Clinica Chimica Acta 453 (2016) 42–47

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/clinchim

First report of imatinib measurement in hair: Method development and preliminary evaluation of the relation between hair and plasma concentrations with therapeutic response in chronic myeloid leukemia

Arnaud Capron [a,b], Marina Venzon Antunes [c], Sandrine Comparsi Wagner [d], Vanessa Suñe Mattevi [d], Natalia Vieira [d], Renata Leite [d], Fabiola Reginato [d], Marcelo Capra [e], Laura Fogliatto [f], Pierre Wallemacq [a,b], Rafael Linden [c,*]

[a] *Clinical Chemistry Department, Cliniques Universitaires St Luc, Brussels, Belgium*
[b] *Louvain Center for Toxicology and Applied Pharmacology, Université Catholique de Louvain, Brussels, Belgium*
[c] *Instituto de Ciências da Saúde, Universidade Feevale, Novo Hamburgo, RS, Brazil*
[d] *Departamento de Métodos Diagnósticos, Universidade Federal de Ciências da Saúde de Porto Alegre, RS, Brazil*
[e] *Serviço de Hematologia, Hospital Nossa Senhora da Conceição, Porto Alegre, RS, Brazil*
[f] *Serviço de Hematologia, Hospital de Clínicas de Porto Alegre, Porto Alegre, RS, Brazil*

ARTICLE INFO

Article history:
Received 23 October 2015
Received in revised form 16 November 2015
Accepted 30 November 2015
Available online 3 December 2015

Keywords:
Imatinib
Chronic myeloid leukemia
Hair analysis
Liquid chromatography–mass spectrometry
Therapeutic drug monitoring
Adherence evaluation

ABSTRACT

Background: Imatinib (IM) is a first choice drug for treatment of chronic myeloid leukemia (CML), with a widely accepted concentration threshold of 1000 ng/ml being used as a target for therapeutic drug monitoring. Once adherence to the pharmacotherapeutic regimen is of paramount importance during the long treatment course of CML, the measurement of hair IM concentrations could be a surrogate of the patient's exposure to the drug.
Methods: IM was extracted from a 5 mg hair sample by a liquid–liquid extraction with ethyl acetate, and IM-d8 was used as internal standard (IS). After evaporation, and reconstitution in acetonitrile, the extract was injected into a LC–MS/MS system. Compounds were eluted on a C8 column in isocratic mode. IM and IS were identified in positive electrospray ionization mode using ion transitions of m/z 494.5 > 394.5 and 503.0 > 394.3 respectively. The method was applied to 102 paired hair and samples obtained from CML patients. Treatment response was evaluated according to the European LeukemiaNet recommendations.
Results: The assay was validated in the concentration range of 0.5–25 ng/mg, with intra- and inter-assay imprecisions of <13.1% and <9.3%, respectively. The limits of quantification and detection were 0.5 and 0.15 ng/mg, respectively. Median hair IM concentrations are significantly smaller in patients with therapeutic failure when compared with patients with partial or optimal response (4.63 vs. 7.93, $p = 0.040$), the same trend presented by median plasma IM concentrations (629.5 vs. 1084.8, $p = 0.009$). An IM hair concentration below 5.8 ng/mg has 83% sensibility and 70% specificity to identify patients with therapeutic failure.
Conclusions: A fast, sensitive, and selective LC–MS/MS method allowing quantification of IM in hair samples was developed and validated. CML patients with therapeutic failure had significantly lower hair IM concentrations when compared with patients with optimal response. These preliminary findings may support the use of hair as a matrix for IM monitoring in clinical settings, with significant logistic advantages over the collection of venous blood, particularly in developing countries.

© 2015 Elsevier B.V. All rights reserved.

1. Introduction

Chronic myeloid leukemia (CML) is characterized by the presence of an abnormal chromosome, which presents a fusion gene named *BCR-ABL1* [1]. *BCR-ABL1* transcript levels are used as an indicator of therapeutic response during CML treatment with imatinib (IM) and its 3-log reduction, classified as Major Molecular Response (MMR), usually occurs for approximately 50% of patients within 5-year of treatment, being considered as a marker of adequate response [2]. Despite the initial recommendation of a standard and empirical dosage of IM for all CML patients, several studies had identified a relationship between plasma IM levels and treatment efficacy [3]. In the study performed by Larson et al., patients with complete cytogenetic response (CCR) had mean IM plasma trough levels of 1009 ± 544 ng/ml, while those who did not attain CCR presented levels of 812 ± 409 ng/ml [4]. Several other studies confirmed the relation between IM through plasma concentrations and therapeutic response, with a widely accepted

* Corresponding author at: Laboratório de Análises Toxicológicas, Instituto de Ciências da Saúde, Universidade Feevale, Rodovia ERS 239, n. 2755, CEP 93352-00 Novo Hamburgo, RS, Brazil.
E-mail address: rafael.linden@feevale.br (R. Linden).

http://dx.doi.org/10.1016/j.cca.2015.11.032
0009-8981/© 2015 Elsevier B.V. All rights reserved.

concentration threshold of 1000 ng/ml being used as a target for therapeutic drug monitoring (TDM) [5,6].

The achievement of therapeutic concentrations is dependent of both biological and behavioral factors. In this context, patient adherence to the prescribed dosage regimen plays a major, and frequently underestimated role, as previously demonstrated for IM. Using microelectronic monitoring systems, Marin et al. in 2010 observed a strong correlation between adherence rate and the 6-year probability of a 3-log reduction in *BCR-ABL1* transcripts. In this study, multivariate analysis even identified adherence as the major independent predictor for MMR [7]. Similar trends were observed in several other studies [8–10].

Patients who are persistently non-compliant tend to take more rigorously their medication on the days before they visit their physician, thereby giving a false impression of adherence when blood drug levels are measured. Adherence assessment through biological measures could be obtained by random dried blood spots, using fingerpricks, as already described for IM [11], or by hair samples. The latest approach is regularly used in toxicology or more recently in HIV pharmacotherapy (another chronic treatment with significant adverse effects). In previous studies, hair concentrations of antiretroviral drugs were strongly correlated with therapeutic response [12–14]. In this context, the determination of hair concentration of IM could be a promising strategy to evaluate exposure to IM, taking into account the ease of collection and transportation in limited resource settings.

2. Material and methods

2.1. Reagents, materials and reference standard samples

IM mesylate and IM-D8 were obtained from Novartis. Ammonium acetate, formic acid, methanol, acetonitrile, acetone, isopropanol, ethyl acetate and hexane (60% n-hexane) were from Merck. Purified water was obtained from an Elga Purelab Ultra® system from Veolia Labwater. IM-free human hairs for analytical development were obtained from healthy volunteers in agreement with local ethics committee.

2.2. Preparation of solutions and standards

IM stock solution was prepared in methanol to obtain a concentration of 100 μg/ml. Stock solution was diluted with methanol to obtain a working solution at 1 μg/ml. Further dilution of the working solution was prepared to obtain additional working solution at 0.1 μg/ml. IM-D8 (internal standard, IS) stock solution was prepared in methanol at 100 μg/ml concentration. The working IS solution was prepared by dilution of the IM-D8 stock with methanol to obtain a concentration of 1 μg/ml. Stock, intermediate and working solutions were stored at −20 °C. Calibration curves included a zero sample and 7 spiked drug-free pulverized hair samples covering the ranges from 0.5 to 25 ng/mg (0.5, 1, 2.5, 5, 10, 20, and 25 ng/mg). Hair IM homemade quality controls (QCs) were prepared using an independent stock solution to achieve concentrations of 0.5 (quality control at the limit of quantification, QCLOQ), 1 (quality control low, QCL), 4 (quality control medium, QCM), and 12.5 ng/mg (quality control high, QCH). For calibrators and QCs, various hair types have been used.

2.3. Sample preparation

Hair strand was decontaminated by washing it successively in water and acetone baths for 15 min at 37 °C. After drying under nitrogen flow, hair powder was obtained by means of a ball mill pulverizer (Mixer Mill MM400, Retsch®, Switzerland) for 5 min at a 40 Hz frequency. An aliquot of 5 mg of pulverized sample was then precisely weighted in an 8-ml glass tube and 10 μl of IM-d8 (1 μg/ml) was added. Sample was treated with 500 μl of ethyl acetate and drug extraction was carried out by sonication (2 h at 60 °C). The mixture was centrifuged for 5 min at 1100 g. The organic phase was quantitatively transferred to a 5-ml glass tube and evaporated to dryness, at room temperature under nitrogen flow. The dried extract was recovered in a 100 μl of mobile phase and transferred into vials for LC–MS/MS analysis and 20 μl was injected into the HPLC system.

2.4. LC–MS/MS equipment and conditions

IM measurement was performed by LC–MSMS, using a Quattro micro tandem-mass spectrometer (Micromass) fitted with a Z-spray ion source. The instrument was directly coupled to a Waters 2795 Alliance (high throughput) HT LC system, with an integrated auto sampler (Waters). Chromatographic separation was performed on a cartridge column XTerra® C8 (2.1 mm × 50 mm, 3.5 μm) (Waters) maintained at 50 °C. The 20 μl injected aliquot was eluted, in isocratic conditions, at a mobile phase flow rate of 0.3 ml/min. The mobile phase consisted of a mixture (45:55%) of solvents A (2 mmol/l ammonium acetate buffer; 0.1% formic acid) and B (0.1% formic acid in acetonitrile). The total run time was 6.5 min. Ionization was in the positive ion mode using the following settings: capillary voltage at 3.2 kV, cone voltage at 40/42 V (IM/IM-d8), source temperature at 125 °C, desolvation temperature at 300 °C at a nitrogen flow of approximately 650 l/h, and collision gas (high-purity argon) pressure at 3×10^{-3} bar. IM and IM-d8 were monitored in MRM mode by detecting specific daughter ions: IM m/z 494.5 → 394.5 (quantitation) and m/z 494.5 → 378.2 (qualification); IM-D8 m/z 503.0 → 394.3 (quantitation) and m/z 503.0 → 377.6 (qualification). Collision energies were 26 and 23 eV for IM product ions m/z 394.5 and 378.2, and 28 and 25 eV for IM-d8 product ions m/z 394.3 and 377.6, respectively. The quantification of IM in hair was performed at the Louvain Center for Toxicology and Applied Pharmacology, Université Catholique de Louvain, Brussels, Belgium.

2.5. Method validation

The assay was fully validated according to the U.S. Food and Drug Administration (FDA) and the Society of Hair testing guidelines for drug testing in hair (SOHT) guidelines [10]. Statistics were performed using JMP software (SAS Institute). Linearity has been assessed over the seven calibrators processed in replicates of 3 over 5 days, using the weighting factor 1/x. The limit of quantification (LOQ) was calculated as the minimum concentration at which IM can be reliably quantified with an imprecision ≤20% and accuracy within 80%–120%. The limit of detection (LOD) was determined as the smallest detectable peak above baseline noise (signal-to-noise ratio > 3:1). As no reference method is available, the real accuracy of this assay could not be assessed. Therefore, accuracy mentioned in this study corresponds to the deviation from target values of control samples (1, 4, 12.5 ng/mg, and at the LOQ). Inter-assay imprecision and accuracy were assessed at QC and LOQ concentrations, in separate replicates of 3 for 5 days. To determine intra-assay accuracy and imprecision, the same samples were analyzed 6 times. Extraction efficiency of IM from hair was assessed by comparing the results for extracted QC samples in replicates of 6, with unextracted standards. Postextraction addition technique has been used to estimate matrix effect (ME) [15,16]. This technique requires sample extracts with IM added postextraction compared with pure solutions prepared in mobile phase containing equivalent amounts of IM. The difference in response between the postextraction sample and the pure solution divided by the pure solution response will determine the degree of ME occurring to IM under chromatographic conditions. ME has been determined at the three QC levels and LOQ in replicates of 6, using 6 different drug-free hair samples (obtained from different healthy individuals) for each aliquot. As hair sample are usually kept at room temperature, the stability assessment of IM was limited to this particular temperature under a short (12 h before extraction) and long time (2 month before extraction) storage in replicates of 10

Download English Version:

https://daneshyari.com/en/article/1965130

Download Persian Version:

https://daneshyari.com/article/1965130

Daneshyari.com