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Original Research Paper

Development and validation of a UPLC–MS/MS assay for the determination of gemcitabine and its L-carnitine ester derivative in rat plasma and its application in oral pharmacokinetics

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ABSTRACT

A simple and rapid UPLC–MS/MS method to simultaneously determine gemcitabine and its L-carnitine ester derivative (2'-deoxy-2', 2'-difluoro-N-((4-amino-4-oxobutanoyl) oxy)-4-(trimethyl amm-onio) butanoate-cytidine, JDR) in rat plasma was developed and validated. The conventional plasma sample preparation method of nucleoside analogues is solid-phase extraction (SPE) which is time-consuming and cost-expensive. In this study, gradient elution with small particles size solid phase was applied to effectively separate gemcitabine and JDR, and protein precipitation pretreatment was adopted to remove plasma protein and extract the analytes with high recovery(>81%). Method validation was performed as per the FDA guidelines, and the standard curves were found to be linear in the range of 5–4000 ng/ml for JDR and 4–4000 ng/ml for gemcitabine, respectively. The lower limit of quantitation (LLOQ) of gemcitabine and JDR was 4 and 5 ng/ml, respectively. The intra-day and inter-day precision and accuracy results were within the acceptable limits. Finally, the developed method was successfully applied to investigate the pharmacokinetic studies of JDR and gemcitabine after oral administration to rats.

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Abbreviations: JDR, 2'-deoxy-2', 2'-difluoro-N-((4-amino-4-oxobutanoyl)oxy)-4-(trimethyl amm-onio) butanoate-cytidine; SPE, solid-phase extraction; LLOQ, lower limit of quantification; PK, pharmacokinetic; OCTN2, organic cation/carnitine transporters 2; ESI, electrospray ionization; THU, Tetrahydrouridine; IS, internal standard; QC, quality control.

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1. Introduction

Gemcitabine is a water-soluble pyrimidine nucleoside analogue with significant anticancer activity for several types of cancers, such as pancreatic adenocarcinoma, bladder cancers and breast cancers [1,2]. Unfortunately, due to high hydrophilicity and poor membrane permeability, it is difficult for gemcitabine to enter the blood circulation after oral administration. Therefore, gemcitabine is administered by intravenous injection in clinic due to its low oral bioavailability [3]. Although many efforts have been made to design advanced intravenous drug delivery system, oral route is still the most preferred route due to its patient compliance, therapeutic efficacy and convenience [4].

Prodrug strategies based on various gastrointestinal nutrient transporters have been utilized to overcome undesirable and pharmacokinetic properties of drug, since these transporters play an important role in the oral absorption of nutrients and therapeutic drugs. It has been reported that valdidanosine and valdecitabine could be transported across intestinal epithelium by oligopeptide transporter 1 [5–7]. In order to further improve the oral bioavailability of nucleoside drugs, some new attempts are required. For example, prednisolone–carnitine conjugate was studied for nasal delivery mediated by organic cation/carnitine transporters 2 (OCTN2) [8]. This high-affinity carnitine transporter is highly expressed in kidney, trachea and intestine and is a promising target protein because it can transport organic cations and nutrients, such as carnitine and ergothioneine [9–13]. OCTN2 is also crucial for the β-oxidation and energy metabolism of fatty acids, and responsible for a primary systemic carnitine deficit. Following this idea, we synthesized the L-carnitine ester derivative of gemcitabine as shown in Fig. 1, namely JDR, to enhance the oral bioavailability of gemcitabine. To compare oral pharmacokinetics of gemcitabine and JDR, it was necessary to develop a sensitive method for simultaneous quantification of both drugs.

Various quantitative methods have been developed for the determination of gemcitabine and its derivatives. Wickremsinhe et al. developed a method for the determination of LY2334737 (gemcitabine prodrug), gemcitabine and its metabolite (dFdU) by LC–MS/MS and column switching chromatography [14]. Bowen et al. had validated a method for the analysis of gemcitabine and dFdU using solid phase extraction (SPE) [15]. However, these methods have some notable limitations, such as time-consuming and cost-expensive features of SPE sample preparation, unconventional column switching chromatography.

There are several challenges in the quantification of gemcitabine and its analogues. The similar chemical structure requires highly selective methods for their quantification. What is more, it is difficult to be simultaneously retained in RP system for the compounds with different polarity. Last, the current available quantification methods have been developed based on SPE of a large-volume sample and long analytical time. However, a simple and efficient protein precipitation method with short analytical time was rarely used in nucleoside analogue pretreatment due to low extraction recovery and matrix effect. Therefore, developing a simple and reliable method to simultaneously determine gemcitabine and its analogue is critical to the pre-clinical study of gemcitabine prodrugs.

In the present study, a rapid, sensitive UPLC–MS/MS method was developed to simultaneously determine JDR and gemcitabine. Compared with the previous assay methods of plasma volume of 100 μl and single run time of 5 min, this method provided shorter analysis time (3 min), reduced volume requirements (50 μl) and simplified plasma sample pretreatment. The lower limit of quantification (LLOQ) of gemcitabine was 4 ng/ml, which was sensitive enough to detect relatively low concentration of gemcitabine in rat plasma. This method was successfully applied to characterize the pharmacokinetic profiles of JDR and gemcitabine after a single dose of oral administration.

2. Materials and methods

2.1. Chemicals and reagents

Gemcitabine (98.8% purity) was purchased from Nanjing Chemlin Chemical Industry Co., Ltd (Jiangsu, Nanjing, PR China). L-carnitine was obtained from Kaiyuan Hengtai Chemical Co., Ltd (Liaoning, Shenyang, P R China). IS (99% purity, didanosine) was supplied by JiaXing I sen Chemical Co., Ltd (Zhejiang, Jiaxing, PR China). JDR (97.2% purity) was synthesized in Shenyang Pharmaceutical University (Shenyang, China). Ammonium acetate (HPLC grade) was obtained from Tianjin Kemiou Chemical Reagent Co. (Tianjin, China). Ultra pure water was prepared by EASYPURE®II RF/UV system (Boston, MA, USA). Tetrahydrouridine (THU), the cytidine deaminase inhibitor, was purchased from J&K Scientific (HPLC grade). HPLC-grade methanol was purchased from Fisher Scientific (Fairlawn, NJ, USA). All other chemicals were of analytical grade.

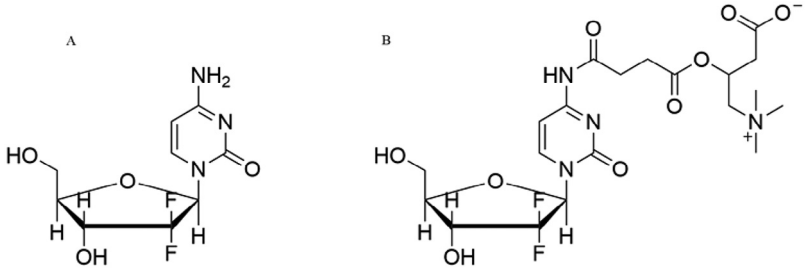

Fig. 1 – Structure of JDR and gemcitabine. (A) gemcitabine, (B) JDR.