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Cholesteryl hemisuccinate as a membrane stabilizer in dipalmitoylphosphatidylcholine liposomes containing saikosaponin-d

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Abstract

In the present study, cholesteryl hemisuccinate (CHEMS) was evaluated for use as a membrane stabilizer in dipalmitoylphosphatidylcholine (DPPC) liposomes. Differential scanning calorimetry (DSC) and a calcein release study showed that CHEMS was more effective than cholesterol (CHOL) in increasing DPPC membrane stability. The findings of Fourier transform infrared spectroscopy (FT-IR) also suggested that CHEMS interacts with DPPC via both hydrogen bonding and electrostatic interaction. More importantly, CHEMS did not interact with saikosaponin-d (SSD), a triterpene saponin from Bupleurum species, unlike CHOL. SSD-containing liposomes with DPPC, CHEMS and DSPE-PEG could greatly decrease the hemolytic activity of SSD. This study demonstrated that CHEMS has more stabilization ability than CHOL since CHEMS may exhibit both hydrogen bond interaction and electrostatic interaction with DPPC membrane while CHOL only has hydrogen bond interaction, resulting in stable and low-hemolytic SSD-liposomes.

Keywords: Cholesteryl hemisuccinate; Saikosaponin-d; Saponin; Liposome; Differential scanning calorimetry; Fourier transform infrared spectroscopy

1. Introduction

Saikosaponin-d (SSD) (Fig. 1A), a triterpene saponin from Bupleurum species, has shown corticosterone-like activity (Yokoyama et al., 1984), Na^+-, K^+-ATPase inhibiting action (Zhou et al., 1996), immunoregulatory action (Ushio and Abe, 1991) and anti-platelet activating factor activity (Nakamura et al.,

Abbreviations: DPPC, dipalmitoylphosphatidylcholine; CHEMS, cholesteryl hemisuccinate; CHOL, cholesterol; SSD, saikosaponin-d; DSPE-PEG, methoxypolyethyeneglycol (Mr2000)-distearoylphosphatidylethanolamine; DSC, differential scanning calorimetry; FT-IR, Fourier transform infrared spectroscopy

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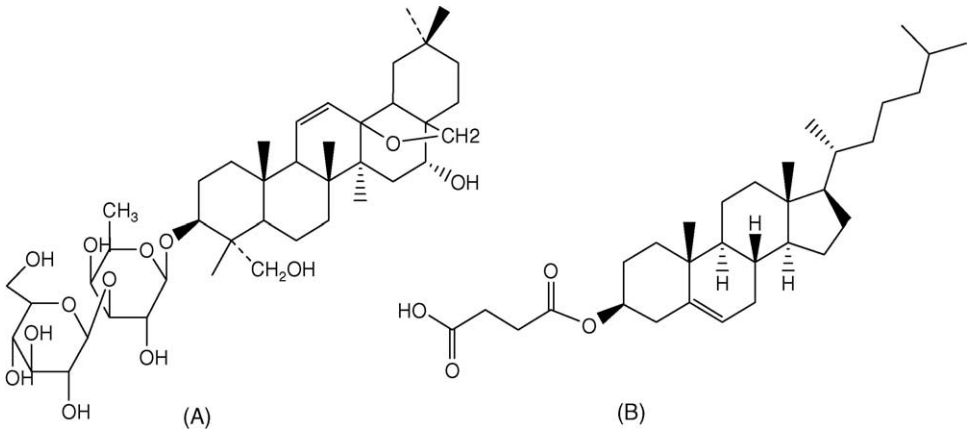

Fig. 1. Structures of saikosaponin-d (A) and cholesteryl hemisuccinate (B).

1993). It has been widely studied as a potential medication in the treatment of nephritis, nephrosis syndrome (Abe et al., 1986) and hepatic fibrosis (Cheng et al., 1999). Furthermore, bupleurum soup and particles for oral administration, the main active constituent of which is SSD, have achieved great success in the treatment of chronic glomerulonephritis and glomerulosclerosis (Zhang, 1993; Cheng, 1994).

SSD and other saikosaponins given in oral dosage form are not readily absorbed in the gastrointestinal tract and easily metabolized by glycosidase to less potent prosaikogenins before absorption occurs (Kida et al., 1998), leading to a dose of 200–300 mg and the need for treatment three times per day for adults. Therefore, the low level (less than 0.01%) of saikosaponins in Bupleurum species and their tendency to transform during separation and purification (Wen, 1993) hinder practical application in the clinic.

Other routes such as intraperitoneal and intramuscular administration have also been explored and are thought to enhance the corticosterone level in serum (Yokoyama et al., 1984; Zhong et al., 1993), however the risk of hemolysis should also be carefully considered. It is widely recognized that the hemolytic activity of SSD is caused by its complex with cholesterol (CHOL) on erythrocyte membrane, leading to membrane disruption and cell lysis. Driven by the need to reduce the hemolytic activity and make possible injections with less SSD, the liposome was chosen as a carrier for the present research because of its non-toxic, enhanced therapeutic efficacy and reduction of drug toxicity (Gregoriads, 1988).

It has been mentioned (Wang, 1992) that sterols with C_3-β-OH (including CHOL) form an insoluble complex with SSD, but, sterols with C_3-α-OH and esterified or glycosidated at C_3-OH do not. It is difficult to prepare liposomes containing SSD (SSD-liposomes), since SSD will form an insoluble complex with CHOL, which is routinely used to stabilize liposomes. Therefore, there is a great need for other membrane stabilizers for the preparation of SSD-liposomes.

Cholesteryl hemisuccinate (CHEMS) (Fig. 1B) is a CHOL-derivative esterified to the 3-hydroxyl group of CHOL and is supposed not to form a complex with SSD. Until now, no toxicity profiles about CHEMS have been reported. On the other hand, it has been found to increase specific immunogenicity of tumor cells by pretreating tumor cells with CHEMS (Skornick et al., 1986). And also, CHEMS was thought to protect against acetaminophen-induced hepatocellular apoptosis (Ray et al., 1996) and carbon tetrachloride-induced hepatotoxicity. It was proved to be a powerful cytoprotective agent against carbon tetrachloride hepatotoxicity in vivo (Fariss et al., 1993). CHEMS can form pH-sensitive fusogenic vesicles when incorporated into phosphatidylethanolamine bilayers and the pH is lowered to 5.5, resulting in H-II phase formation (Ismail et al., 2000; Se'rgio et al., 2004). CHEMS has also been demonstrated to alter acyl chain motion or fluidity in cell membranes (Dumas et al., 1997; Lai et al., 1985). It was proved by fluorescence polarization to be equally effective as CHOL in reducing the acyl chain mobility of DPPC above the phase transition temperature (Massey, 1998) and reported