RAPID COMMUNICATION

Analytical Approach to Characterize the Intratumoral Pharmacokinetics and Pharmacodynamics of Gefitinib in a Glioblastoma Model

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ABSTRACT: Heterogeneity in brain tumors can result in variable drug distribution and possibly drug response; however, there are no readily accessible means to obtain regional pharmacokinetic (PK)/pharmacodynamic (PD) information in preclinical tumor models that typically rely on average drug concentration measurements. On the basis of a novel serial brain tumor sectioning protocol, sensitive and robust methods were developed to characterize the intratumoral PK [liquid chromatography with tandem mass spectrometry detection (LC/MS/MS)] and PD (phosphorylated extracellular-signal-regulated kinase, antibody-based detection) of gefitinib in small amounts of glioblastoma tumor samples obtained from mice bearing intracerebral tumors administered 150 mg/kg of gefitinib. LC/MS/MS method was accurate (±15%) and precise (coefficient of variation ≤15%). For PD analysis, two antibody-based assay systems [enzyme-linked immunosorbent assay and meso scale discovery (MSD)] were compared and the more sensitive method (MSD) was selected. Gefitinib concentrations showed up to 2.4 ± 0.7-fold intratumoral variability in PK and 1.5 ± 0.20-fold variability in PD. The methods are sufficiently accessible and could be applied to other anticancer drugs and tumor models to obtain greater resolution of intratumoral PKs and PDs. © 2012 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci

Keywords: preclinical pharmacokinetics; pharmacodynamics; cancer; blood brain barrier; liquid chromatography; mass spectrometry; LC/MS/MS; MSD assay; gefitinib; mouse orthotopic glioblastoma model; intratumoral pharmacokinetics and pharmacodynamics

INTRODUCTION

Glioblastoma multiforme (GBM) is a malignant brain tumor that is known to be highly disorganized and invasive with a compromised blood–brain barrier (BBB).[1] GBMs show intratumoral variability in the morphology and functioning of the vasculature,[2–3] vessel permeability,[4] and other biological and histological features such as interstitial fluid pressure,[5] necrosis and cell proliferation and density,[6–9] that could cause heterogeneous drug distribution. In a limited number of cases, variable intratumoral drug concentrations have been documented that were attributed to inconsistent BBB integrity,[10–11] necrosis, and high interstitial fluid pressure.[5,12–14] Even though it can be appreciated that the lack of uniformity in drug distribution in brain tumors can profoundly influence a drugs' pharmacodynamic (PD) behavior and efficacy, efforts to examine regional variation in both pharmacokinetics (PK) and PD are quite limited. Most often preclinical studies in brain tumors are based on the quantification of such characteristics from whole tumor homogenates that are compelled to average potential differences, and thus, limit insights on why drugs may be inactive. The goal of this investigation was to develop analytical methods that could better distinguish regional intratumoral PK/PD properties in a preclinical brain tumor model for the anti-human epidermal growth factor receptor (anti-EGFR) inhibitor gefitinib, a low-molecular-weight tyrosine-kinase inhibitor. The methods make use of a tumor sectioning protocol and relatively conventional liquid

Additional Supporting Information may be found in the online version of this article. Supporting Information

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chromatography with tandem mass spectrometry detection (LC/MS/MS) and antibody-based techniques that are likely to be available to a broader community than those based on emerging matrix-assisted laser desorption/ionization mass spectrophotometric imaging (MALDI-MSI) approaches.[15] The EGFR (also called HER 1) pathway has been found to be commomnly dysregulated in glioblastomas, either because of the overexpression of EGFR (40%–50% of the glioblastomas)[16] or because of the presence of a constitutively active EGFR mutant, EGFRVIII (about 67% of all mutants in glioblastoma).[17] Given the high frequency of dysregulation of this receptor in GBMs, it is an attractive target for therapeutic intervention by small-molecule inhibitors of EGFR such as gefitinib. Despite promising results in preclinical studies, the results from various clinical trials investigating the role of gefitinib in GBMs have been disappointing. There is very limited information available on the intratumoral distribution of this drug. To address the question whether gefitinib is able to reach therapeutic concentrations and inhibit its target within various regions of tumor, experimental methods were developed to explore its intratumoral PK and PD characteristics.

MATERIALS AND METHODS

Chemicals and Standards

Gefitinib and the internal standard (IS) vandetanib were purchased from LC Laboratories (Woburn, Massachusetts). Ammonium formate was purchased from Sigma–Aldrich, Inc. (St. Louis, Missouri). High-performance liquid chromatography (HPLC)-grade acetonitrile and methanol were purchased from Fisher Scientific (Fair Lawn, New Jersey). Deionized water (Nanopure deionization system; Barnstead/Thermolyne, Dubuque, Iowa) was used for all aqueous solutions.

Chromatographic and Mass Spectroscopic Conditions

Method development and validation was performed with an LC/MS/MS system (HPLC—Shimadzu, Kyoto, Japan; QTrap 5500—Applied Biosystems, Foster City, California) using an electrospray ionization (ESI) interface and operated in positive ion mode. Instrument control, data acquisition, and processing for both chromatography and MS were performed using the Analyst 1.5.1 software (Applied Biosystems MDS Sciex, Ontario, Canada). The chromatographic separation system consisted of a guard cartridge (C18, 4.0 × 2.0 mm^2; Phenomenex, Torrance, California), an analytic column (Luna C18, 3 μm particle size, 50 × 2.0 mm^2; Phenomenex), and a mobile phase of acetonitrile/10 mmol/L ammonium formate (65:35, v/v), delivered isocratically at a flow rate of 0.2 mL/min.

Drug quantification was performed by ESI–selected reaction monitoring. The column effluent was monitored at the following precursor–product ion transitions: *m/z* 447.2 → 100.2 for gefitinib and *m/z* 477.2 → 112.1 for vandatinib with a dwell time of 100 ms for each ion transition. The retention time was 1.5 min for gefitinib and 1.4 min for vandatinib with a total run time of 3 min per sample.

Preparation of Stock Solution, Calibration Standards, Quality Control Samples, and Brain Tumor Samples from the PK Study

Stock solutions of gefitinib and vandatinib (the IS) were prepared separately in methanol at a target concentration of 200 μg/mL as free base. The stock solution of gefitinib was then used to prepare calibration standards and quality control (QC) samples in brain tumor matrix. Blank brain tumor samples were obtained from untreated nude mice bearing U87 human glioma xenografts. To each gram of brain tumor tissue, 20 mL of deionized water (5%, w/w) was added followed by homogenization at 30,000 rpm for 5 s (Polytron PT2100, Kinematica AG, Littau/lucerne, Switzerland). The blank brain tumor homogenate was then used for preparation of standard curves and QC samples. Calibration standards were prepared in the concentration range of 1.2–2600 ng/g by adding stock solutions of gefitinib obtained through serial dilutions to the blank brain tumor homogenate. Similar to the calibration standards, QC samples were prepared in replicates ($n = 5$ for the intraday and interday validation) at four concentration levels representing the entire range of concentrations (1.2, 16, 144, and 1300 ng/g). All the solutions were sonicated for 20 min in a bath sonicator.

Tumor tissue samples (about 1 mg) from the PK study were collected in preweighed 0.5 mL plastic tubes according to a specific tumor sectioning protocol as described below in the section *In Vivo Gefitinib Treatment and Sampling* and illustrated in Figure 1. The tumor samples were then homogenized by adding water [5% (w/w) tumor/water], followed by sonication in a bath sonicator for 20 min at room temperature.

Gefitinib was extracted from mouse brain tumor matrix by a protein precipitation method as reported previously.[18] To 10 μL of tumor homogenate [5% (w/w) tumor/water], 40 μL of methanol containing vandatinib (100 ng/mL) was added, vortexed for 1 min, and then centrifuged at 20,817 x g (eppendorf centrifuge 5430R, Eppendorf AG,Hamburg, Germany) for 5 min. Aliquots of 1 μL of the resultant supernatant were injected into the LC/MS/MS system.

Method Validation

Linearity of the method was evaluated in three sets of matrix-matched (blank brain tumor homogenates) calibration standards. It was considered satisfactory