients significantly

higher concentrations of imatinib were observed than in the other patients (2312, 2482, 3885 ng/ml). However, only in the first of them the high concentration of imatinib may have been the consequence of the application of omeprazole, which is an inhibitor of the CYP1A2, CYP2C9, CYP 2C19 isoenzymes.

The patients with very low imatinib concentrations were particularly thoroughly analysed. In three smoking patients low concentrations of imatinib in the plasma were observed (751, 688, 484 ng/mL). This may have been caused by the inductive influence of nicotine on hepatic enzymes, which accelerates the elimination of imatinib, although CYP1A2 plays a less important role in the metabolism of the drug [18,26-28]. The medical history of the patient with the imatinib concentration of 233 ng/mL revealed regular consumption of alcohol, which may have had inductive influence on the metabolism of the drug [29]. Probably the low C_{trough} values in the patient receiving metformin (190 ng/mL) and in the patient receiving carbamazepine (228 ng/mL) are the result of interaction between these drugs and imatinib. Because of the inhibition of the hOCT1 protein metformin may cause lower concentration of imatinib [30]. On the other hand, carbamazepine is an inductor of the CYP3A4, CYP1A2, CYP2D6, CYP2C9, CYP2C19 and Pgp enzymes, as a result of which the metabolism of imatinib may be accelerated [31].

The drugs applied in a therapy of chronic diseases, which are the inductors and inhibitors of the enzymes participating in the metabolism of imatinib (e.g. omeprazole, fenofibrate, paroxetine, ranitidine, carbamazepine) were the sources of interaction, which more or less significantly may have influenced the observed concentrations. The concomitant diseases and pathological states of the organism, such as obesity (8 patients), diabetes (4 patients), hypertension (5 patients), lipid and hormonal management disorders also may significantly have changed the activity of the enzymes participating in the biotransformation of imatinib. The influence of genetically conditioned differences in the activity of those enzymes and transport proteins may also have proved to be significant, i.e. the patient's pharmacogenetic profile [6,32]. Another factor which may have affected the observed differences in the concentrations may have been the patients' negligence of the indications concerning dosage of the drug – different times of administration, longer than 24-hour intervals between consecutive doses, omission of individual doses. In such situations there is higher likelihood of subtherapeutic concentrations.

Undoubtedly, a considerable limitation to the investigation is the absence of correlations between imatinib concentrations and the pharmacodynamic effect of the drug. However, the study encompassed the patients with different periods of imatinib therapy (even for 1 month). Therefore, the assessment of the pharmacological effect would not be reliable. Nevertheless, Larson et al. [14] proved that after one month of the imatinib therapy the minimum concentration at steady state, (on the 29th day) is a significant prognostic factor of a long-term response to the therapy. The steady state of imatinib is achieved within one week ($t_{0.5}$ ~20 h), and the steady state of its active metabolite is achieved within two weeks ($t_{0.5}$~40 h), which justifies testing the minimum concentration after such a short period of therapy.

The authors focused only on the analysis of concentrations in order to draw our attention to the problem that most patients with CML do not achieve the desirable C_{trough} values, which should induce verification of the rigid dosage scheme of the drug with such considerable intersubject variability, as the authors of other studies also indicate. The variability of imatinib concentration in patients receiving the same dose of the drug, which is observed in clinical practice, is a strong argument confirming the need to monitor the therapy with this drug.

Conlusions

The recommended steady-state trough concentration of imatinib was reached only in 27% of analysed patients. Considerable inter-subject variability for C_{trough} in CML patients requires systematic monitoring.

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Konflikt interesów / Conflict of interest
Brak/None

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