An Alternative Approach for Quantitative Bioanalysis using Diluted Blood to Profile Oral Exposure of Small Molecule Anticancer Drugs in Mice

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ABSTRACT: A quantitative bioanalytical method for pharmacokinetic studies using diluted whole blood from serially bled mice was developed. Oral exposure profiles in mice for five model anticancer compounds dacarbazine, gefitinib, gemcitabine, imatinib, and topotecan were determined following discrete and cassette (five-in-one) dosing. Six micro blood samples per animal were collected and added to a fixed amount of water. This dilution served several purposes: the red blood cells were lysed; an anticoagulant was unnecessary and the fluid volume of diluted sample was sufficient for bioanalytical assays. AUC values obtained from blood concentrations were within twofold for discrete and cassette dosing except for imatinib (2.1-fold difference) and in agreement with those obtained from plasma concentrations after discrete dosing. All compounds were stable in plasma and diluted blood samples for at least 2 weeks at approximately −80°C. Matrix and intermatrix effects were evaluated to ensure robustness and integrity of the bioanalytical assays. This method provides significant process improvement by enhancing efficiency for sample collection and processing and reducing resources (e.g., reduced compound, cost, and animal requirement) compared with conventional methods. Our study demonstrates the applicability of using diluted micro blood samples for small molecule quantitative bioanalysis to support mouse studies in drug discovery. © 2012 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 102:750–760, 2013

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INTRODUCTION

Conventionally, pharmacokinetic (PK) parameters have been assessed using plasma samples based on the relative simplicity of plasma as a bioanalytical matrix compared with whole blood. In larger animals, such as rat or dog, collection of plasma for quantitative bioanalysis is a relatively easy process as each animal can be serially bled to obtain a sufficient volume of plasma. However, in smaller rodents, such as mice, collection of plasma is more complicated, labor intensive, and time consuming. Each mouse can be bled for only a limited amount of time, as the blood collection volume should not exceed approximately 10% of the blood volume over a 24 h period. For a mouse with a bodyweight of 20 g, only about 0.125 mL of blood can be withdrawn (assuming that the total blood volume is approximately 6% of body weight), limiting blood collection to about one to three samples depending on blood volume drawn. Therefore, a large number of mice per study are needed, increasing compound requirement, animal handling, and costs. Obtaining PK parameters in mice during drug discovery and development is often essential to support lead optimization by identifying molecules with desirable PK properties, to support preclinical toxicology studies and in anticancer drug research to support efficacy studies in mouse human tumor xenograft models to better understand PK–pharmacodynamic relationships. In addition, mouse models such as hepatic cytochrome P450 reductase null mice, chimeric mice with humanized livers or knockout mice are often also used for basic research studies. To alleviate some of these issues, cassette (*N*-in-one) dosing, which involves the simultaneous administration of several compounds to a single animal, and/or serial bleeding in mice has been utilized with the advent of more sensitive and specific bioanalytical methods.[1–6]

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Although potential concerns are associated with blood PK and cassette dosing, such as matrix effects, drug—drug interactions, and formulation challenges (e.g., the same vehicle is required for all compounds), many programs have used this technique very successfully to enhance the drug discovery process.[7–9] However, published data on mouse studies utilizing any of these methods are limited and some of these methods have limitations as discussed by Watanabe et al.[10] Moreover, although oral drug administration reflects the most clinically and commercially desirable and patient-friendly route of administration and cassette dosing has a very high impact on process improvement (e.g., reduced compound, cost, and animal requirement), very limited data have been published on oral cassette dosing in mice. The majority of these studies determined drug concentrations in plasma rather than whole blood. Besides the disadvantage of requiring a larger blood volume for plasma analysis, for compounds that preferentially distribute into red blood cells, a blood analysis would be more appropriate than plasma analysis as PK parameters determined based on measured plasma concentrations can be misleading (e.g., overestimation of clearance and underestimation of oral exposure). To our knowledge only one published study reported using blood PK for oral cassette (four-in-one) dosing in mice.[10] In that study, four epoxide hydrolase inhibitors were evaluated; however, discrete and cassette PK parameters and plasma concentration profiles could be compared for only two compounds due to the low oral exposure of the other two compounds. The blood sample preparation was also fairly involved and included weighing, drying, and reconstitution of the blood samples. Mouse PK studies appear to be especially relevant to anticancer drug discovery and development due to the use of xenograft tumor models in mice to assess responses of tumor to potential novel anticancer drugs but very few publications are focused on this therapeutic area.[11–13]

Here, we evaluate a quantitative bioanalytical method for discrete or cassette dosing PK studies using diluted whole blood from serially micro bled (10 or 15 μL) mice. Because each mouse gives little blood, collected blood was added to a fixed amount of water. This dilution served several purposes: the red blood cells are lysed; an anticoagulant was unnecessary and the fluid volume of diluted sample was sufficient for bioanalytical assays. Bioanalytical methods were developed for five small molecule anticancer model compounds: dacarbazine, gemcitabine, gefitinib, imatinib, and topotecan (Fig. 1). Both matrix effects and intermatrix studies were conducted to ensure robustness and integrity of the bioanalytical assays. These compounds were then dosed orally in mice at 10 mg/kg either as a cassette (five-in-one) or discretely. The drug concentrations in both blood (after discrete and cassette dosing) and plasma samples (after discrete dosing) were measured and the PK parameters were determined. Measurements of drug blood concentrations may not be sufficient for the consistent calculation of PK parameters or for physiologically based PK modeling and, consequently, the interpretation of PK and pharmacodynamic data of drugs between blood and plasma may vary. For this reason, the determination of the blood–plasma ratio is needed. Possible ways of measuring this parameter are suggested.

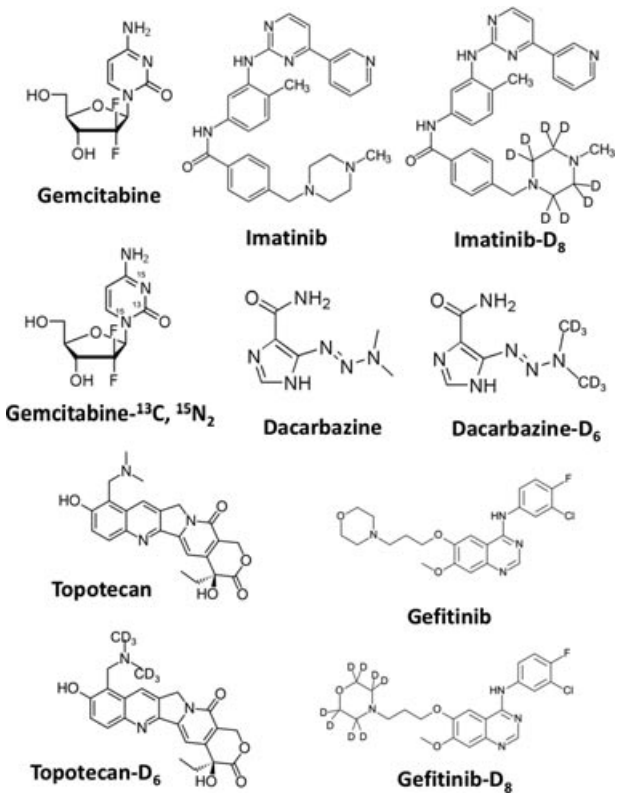

Figure 1. Chemical structures of dacarbazine, gefitinib, gemcitabine, imatinib, and topotecan, and their respective internal standards dacarbazine-D_6, gefitinib-D_8, gemcitabine-^{13}C, $^{15}N_2$, imatinib-D_8, and topotecan-D_6.

MATERIALS AND METHODS

Chemicals, Reagents, and Materials

The chemical structures of dacarbazine [molecular weight (MW) = 182Da], gefitinib (MW = 447Da), gemcitabine (MW = 263Da), imatinib (MW = 494Da), topotecan (MW = 421Da), and their stable label internal standards (ISs) are shown in Figure 1. Gemcitabine hydrocholoride, gemcitabine-^{13}C, $^{15}N_2$ hydrochloride (IS), topotecan hydrochloride, topotecan-D6 (IS), imatinib methanesulfonate, and gefitinib were purchased from US Biologicals (Swampscott, Massachusetts). Dacarbazine was purchased from Sigma–Aldrich (St. Louis, Missouri).