Characterization of the pharmacokinetics of a liposomal formulation of eribulin mesylate (E7389) in mice

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ABSTRACT

Eribulin mesylate (E7389), a tubulin and microtubule inhibitor, has been approved to treat metastatic breast cancer in certain patient populations. A liposomal formulation of E7389, E7389-LF, aims to increase the therapeutic profile of E7389. As determining the free drug concentration is crucial for the assessment of efficacy and toxicity of liposomal drug, in this study, an ultracentrifugation method coupled with LC–MS/MS was developed to separate the free E7389 from liposomal and protein bound E7389. The pharmacokinetics of the free E7389 after dosing either E7389 or E7389-LF was characterized. The concentration ratio of E7389 in ultracentrifuged mice plasma (UCM) vs E7389 in plasma after a 2 mg/kg i.v. of E7389 ranged from 54.19% to 65.41%, which was similar to the free fraction in the mouse plasma. The respective concentration ratio of E7389 in UCM vs E7389 in plasma after a 2 mg/kg i.v. of E7389-LF ranged from 0.07% to 0.59%, and the exposure, expressed as AUC, of UCM/plasma ratio was determined to be 0.2%. Pharmacokinetic modeling was performed to estimate the release kinetics of E7389 from E7389-LF, and the release was best described by a first order rate constant k_{rel} 0.078 h^{-1}. Sensitivity analysis demonstrated that further decrease the release rate constant by adjusting liposome formulation would lead to decreased C_{max} and much longer half-life of UCM E7389, which might result in better efficacy and lower toxicity.

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1. Introduction

Eribulin mesylate (E7389, Halaven™, Eisai) is a synthetic macrocyclic ketone analog of halichondrin B, a natural product isolated from marine sponge *Halichondria okadai* (Goel et al., 2009; Gourmelon et al., 2011; Hussar and Pasco, 2011). It was approved by the FDA in November 2010, for metastatic breast cancer patients who have received at least two prior chemotherapy regimens for late-stage breast cancer (FDA, 2010). Eribulin mesylate acts as a tubulin and microtubule inhibitor with a distinct mechanism from other microtubule targeting agents such as paclitaxel and vinblastine, inhibiting the microtubule growth phase without affecting the shortening phase of microtubule dynamic instability (Aftimos and Awada, 2011; Jordan et al., 2005; Okouneva et al., 2008). Eribulin binds soluble tubulin at a single site with low affinity; whereas, it also binds a small subset of the tubulin with high affinity at microtubule ends (vinca-site binding) (Smith et al., 2010). Eribulin inhibits dynamic instability at microtubule plus ends but not

minus ends, suppresses microtubule polymerization, and induces microtubule depolymerization (Smith et al., 2010). The inhibition of microtubular function results in cell cycle G2-M phase arrest, mitotic spindles disruption, irreversible mitotic block, and tumor cell apoptosis (Donoghue et al., 2012). In a phase 3 open-label study, women with locally recurrent or metastatic breast cancer treated with eribulin had median survival of 13.1 months, significantly improved compared to the median survival of 10.6 months of subjects received treatment of physician's choice such as vinorelbine, gemcitabine, or capecitabine (Cortes et al., 2011). Neutropenia and asthenia or fatigue were the most common adverse events observed for eribulin treatment, occurring in about 52% and 54% of subjects, respectively; and about 5% of the subjects need to be withdrawn from the treatment due to peripheral neuropathy (Cortes et al., 2011).

Liposome has been employed as a drug delivery vehicle for various chemotherapeutic agents. Up to date, 12 liposomal drug products are in the market including liposomal formulations of Doxorubicin, Daunorubicin, Cytarabine, Amphotericin B, Verteporfin, Morphine, and Vincristine (Zhang et al., 2008). Liposomal drugs offered the following advantages: targeted delivery (passive targeting through enhanced permeability and longer retention to tumor tissues for anticancer drugs, or targeted delivery through the conjugation of tumor targeting agents), controlled and sustained release of the drugs, decreased systemic toxicity,

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and improved efficacy (Fanciullino and Ciccolini, 2009). Liposomal drugs existing in three forms after injecting into the blood stream: liposome-encapsulated drug, protein-bound nonliposomal drug, and completely free drug. Due to the technical difficulties, most of the pharmacokinetic studies of liposomal drugs only determined the total drug concentrations. As only free fraction exerts the pharmacological and toxicological effects, understanding the pharmacokinetics of free fraction would lead to better assessment and prediction of the efficacy and toxicity of liposomal drugs in vivo.

Currently, a liposomal formulation of eribulin mesylate (E7389-LF) is under clinical development and entering a Phase 1 clinical trial. In the present study, we have developed an ultracentrifugation method for the separation of free E7389 from the liposome-encapsulated E7389 and protein bound E7389. Using this method, we characterized the pharmacokinetics of the free E7389 and total E7389 in mice plasma after either E7389-LF or E7389 administration.

2. Materials and methods

2.1. Chemicals and reagents

Eribulin mesylate (E7389), and an analog of eribulin, ER-076349, used as internal standard (IS) were synthesized at Eisai Inc. (Andover, MA, USA). The chemical structures of E7389 and IS are shown in Fig. 1. E7389-LF was formulated by Eisai Inc. (Research Triangle Park, NC, USA) (Kibuchi et al., 2012). The liposomes are pegylated. The main liposome formulation includes hydrogenated soy phosphatidylcholine (HSPC), cholesterol, and 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] (MPEG2000-DSPE). Ammonium sulfate and citric acid monohydrate are the buffering agents in the interior E7107 phase of the liposome. The median particle size ranged from 60 to 100 nm. The encapsulation efficiency of liposomal E7389 was approximately 96%. Methanol (MeOH), tetrahydrofuran (THF), acetonitrile (ACN), and formic acid (FA) were purchased from EMD Chemicals (Gibbstown, NJ, USA). All material and solvents used for this study were of either analytical or HPLC grade. Water was purified with a Milli-Q water system (Millipore, Bedford, MA, USA). Blank male mouse plasma containing sodium heparin as the anticoagulant was purchased from Bioreclamation Inc. (Westbury, NY, USA).

2.2. Equipment

The quantitative LC–MS/MS analysis was performed on an API4000 mass spectrometer (Applied Biosystems, Foster City, CA) coupled with a Shimadzu HPLC system (Shimadzu Scientific Instruments, Columbia, MD).

2.3. LC–MS/MS method for quantification of E7389

The LC–MS/MS method for the quantification of the freebase of E7389 was developed and validated previously (Desjardins et al., 2008). Briefly, the liquid chromatography was performed using a Polaris® (MetaChem) C18 (3 μm, 2.0 mm × 30 mm) column. The injection volume was 30 μL and the flow rate was 300 μL/min. Mobile phases A and B were 13% acetonitrile in water containing 0.1% formic acid and 30% tetrahydrofuran in acetonitrile containing 0.1% formic acid, respectively. The flow gradient started with 100% mobile phase A and held for 1.5 min, was then linearly ramped up to 60% mobile phase B over 0.1 min, and held for 3.9 min, then returned to 100% mobile phase A in 1.5 min, and held for an additional 3 min.

The mass spectrometer was operated under positive ion mode using electrospray ionization with multiple reaction monitoring.

The MS/MS transitions were: eribulin, 730.5/712.5; IS, 731.5/681.5. The iron spray voltage was set at 5500 V, source temperature set as 450 °C, and CAD was set as 4. The curtain gas, gas 1 and gas 2 were set at 20, 45, and 80, respectively. The declustering potential, collision energy, entrance potential, and collision exit potential were 110, 41, 9, and 24 V for E7389; and 85, 24, 8, and 25 V for IS, respectively. Analyst 1.6 software (AB Sciex, Foster City, CA) was used for data acquisition and quantitation.

2.4. Ultracentrifugation method

One milliliter mouse plasma samples were transferred to Beckman ultracentrifuge tubes place in Beckman TL-100 Ultracentrifuge rotors (Beckman Coulter, Brea, CA), and centrifuged at 10 °C for 4 h at approximately 100,000 rpm ($355,040 \times g$). After centrifugation, 150 μL ultracentrifuged mouse plasma (UCM) was removed from the top layer using a 16 gauge syringe carefully to avoid contamination from the bottom layers which contained liposomal E7389 (E7389-LF) and protein bound E7389. The prepared UCM was then subject to LC–MS/MS analysis. Additional blank UCM was prepared for calibration standards, and quality control (QC) samples. The protein concentration of UCM was determined by BCA protein assay method (Pierce, Rockford, IL, USA) (Yu et al., 2010).

2.5. Preparation of stock solution, calibration standard, and quality control samples

The 1 mg/mL freebase of E7389 primary standard solution was prepared in methanol (MeOH). The primary standard solution was serially diluted with a mixture of MeOH and H_2O (50:50) to produce E7389 standard solutions of 5000, 1000, 500, 100, 50, 20, 10, and 5 ng/mL. The 1 mg/mL IS was dissolved in MeOH and was further diluted to 5000 ng/mL with a mixture of MeOH and H_2O (50:50) as the working solution. These solutions were stored at 4 °C. The QC samples were prepared in a similar manner with concentrations of 5000, 1000, 50, and 10 ng/mL.

Ten microliters of the E7389 standard or QC solution was added to 100 μL of blank UCM in a clean 15 mL polypropylene tube, to prepare calibration standards or QC samples. For the blank and blank with IS, 10 μL of MeOH and H_2O (50:50) was added to the UCM. The calibration standard curves consisted of a blank sample, blank with IS, and at least six non-zero samples covering the expected range.

2.6. Sample extraction

To 100 μL of sample, 10 μL of the IS solution were added to the sample in a 15 mL polypropylene tube. For plasma samples, 25 μL of 0.1 N NaOH was added, and the tubes were vortexed. Five hundred microliters of water was added to each sample and the mixture was vortexed for 10 s. Five milliliters of ethyl acetate:methanol:ethanol (90:5:5) was added into the mixture, shaken horizontally at high speed for 10 min and then centrifuged at approximately 3000 rpm ($1000 \times g$) (5 min, 4 °C). The aqueous layer was frozen in a dry ice/isopropanol bath. The organic phase was transferred to a clean 15 mL polypropylene tube and dried under nitrogen gas at approximately 35 °C in a TurboVap® evaporator. The plasma extracts were reconstituted with 200 μL of MeOH:H_2O (50:50) containing 0.1% formic acid. The reconstituted sample was injected onto the LC/MS/MS system.

2.7. Preparation of samples for UCM recovery

Plasma samples were prepared in duplicate sets at 3, 100, and 750 ng/mL of E7389. The samples were centrifuged at 10 °C for 4 h at 100,000 rpm. The recoveries of E7389 in plasma were determined