International Journal of Pharmaceutics 490 (2015) 173–179

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

Pharmaceutical nanotechnology

Biocompatible anionic polyelectrolyte for improved liposome based gene transfection

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ARTICLE INFO

Article history:
Received 23 February 2015
Received in revised form 4 May 2015
Accepted 17 May 2015
Available online 21 May 2015

Keywords:
Anionic polyelectrolyte
Liposome
Biocompatibility
Endosomal escape
Gene transfection

ABSTRACT

Cationic liposomes have been widely used as efficient gene carriers. However, the serious cytotoxicity caused by exposed positive charges restricts the further application of those kinds of gene vectors. Thus, it is challenging to develop biocompatiable non-positive charge carriers to achieve high gene transfection efficiencies. Herein, we report a novel design by pasting biocompatible anionic polyelectrolyte, namely alginic acid, hyaluronic acid, pectin and polyglutamic acid, to the positive charge surface of liposome/pDNA complex. Through shielding the positive charges, the new gene carriers show decreased cytotoxicity while still maintaining high transfection efficiency. To be noted, the complex formed by coating polyglutamic acid to the surface of liposome/pDNA greatly enhanced the transfection efficiency in HepG2 cells, and the use of pectin shows increased transfection in MCF-7 cells. Hemolysis assay proved a possible mechanism that when the new gene complex was internalized into cells, as acidity increases, more side chains become hydrophobic, and thus destabilizing the endosomal membrane to accelerate DNA escape. The present results suggest that such anionic polyelectrolyte covered liposome based carrier possess promising application for clinical gene delivery.

1. Introduction

Gene therapy is a very promising method for the treatment of cancers comparing to traditional chemotherapeutic agents (El-Aneed, 2004; Merdan et al., 2002). However, it has not been widely adopted especially in clinial applications owing to its instability, poor cellular uptake and limited transfection efficiency. Hence, an effective carrier is of vital importance for successful gene therapy. Viral vectors show high efficient gene delivery; however, the drawbacks such as immunogenicity and carcinogenicity restrict their further application (Ferber, 2001; Flotte et al., 2007; Kuo et al., 2009). Thus, non-viral gene delivery systems with less immunotoxic and easier preparation are widely explored (Gargouri et al., 2009; Guo and Huang, 2012; Fortier et al., 2014). Most non-viral gene vectors investigated are positive charged materials, such as cationic liposome (Naicker et al., 2014; Mahmoudi et al., 2014; Junquera and Aicart, 2014; Caracciolo and Amenitsch, 2012), polyethylenimine (PEI) (Zhang et al., 2004; Nakayama, 2012; Wang et al., 2014), polyaminoamine dendrimers (Zhu et al., 2012; Pandita et al., 2011 Shcharbin et al., 2013). Among these various cationic carriers, cationic liposome based gene delivery has attracted considerable attention and become a routine technique in basic research (Mallick and Choi, 2014; Gao and Huang, 1991). As we know, cationic surface can facilitate the interaction between the compounds and cells, and thereby enhance the cell endocytosis (Ewert et al., 2010; Duan et al., 2009). However, positive charges can also lead to serious cytotoxicity, which is the main reason limiting the clinical use of cationic liposome (Miller, 2003; Masotti et al., 2009; Audouy et al., 2002; Lv et al., 2006; Akhtar, 2010). Therefore, it is necessary to modify cationic liposomes to minimize their toxicity for further clinical applications.

Biocompatible anionic polyelectrolytes, such as alginic acid (AA), hyaluronic acid (HA), pectin (PC) from citrus peels, and polyglutamic acid (PG), has been widely used in gene delivery (Ran et al., 2014). Alginic acid, a popularly used polysaccharide in food and pharmaceutical industries, contributes to the decrease of cytotoxicity in nanocomposites. PEI-alginic acid nanoparticles has been reported to have reduced cytotoxicity and improved gene transfection in the presence of serum (Jiang et al., 2006; He et al., 2012). Hyaluronic acid is an endogenous polyanionic polysaccharide distributed widely in the extracellular matrix and approved for injections by the Food and Drug Administration (FDA). HA can be efficiently taken up by the HA receptor-mediated endocytosis in the body (Ito et al., 2008; Yao et al., 2010; Zhang et al., 2013). Pectin is composed of complex polysaccharides rich in galacturonic acid residues, and it is abundant in plant-derived diet. Pectin and modified pectin have been found to exhibit anti-mutagenic activity

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http://dx.doi.org/10.1016/j.ijpharm.2015.05.046
0378-5173/

and block cancer cell proliferation, with no evidence of toxicity or other serious side effects. Citrus pectin modified with different amine groups has been used for gene delivery (Munarin et al., 2012; Nangia-Makker et al., 2002; Katav et al., 2008). Polyglutamic acid is a biopolymer made up of repeating units of L-glutamic acid. Owing to its biodegradable, non-toxic and non-immunogenic properties, it has the potential to be used as non-viral vector for safe gene delivery (Wang et al., 2010; Liao et al., 2012). PG containing the Arginine-Glycine-Aspartic Acid (RGD) were coated onto positively charged nanoparticles and shown to increase in vitro gene delivery to endothelial cells compared to scrambled sequence coated particles containing RDG (Lin et al., 2011).

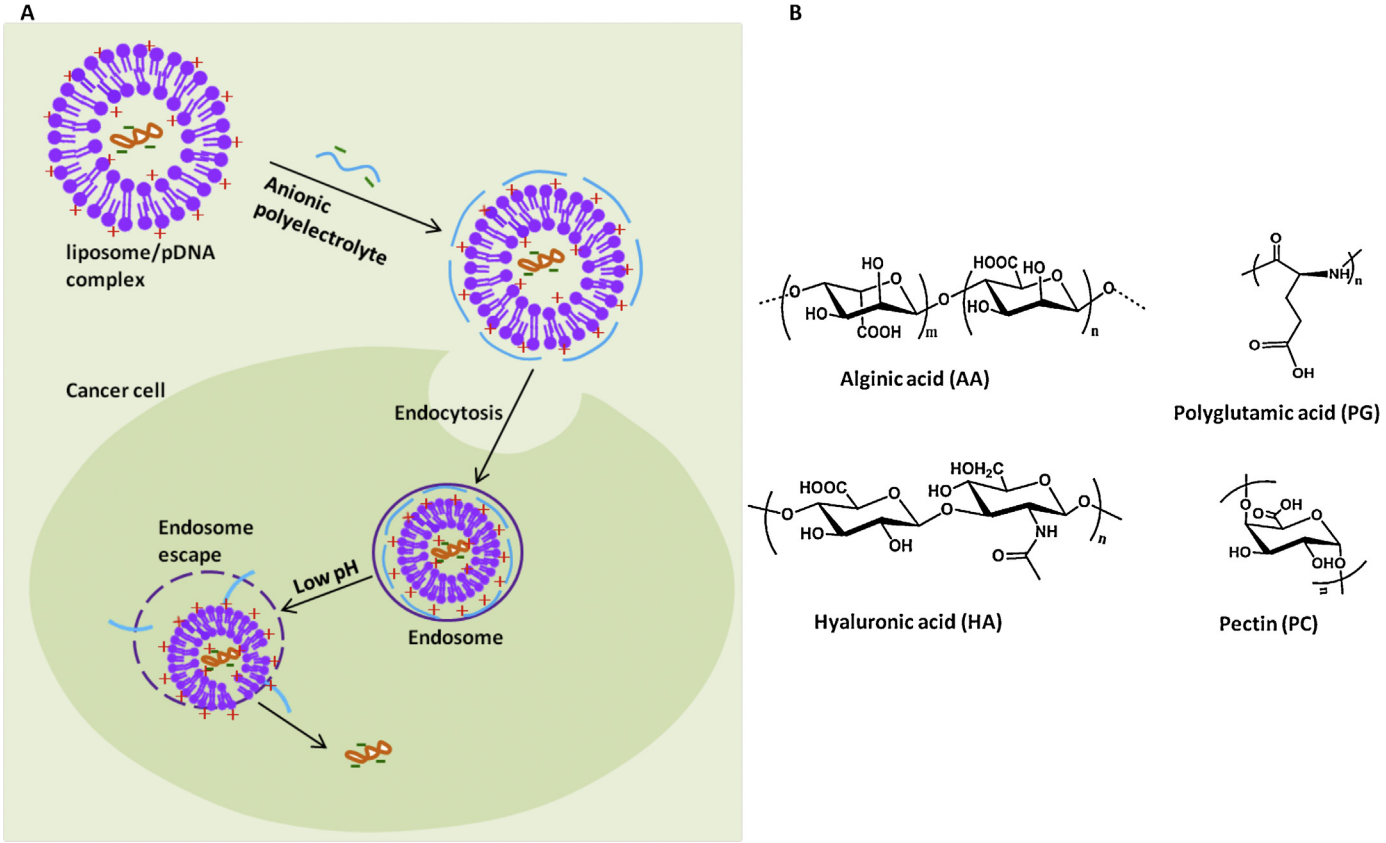
Polyanionic coatings on polycation/DNA complexes could efficiently reduce their non-specific interaction. However, it may also suppress the electrostatic interactions required for cellular uptake and cause lag behind endosomal escape, which is very important step to achieve high transfection efficiency (Hwang et al., 2014; Khalil et al., 2006; Mishra et al., 2004; Shete et al., 2014). Those gene cargoes which cannot escape from endosome may face degradation as endosome mature and fuse with lysosome. Thus, it is of vital importance to choose appropriate coating materials to get high transfection efficiency. Based on these, the biodegradable anionic polyelectrolytes (AA, HA, PC, PG) we chose are pH sensitive. Their side chains will be neutralized and peel off when pH gets down. At the same time, the noncharged hydrophobic side chains will insert into the hydrophobic part of membrane and decrease the stability of endosome, which is helpful for enhancing gene transfection (Lackey et al., 1999; Cheung et al., 2001; Steele and Shier, 2010) (as shown in Scheme 1).

In this study, we evaluated the transfection efficiency of AA, HA, PC, PG coated cationic liposome/pDNA complexes to Hela, MCF-7 and HepG2 cells. All the three cell lines have stable characters in vitro and have different transfection property. Hela cell is one of easiest to be transfected cell lines widely used in gene transfection; MCF-7 cell can achieve high gene expression with most available transfection agents; HepG2 cell, however, is known hard to be transfected. So we want to test the transfection efficiency in these three different cell lines to prove the advantage of the new gene carriers. The cytotoxicity, stability, pH sensitive hemolytic properties of the materials were also studied .We found that by partial or even reverse the charge of liposome/pDNA complex could still achieve high or even better transfection efficiency. These promising results demonstrated that electrostatically coating of biocompatible negatively charged materials to the positively charged surface of liposome/pDNA is a simple and efficient alternative to push the liposome based gene therapy to the clinical application.

2. Materials and methods

2.1. Materials

Lipofectamine 2000 was purchased from Invitrogen (Carlsbad, CA) and used according to manufacturer's instructions. Alginic Acid and Hyaluronic Acid were purchased from Sangon Biotech Shanghai Co. Ltd., Pectin from citrus peel and Poly-L-glutamic acid sodium (wt 15,000–50,000) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Thiazolyl blue tetrazolium bromide(MTT) was purchased from reagent Beyotime Institute of Biotechnology (Haimen, China). Plasmid DNA encoding enhanced green fluorescent protein (pCX-EGFP), human breast cancer cells (MCF-7) and Human cervix carcinoma cells (Hela) were gifts from Dr. Libing Liu (Institute of Chemistry, Chinese Academy of Sciences, Beijing, China), human hepatocellular carcinoma cell lines (HepG2) were kindly given by Gene Function and Regulation lab (Hunan Normal University, Hunan, China).



Scheme 1. Schematic illustrations showing (A) the self-assembly of various anionic polyelectrolytes (AA, HA, PC and PG) with cationic liposome/pDNA complex and the process of cellular uptake and gene release; (B) chemical structures of AA, HA, PC and PG.