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Short communication

LC–MS/MS assay for the quantitation of the ATR kinase inhibitor VX-970 in human plasma

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ABSTRACT

DNA damaging chemotherapy and radiation are widely used standard-of-care modalities for the treatment of cancer. Nevertheless, the outcome for many patients remains poor and this may be attributed, at least in part, to highly effective DNA repair mechanisms. Ataxia-telangiectasia mutated and Rad3-related (ATR) is a key regulator of the DNA-damage response (DDR) that orchestrates the repair of damaged replication forks. ATR is a serine/threonine protein kinase and ATR kinase inhibitors potentiate chemotherapy and radiation. The ATR kinase inhibitor VX-970 (NSC 780162) is in clinical development in combination with primary cytotoxic agents and as a monotherapy for tumors harboring specific mutations. We have developed and validated an LC–MS/MS assay for the sensitive, accurate and precise quantitation of VX-970 in human plasma. A dilute-and-shoot method was used to precipitate proteins followed by chromatographic separation with a Phenomenex Polar-RP 80 Å (4 μm, 50 × 2 mm) column and a gradient acetonitrile-water mobile phase containing 0.1% formic acid from a 50 μL sample volume. Detection was achieved using an API 4000 mass spectrometer using electrospray positive ionization mode. The assay was linear from 3 to 5,000 ng/mL, proved to be accurate (94.6–104.2%) and precise (<8.4% CV), and fulfilled criteria from the FDA guidance for bioanalytical method validation. This LC–MS/MS assay will be a crucial tool in defining the clinical pharmacokinetics and pharmacology of VX-970 as it progresses through clinical development.

1. Introduction

Cytotoxic chemotherapies and ionizing radiation that induce DNA damage are widely used standard-of-care modalities for solid malignancies. For many patients the benefit of these therapies is limited, at least in part, by the DNA damage response (DDR), the signaling that orchestrates a wide array of mechanisms that detect and repair DNA damage in cancer cells as well as normal cells [1]. Cancer cells frequently acquire mutations that inactivate specific DNA repair mechanisms and these changes that are not present in normal cells, may be exploited to achieve significant therapeutic index with small molecules such as PARP inhibitors. Furthermore, the DDR may be a barrier to oncogenic transformation that may be elevated in cancer cells and therefore targeted to achieve significant therapeutic index [2].

Ataxia telangiectasia mutated (ATM) and ataxia telangiectasia mutated and Rad3-related (ATR) are two serine/threonine kinases that have been identified as primary activators and coordinators of DDR [3,4]. ATM recognizes DNA double-strand breaks (DSBs) and ATR recognizes single-strand DNA at damaged replication forks and resected (DSBs) [1]. ATM and ATR initiate complex and overlapping

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signaling cascades [5]. Up to 70% of tumors display inactivating mutations in either ATM or tumor suppressor protein p53 and it is hypothesized that cells harboring defects in this signaling axis acquire a selective advantage during carcinogenesis that is associated with loss of the G1 cell cycle checkpoint and reduced DNA repair [6]. Cancer cells that have lost the ATM/p53 axis have an increased dependence on the ATR signaling pathway and correspondingly both ATM- and p53-deficient cancer cells are selectively killed by ATR kinase inhibitors *in vitro* an *in vivo* [7–9].

VX-970 has recently entered clinical development as a potent and selective inhibitor of ATR [10]. VX-970 has anti-proliferative qualities as a single agent and it potentiates the effects of DNA damaging therapies in ATM-deficient xenograft models [11–13]. Radiation and chemotherapy are typically administered at or near their maximum tolerable dose and potentiating these standard-of-care therapies with VX-970 is a rational approach to increase the efficacy of the primary DNA damaging modality without creating additional toxicity. As VX-970 progresses through clinical development, an accurate description of its pharmacokinetics and potential alterations by primary chemotherapies is crucial. To support these endeavors we developed a sensitive and accurate LC–MS/MS assay to quantify VX-970 in human plasma. The calibration curve ranges from 3 to 5000 ng/mL to accommodate expected clinical concentrations.

2. Experimental

2.1. Chemicals and reagents

VX-970 (NSC 780162, VRT-0768079, VE-822) ($M/(M+M_{+7})$ >99.99%) was obtained from Vertex Pharmaceuticals (Boston, MA) as the free base. Because the drug substance used clinically, and therefore the reported doses, reflect the hydrochloride salt, all concentrations described in this report are expressed as hydrochloride salt. The internal standard, [d_7]-VX-970 (VRT-1135046) ($M_{+7}/(M+M_{+7})$ >99.66%), was obtained from Vertex Pharmaceuticals (Boston, MA). Bile from VX-970 treated rat was provided by Vertex Pharmaceuticals, London, UK. Acetonitrile, water (both HPLC grade) and dimethyl sulfoxide were purchased from Fisher Scientific (Fairlawn, NJ). Formic acid was purchased from Sigma-Aldrich (St. Louis, MO). Control human plasma was purchased from Valley Biomedical (Winchester, VA) and Lampire (Everett, PA). Bovine serum albumin (BSA), pooled human liver microsomes, pooled female rat microsomes, UDPGA, $MgCl_2$, alamethicin and formic acid were purchased from Sigma-Aldrich (St. Louis, MO).

2.2. Chromatography

The LC system consisted of an Agilent (Palo Alto, CA, USA) 1200 SL autosampler and binary pump and a Phenomenex (Torrence, CA USA) Synergi Polar-RP 80 Å (4 μm, 50 × 2.0 mm) column using a gradient style elution program. Mobile phase solvent A consisted of 0.1% formic acid in acetonitrile and mobile phase solvent B consisted of 0.1% formic acid in water. The initial mobile phase composition was 30% solvent A and 70% solvent B. From 0 to 2.2 min, at a 0.4 mL/min flowrate, solvent A was increased to 80% and conditions held until 2.3 min. Between 2.3 and 3.0 min, solvent A was maintained at 80% and the flowrate increased to 0.8 mL/min and conditions held until 3.1 min. At 3.1 min he mobile phase composition was then returned to 30% solvent A until 4 min at a flowrate of 1.0 mL/min. The total run time was 4 min with an injection volume of 5 μL.

2.3. Mass spectrometry

Mass spectrometric detection was carried out using a SCIEX (Framingham, MA) 4000 hybrid linear ion trap tandem mass spectrometer utilizing electrospray ionization in positive-ion multiple reaction monitoring (MRM) mode. The settings of the mass spectrometer in positive mode scanning parameters were as follows: curtain gas 40, IS voltage 5000 V, probe temperature 500 °C, GS1 65, GS2 65, DP 50 V, CE of 25 eV, and an exit potential of 10 V. The temperature of the autosampler was 4 °C. The MRM *m/z* transitions monitored were: 464.3 > 433.5 for VX-970 and 471.3 > 440.3 for [d_7]-VX-970. Control of the LC system and mass spectrometer as well as data collection was accomplished with Analyst software (version 1.4.2).

2.4. Preparation of calibration standards and quality control samples

Stock solutions of VX-970 and internal standard [d_7]-VX-970 (see Fig. 1 for structures) were prepared independently at 1.0 mg/mL in dimethyl sulfoxide (DMSO) and stored at −80 °C. Working stock solutions of 0.1 mg/mL VX-970 and 0.1 mg/mL [d_7]-VX-970 were prepared in DMSO. On the days of analysis, the working stock solution of VX-970 was serially diluted in 10 fold steps using DMSO, and the internal standard was diluted 200 fold to a final concentration of 0.5 μg/mL. VX-970 calibration working stock solutions were then diluted in human plasma to produce the following analyte concentrations: 3, 10, 30, 100, 300, 1000, 3000 and 5000 ng/mL. For each calibration series zero and blank samples were also prepared from 50 μL of control plasma.

Quality control (QC) stock solutions were stored at −80 °C. These solutions were diluted in human plasma to produce the following QC samples of either: Lower Limit of Quantification (LLOQ) 3 ng/mL, QC Low (QCL) 5 ng/mL; QC Mid (QCM) 150 ng/mL, and QC High (QCH) 4000 ng/mL.

2.5. Sample preparation

Ten μL of 0.5 μg/mL [d_7]-VX-970 was added to each microcentrifuge tube except blank plasma samples. This was followed by addition 50 μL plasma (standard, QC or sample plasma). Protein precipitation was sequentially accomplished using 200 μL of acetonitrile (aqueous to organic ratio of 1:4 *v/v*). Samples were vortexed for 1 min on a Vortex Genie-2 set at 9 (Model G-560 Scientific Industries, Bohemia, NY) and then centrifuged at 13,500 × *g* at room temperature for 5 min. A 100 μL aliquot of the resulting supernatant was transferred to an autosampler vial followed by addition of 150 μL of water (final organic composition 32%), vial capping and brief vortexing. A 5 μL volume of each sample was then injected into the LC–MS/MS system.

2.6. Validation procedures

2.6.1. Calibration curve and lower limit of quantitation (LLOQ)

Calibration standards and blanks were prepared and analyzed (see paragraph 2.3 and 2.4) in triplicate to establish a calibration range with acceptable accuracy and precision, as previously described [14].

2.6.2. Accuracy and precision

The accuracy and precision of the assay were determined by analyzing samples at the LLOQ, QCL, QCM, and QCH concentrations, as previously described [14].