European Journal of Pharmaceutical Sciences 52 (2014) 1–11

Contents lists available at ScienceDirect

European Journal of Pharmaceutical Sciences

journal homepage: www.elsevier.com/locate/ejps

Improving cabazitaxel chemical stability in parenteral lipid emulsions using cholesterol

Yanjie Shao, Chungang Zhang, Qing Yao, Yueqi Wang, Bin Tian, Xing Tang, Yanjiao Wang *

School of Pharmacy, Shenyang Pharmaceutical University, 103 Wenhua Road, Shenyang 110016, China

ARTICLE INFO

Article history:
Received 9 July 2013
Received in revised form 23 September 2013
Accepted 23 September 2013
Available online 20 October 2013

Chemical compounds studied in this article:
Cabazitaxel
Cholesterol
Triglyceride
Phosphatidylcholine
Sphingomyelin
Alcohol
Acetonitrile
Phosphatidylethanolamine
Poloxamer 188

Keywords:
Cabazitaxel
Cholesterol
Intravenous lipid emulsion
Formulation
Chemical stability
Degradation kinetics

ABSTRACT

Intravenous lipid emulsions of cabazitaxel (CLEs) with a high stability were prepared by adding cholesterol (CH) to provide a new and more suitable delivery system for its administration. The factors affecting CLEs, such as the solubility of cabazitaxel in various oils, different kinds of lecithin, pH, different types of oil phases, and different concentrations of lipoid E80®, CH and poloxamer 188 were investigated systematically. The degradation of cabazitaxel in aqueous solution and lipid emulsion both followed pseudo first-order kinetics. A degradation mechanism was suggested by the U-shaped pH-rate profile of cabazitaxel. A formulation containing 0.5% (w/v) CH and another formulation without CH were made to investigate the protective influence of CH on the chemical stability of CLEs. The activation energy of the two formulations was calculated to be 65.74 ± 6.88 and 54.24 ± 1.43 kJ/mol (n = 3), respectively. Compared with the untreated CH, the shelf-life of cabazitaxel with added CH was longer, namely 134.0 ± 23.4 days versus 831.4 ± 204.4 days (n = 3) at 4 °C. This indicates that the addition of CH significantly improved the lifetime of cabazitaxel in intravenous lipid emulsions. The hydrogen bonding that takes place between cabazitaxel and CH accounts for the protective effect of CH on the chemical stability of CLEs in two ways: preventing cabazitaxel from leaking and hydrolyzing in aqueous solution and hindering hydrolysis in the oil phase. Finally, the hypothesis was confirmed by LC/TOFMS and Fourier-transform infrared-spectroscopy. As a result, CLEs were obtained successfully by the addition of CH and were stable enough to allow further research.

1. Introduction

Taxanes, such as paclitaxel and docetaxel (i.e., the two approved first generation taxanes), are widely used for the daily clinical treatment of cancers as a result of their beneficial effects and broad-spectrum of efficacy (Francis et al., 1995; Pazdur et al., 1993). However, intrinsic and acquired resistance to docetaxel and paclitaxel has become an urgent issue, following the emergence of multi-drug resistant tumors caused by overexpression of P-glycoprotein (Pgp). Consequently, the semi-synthetic taxane derivatives, cabazitaxel (Jevtana®; XRP6258; TXD258; XRP116258A) and larotaxel (RPR 109881A), are being used for cancer therapy because of their superior pharmacological properties compared with docetaxel and paclitaxel. In addition, it has been proven that both compounds have improved activity against drug-resistant human cancers (Froehner and Wirth, 2011; Metzger-Filho et al., 2009).

In June 2010 (Oudard, 2011), cabazitaxel (Jevtana®; Sanofi–Aventis), in combination with prednisone, was approved by the US Food and Drug Administration (FDA) for the treatment of patients with hormone-refractory metastatic prostate cancer who had been previously treated with a regimen containing docetaxel. Cabazitaxel was active against both docetaxel-sensitive and docetaxel-resistant cancers in preclinical testing and in initial clinical trials, providing a rationale for further clinical development in cancers such as metastatic castration-resistant prostate cancer (CRPC) (de Bono et al., 2010; Walsh, 2011). However so far, the optimum route of administration has not been fully established, since cabazitaxel is lipophilic, practically insoluble in water and is chemically unstable. The only commercial product is the aforementioned Jevtana®, which is available in single-use vials containing 60 mg

* Corresponding author. Tel.: +86 24 23986343; fax: +86 24 23911736.
E-mail address: tangpharma@gmail.com (Y. Wang).

0928-0987/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.ejps.2013.09.024

cabazitaxel (anhydrous) and 1.56 g polysorbate 80 according to the manufacturer's instructions. However, there are two major problems associated with its use. On one hand, the need to prepare solutions is time-consuming and requires significant effort, because Jevtana® requires two dilutions prior to administration. In addition, severe hypersensitivity reactions thought to be caused by polysorbate 80 can occur and these may include generalized rash/erythema, hypotension and bronchospasm (Norris et al., 2010).

To help reduce such adverse reactions and increase the solubility of cabazitaxel, a suitable carrier is needed. Lipid emulsions are widely used in parenteral nutrition (Mizushima, 1996) and more attention has been devoted to developing lipid emulsions as carriers for the hydrophobic drugs (Date and Nagarsenker, 2008) due to their advantages which include the ability to solubilize considerable amounts of drugs, the ability to prevent hydrolysis of the drugs such as cinnarizine (Shi et al., 2009), reduce irritation and toxicity, and the simple large-scale manufacture in comparison with other carriers (Collins-Gold et al., 1990). As a carrier, an intravenous lipid emulsion is expected to be a novel and suitable delivery system for the administration of cabazitaxel, reducing the incidence of adverse actions and offering more rapid preparation of solutions. Most studies on cabazitaxel have reported on its use in clinical antitumor trials (Diéras et al., 2013), the introduction of Jevtana® and the quantification of cabazitaxel in human plasma (de Bruijn et al., 2012). However, it appears that no studies have characterized the stable intravenous lipid emulsion containing cabazitaxel.

In this study, cabazitaxel intravenous lipid emulsions (CLEs) were prepared by high-pressure homogenization. Detailed studies were performed to determine the degradation of cabazitaxel in different oils and the effect of a series of lecithins on the chemical stability of cabazitaxel in order to prevent its degradation in initial studies. The solubility of cabazitaxel in different oils was also monitored by HPLC to ensure the optimum drug loading. The influence of different concentrations of lecithin (lipoid E80®), cholesterol (CH) and poloxamer 188 (pluronic F68®) on the particle size after sterilization was investigated using response surface methodology (RSM). Furthermore, the degradation kinetics of cabazitaxel in aqueous solution were studied over a wide range of pH values (2.40–10.30) to find the most appropriate pH value and suggest a possible degradation mechanism demonstrated by LC/TOFMS. Lastly, studies on the influence of CH on the degradation kinetics of CLEs were carried out to estimate the shelf-life of the product. While CH is regarded as a good W/O type emulsifier, CH is also useful in O/W type emulsions as a co-emulsifier. In this paper, we describe detailed investigations of how to improve the chemical stability of cabazitaxel in intravenous lipid emulsions using CH.

2. Materials and methods

2.1. Materials

Cabazitaxel (98.6%) was kindly supplied by the Medicinal Chemistry Lab of Yantai University (Yantai, China). Lipoid E80® contained five main ingredients (82.1% PC (phosphatidylcholine), 8.1% PE (phosphatidylethanolamine), 1.9% TG (triglyceride), 2.2% SPM (sphingomyelin), and 0.9% CH (cholesterol)), lipoid S75 (69.2% PC, 9.8% PE, 0.5% TG) and medium-chain triglycerides (MCT) were purchased from lipoid KG (Ludwigshafen, Germany). Soybean oil was obtained from TieLing BeiYa Pharmaceutical Corporation (TieLing, China), while poloxamer 188 (pluronic F68®) and glycerin for parenteral use were obtained from BASF AG (Ludwigshafen, Germany) and Suichang Glycerin Company (Zhejiang, China), respectively. In addition, cholesterol of injection grade (CH) was provided by Shanghai Advanced Vehicle Technology Ltd. Co. (Shanghai, China). All chemicals and reagents used were of analytical or chromatographic quality. Deionized water was used throughout the study.

2.2. Preparation of CLEs

All the cabazitaxel lipid emulsions were prepared by high-pressure homogenization. Cabazitaxel and 10% (w/v) oil containing a mixture of MCT and soybean oil in a ratio of 5:5 were heated at 70 °C until complete fusion was achieved. Next, lecithin and/or CH were dissolved in this mixture and the mixture was then agitated until uniform dissolution was obtained. Then, the aqueous phase consisting of 2.25% (w/v) glycerin, and different amounts of F68® was heated at 72 °C until uniform dispersion was achieved. The coarse emulsion was prepared by high shear mixing (ULTRA TURRAX® T18 basic, IKA® WORKS, Germany) by adding the aqueous phase to the oil phase slowly at 10,000 rpm over 4 min. The temperature of the entire homogenization process was controlled at ~40 °C using an ice-water bath. The pH of the coarse emulsion was adjusted to 6.85 with 0.1 mol/L NaOH, and the final volume was made up to 100 mL with purified water. The final emulsion was obtained by using a high-pressure homogenizer (Niro Soavi NS10012k, Niro Soavi S.p.A., via M.da Erba, Italy) at 800 bar for 8 cycles. Then, the CLEs were sealed in vials after adding nitrogen gas, and autoclaved at 121 °C for 8 min. The resulting products had a pH of about 6.20.

2.3. Characterization of CLEs

2.3.1. Particle size distribution

The particle size distribution of the CLEs was measured by photon correlation spectroscopy, using a Nicomp™ 380 Particle Sizing system (Zeta Potential/Particle Sizer NICOMP™ 380ZLS, Santa Barbara, California, USA), which covered the range from 5 nm to approximately 3 μm and provided two parameters, a mean diameter and standard deviation (S.D.). Samples were immediately diluted 1:5000 with purified water to avoid multiple scattering effects before measurements were carried out at 25 °C.

2.3.2. Measurement of the entrapment efficiency (EE)

The EE of the CLEs was obtained by measuring the free cabazitaxel concentration in the dispersed medium. Ultrafiltration was carried out three times using a Vivaspin 4 apparatus (Beijing Genosys Tech-Trading Co. Ltd., Beijing, China) that contained a filter membrane with a molecular weight cut-off of approximately 10,000 Da and was operated at 3000 rpm for 15 min. The centrifuged free drug in the aqueous phase was determined by HPLC and the EE was calculated according to the following equation (Zurowska-Pryczkowska et al., 1999):

$$\mathrm{EE}(\%) = \frac{C_{\mathrm{total}}V_{\mathrm{total}} - C_{\mathrm{water}}V_{\mathrm{water}}}{C_{\mathrm{total}}V_{\mathrm{total}}} \times 100 \quad (1)$$

2.4. Analysis of cabazitaxel and its degradation products by HPLC

Samples containing all the important substances derived from CLEs and cabazitaxel aqueous solutions (pH 6.20) were detected by HPLC. The HPLC system was composed of an L-2130 pump, an L-2200 autosampler, an L-2300 column oven, an L-2400 UV detector (Hitachi Company, Japan) and a Agilent TC-C18 column (5 μm, 4.6 mm × 250 mm, Agilent Technologies, America). The mobile phase consisted of acetonitrile and deionized water in a ratio of 3:2 (v/v). The flow rate was 1.0 mL/min, the column temperature and wavelength of the UV detector was 25 °C and 230 nm, respectively, and the injection volume was 20 μL.