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Tumor-targeting and pH-sensitive lipoprotein-mimic nanocarrier for targeted intracellular delivery of paclitaxel

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ABSTRACT

In the present study, we constructed a tumor-targeting and pH-sensitive lipoprotein-mimic nanocarrier containing paclitaxel (FA-BSA-LC/DOPE-PTX), by adding 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE) and oleic acid as pH-sensitive components into the formulation of lipid core and then coating with folic acid modified bovine serum albumin (FA-BSA) for tumor targeting activity. In vitro drug release study demonstrated that paclitaxel (PTX) was released from FA-BSA-LC/DOPE in a pH-dependent manner. The vitro cytotoxicity assays showed that all the blank nanocarriers were nontoxic. However, MTT assay showed that FA-BSA-LC/DOPE-PTX was highly cytotoxic. Cellular uptake experiments analyzed with flow cytometry and laser scan confocal microscope (LSCM) revealed that FA-BSA-LC/DOPE was taken up in great amount via folate receptor-mediated endocytosis and pH-sensitive release of drug to cytoplasm. Furthermore, the study of intracellular drug release behavior demonstrated that the FA-BSA-LC/DOPE escaped from lysosomes and released drug into cytoplasm. The in vivo targeting activity showed that the nanocarrier selectively targeted tumor and had long residence time for BSA layer increased the stability in blood. Moreover, FA-BSA-LC/DOPE-PTX produced very marked anti-tumor activity in tumor-bearing mice in vivo. Therefore, FA-BSA-LC/DOPE as biocompatible, tumor-targeting and pH-sensitive lipoprotein-mimic nanocarrier is a promising system for effective intracellular delivery of PTX to tumor.

1. Introduction

Studies of suitable targeting carriers have become an important field of pharmaceutical research. Recently, use of lipoproteins as potential drug carriers has attracted a great deal of attention. Lipoproteins are spherical particles consisting of a core of apolar lipids surrounded by a phospholid monolayer, in which cholesterol and apoproteins are embedded. Highly hydrophobic drugs can be incorporated into the apolar core and thus be transported and hidden inside the particles (Rensen et al., 2001). Lipoproteins are endogenous molecules, as drug carriers, these particles are completely biodegradable, do not trigger immunological responses, and can escape recognition and elimination by the reticuloendothelial system (Feng et al., 2008). Because of the specific structural characteristics and the selective receptor-mediated uptake of their core components, lipoproteins have been widely used as an effective drug delivery model (Hamidi et al., 2006; Huntosova et al., 2012; Wasan et al., 2008; Zhang and Chen, 2010; Zhang et al., 2010).

However, the lipoprotein and apo are difficult to isolate from human serum in large quantities (Nikanjam et al., 2007). Although the full synthesis technology of related peptide has been developed recently, its use as a medicinal nanocarrier has a long way to go to produce the peptides that can be used as clinical nanocarrier (Xu et al., 2010). Moreover, LDLR-mediation is probably not an ideal targeting delivery route; many tumors do not overexpress the LDLR, whereas some normal tissues do (Zheng et al., 2005). Thus, there is a critical need to construct a lipoprotein-mimic nanocarrier, which is expected to inherit the advantages and to overcome the shortages of lipoprotein.

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Albumin has been shown to be non-toxic, non-immunogenic, biocompatible, biodegradable and metabolizable in vivo into innocuous degradation products (Kratz, 2008; Li et al., 2008). In addition, it is likely that endogenous albumin, with the half-life of 19 days in the blood circulation, may play an important role for improving the pharmacokinetic profile (Furumoto et al., 2007; Ogawara et al., 2004; Yokoe et al., 2008). Albumin-based nanoparticles as drug delivery systems represent a promising strategy for targeted delivery of anticancer drugs to tumor cells. Their enhanced uptake in solid tumors is mediated by binding of albumin to albumin-binding proteins, such as membrane-associated gp60 (albondin) and secreted protein, acidic and rich in cysteine (SPARC). Albondin receptor on the endothelial cells of tumor vessels allows transcytosis of albumin across continuous endothelium while overexpressed SPARC results in accumulation of albumin within the tumor interstitium (Hawkins et al., 2008; Park, 2012). Therefore, bovine serum albumin (BSA) was used as the protein model for constructing the lipoprotein-mimic nanocarrier in this study.

Folate is a low-molecular-weight vitamin that can selectively bind to folate receptor (FR), which has been widely employed as a targeting moiety for various anticancer drugs and nanocarriers (Leamon and Reddy, 2004; Sabharanjak and Mayor, 2004). FR is overexpressed by many primary and metastatic cancers, including ovarian and breast cancers, while its expression is highly restricted in normal cells (Elnakat and Ratnam, 2004; Ross et al., 1994). Moreover, the folate and its conjugates can bind FR with high affinity and enter cancer cells via a receptor-mediated endocytic process (Antony, 1992). Therefore, FR is a potential target for tumor-specific drug delivery, and folic acid has been successfully used to deliver drug into FR-positive cancer cells for imaging and therapy purpose (Hilgenbrink and Low, 2005).

It is well known that the physiological pH in cancer cells is lower than that in blood and normal tissues, and is about 6.0 and 5.0 in intracellular early lysosomes and late lysosomes, respectively (Fan et al., 2012). Acid triggering of drug delivery systems takes advantage of the decrease in the pH during endosomal localization (Watson et al., 2005) and has previously been shown to increase the efficacy of drug delivery systems when compared to pH-stable systems (Duncan, 2003; Guo and Szoka, 2003). Nanocarriers without special function lack the ability to escape from endo/lysosome timely, resulting in poor therapeutic index of the loaded active agents (Singh et al., 2008; Van den Bossche et al., 2011). One strategy was adding fusogenic lipid or fusogenic peptide, called "helper lipid", in formulation to facilitate their endo/lysosomal escape (Chhabra et al., 2014). pH sensitive dioleoyl-phosphatidylethanolamine (DOPE) was the representative "helper lipid". It has been suggested that under mild acidic environment of endosome–lysosome, DOPE might induce fusion between DOPE composed lipoplexes and endosome–lysosome membrane, resulting in the leakage of entrapped agents to cytoplasm (Vanic et al., 2012). DOPE promotes the flip–flop mechanism, commonly used to initiate the fusion of the lipoplexes with the endosomal membrane. The role of DOPE in destabilizing the endosome, enhancing the transfection efficiency, was attributed to its ability to undergo transition from bilayer to inverted hexagonal structures at low pH, which is known to catalyze the fusion process with the endosomal membrane (Elouahabi and Ruysschaert, 2005).

In our study, the bovine serum albumin (BSA) was used as the protein model for constructing the lipoprotein-mimic nanocarrier. BSA specifically target to tumor by increased transendothelial gp60-mediated transport and increased intratumoral accumulation as a result of the SPARC–albumin interaction. The further conjugated folic acid selectively binding to FR enhance the tumor-targeting activity. The lipid core (LC/DOPE) of lipoprotein-mimic nanocarrier was consisted of the pH-sensitive lipid dioleoyl-phosphatidylethanolamine (DOPE) and a stabilizer amphiphile oleic acid (Fattal et al.,

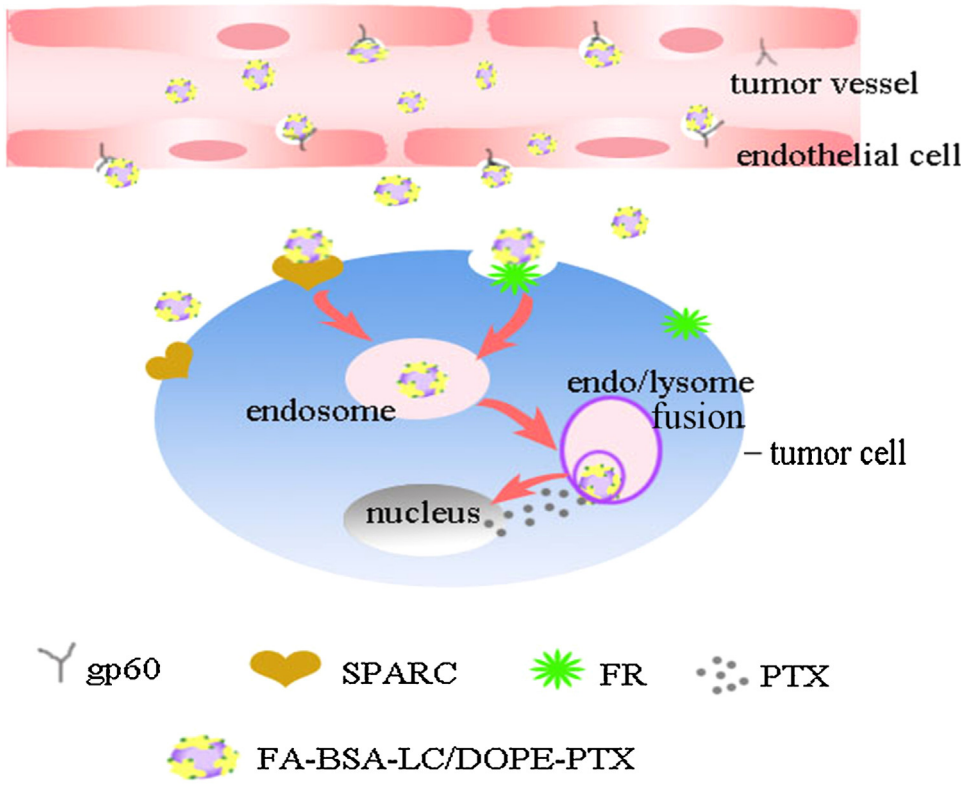

Fig. 1. The mechanism of the FA-BSA-LC/DOPE-PTX deliver PTX to tumor cell. FA-BSA-LC/DOPE-PTX are selectively taken up by tumor cells via the accumulation ability of BSA in tumor and folate receptor-mediated endocytosis, then induced fusion between FA-BSA-LC/DOPE-PTX and endosome–lysosome membrane, releasing of PTX into the cytoplasm.