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A comparison between conventional liposome and drug-cyclodextrin complex in liposome system

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ABSTRACT

A drug-cyclodextrin complex in liposome system was prepared in order to make a comparison with conventional risperidone-loaded liposome. Thin film hydration, reverse phase evaporation and ethanol injection methods were taken as preparation means to obtain the two types of liposome. Differential thermal analysis (DTA) and transmission electron microscopy (TEM) were used to investigate the thermal characters of inclusion complexes and morphology of liposome, respectively. Particle size, zeta potential and encapsulation efficiency were studied by light scattering analysis and ultrafiltration. In vitro release was carried out in the pH 7.4 phosphate buffer solution and samples were collected at the certain time. As a result, drug-cyclodextrin complex in liposome prepared by various methods displayed lower encapsulation efficiency than conventional liposome. However, size was larger and its stability was better than the latter. The second release phase of novel delivery system was slightly slower after initial burst release at the first phase, while the conventional liposome displayed a more regular trait. Thus, the novel liposome have potential to be developed as co-administration formulation with long-acting injection.

1. Introduction

Risperidone(RS), a benzisoxazole derivative, is atypical antipsychotic agent with approval in the USA (Stathis et al., 1996). It was firstly put into the UK market as risperidone tablet in 1993. And it is recently studied as several dosage forms such as tablet (Thyssen et al., 2007), oral solution (Laks et al., 2001) and long-acting injection (Nick et al., 2006). Due to the lack of bioequivalence and low bioavailability for oral administration (Van Os et al., 2007), long-acting injectable risperidone microsphere (D'souza et al., 2013) was developed as an alternative formulation. However, it was reported that the marketed microsphere dosage form exhibited a delayed response profile and drug begun to release after 3 weeks (Knox and Stimmel, 2004). It resulted in a fact that patients had to take oral risperidone agents to stabilize the plasma level of drug during the latency. Thus, it is necessary to develop a high-efficiency and low-toxicity formulation as co-administration therapy with long-acting injection.

In order to improve the solubility of risperidone, water-soluble inclusion complex is used to increase the bioavailability. Cyclodextrins (CDs) are cyclic oligosaccharides with six or more(α-1,4)-linked α-D-glucopyranose units which can form a hydrophobic central cavity and

hydrophilic outer surface (Ouyang et al., 2012), as shown in Fig. 1(a). Hydroxypropyl-beta-Cyclodextrin (HP-β-CD) is a derivative of β-CD which is widely used in improving the solubility of hydrophobic drugs with its better aqueous solubility and higher safety (Weina et al., 2015).

Liposomes are spherical vesicles which have been extensively studied as nanocarrier due to the special structure consisting of a lipid bilayer and aqueous inner cavity (Torchilin, 2005), as shown in Fig. 1(b). For the hydrophilic drugs, they are entrapped in the aqueous core and release gently with little effect on the stability of liposome. However, some of the lipophilic drugs entrapped into the lipid bilayer can interfere with the stability of liposomes (Alomrani et al., 2014). Hence, McCormack and Gregoriadis (1994))

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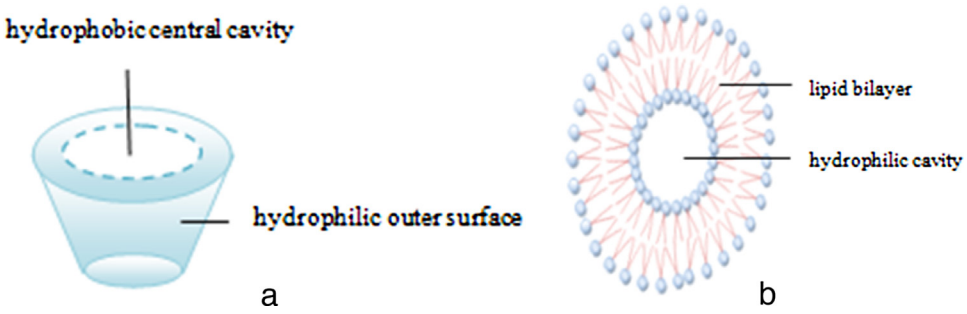

Fig. 1. Diagram of (a)Cyclodextrin inclusion complex and (b)liposome.

developed a novel liposome loaded drug-cyclodextrin complex in 1994. CDs have capacity to form inclusion complexes with lyophobic drugs which are then entrapped in the aqueous center of liposome (Chen et al., 2014).

In this study, several comparative measures were taken to investigate the difference between conventional liposome and drug-cyclodextrin complex in liposome system prepared by various methods. Meanwhile, one of them was chosen as the optimum for co-administration therapy with long-acting injection.

2. Materials and methods

2.1. Materials

Soya phosphatidylcholine (LIPOID S100) and cholesterol were purchased from Lipoid Gmbh and klamar® (China), separately. HP-β-CD was provided by DELI Biochemical Industry Co., Ltd; China. Risperidone was purchased from Dalian Meilun Biotech Co., Ltd; China. And all other chemicals were obtained commercially as analytical-grade reagents.

2.2. Determination of risperidone by HPLC

The analysis was performed on a Agilent Edipse × DB-C_{18} column at a wave-length of 279 nm at 25 °C. The mobile phase was made up of 0.1 M ammonium acetate and acetonitrile (75:25, v/v) and its flow rate was 1.0 mL/min. To obtain the risperidone standard solution, 10 mg risperidone was dissolved in 50 mL methanol as the stock solution. It was then diluted by methanol to desired concentrations. The samples were analyzed at 20 μL injection volume.

2.3. Phase solubility

Phase solubility study was performed according to a reported method (Weina et al., 2015). Excess amount of risperidone was added to 10 mL phosphate buffer (pH 6.0) which contained gradient concentration of HP-β-CD from 0 to 70 mmol/L. The systems were put into a shaker at 25 °C for 48 h. Then samples were taken out and filtered through 0.45 μm pore size filters, and the filtrates were analyzed by the HPLC method. The apparent stability constant (Kc) of risperidone and HP-β-CD complex was obtained based on the slope and intercept of phase solubility diagram.

$$K_c = \frac{[\text{slope}]}{[\text{intercept}](1 - [\text{slope}])}$$

2.4. Preparation and characterization of RS-CD solid dispersion

The inclusion complex of risperidone with HP-β-CD(RS-CD) was prepared by a modified neutralization and freeze-dry method (Garnero et al., 2012). Drug and cyclodextrin at a molar rate of 1:1 were dissolved in 30 mL distilled water whose pH value was adjusted to 2 by HCl solution. After stirring for 1 h, proper volume of NaOH solution was added to adjust the pH value to 5.5 according to the literature (He et al., 2012). The resultant solution was stored in −20 °C for 48 h and then filtered through a 0.45 μm pore size cellulose acetate filter. The filtrate was lyophilized in the end.

Differential Thermal Analysis(DTA) was used to investigate the thermal characters of risperidone, HP-β-CD, their mixture and inclusion compounds. The samples were heated at a 10 °C min^{-1} rate from 20 to 250 °C.

2.5. Liposome preparation

2.5.1. Thin film hydration(TH)

A general method (Zhang et al., 2015) was used in the preparation. Briefly, 0.2 g soya bean lecithin and cholesterol (8:1, w/w) were dissolved in 10 ml chloroform, which was then removed completely under reduced pressure. Then the obtained film was hydrated by glucose water solution(5%,w/v) containing 60 mg RS-CD under 50 °C for 2 h. By the way, coagulation phenomenon of liposome could be decreased by glucose water solution compared to PBS and pure water according to our pre-experiment. For the conventional liposome, drug was directly added into the organic phase without HP-β-CD in the first step and the hydration was only in the glucose water solution. The obtained liposomes were sonicated (2 s duration and 2 s interval; 400 W) in ice bath for 6 min by probe-trasonic cell disruptor (JY. 92-IIDN; Ninbo Scientz Biotechnology Co., Ltd; China) to gain small and homogenous vesicles. Finally, the resultant liposomes were stored at 4 °C. RS-TH and RS- HP-β-CD-TH denoted separately the conventional and novel liposomes prepared by this method.

2.5.2. Reverse phase evaporation(RE)

In this process (Ko and Bickel, 2012), a certain amount of soya bean lecithin and cholesterol (8:1,w/w) were dissolved in chloroform and RS-CD was dissolved in glucose water solution. For the conventional liposome, drug was added into the organic phase. Then the organic phase was mixed with the aqueous phase to form W/O emulsion using probe sonication for 4 min. The mixture was placed in a round-bottom flask and a gel was formed by evaporating the organic solvent under reduced pressure at 35 °C. Then 20 mL glucose water solution was added and stirred for another 30 min. Finally, The obtained liposomes were sonicated in ice bath for 6 min and stored at 4 °C. RS-RE and RS- HP-β-CD-RE denoted separately the conventional and novel liposomes prepared by this method.

2.5.3. Ethanol injection(EI)

Liposome was prepared by a modified method (Charcosset et al., 2015). In brief, 0.2 g soya bean lecithin and cholesterol (8:1,w/