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Original Research Paper

Preparation, stability and pharmacokinetics evaluation of lipid microspheres loading a promising antitumor candidate, Timataxel

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ABSTRACT

Timataxel (13-(N-Boc-3-*i*-butylisoserinoyl-4,10-β-diacetoxy-2-α-benzoyloxy-5-β-20-epoxy-1,13-α-dihydroxy-9-oxo-19-norcyclopropa[g]tax-11-ene), used to be called TM-2, is a novel semi-synthetic promising candidate for cancer treatment. However the preformulation study showed that TM-2 was insoluble and chemically instable in water, which would limit its application. This study aimed at the preparation of Timataxel lipid microspheres (TM-2 LMs) and investigated the difference between TM-2 LMs and TM-2 solution in pharmacokinetics. In this work, the final formulation was as follows: 0.10% (w/v) TM-2; 10.00% (w/v) oil phase (long chain triglyceride:media chain triglyceride = 2.50%:7.50%); 1.40% (w/v) phospholipid; 0.02% (w/v) NaH_2PO_4; 2.25% (w/v) glycerin and water to a total volume of 100 ml. The particle size distribution, content and entrapment efficacy were 205.0 ± 43.3 nm, 101.00%, and 99.12%, respectively. TM-2 LMs were stable during storage at 25 °C for 3 months, even under the condition of 60 °C and 4500 lx for 10 d. Phosphatidylethanolamine (PE) in phospholipid may contribute to the stability of TM-2 LMs. The pharmacokinetic parameters for TM-2 LMs were as follows: $AUC_{(0-\infty)}$ 3663.71 μg/l h and the clearance 2.26 l/h/kg. As for solution, these parameters were 1712.52 μg/l h and 4.77 l/h/kg, respectively. The $t_{1/2}$ of TM-2 LMs was similar to TM-2 solution. The pharmacokinetic results indicated that the AUC of TM-2 LMs was larger, the clearance was smaller than that of TM-2 solution. In a word, lipid microspheres were a promising drug delivery system for TM-2.

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1. Introduction

Paclitaxel, which was first extracted from the stem bark of *Taxus brevifolia*, exhibited excellent anti-tumor effect. The antitumor mechanism of paclitaxel is to combine with β-tubulin subunit, inhibit microtubule depolymerization, and finally lead to apoptosis [1,2]. Considering its superiority in the treatment of cancer, taxanes had attracted interests all over the world. Currently, taxane derivatives like paclitaxel, docetaxel and cabazitaxel have been commercialized as intravenous injections for years [3]. Multidrug resistance, mainly caused by overexpression of P-glycoprotein (P-gp), has limited the application of docetaxel and paclitaxel [4]. P-gp acts as an energy dependent drug efflux pump which could transport drugs to the extracellular medium [5], and meanwhile, paclitaxel and docetaxel are substrates of P-gp. Thus, it is essential to modify the structure of taxanes to improve activity especially against multidrug resistant tumor.

Timataxel (TM-2, $C_{43}H_{57}NO_{14}$), one of the novel semi-synthetic taxanes, has lower affinity to P-gp than docetaxel, and its structure was listed in Fig. 1. *In vitro* study has revealed that TM-2 exhibits more promising efficacy on a variety of human tumor lines than docetaxel or larotaxel, especially on multidrug resistant cancer cell lines involving KB/VCR and MCF-7/ADR [6,7]. The inhibition rate of A549 human lung xenografts was up to 82.24% [6,8]. Based on these encouraging results, TM-2 was selected for further preclinical study.

For many drugs, insufficient solubility and less stability in water limit their clinical use. Usually, the commercial taxane formulations are made up of Cremophor EL or Polysorbate 80, ethanol and water. Cremophor EL and Polysorbate 80 are nonionic surfactants that are responsible for the occurrence of side effects, including acute hypersensitivity reactions and peripheral neurotoxicity [3]. What's more, these solvents were supposed to be bound with alterations in the pharmacokinetic characteristics after *i.v.* administration [9]. So a suitable carrier is required to reduce these adverse reactions.

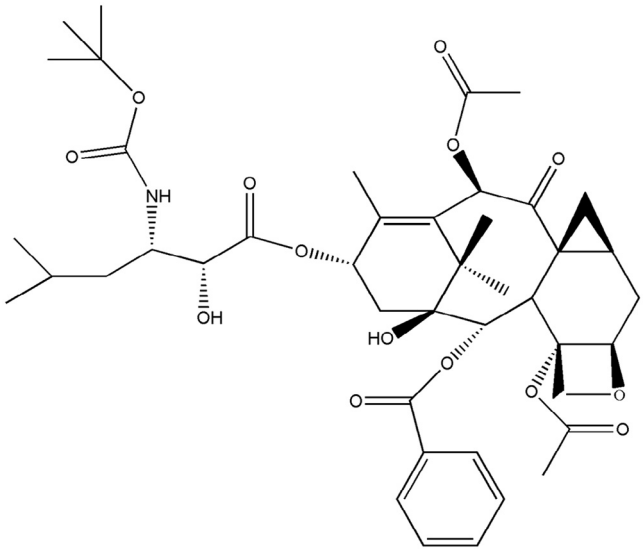

Fig. 1 – Chemical structure of Timataxel.

Lipid microspheres (LMs) mainly consist of oil, phospholipid and water. Usually, drugs are incorporated in oil which is also formed in the internal phase. This kind of structure could protect drugs from hydrolysis, reduce irritation and other side effects, and sustain drug release [10,11]. LMs are biocompatible, biodegradable, and simply applied for large-scale manufacture [12]. In addition, tumor is different from normal tissue that could increase drug retention due to enhanced permeation and retention effect [13]. Besides, tumors need higher energy which could be supplied by LMs [14]. Thus, the encapsulation of TM-2 into LMs could increase drug accumulation in tumor and improve therapeutic efficacy. What's more, our previous study had shown that TM-2 was insoluble in water but lipophilic, so LMs seem to be an attractive carrier for it [6].

The stable pH for TM-2 is 5.5–6.0, and TM-2 is liable hydrolysis in excessive acid media and alkaline media [6]. So pH is a key factor during the preparation of TM-2 LMs. While in our previous study, when TM-2 LMs were prepared, the final pH varied within a wide range, which made the quality of the final product uncontrollable. Moreover, the oil phase of previous TM-2 LMs was media chain triglyceride (MCT) only; there was some disadvantage compared with mixed oil. In this paper, a new formulation containing MCT and long chain triglyceride (LCT) for Timataxel lipid microspheres (TM-2 LMs) was prepared. The pH was well controlled by NaH_2PO_4 and phospholipid, and the effect of different phospholipid on the stability of TM-2 LMs was studied. The stability of TM-2 LMs was investigated in detail, including autoclaving stability, freezing and thawing stability, dilution stability and acceleration stability. Finally, the pharmacokinetic characteristics of TM-2 LMs were investigated.

2. Materials and methods

2.1. *Materials, reagents, and animals*

Timataxel (TM-2, purity >99%) was kindly supplied by School of Pharmacy, Fudan University (Shanghai, China). Cabazitaxel (CBZ, purity >98%) was synthesized in the Medicinal Chemistry Lab of Yantai University (Yantai, China). Long chain triglyceride (LCT) and media chain triglyceride (MCT) were purchased from TieLing Beiya Pharmaceutical Co. (Tieling, China). Egg lecithin PL-100M was provided by Shanghai Advanced Vehicle Technology Pharmaceutical Co., Ltd. (China). Lipid E80 and Lipid S100 were purchased from Lipid KG (Ludwigshafen, Germany). Glycerol was purchased from Zhe-Jiang Suichang Glycerol Plant (Zhejiang, China). All other chemicals and reagents were of HPLC or analytical grade.

Male Sprague-Dawley (SD) rats, weighing 200 ± 10 g, were kindly provided by the Experimental Animal Center of Shenyang Pharmaceutical University (Shenyang, China). The animal study was conformed to the Guideline for Animal Experimentation of Shenyang Pharmaceutical University.

2.2. *Preparation of TM-2 LMs*

TM-2 LMs were prepared by high-pressure homogenization method. Considering the drug loading (1 mg/ml), 10% (w/v) oil phase was selected for further study according to our previous