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Quantification of the next-generation oral anti-tumor drugs dabrafenib, trametinib, vemurafenib, cobimetinib, pazopanib, regorafenib and two metabolites in human plasma by liquid chromatography-tandem mass spectrometry

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ABSTRACT

A sensitive and selective method of high performance liquid chromatography (HPLC) coupled to tandem mass spectrometry (MS/MS) has been developed for the simultaneous quantification of six anticancer protein kinase inhibitors (PKIs), dabrafenib, trametinib, vemurafenib, cobimetinib, pazopanib, regorafenib, and two active metabolites (regorafenib-M2 and regorafenib-M5) in human plasma. Plasma protein precipitation with methanol enables the sample extraction of 100 µL aliquot of plasma. Analytes are detected by electrospray triple-stage quadrupole mass spectrometry and quantified using the calibration curves with stable isotope-labeled internal standards. The method was validated based on FDA recommendations, including assessment of extraction yield (74–104%), matrix effects, analytical recovery (94–104%) with low variability (< 15%). The method is sensitive (lower limits of quantification within 1 to 200 ng/mL), accurate (intra- and inter-assay bias: −0.3% to +12.7%, and −3.2% to +6.3%, respectively) and precise (intra- and inter-assay CVs within 0.7–7.3% and 2.5–8.0%, respectively) over the clinically relevant concentration range (upper limits of quantification 500 to 100,000 ng/mL). This method is applied in our laboratory for both clinical research programs and routine therapeutic drug monitoring service of PKIs.

1. Introduction

Since the beginning of oral targeted anticancer therapy, with imatinib as emblematic archetype, a considerable number of new protein kinase inhibitors (PKIs) have been developed, targeting cellular signaling pathways implicated in cancer. Some PKIs approved for the treatment of various solid tumors are multikinases inhibitors, such as pazopanib and regorafenib, while others selectively target a protein kinase harboring specific genetic mutations, like vemurafenib for BRAF V600 and cobimetinib for MEK1/2.

Dabrafenib, trametinib, vemurafenib, cobimetinib, pazopanib and regorafenib are recent PKIs approved for the treatment of solid tumors, namely melanoma, advanced renal cell carcinoma (RCC), colorectal cancer (CRC) and gastrointestinal stromal tumor (GIST). The concept of *combination* of PKIs simultaneously inhibiting different targets within the same cellular signaling pathway has progressively emerged in the clinics. Similarly to what was developed in antimicrobial therapy, this strategy aims at decreasing the likelihood of drug resistance and appears to improve the clinical efficacy as compared to using single PKI [1,2]. This approach is currently being applied notably to the treatment of melanoma, with the association of BRAF and MEK inhibitors. Moreover, targeted anticancer drugs are increasingly used beyond their

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approved indications, based on tumor molecular profiling, especially in patients with advanced cancer escaping from conventional treatment or in pediatric patients. This trend is supported by recent clinical studies [3,4] and shows a promising potential, especially for PKIs targeting the MAPK pathway [5].

The laboratory of clinical pharmacology of the University Hospital of Lausanne (CHUV) pioneered the development of a measurement method for first-generation protein kinase inhibitors (i.e. imatinib, nilotinib, dasatinib, sunitinib, sorafenib and lapatinib) [6]. Since then, we continuously updated our routine services for the therapeutic drug monitoring (TDM) of targeted anticancer drugs, which has been extended to newer PKIs notably i) erlotinib and gefitinib, used against the non-small cell lung cancer (NSCLC) harboring activating EGFR mutations, ii) bosutinib and more recently iii) ponatinib, used against imatinib-resistant chronic myeloid leukemia (CML). Most PKIs meet criteria indicating the relevance of a TDM program [7], notably marked inter-individual pharmacokinetic variability on standard dosage regimens and correlation between drug plasma concentrations and clinical effects (efficacy and/or toxicity). Part of the inter-individual pharmacokinetic variability observed for PKIs can be explained by various pathways affecting PKIs exposure in the body, mediated by cytochromes CYP450, and also by drug transporters [8], known to be polymorphic and characterized by large differences in expression and activity. For instance, regorafenib is metabolized by CYP3A4 yielding two major pharmacologically active metabolites (regorafenib-M2 and regorafenib-M5), and is also substrate of UGT1A9 [9]. The metabolism of dabrafenib is altered by concomitant administration of strong inhibitors or inducers of CYP3A or CYP2C8, which are not recommended during treatment with dabrafenib [10]. For example for the first PKI imatinib, a number of clinical studies found a relationship between plasma levels – rather than drug dose – and efficacy and/or toxicity [7,11–13]. Thus inadequate drug exposure might explain the occurrence of adverse drug effects and treatment failures, not infrequently seen in some patients receiving PKIs. Thus, treatment individualization by dose adjustment according to plasma concentrations has been advocated for various PKIs, notably to increase the probability of clinical response in case of insufficient exposure, or to avoid unnecessary toxicity in case of excessive plasma levels [7,8]. It must be acknowledged that information on concentration-effect relationships for the more recent PKIs [14] are scarce, except possibly for pazopanib [15], and therapeutic windows mostly remain to be established. Overall, despite the growing interest in personalized medicine, the clinical benefit of TDM of PKIs remains to be formally established. In this context, considerable efforts have to be undertaken to improve our knowledge on clinical pharmacokinetics of the last-generation PKIs in the real-life setting (i.e. outside the stringent frame of clinical trials). A rapid method for the simultaneous quantification of the recent PKIs in patients' samples is needed to that endeavor.

Many analytical methods using high performance liquid chromatography coupled to tandem mass spectrometry (HPLC-MS/MS) have been published for the quantification of PKIs, mostly alone (regorafenib [16], vemurafenib [17], cobimetinib [18] and pazopanib [19]), in combination (dabrafenib-trametinib [20]) or sometimes in association [21,22]. Recently, an assay has been reported to analyze 14 PKIs in < 5 min, all PKIs being eluted within 1.45–1.79 min [23]. With such a short chromatographic step, selectivity may not be optimal since metabolites may co-elute with parent compounds, which is an issue notably for phase II metabolites (i.e. glucuronides, sulfates, etc.) that might regenerate the parent compound via in-source dissociation during the ionization step.

Our objective was to develop and validate a sensitive and selective method by HPLC-MS/MS for the simultaneous quantification of six recent anticancer protein kinase inhibitors (dabrafenib, trametinib, vemurafenib, cobimetinib, pazopanib, regorafenib) and the two clinically relevant (i.e. active) metabolites of regorafenib (regorafenib N-oxide (M2) and *N*-desmethyl-regorafenib N-oxide (M5)) in human plasma.

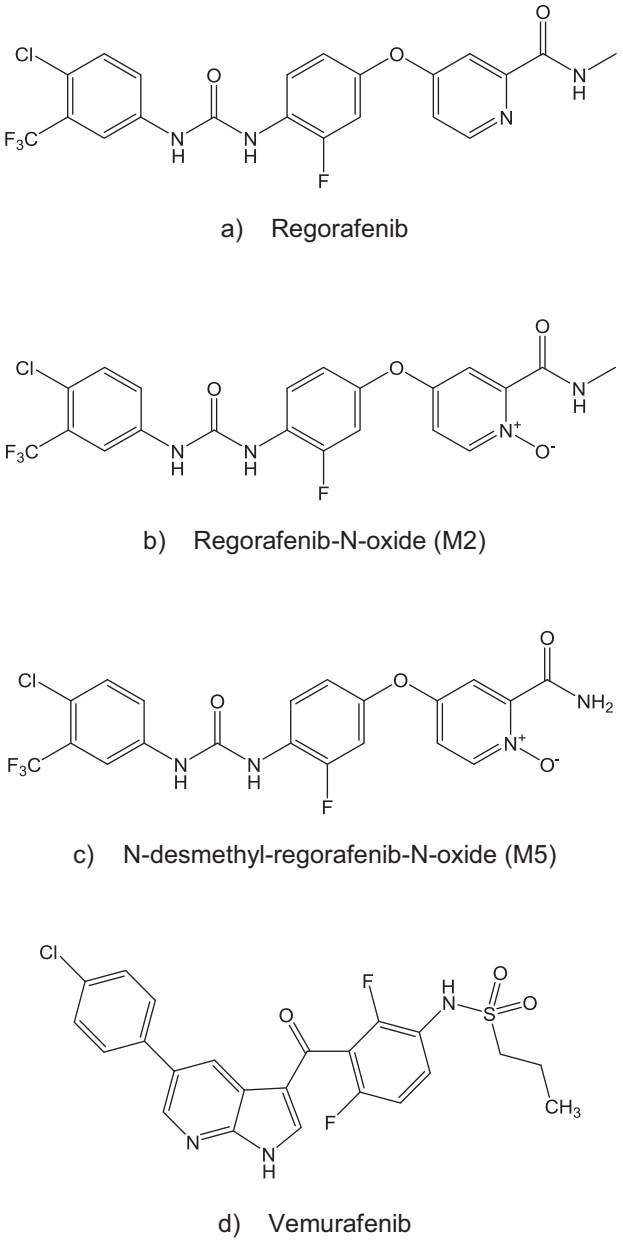

Fig. 1. Chemical structures of the six PKIs and the two metabolites under study. Regorafenib (a), Regorafenib-M2 (b), Regorafenib-M5 (c), Vemurafenib (d), Cobimetinib (e), Dabrafenib (f), Trametinib (g) and Pazopanib (h).

2. Material and methods

2.1. Chemicals, reagents and plasma

All analytes – dabrafenib, trametinib, vemurafenib, cobimetinib (racemic mixture), pazopanib, regorafenib and its metabolites M2 and M5 – as well as the corresponding Internal Standards (IS) – dabrafenib-D_9, trametinib-$^{13}C_6$, vemurafenib-$^{13}C_6$, cobimetinib-$^{13}C_6$, pazopanib-^{13}C D_3, regorafenib-^{13}C D_3 – were obtained from Alsachim (Illkirch, France). The chemical structures of the analytes are shown in Fig. 1.

Chromatography was performed with Lichrosolv® HPLC-grade acetonitrile (ACN) and methanol (MeOH) purchased from Merck (Darmstadt, Germany). Formic acid (FA, 98% pure) and ammonium acetate for chromatography were purchased from Merck (Darmstadt, Germany). Dimethylsulfoxide (DMSO) was purchased from Sigma-Aldrich (Steinheim, Germany). All other chemicals were of analytical