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Liquid chromatography–tandem mass spectrometric assay for the simultaneous determination of the irreversible BTK inhibitor ibrutinib and its dihydrodiol-metabolite in plasma and its application in mouse pharmacokinetic studies

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ABSTRACT

A validated simple, fast and sensitive bio-analytical assay for ibrutinib and its dihydrodiol metabolite in human and mouse plasma was set up. Sample preparation was performed by protein precipitation, and addition of the respective deuterated internal standards, followed by LC–MS/MS analysis. Separation was performed on a 3.5 μm particle-size, bridged ethylene hybrid column with gradient elution by 0.1% v/v formic acid and acetonitrile. The full eluate was transferred to an electrospray interface in positive ionization mode, and subsequently analyzed by a triple quadrupole mass spectrometer by selected reaction monitoring. The assay was validated in a 5–5000 ng/ml calibration range. Both ibrutinib and dihydrodiol-ibrutinib were deemed stable under refrigerated or frozen storage conditions. At room temperature, ibrutinib showed a not earlier described instability, and revealed rapid degradation at 37 °C. Finally, the assay was used for a pharmacokinetic study of plasma levels in treated FVB mice.

1. Introduction

Ibrutinib is an orally administered tyrosine kinase inhibitor (TKI) for the treatment of B-cell malignancies. It was approved by the US FDA in November 2014 [1] and by the EMA as an orphan medicinal product in 2012 for treatment of mantle cell lymphoma (MCL), in 2013 for treatment of chronic lymphocytic leukemia (CLL), and in 2015 as the first registered drug for Waldenström macroglobulinemia (WM). The European Committee for Medicinal Products for Human Use (CHMP) adopted a positive opinion, recommending the granting of an initial marketing authorization in 2014 [2,3]. Ibrutinib covalently binds and inhibits the Bruton's tyrosine kinase (BTK) protein. BTK is a signaling molecule of the B-cell antigen receptor (BCR) and cytokine receptor pathways [4]. Since the market authorization, several research articles have been published on the use of Ibrutinib for diseases other than B cell malignancies, such as overactive immune response related (rheumatic) diseases [5] and even more possible drug-targets have been described (MRP1, ErbB2) [6,7]. Pharmacokinetic evaluations of ibrutinib, however, are still scarce. In the first pharmacokinetic studies there was very limited information concerning the quantitative bio-analytical method [8–11]. Since then, some studies have been completed using more detailed methods, although they do not analyze the main metabolite or do not make use of a satisfying internal standard. In addition, they use long run times, high sample volume and laborious sample preparation methods [12,13]. Although one method has been described analyzing ibrutinib in dog, rat and monkey plasma (but

Abbreviations: BCR, B-cell antigen receptor; BSA, bovine serum albumin; BTK, Bruton's tyrosine kinase; CHMP, Committee for Medicinal Products for Human Use; DHI, dhihydrodiol-ibrutinib; DMSO, dimethyl Sulphoxide; EMA, European Medicines Agency; ErbB2, receptor tyrosine-protein kinase erbB-2; ESI, electro spray ionization; FDA, Food and Drug Administration; LC–MS/MS, liquid chromatography–tandem mass spectrometry; LLOQ, lower limit of quantitation; MRP1, multidrug resistance protein 1; PP, polypropylene; QC, quality control; SRM, selective reaction monitoring; ULOQ, upper limit of quantitation; WM, Waldenström macroglobulinemia.

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not the metabolite) [13], none have been described for the analysis in mouse plasma. Therefore, the development and validation of a simple, fast and sensitive bio-analytical assay for ibrutinib and the dihydrodiol metabolite in human and mouse plasma was set up. This method uses a simple protein precipitation step, followed by LC–MS/MS analysis. This assay proves a useful tool in pharmacokinetic studies pertaining to ibrutinib, and was subsequently used for the evaluation of plasma levels in mice and in the assessment of the not earlier described breakdown of ibrutinib in human plasma.

2. Materials and methods

2.1. Chemicals and reagents

Ibrutinib (>99.9%, mw: 440.51), the main metabolite dihydrodiol-ibrutinib (95.9%, mw: 474.52) and the respective [$^2H_5$]-labeled ibrutinib (acetate salt, >99.9%, mw: 445.54), and dihydrodiol-ibrutinib (>99.9%, mw: 479.23) were obtained from Alsachim (Illkirch Graffenstaden, France). LC–MS grade water, methanol of HPLC quality and acetonitrile of HPLC-S gradient grade quality were acquired from Biosolve (Valkenswaard, The Netherlands). Water not used as eluent was home-purified by reversed osmosis on a multi-laboratory scale. Formic acid was of analytical grade and originated from Merck (Darmstadt, Germany) and analytical grade dimethyl sulfoxide (DMSO) was supplied by Acros (Geel, Belgium). Human and mouse lithium-heparinized plasma (pooled human and mouse, and from individual mice) were obtained from Sera Laboratories International (Haywards Heath, West Sussex, UK). For the evaluation of selectivity and matrix effect, human lithium-heparinized plasma of six individual donors were obtained from Innovative Research (Novi, MI, USA).

2.2. Chromatography and MS/MS method

The LC-system consisted of a DGU-14A degasser, a CTO-10Avp column oven, a Sil-HTc auto sampler and two LC10-ADvp-μ pumps (all from Shimadzu, Kyoto, Japan). 5 μl of the plasma extract was injected on a Waters XBridge BEH300 column (2.1 × 50 mm, dp = 3.5 μm, Waters, Milford, USA), protected by a Agilent Polaris3C18-A MetaGuard pre-column (10 × 2.0 mm, dp = 3.0 μm, Agilent, Santa Clara, USA). The column temperature was maintained at 40 °C and the auto injector at 4 °C. Elution was performed by a gradient of (**A**) 0.1% (v/v) formic acid in water and (**B**) methanol at a flow of 0.5 ml/min. The gradient started at 40% methanol, increasing linearly to 85% methanol in 2.0 min, after which an isocratic plateau was held for 0.5 min, till 2.5 min. At 2.5 min, the level of methanol was decreased back to 40%, at which the column was equilibrated for 0.5 min until the following injection. The whole eluate was transferred into the electrospray probe, starting at 1.2 min after injection by switching the MS inlet valve, until 2.4 min of the 3 min analytical run. For detection of all compounds, a TSQ Quantum Discovery Max quadrupole mass spectrometer with electrospray ionization (Thermo Fisher Scientific, San Jose, CA, USA) was used. Data were recorded on, and the system was controlled by the Thermo Fisher Xcalibur software (Version 2.0.7 SP1). The electrospray was tuned in the positive ionization mode by introducing 0.5 ml/min of a mixture of Methanol and 0.1% v/v formic acid in water (50/50, v/v), while infusing 5 μg/ml ibrutinib or dihydrodiol-ibrutinib at 5 μl/min. The highest response was obtained with a 5000 V spray voltage, a 400 °C capillary temperature and the nitrogen sheath, ion sweep and auxiliary gasses set at 50, 0 and 5 arbitrary units, respectively; the skimmer voltage was set off (0 V). The SRM mode was used with argon as the collision gas at 1.5 mTorr. The tube lens off-set was 103 V for both compounds. Ibrutinib was monitored at m/z 441.2 → 304.2 and 84.1 at −27 and −33 V collision energies and 0.100 s dwell time, the dihydrodiol-metabolite at m/z 475.2 → 304.2 and 84.1 at −27 and −33 V collision energies and 0.100 s dwell time. The mass resolutions were set at 0.7 for both separating quadrupoles.

2.2.1. Qualitative analysis

To chromatographically separate unknown metabolites of ibrutinib, a slower gradient method was set up. Ten microliter was injected onto column, followed by a 15 min gradient, starting at 5% methanol, increasing linearly to 100% methanol in 13 min, after which an isocratic plateau was held for 1 min, till 14 min. At 14 min, the level of methanol was decreased back to 5%, at which the column was equilibrated for 1 min until the following injection. For detection of any unknown metabolites, various scanning methods were used for MS and MS/MS: the aforementioned SRM, a parent ion scan of product m/z 84.1, neutral loss scan of m/z 84.1 and full ESI scans ranging between m/z 50–650 (coarse) and 200–500 (fine).

2.2.2. Data processing

For chromatographic data, Thermo Fisher Xcalibur software (Version 2.0.7 SP1) was used. For further data processing Microsoft Excel (Office 2010, Version 14.0.7147.5001) and GraphPad Prism 6 (Version 6.05) were used. Calculations of the pharmacokinetic data were made using the PK-solver add-in for Excel [14]. Averages (±SD) were calculated using MS Excel.

2.3. Standard solutions and quality controls

Stock solutions of 500,000 ng/ml were made by weighing 300–500 μg, and dissolving this in 600–1000 μl methanol (ibrutinib) or DMSO (dihydrodiol-ibrutinib, and both internal standards). Dual calibration standards were prepared from a 5000 ng/ml standard solution in human plasma, and stored in aliquots at −30 °C until further use. For this, the 5000 ng/ml standard solution was diluted to produce the calibration curve of 5000, 2000, 500, 200, 50, 20 and 5 ng/ml using blank human plasma.

Quality control (QC) samples were prepared from separate 500,000 ng/ml stock solutions. The 500,000 ng/ml solutions were diluted using blank plasma to produce QCs of 4000 (high), 400 (medium), 15 (low) and 5 (lower limit of quantitation, LLOQ) ng/ml in blank human or mouse plasma. QCs were stored in a similar fashion to calibration samples.

2.4. Sample preparation

Plasma samples were pretreated by protein precipitation. For all matrices, in a polypropylene (PP) reaction tube, 30 μl 150 ng/ml internal standard solution in acetonitrile was added to 20 μl plasma, followed by vortex mixing for approximately 5 s. The samples were centrifuged at 15,000 × g for 5 min at 15 °C. Fourty microliter of the supernatant was transferred to a 1.5 ml vial with a 250 μl glass micro-insert. Before closing the vial, 40 μl of 50% v/v methanol was added. For all samples, 5 μl sample was injected onto the column.

2.5. Degradation study of ibrutinib in human plasma

Degradation of ibrutinib was investigated in human and murine plasma. To exclude the influence of anticoagulant, multiple anticoagulants were tested, namely lithium-heparin, sodium-EDTA and the absence of one by testing serum, as EDTA can have a possible effect on catalysis by capturing metal ions [15,16]. Samples of 5000 ng/ml in each matrix were subjected to incubation at 37 °C, and aliquots were taken at 0, 3 and 18 h.

For the determination of the conversion rate, blank human serum was used. Serum was chosen over plasma for the above