HOSTED BY

ELSEVIER

Available online at www.sciencedirect.com

ScienceDirect

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

Original Research Paper

An intravenous clarithromycin lipid emulsion with a high drug loading, H-bonding and a hydrogen-bonded ion pair complex exhibiting excellent antibacterial activity

Haoyu Gong, Sicong Geng, Qi Zheng, Puxiu Wang, Lifeng Luo, Xiuzhi Wang, Yan Zhang, Yu Zhang, Haibing He, Xing Tang *

Shenyang Pharmaceutical University, No.103, Wenhua Road, Shenyang 110016, China

ARTICLE INFO

Article history:
Received 24 February 2016
Received in revised form 13 April 2016
Accepted 22 April 2016
Available online 10 May 2016

Keywords:
Clarithromycin
Cholesteryl hemisuccinate
H-bonding and hydrogen-bonded ion pair complex
Antibacterial activity
Thin-film dispersed homogenization

ABSTRACT

The aim of this study was to develop an intravenous clarithromycin lipid emulsion (CLE) with good stability and excellent antibacterial activity. The CLE was prepared by the thin-film dispersed homogenization method. The interaction between clarithromycin (CLA) and cholesteryl hemisuccinate (CHEMS) was confirmed by DSC, FT-IR and ^{1}H NMR analysis. The interfacial drug loading, thermal sterilization, freeze–thaw stability, and *in vitro* and *in vivo* antibacterial activity were investigated systematically. DSC, FT-IR and ^{1}H NMR spectra showed that CHEMS (CLA: CHEMS, M ratio 1:2) could interact with CLA through H-bonding and a hydrogen-bonded ion pair. The CHEMS was found necessary to maintain the stability of CLE. Ultracentrifugation showed that almost 88% CLA could be loaded into the interfacial layer. The optimized CLE formulation could withstand autoclaving at 121 °C for 10 min and remain stable after three freeze–thaw cycles. The *in vitro* susceptibility test revealed that the CLA–CHEMS ion-pair and CLE have similar activity to the parent drug against many different bacterial strains. The *in vivo* antibacterial activity showed that the ED_{50} of intravenous CLE was markedly lower than that of CLA solution administrated orally. CLE exhibited pronounced antibacterial activity and might be a candidate for a new nanocarrier for CLA with potential advantages over the current commercial formulation.

* *Corresponding author.* Shenyang Pharmaceutical University, No.103, Wenhua Road, Shenyang 110016, China. Fax: +86 24 23911736.
E-mail address: tanglab@126.com (X. Tang).

Abbreviations: CFU, colony-forming units; CHEMS, cholesteryl hemisuccinate; CHO, cholesterol; CLA, clarithromycin; CLE, clarithromycin lipid emulsion; CP, complex; DDW, double-distilled water; DMSO, deuterated dimethyl sulfoxide; ED_{50}, 50% effective dose; ELS, electrophoretic light scattering; HHIPC, H-bonding and HIP CLA–CHEMS complex; HIP, hydrogen-bonded ion pair; HTM, Haemophilus test medium; MAC, *Mycobacterium avium* complex; MCT, medium chain triglyceride; MHA, Mueller-Hinton agar; MIC, minimal inhibitory concentration; MLD, minimum lethal dose; LCT, long chain triglyceride; PA, phosphatidic acid; PC, phosphatidylcholine; PCS, photon correlation spectroscopy; PI, phosphatidylinositol; PI, polydispersity index; PM, physical mixture; PS, phosphatidylserine; PSD, particle size distribution; TMS, tetramethylsilane; TS, tocopherol succinate.

http://dx.doi.org/10.1016/j.ajps.2016.04.002

1. Introduction

Clarithromycin (CLA), an erythromycin analogue with a 14-membered ring structure, exhibits important new properties following substitution of a methoxy group (-OCH_3) for the C_6 hydroxy group (-OH) of erythromycin [1]. These structural modifications allow CLA to perform better than erythromycin in terms of acid stability, pharmacokinetics, gastrointestinal adverse effects and antibacterial spectrum. Clarithromycin is widely used in the treatment of bacterial infections, and it has been proved to be effective in *Mycobacterium avium* complex (MAC) infections both *in vitro* and *in vivo* [2–4]. Currently, clarithromycin lyophilized powder for injection contains ingredients like lactobionic acid and sodium hydroxide, which causes serious irritation at the injection site. Therefore, the development of an alternative formulation for intravenous injection with less irritation is necessary [5].

To obtain a less painful and more stable CLA treatment, various drug delivery systems for CLA have been studied, such as micelles [5], liposomes [6,7], emulsions [8–10], and nanoparticles [11]. However, there are still many problems limiting the clinical applications of these formulations, such as low entrapment efficiency, intolerable pain, a complex manufacturing process, poor physiochemical stability during long-term storage and the very high cost.

Lipid emulsions have been proven to be ideal carriers for parenteral drug delivery, due to their unique properties such as being biodegradable, biocompatible, physically stable, with low toxicity, and being easy to prepare even on a large scale. In addition, it had been shown that the oil-in-water emulsion system can significantly lower the incidence and the intensity of pain on injection because direct contact of the drug with body fluids and tissues is prevented by incorporating the drug into the oil phase or the interfacial layer [12,13]. So, the possibility of reducing irritation by preparing CLA lipid emulsions has been demonstrated and a number of related research studies have been launched. Lovell et al. developed a less painful clarithromycin emulsion with the aid of lipophilic counterions, like hexanoic acid and oleic acid, which improved the drug solubility in the oil phase [8]. However, the final emulsion was unable to withstand thermal sterilization which limited its industrial application. Yan et al. and Jie et al. reported clarithromycin emulsions containing vitamin E, a phospholipid complex, and tocopherol succinate (TS) respectively [9,10]. The final emulsions remained stable after undergoing sterilization at 100 °C in a rotating water bath for 30 min. However, too many excipients were used in the formulation, which limited the safety and tolerability of the preparation. Currently, many studies are being conducted on ion-pair, which is a suitable chemical approach to increase the lipophilicity of drugs, making it easier to incorporate drugs into the lipid matrix [14–16]. According to Brønsted–Lowry acid–base theory, strong acids and bases tend to form hydrogen-bonded ion pair (HIP) by proton transfer, especially in solvents with a low dielectric constant [17].

$$R' - OH + :NR_3 \rightleftharpoons R' - \overset{\ominus}{O} \cdots H - \overset{\oplus}{N}R_3$$

In the case of CLA, it is expected that the dimethylamino group will probably form strong hydrogen bonds like OH. . .N followed by proton transfer: OH. . .N ⇌ O^-. . .HN^+, with the excipient carrying the hydroxyl group. Therefore, it is essential to find an appropriate counter-ion to interact with CLA. In addition, to effectively localize the drug in the interfacial lecithin-rich layer, a thin-film dispersed homogenization method was developed. The resulting lipid emulsion exhibited good physical stability.

Based on the above factors, a novel parenteral clarithromycin lipid emulsion (CLE) with cholesteryl hemisuccinate (CHEMS) was developed and investigated. Considering that CLA has poor solubility in both oil and water media, efforts were made to localize the drug in the interfacial film by H-bonding and the formation of a hydrogen-bonded ion-pair between CLA and CHEMS. Also, characterization by DSC and FT-IR analysis was performed to examine a possible interaction between CLA and CHEMS. Furthermore, a stability test was carried out after sterilization and freeze–thawing, which proved that the clarithromycin lipid emulsion was of high quality. Finally, the antibacterial activity of the drug was assessed both *in vitro* and *in vivo*. The experimental results obtained showed that CLE exhibited good physical stability, thermal stability, and excellent antibacterial activity, suggesting that it had great prospects for clinical applications and its production could also be scaled up without great expense.

2. Materials and methods

2.1. Materials

The following materials were purchased from or provided by the sources in parentheses: CLA (Zhejiang Huayi Pharma Ltd. Co., Zhejiang, China), cholesterol (CHO), CHEMS, and PL-100M (Shanghai Advanced Vehicle Technology Ltd. Co., Shanghai, China), Lipoid E80, Lipoid S100, medium chain triglyceride (MCT) and long chain triglyceride (LCT) (Lipoid KG, Ludwigshafen, Germany), PC-98T (Q.P. Corporation, Tokyo, Japan), potassium bromide (FTIR grade) and DMSO-d6 (Sigma Aldrich), Poloxamer 188 (Pluronic F68) (BASF AG, Ludwigshafen, Germany), and glycerol (ZheJiang Suichang Glycerol Plant, Zhejiang, China). Double-distilled water (DDW) was used throughout the study. All other chemicals and reagents were of analytical or chromatographic grade.

2.2. Degradation of clarithromycin in different oils

Clarithromycin was dissolved in MCT, LCT and an MCT–LCT mixture (1:1) by agitation in a water bath maintained at 80 °C. Then, the oils containing 0.2% CLA were sealed in vials and incubated at 80 °C in a thermostatically controlled water bath. The samples were then withdrawn at intervals of 0, 1, 2, 4, 6, 8, 12, and 24 h and cooled to room temperature immediately to terminate the reaction. The drug content was determined by HPLC as illustrated in Section 2.4, and the percentage CLA degradation was calculated by the changes in CLA content.