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Journal of Chromatography B, 835 (2006) 136–142
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Short communication

High-performance liquid chromatographic method for the determination of gemcitabine and 2′,2′-difluorodeoxyuridine in plasma and tissue culture media

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Received 29 March 2005; accepted 9 March 2006

Abstract

Gemcitabine, a pyrimidine antimetabolite undergoes metabolism by plasma and liver cytidine deaminase to form the inactive compound, 2′,2′-difluorodeoxyuridine (dFdU). The parent molecule is activated by intracellular phosphorylation. To evaluate the population pharmacokinetics in patients receiving gemcitabine, and to test the relation between gemcitabine infusion rate and antitumor activity in an in vitro bioreactor cell culture system, we developed and validated a sensitive and specific HPLC-UV method for gemcitabine and dFdU. Deproteinized plasma is vortexed, centrifuged, and 25 μL of the acidified extract sample is injected onto a Waters Spherisorb 4.6 mm × 250 mm, 5 μm C18 column at 40 °C. The mobile phase (flow rate, 1.0 mL/min) consists of 10:90 (v/v) acetonitrile-aqueous buffer (50 mM sodium phosphate and 3.0 mM octyl sulfonic acid, pH 2.9). Gemcitabine, dFdU, and the internal standard, 2′-deoxycytidine (2′dC) were detected with UV wavelength set at 267 nm. The standard curves for gemcitabine in both matrices ranged from 2 to 200 μM, and for dFdU in plasma, from 2 to 100 μM. Within-run and between-run component precision (CV%) was ≤6.1 and 5.7%, respectively for both human plasma and tissue culture media, and for dFdU, 2.3 and 2.7%. Total accuracy ranged from 98.7 to 106.2% for human plasma and from 96.9 to 99.2% for tissue culture media, respectively, and for dFdU, from 96.5 to 99.6%. Tetrahydrouridine (THU), an inhibitor of cytidine deaminase is used to prevent breakdown in human plasma. With one method we can measure gemcitabine in both plasma and tissue culture media. Utility is demonstrated by evaluation of the disposition of gemcitabine in an in vitro bioreactor cell culture system.

Keywords: HPLC; Gemcitabine; dFdU; In vitro bioreactor cell culture; UV

1. Introduction

Gemcitabine (2′,2′-difluorodeoxycytidine, dFdC, Gemzar) a pyrimidine antimetabolite, is approved for treatment of pancreatic and non-small cell lung cancer, and second-line therapy in combination with paclitaxel for treatment of breast cancer. After intravenous injection, gemcitabine undergoes metabolism (Fig. 1) by plasma and liver cytidine deaminase to form 2′,2′-difluorodeoxyuridine (dFdU), a compound with little antitumor activity. Overall, approximately 77% of administered gemcitabine is excreted either unchanged, or as the dFdU metabolite into the urine within 24 h. Gemcitabine also undergoes intracellular phosphorylation by deoxycytidine kinase at the tumor site to form difluoro-dCMP, and is phosphorylated further by other intracellular kinases to form difluoro-dCDP and difluoro-dCTP [1–3]. The diphosphate metabolite (dFdCDP) inhibits ribonucleotide reductase, an enzyme that catalyzes formation of deoxynucleosides required for DNA synthesis [4]. The triphosphate (dFdCTP) is incorporated into DNA, resulting in chain termination [5,6].

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doi:10.1016/j.jchromb.2006.03.023

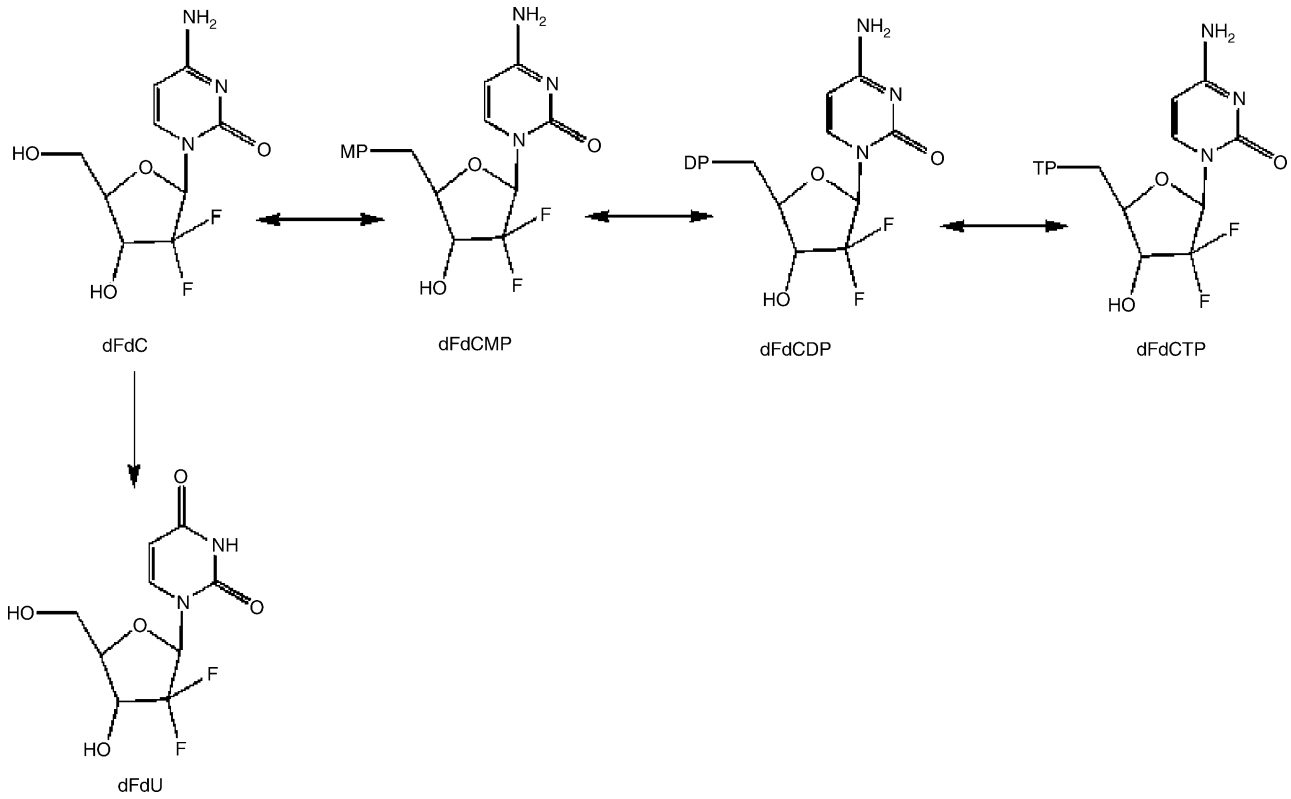

Fig. 1. Metabolic schema for gemcitabine showing conversion to its inactive metabolite, dFdU by cytidine deaminase, and also phosphorylation to the mono-, di-, and triphosphate metabolites.

Plasma gemcitabine clearance varies 4–30-fold between patients receiving the same dose [7,8]. It is unclear how this variability affects active metabolite production and antitumor activity, since dFdU production also varies 2–11-fold between patients [8]. Patient-specific characteristics (i.e., covariates, genetic polymorphisms, etc.) can influence drug and metabolite disposition [9], and could affect pharmacologic activity (i.e., antitumor activity, toxicity). Thus, identification of covariates associated with variable gemcitabine distribution in patients may provide a rationale for individualized drug dosing and treatment regimens. Hence, further pharmacokinetic studies that evaluate the importance of gemcitabine disposition on pharmacologic activity in human subjects are warranted.

Infusion rates also influence gemcitabine antitumor activity. Tempero and colleagues compared gemcitabine efficacy when administered as a 30-min infusion versus a fixed dosage rate infusion (10 mg/m^2/min) to patients with pancreatic adenocarcinoma [10]. It is theorized that the prolonged infusion results as an increase of active metabolite production at the tumor site, leading to an improved survival rate. While these results appear promising, additional investigation to clarify underlying mechanism(s) responsible for undefined dFdCTP concentration-effect relationships of gemcitabine are needed, especially in tumor cells. Studies that establish optimal dose-infusion rates that lead to maximum intracellular active metabolite concentrations are needed to enhance response rates during therapy with gemcitabine. To allow for testing of multiple gemcitabine treatment regimens and measurement of active metabolite production under controlled conditions, an in vitro bioreactor cell culture system will be used [11–13]. Mammalian cells are grown in a sterile-enclosed space that receives media driven by a pump. This system will allow for controlled gemcitabine infusion, which simulates human concentration–time profiles. The resultant accumulation of gemcitabine intracellular phosphorylated metabolites leading to cell death can then be studied. From these studies, we anticipate that we can develop dosing regimens that will lead to greater intracellular active metabolite exposure and enhanced antitumor activity. Once monotherapies are optimized, work can begin to test the utility of adjunct agents in improving efficacy. For phase I trials, Abbruzzese et al. [7] developed an ELISA method for determination of gemcitabine in plasma. Several high performance liquid chromatography assays with ultraviolet detection to measure gemcitabine and dFdU in human plasma have been described [14–18]. Reversed-phase columns were used by most investigators, although Freeman et al. initially employed a normal phase aminopropyl silica column, an approach also used by other investigators [16]. Sottani et al. [20] and Xu et al. [19] developed an LC–MS methods for gemcitabine and its major metabolite, 2′,2′-difluorodeoxyuridine. Many of these reported assays require solid-phase [19,20], or liquid–liquid extractions [16]. Direct plasma protein precipitation procedures with acids [15] or water-miscible organic solvents [14] have also been used for sample preparation. Mobile phases used for reversed-phase columns employ either gradient elution [8,17,19] or ion-pairing procedures [15] for efficient separation of gemcitabine, dFdU, and an internal standard. To