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Investigation of the effect of plasma albumin levels on regorafenib-induced hepatotoxicity using a validated liquid chromatography-tandem mass spectrometry method

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ABSTRACT

Regorafenib is an oral multikinase inhibitor indicated for metastatic colorectal cancer and gastrointestinal stromal tumour. Due to its extensive plasma protein binding and low calculated hepatic extraction ratio, the hepatotoxicity observed with usage of the drug may be related to its plasma exposure. To investigate the highly dynamic free:bound drug concentration for regorafenib in the plasma, a bioanalytical liquid chromatography-tandem mass spectrometric assay was developed and validated in human plasma. The concentration range of the assay was 2–1000 ng/mL. Sample preparation was via protein precipitation using acetonitrile with sorafenib as the internal standard. The supernatant was injected into an ultra-performance liquid chromatographic system coupled to a triple quadrupole mass spectrometer. The analytes were separated on an AQUITY UPLC BEH C_{18} column (120 Å, 1.7 μm, 2.1 mm × 50 mm) and eluted with a gradient elution system. The ions were detected in multiple reaction monitoring mode. The linearity, lower limit of quantification, intra-day and inter-day precision and accuracy conformed to FDA guidelines. The validated method was successfully applied to determine the effect of albumin levels in plasma on the extent of protein binding of regorafenib. The results indicated that physiologically-relevant levels of albumin were found to have no significant effect on the extent of protein binding of regorafenib, hence imposing minimal effect on drug disposition.

1. Introduction

Regorafenib (BAY73-4506, Fig. 1) is an oral multikinase inhibitor indicated for metastatic colorectal cancer (mCRC) and gastrointestinal stromal tumour. It acts by targeting the receptors of vascular endothelial growth factor (VEGF), platelet derived growth factor (PDGF) and fibroblast growth factor (FGFR) as well as tyrosine kinases such as TIE2, KIT, RET and BRAF [1].

Current dosing guidelines recommend that regorafenib be given at 160 mg daily for 21 days, followed by 7 days drug-free in 28-days repeating cycles [2]. Despite administering regorafenib at optimal dose, various adverse effects were still observed. The most serious adverse reaction was drug-induced hepatotoxicity and a black box warning has been issued by the US Food and Drug Administration (FDA) [3]. While severe regorafenib-induced hepatotoxicity had a low incidence of 0.3%, its actual incidence could be much higher than its post-marketing reports [4]. Confirmation of drug-induced hepatotoxicity can be challenging in the presence of hepatic metastases. Most clinical studies had the inclusion criteria of adequate liver function, hence under-estimating the true incidence of possible drug-induced hepatotoxicity risk amongst patients with pre-existing liver impairment [2,5–9]. Also, a meta-analysis of clinical trials suggests a risk of hepatotoxicity associated with the wider use tyrosine kinase inhibitors (TKI) [10]. This provides grounds to believe that regorafenib, would have a stronger association with drug-induced hepatotoxicity than what is reported in clinical trials.

Most of the adverse effects associated with regorafenib could be managed with dose reduction or termination [11]. Regorafenib exhibits high plasma protein binding (i.e. 99.5%) and low calculated hepatic extraction ratios (i.e. < 0.3) [12,13]. Based on well-established pharmacokinetics models, this suggests that regorafenib's drug-induced hepatotoxicity could be related to its plasma exposure due to limited

Abbreviations: ACN, acetonitrile; CE, collision energy; CS, calibration standards; CXP, collision cell exit potential; ED, equilibrium dialysis; EP, entrance potential; ESI, electrospray ionization; DP, declustering potential; FDA, US Food and Drug Administration; IS, internal standard; LC–MS/MS, liquid chromatography tandem mass spectrometry; LLOQ, lower limit of quantification; MRM, multiple reaction monitoring; PBS, phosphate buffered saline; QC, quality control; RED, Rapid Equilibrium Dialysis; SD, standard deviation; ULOQ, upper limit of quantification

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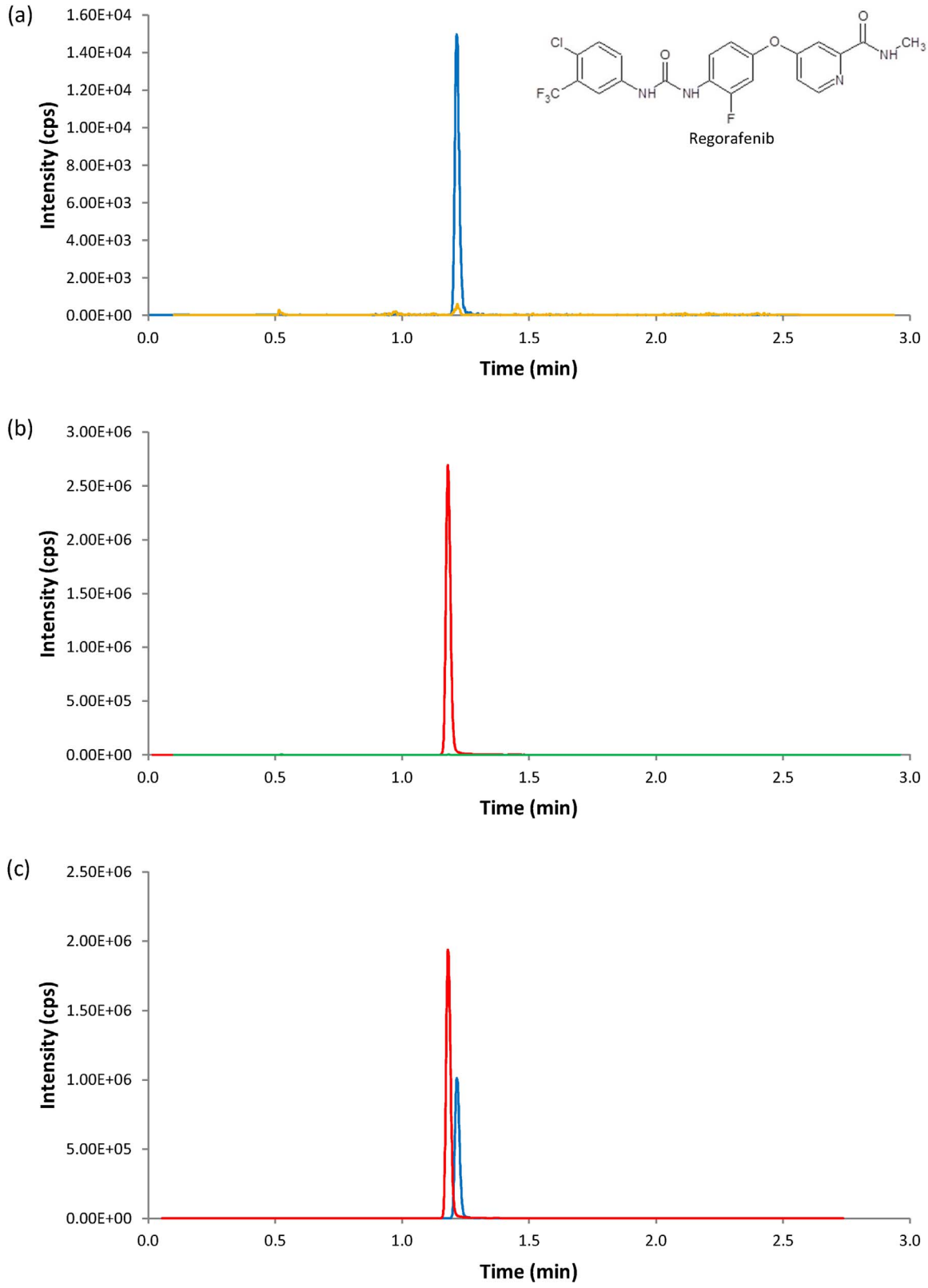

Fig. 1. Chromatograms of regorafenib and IS human plasma (a) regorafenib at LLOQ level (blue) and in blank human plasma (yellow); (b) IS at the 100 ng/mL (red) and in blank human plasma (green) and (c) regorafenib at 250 ng/mL (blue) and IS at 100 ng/mL (red). The retention times of regorafenib and IS are 1.21 min and 1.18 min respectively.

drug clearance capacity that is sensitive to both changes in protein binding and liver intrinsic metabolic activity. Accordingly, the variation in free regorafenib concentration amongst patients receiving the same dose could thus be accentuated by inter-patient variability in the level of plasma proteins, namely albumin. Therefore, the selective occurrence of hepatotoxicity could be due to inter-patient variability in plasma albumin levels, resulting in variation in free drug concentration, especially in patients with underlying liver dysfunction.

To be able to accurately determine the highly dynamic free drug concentration for regorafenib based on this clinical context, this study