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Development and validation of an high performance liquid chromatography–tandem mass spectrometry method for the determination of imatinib in rat tissues

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ABSTRACT

An high performance liquid chromatography–tandem mass-spectrometry (HPLC–MS/MS) method was developed and validated for the determination in rat heart and liver of the tyrosine kinase inhibitor imatinib (IM), an anticancer drug approved for the treatment of chronic myeloid leukemia and gastrointestinal stromal tumors. Extraction of the drug from tissues was performed by solvent extraction and the obtained extracts were analyzed by HPLC–MS/MS in selected reaction monitoring mode. The developed method was validated according to the criteria for bioanalytical method, showing good performances in terms of lower limit of quantification (LLOQ = 0.02 μg ml^{-1}), linearity (R^2 = 0.998), repeatability (RSD < 3%), reproducibility (RSD < 13%) and recovery (RR > 89%). The developed method was then applied to the analysis of heart and liver of rats treated with different doses of IM, with and without the simultaneous administration of carvedilol, a beta-blocking agent with cardioprotective effect, in order to evaluate tissue levels of the tyrosine kinase inhibitor. The obtained results revealed that the amount of IM in the rat heart was significantly affected by the administered dose, whereas carvedilol had no effect on IM concentrations. Thus, we have developed a method that allows the detection of IM traces in complex tissues such as the heart and liver and that may be proposed for the determination of the drug in other clinically relevant biological samples.

1. Introduction

Imatinib (IM), also known as Glivec™ or STI-571, is a 2-phenylaminopyrimidine-type selective tyrosine kinase inhibitor (TKI) developed at the end of 1990 for the treatment of chronic myeloid leukemia and gastrointestinal stromal tumors [1–5].

In recent years, several analytical methods have been developed for the determination of IM in human and animal matrices of biological and clinical interest. Whereas several studies have been performed to determine IM concentrations in biological fluids, as blood, plasma, urine and cerebrospinal fluid [6–18], only few observations have been addressed to assess IM concentration in biological tissues as liver, brain, spleen, kidney, heart and tumor tissue [7,11,19]. Before the instrumental analysis, a preliminary treatment of the sample is required, often consisting in protein precipitation and/or solid phase extraction when liquid matrices are considered [8–10,12,14], while in the case of solid samples as biological tissues a preliminary homogenization step followed by solvent extraction should be performed in order to obtain a liquid extract to be further analyzed [7]. Instrumental analysis has been usually carried out by high performance liquid chromatography equipped with an ultraviolet–visible [7,8,11–13,15] or a mass spectrometer [6,9,10,14,16–19] detector.

On clinical ground, IM is a well tolerated drug, being fluid retention, muscle cramps, diarrhea, nausea, musculoskeletal pains and skin rash the most common non-hematological adverse events [20]. Among potential IM side effects, cardiotoxicity remains a controversial issue [21,22]. Clinical observations have indicated the development of congestive heart failure in patients treated with IM [23,24], whereas other studies have not provided relevant evidences for IM related cardiovascular events [25,26]. However, side effects involving the cardiovascular system associated with the use of TKIs have been widely reported and recently reviewed [27].

Our previous studies, based on the evaluation of the ventricular function and on the structural characterization of the myocardium of rats treated with IM in presence or in absence of carvedilol, a beta-blocking agent, add contribution to assess both the cardiotoxicity of IM and the cardioprotective effect of carvedilol [28]. However, the mechanism of imatinib-related cardiac dysfunction is not yet fully elucidated and requires a deeper investigation in

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view of the ability of TKIs to change the history of some neoplastic diseases. On the basis of these considerations, we developed and validated an high performance liquid chromatography–tandem mass spectrometry (HPLC–MS/MS) method characterized by high selectivity and sensitivity for the determination of imatinib in heart and liver of rats treated with different IM doses. In addition, in order to evaluate the effect of carvedilol on imatinib accumulation in rat tissues, the developed method was applied to the analysis of heart and liver of rats treated simultaneously with IM and carvedilol.

The developed HPLC–MS/MS method proved to be reliable to detect IM traces in complex matrices such as tissues thus suggesting its use for the determination of IM in other substrates of clinical interest.

2. Materials and methods

2.1. Chemicals

Imatinib (IM) used for rat administration was a commercially available drug 100 mg capsules (Novartis, Switzerland) Carvedilol (CAR) was a 6.25 mg tablet commercially available drug (Carvedilol EG, Eurogenerici, Milan, Italy).

Imatinib for standard solutions preparation was from Alexis Biochemical (Switzerland). The internal standard clofazimine (CLOF) was from Sigma–Aldrich, USA. Sterile distilled water was from Bieffe Medital (SO, Italy). HCl was from J.T. Baker (Deventer, The Netherlands).

HPLC grade organic solvents (methanol, acetonitrile and formic acid) used for standard solution preparation, sample extraction and HPLC analysis were from Sigma–Aldrich (St. Louis, MO, USA).

Deionized water was produced with an Element A10 equipment (Millipore, Bedford, MA, USA).

Ketamine was from Merial Italia (Milan, Italy) and medetomidine was from Pfitzer Italia (Roma, Italy).

2.2. Standard solutions preparation

Imatinib solutions for 100 mg kg^{-1} dose rat administration were freshly prepared by dissolving the content of four 100 mg imatinib capsules in 20 ml distilled water acidified at pH 2 with HCl 10 N; after 30 min shaking at room temperature, solution was bring to pH 6.4 adding NaOH 10 N. Likewise, imatinib solutions for 50 mg kg^{-1} dose rat administration were prepared as previously described, by dissolving the content of two 100 mg imatinib capsules in 20 ml water. In this way, similar volumes of imatinib solution were administrated to rats treated with 100 mg kg^{-1} or 50 mg kg^{-1} dose.

Carvedilol solutions for rat administration were freshly prepared, by dissolving 1 tablet of carvedilol in 50 ml water.

Stock standard solutions of imatinib and clofazimine were prepared by dissolving an accurately weighted amount of drug in methanol to obtain a final concentration of 1 mg ml^{-1}. All stock solutions were stored at −20 °C for a maximum of three months. Working standard solution were prepared daily by diluting stock standard solutions with methanol until the desired concentration.

2.3. Experimental design and animal protocol

All handling involving animals were approved by the animal experiment committee of the Institute and carried out in accordance with the guide for the Care and Use of Laboratory Animals (National Research Center, National Academic Press, Washington, DC, 1996). Experiments were approved by the local Ethical Committee.

Fifteen male Wistar rats of about 5 month of age and 200–250 g body weight, born at the Department of Developmental Biology of the University of Parma, were separately housed in plexiglas cages at 22 °C and controlled lighting and provided with water and food ad libitum.

Proper volumes of IM and/or of CAR solutions, prepared as described in the previous paragraph, were administered to the animals considering both the dose and the weight of the animal. IM solutions were administered via intraperitoneal injection, whereas CAR solutions were administered orally.

The experimental design (3 animals for each treatment) was as follows:

- T1: treatment with IM 100 mg kg^{-1} dose three times a week, for 3 weeks
- T2: treatment with IM 50 mg kg^{-1} dose three times a week, for 3 weeks
- T3: pretreatment with CAR 7 mg kg^{-1} day^{-1} dose for one week, followed by simultaneous administration of the same dose of CAR and 100 mg kg^{-1} IM three times a week, for 3 weeks
- T4: treatment with 7 mg kg^{-1} day^{-1} CAR dose, every day for 4 weeks
- T5: treatment with equal volume of physiological salt solution by intraperitoneal injection, three times a week, for 3 weeks

Twelve hours after the last injection, the rats were anesthetized with a mixture of ketamine and medetomidine and killed by cervical dislocation; blood was then collected via cardiac puncture to minimize any residual blood in tissues.

The rats were then dissected to harvest heart and liver. Tissue samples were frozen in liquid nitrogen and kept at −80 °C until analysis.

2.4. Sample treatment

Prior to the extraction, tissue samples were thawed at room temperature, accurately weighted and finely minced with a lancet. As for liver samples, three portions of 0.5 g were obtained and submitted to solvent extraction procedure. As for heart tissues, the whole sample was used, due to its little mass.

Tissue samples were added with 7.5 μl of a 1000 mg l^{-1} methanolic CLOF solution, with 142.5 μl of methanol and homogenized with 3 ml of the water:methanol:acetonitrile 40:30:30 (v/v/v) extraction solution using an Ultra Turrax T25 homogenizer (Jankel & Kunkel IKA, Labortechkink) for 20 s in an ice bath. Aliquots of tissue slurry were transferred into 1.5 ml Eppendorf tubes and centrifuged at 14,000 rpm for 5 min (Microfuge18, Beckmann). Then the supernatant was filtered (Econofilters, Regenerated cellulose-polypropylene 25 mm diameter, 0.20 μm pore size, Agilent Technologies, Germany) and kept at −20 °C until HPLC analysis. Prior to the HPLC analysis, extracts were diluted 1:30 with the extraction solution and transferred into HPLC vials which were maintained at 10 °C until injection in the HPLC system.

2.5. Liquid chromatography–mass spectrometry

Liquid chromatographic separation was carried out on a C18 Waters column (30 mm × 3 mm; 3.5 μm particle size) (Waters, Italy), with a liquid chromatograph Dionex Ultimate 3000 (Dionex, USA) using a gradient solvent elution system consisting of 0.2% formic acid in water (eluent A) and 0.2% formic acid in methanol (eluent B). Optimized gradient elution was as follows: eluent A was initially set at 80%, then delivered at 40% for 8 min; flow rate was 1 ml min^{-1}. Under these conditions, the observed retention times were 2.39 min for IM and 7.18 min for CLOF. After the elution of the analytes, the column was washed with 100% of eluent B for 2 min at a flow rate of 2 ml min^{-1}, then the column was re-equilibrated to the initial conditions.