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Review

Recent development of poly(ethylene glycol)-cholesterol conjugates as drug delivery systems

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ABSTRACT

Poly(ethylene glycol)-cholesterol (PEG-Chol) conjugates are composed of "hydrophilically-flexible" PEG and "hydrophobically-rigid" Chol molecules. PEG-Chol conjugates are capable of forming micelles through molecular self-assembly and they are also used extensively for the PEGylation of drug delivery systems (DDS). The PEGylated DDS have been shown to display optimized physical stability properties in vitro and longer half-lives in vivo when compared with non-PEGylated DDS. Cell uptake studies have indicated that PEG-Chol conjugates are internalized via clathrin-independent pathways into endosomes and Golgi apparatus. Acid-labile PEG-Chol conjugates are also able to promote the content release of PEGylated DDS when triggered by dePEGylation at acidic conditions. More importantly, biodegradable PEG-Chol molecules have been shown to decrease the "accelerated blood clearance" phenomenon of PEG-DSPE. Ligands, peptides or antibodies which have been modified with PEG-Chols are oftentimes used to formulate active targeting DDS, which have been shown in many systems recently to enhance the efficacy and lower the adverse effects of drugs. Production of PEG-Chol is simple and efficient, and production costs are relatively low. In conclusion, PEG-Chol conjugates appear to be very promising multifunctional biomaterials for many uses in the biomedical sciences and pharmaceutical industries.

Contents

1. Introduction

PEGylation applications are extensively used in the pharmaceutical industry today (Harris and Chess, 2003; Pasut and Veronese, 2009, 2012; Veronese and Pasut, 2005; Wei et al., 2009a), especially for selected drug delivery systems (DDS) in order to improve their physical stability in vitro, to avoid engulfment by mononuclear phagocytes, and to prolong circulation times of novel drugs in vivo (Dufort et al., 2012; Fleige et al., 2012; Otsuka et al., 2003;

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Rodriguez et al., 2013; Torchilin, 2007; Zhao et al., 2013). By "passive" or "active" targeting, PEGylated DDS can accumulate the selected drug cargos specifically at the target site, greatly enhance the efficacy of therapeutic agents, to improve the efficacy of imaging agents, and to lower the toxicity or adverse reactions of drugs resulting in enhanced safety profiles in scientific studies or human clinical trials (Dufort et al., 2012; Ni et al., 2014; Obermeier et al., 2011; Pasut and Veronese, 2009; Peng et al., 2013; Zhu et al., 2013). Cholesterol (Chol, Fig. 1) has been shown to reside in the biological membranes of various organisms, and it can modulate the fluidity of biological membranes involved in many important life events (Rayner et al., 2010). Due to relatively good biocompatibility with the host, as well as low toxicity profiles (Hullin-Matsuda et al., 2009), PEG-Cholesterol (PEG-Chol) conjugates are oftentimes used to modify diverse DDS (PEGylated DDS), including micelles (Li et al., 2012), liposomes (Xu et al., 2008), nanoparticles (Stevens et al., 2004), hydrogels (Rao and Taguchi, 2012; van de Manakker et al., 2009), lipoplexes (He et al., 2013b), and polyplexes (Wang et al., 2007). When combined with the oral or injectable administration of PEG-Chol-based therapeutic agents, PEGylated DDS are now used to treat infectious diseases (Anderson et al., 1999; Liu et al., 2008a), central nervous system diseases (Chen et al., 2012; Yang et al., 2008), augmentation of cancer chemotherapies (Xiong et al., 2011), enhance imaging efficiency in vivo (Pan et al., 2007), and to optimize numerous in vivo gene therapy applications (He et al., 2013a). It is now well known that non-degradable 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-*N*-PEG (PEG-DSPE)-modified liposomes or vesicles display "accelerated blood clearance" (ABC) phenomenon (Abu Lila et al., 2013), but biodegradable PEG-Chol conjugates can effectively prolong circulation half-life times of liposomes or vesicles by either limiting or eliminating the ABC phenomenon in vivo (Xu et al., 2010).

This review focuses on PEGylated and dePEGylated DDS by PEG-Chol conjugates (Beugin et al., 1998b; Boomer et al., 2009; Ishiwata et al., 1997; Zhao et al., 2007), and active targeted DDS based on functionalized-PEG-Chol conjugates (Cai et al., 2012; He et al., 2010; Pan et al., 2007). In addition, some novel PEG-Chol conjugates (Hofmann et al., 2010; Rao et al., 2011; van de Manakker et al., 2009) have been reviewed.

2. PEGylation by PEG-Chol in DDS

By using ether (Fig. 2A and B) (Brockerhoff and Ramsammy, 1982; Patel et al., 1984), carbonate (Fig. 2C) (Beugin et al., 1998a,b), carbomate (Fig. 2D) bonds (Bradley et al., 1998) and linkers (Fig. 2E, F and H) (Carrion et al., 2001; Xu et al., 2008; Yang et al., 2008), variable molecular weight PEGs are routinely coupled to Chol to synthesize PEG-Chol conjugates. With a hydrophilic PEG "head group", and a hydrophobic Chol segment, PEG-Chol is an amphiphilic surfactant (Beugin et al., 1998a,b). The critical micelle concentration (CMC) of PEG-Chol is $0.4–12.7 \times 10^{-6}$ μM (PEG with molecular weight in the range of 400–10,000 Da) (Buzova et al., 2013; Li et al., 2012; Xu et al., 2005; Yang et al., 2008). Due to the low CMC values like some Poloxamers (Pluronic®) (Alvarez-Lorenzo et al., 2011; Dumortier et al., 2006; Kabanov et al., 2002, 2003), PEG-Chol micelles have high stability and thereby can maintain their integrity even upon strong dilution during systemic circulation (Gong et al., 2012; Han et al., 2009; Li et al., 2012; Liu et al., 2013; Wang et al., 2012; Wei et al., 2009b; Wu et al., 2013). PEG-Chol (Fig. 2B) has been used to prepare a micelle loading Adriamycin (Xu et al., 2005). The Adriamycin-release behavior demonstrated significant sustained release characteristics since it was found that Adriamycin was loaded into the inner core of the micelle. In addition, selected hydrophobic drugs, including Quercetin and Docetaxel (Li et al., 2012; Yu et al., 2013), were solubilized and encapsulated into PEG-Chol molecules (Fig. 2F), and shown to form stable micelle systems. Since the elimination of PEG-Chol (Fig. 2F) under acidic condition (pH 5.0) is enhanced (Xu et al., 2008), these drugs were quickly released in phosphate buffered saline (PBS) at pH 5.0. These micelles appear to be extremely promising vectors for the controlled and targeted drug delivery to several human diseases shown to display acidic microenvironments (Ge and Liu, 2013; Mura et al., 2013). PEG-Chol (Fig. 2A) or PEG-squalene was capable of encapsulating gemcitabine or deoxycytidine and forming nanoparticulate DDS, which displayed superior anticancer activity on gemcitabine-resistant leukemia cell line (Bekkara-Aounallah et al., 2008; Trung Bui et al., 2013).

Historically, PEG-Chol conjugates were originally synthesized to study liposomal bilayer structures (Brockerhoff and Ramsammy, 1982). As rigid structures, the Chol segments of PEG-Chol provide a stabilizing hydrophobic anchor and are therefore able to elevate the "orderliness" (high degree of structural organization) and stability of phospholipids bilayers (Beugin et al., 1998a,b; Brockerhoff and Ramsammy, 1982; Lingwood and Simons, 2010; Patel et al., 1984); PEG segments of PEG-Chol are flexible structures with "steric barrier" properties when anchored in the surfaces of liposomes or niosomes (Dufort et al., 2012). These elevated flexibility properties have several advantages, including: (1) enhancing lipid bilayer impermeability, (2) stabilization of liposomes or niosomes against aggregation, (3) reducing the adsorption of proteins and macromolecules, (4) inhibition of complement activation, (5) increases their stability in buffer systems and human plasma, and (6) prolonging their circulation half-lives (Beugin et al., 1998a,b; Bradley et al., 1998; Janzen et al., 1996; Xu et al., 2008). Additionally, by introducing a "spacer arm", the flexibility of the PEG chain can be reinforced and the rigid Chol segment can then be inserted deeper into the bilayer of liposomes (Carrion et al., 2001). For example, liposomes loaded with calcein were prepared using a cleavable PEG-Chol (Fig. 2F), and the liposome contents were rapidly released under esterase-acid conditions (Xu et al., 2008). By loading niosomes with nimodipine modified by PEG-Chol (Fig. 2G), the results showed a greater accumulative release of drug than that of plain niosomes alone (Yang et al., 2008). When PEG-Chol (Fig. 2A) modified liposomes, designed with a stable size and bilayer impermeability, were loaded with methotrexate, these structures were observed to avoid being taking up quickly by the liver in vivo, which resulted in methotrexate not being leaked into the blood circulation (Patel et al., 1984). This liposome structure significantly extended the survival of mice bearing hepatoma 129 ascites tumors. It is well known that PEG-DSPE-modified liposomes or vesicles possess the

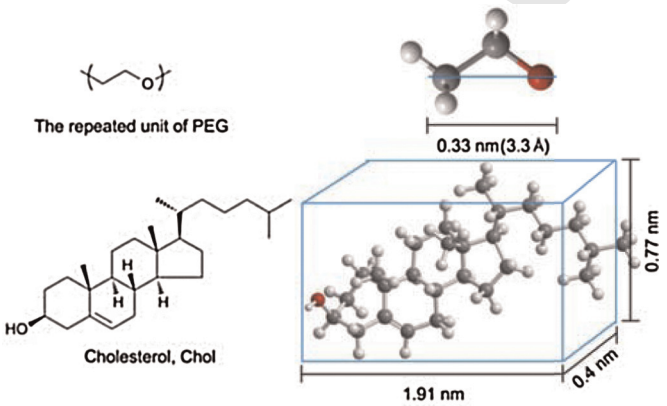

Fig. 1. Structures and dimensions of PEG and Cholesterol. PEG, with a flexible structure, is a hydrophilic molecule (Hansen et al., 2003); Cholesterol (Chol), with a rigid structure, is a hydrophobic molecule (Gimpl and Gehrig-Burger, 2011).

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