Journal of Colloid and Interface Science 529 (2018) 34–43

Contents lists available at ScienceDirect

Journal of Colloid and Interface Science

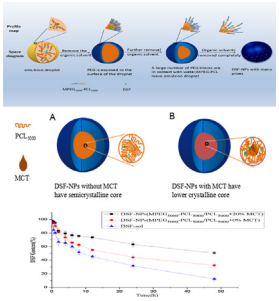
journal homepage: www.elsevier.com/locate/jcis

Disulfiram-loaded mixed nanoparticles with high drug-loading and plasma stability by reducing the core crystallinity for intravenous delivery

Xuezhi Zhuo, Tian Lei, Linlin Miao, Wei Chu, Xiaowen Li, Lifeng Luo, Jingxin Gou, Yu Zhang, Tian Yin, Haibing He *, Xing Tang

Department of Pharmaceutics, College of Pharmacy, Shenyang Pharmaceutical University, Shenyang, Liaoning, PR China

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 23 January 2018
Revised 15 May 2018
Accepted 19 May 2018
Available online 30 May 2018

Keywords:
Disulfiram
Nanoparticles
mPEG-PCL
PCL
Medium chain triglyceride
Flory–Huggins interaction parameters
Blend
Crystallinity

ABSTRACT

To develop an injectable formulation and improve the stability of disulfiram (DSF), DSF was encapsulated into mixed nanoparticles (DSF-NPs) through a high-pressure homogenization method. The Flory-Huggins interaction parameters (χ_{FH}) were calculated to predict the miscibility between DSF and the hydrophobic core, resulting in PCL_{5000} selected as the hydrophobic block to encapsulate the DSF, as PCL_{5000} had a lower χ_{FH} 3.39 and the drug loading of the nanoparticles prepared by $mPEG_{5000}$-PCL_{5000} was relatively higher. $mPEG_{5000}$-PCL_{5000} and PCL_{5000} were blended to reduce the leakage of DSF during preparation, as well as increase the stability of the nanoparticles. The cargo-loading capacity of the nanoparticles was improved from 3.35% to 5.50% by reducing the crystallinity of the PCL nanoparticle core, and the crystallinity decreased from 51.13% to 25.15% after adding medium chain triglyceride (MCT). The DSF-NPs prepared by the above method had a small particle size of 98.1 ± 10.54 nm, with a polydispersity index (PDI) of 0.036, as well as drug loading of 5.50%. Furthermore, DSF-NPs containing MCT showed higher stability than DSF-NPs without MCT and DSF-sol (DSF dissolved in Cremophor EL and ethanol) in water and 90% plasma-containing PBS. The pharmacokinetics proved that DSF-NPs containing MCT enhanced the DSF concentration in the blood. Finally, DSF-NPs effectively inhibited H22 xenograft tumor growth in vivo.

1. Introduction

The FDA-approved small molecule DSF has been used as an anti-alcoholism drug in the clinic for decades, due to its ability to irreversibly inhibit acetaldehyde dehydrogenase (ALDH) [1]. In

Abbreviations: DSF, disulfiram; MCT, medium chain triglyceride; DSF-sol, DSF dissolved in Cremophor EL and ethanol; DSF-NPs, DSF was encapsulated into mixed nanoparticles; χFH, χps, Flory-Huggins interaction parameters; PEG-PCL, methoxy poly(ethylene glycol)-poly(ε-caprolactone); PEG-PLA, methoxy poly (ethyleneglycol-poly (DL-lactide).

* Corresponding author.
E-mail address: hhb_emily@126.com (H. He).

https://doi.org/10.1016/j.jcis.2018.05.057
0021-9797/

addition, in recent years, several investigations of in vitro and in vivo studies have shown that DSF also has antitumor activity towards many cancers, such as breast cancer [2], non-small cell lung cancer [3], colorectal cancer [4], and prostate cancer [5].

DSF can act on multiple intracellular signaling pathways and targets, and in general, there are two mechanisms which can be used to explain the antitumor properties of DSF. Recently, Zdenek Skrott et al proposed a new anti-cancer mechanism [6]. In the body, ditiocarb (diethyldithiocarbamate, DTC) is one of the DSF's metabolites. DTC is able to form a complex with copper, DTC-copper complex (bis (diethyldithiocarbamate)-copper (CuET)), which provides the anti-tumour activity and accumulates in tumours. Within tumor cells, CuET binds with NPL4 (a key component of the P97 segregase) inducing aggregation, which then inhibits the processing of ubiquitylated proteins. This consequently disables the vital p97-NPL4-UFD1 pathway and induces a complex cellular phenotype leading to cell death. In addition, angiogenesis is an important condition for tumorigenesis, growth and infiltration and metastasis. Yi LI et al confirmed that DSF could effectively inhibit angiogenic processes in the absence of Cu, by causing suppression of the EGFR/Src/VEGF pathway [7].

However, DSF is extremely unstable in the acidic gastric environment and the bloodstream, and it has been shown that the $t_{1/2}$ of DSF is only 4 min in vivo [8]. Previously, oral pills of DSF (250 mg/pill) have been studied in Phase I clinical trials (www.clinicaltrials. gov, identifiers NCT00256230) for the treatment of metastatic melanoma, but did not produce positive outcomes. In the blood, DSF quickly degrades to DTC, and then the DTC is metabolized to S-methyl *N,N*-diethyldithiocarbamate (DTC-Me) by thiol methyltransferase. Further, the DTC-Me is then oxidized to S-methyl *N,N*-diethyldithiocarbamate sulfoxide (DTC-*MeSO*) and S-methyl *N,N*-diethyldithiocarbamate sulfone (DTC-$MeSO_2$) (Fig. 1). DTC-*MeSO* and DTC-$MeSO_2$ are also potent ALDH inhibitors in vivo [8,9]. Hence, when the DSF is exposed to blood, it retains its anti-alcoholism activity, but lost its anticancer activity. As a result, the unstability of DSF in the blood is the main obstacle towards its clinical usage for anticancer activity.

Based on this, it is necessary to develop an efficient drug delivery system to maintain the stability of DSF in the blood, ensuring high concentrations in the tumor tissue to active its anticancer activity. Although recent studies have shown that the use of liposomes and micelles to encapsulate DSF show good activity in vitro, they still have the disadvantages of low drug loading and poor stability [10,11]. In order to improve the common problems of low drug loading and unstability of nanoparticles, this research designed and prepared mixed nanoparticles (DSF-NPs) by focusing on the different physical-chemical properties between DSF and the amphipathic carriers. Previous studies have shown that the degree of miscibility between the drug and the hydrophobic block is one of the important factors for both drug loading and stability of the nanoparticles [12,13]. In order to reduce the typically time-consuming experiments required for screening polymer with high miscibility with DSF, the traditional method of calculating the Flory-Huggins interaction parameter (χ_{FH}) between DSF and the hydrophobic core can be used [12,14]. In addition, drug leakage from the core during the preparation of the nanoparticles can also influence the drug loading of nanoparticles. Therefore, it is possible to improve the drug loading of nanoparticles by reducing the leakage of drug in the preparation process. As well, the core crystallinity of the nanoparticles is another factor influencing the drug loading capacity and stability of nanoparticles [15,16]. It is known that if the hydrophobic block is semicrystalline, when increased the crystallinity of the hydrophobic core, the drug loading capacity of polymer will be reduced, due to the tight packing of the polymer chains making it hard for drug molecules to be incorporated into the crystal region [15]. Therefore, disrupting the ordered structure of the hydrophobic block to reduce the core crystallinity should theoretically increase the drug loading of the nanoparticles.

In this study, a high-pressure homogenization method was used for the preparation of DSF-nanoparticles (DSF-NPs). As shown in Fig. 2, the DSF-NPs consisted of $mPEG_{5000}$-PCL_{5000}, PCL_{5000}, MCT and DSF. Hydrophobic block PCL_{5000} was selected as the core hydrophobic segment to encapsulate DSF through calculating Flory-Huggins interaction parameters (χ_{FH}) between polymers and DSF. PCL_{5000} and $mPEG_{5000}$-PCL_{5000} were blended in order to reduce drug leakage from the hydrophobic core during the preparation of DSF-NPs. The hydrophobic crystallization inhibitor medium chain triglyceride (MCT) was added in order to increase the drug loading and stability of DSF-NPs by reducing the core crystallinity of the nanoparticles. MCT is a biocompatible and safe pharmaceutical adjuvant, and is widely used in emulsions [17]. In our previous studies on lipid emulsions, MCT showed certain a solubility for DSF [18]. And MCT has also been shown to reduce the crystallinity of PCL in micelle systems [15]. In vitro and in vivo experiments indicated that nanoparticles formed through the above method were efficient carriers for DSF.

2. Materials and methods

2.1. Materials

Methoxy poly(ethylene glycol)-poly(ε-caprolactone) (mPEG-PCL; Mw: mPEG 5000 Da, PCL 5000 Da), poly(ε-caprolactone) (MW: PCL 5000 Da, 3400 Da), Methoxy poly(ethyleneglycol-poly (DL-lactide) (mPEG-PLA; Mw: mPEG 5000 Da, PCL, 16,000 Da) and poly(DL-lactide) (PLA; Mw: 16,000 Da) were gifts from Jinan Daigang Biomaterial Co., Ltd., China. Disulfiram was synthesized by the Department of Organic Synthesis of Shenyang Pharmaceutical University. Poloxamer188 (Pluronic F68) was purchased from BASFAG (Ludwigshafen, Germany). medium chain triglyceride (MCT) was purchased from Lipoid KG (Ludwigshafen, Germany). All other reagents used were of analytical grade.

2.2. Calculation of flory–huggins interaction parameter χ_{FH}

The Flory-Huggins interaction parameter χ_{FH} is classic way to evaluate the miscibility in polymer-drug systems [19]. Generally, the lower the χ_{FH} value, the higher the miscibility between the drug and polymer in the nanoparticle system [12,14]. The equation

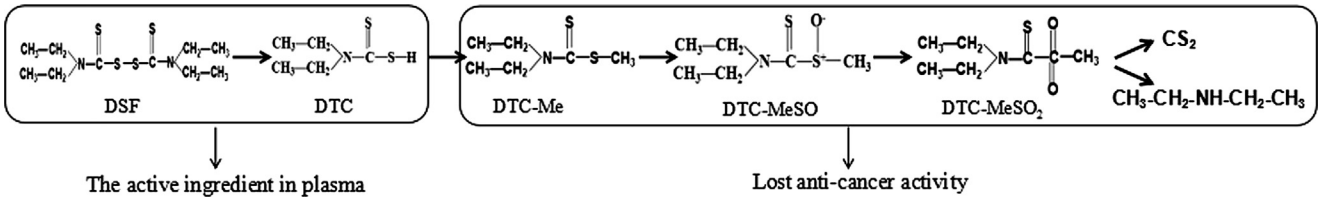


Fig. 1. Metabolism process of disulfiram.