International Journal of Pharmaceutics xxx (2012) xxx–xxx

Contents lists available at SciVerse ScienceDirect

International Journal of Pharmaceutics

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

Pharmaceutical Nanotechnology

Delivery of paclitaxel across cellular barriers using a dendrimer-based nanocarrier

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ARTICLE INFO

Article history:
Received 20 September 2012
Received in revised form 10 October 2012
Accepted 10 October 2012
Available online xxx

Keywords:
Paclitaxel, Dendrimer prodrugs
Caco-2 cells
Blood–brain barrier
Nano carrier
Drug delivery

ABSTRACT

The aim of this study was to investigate the ability of a third-generation (G3) polyamidoamine (PAMAM) dendrimer-based carrier to enhance the permeability of paclitaxel (pac) and to overcome cellular barriers. G3 dendrimers were surface modified with lauryl chains (L) and conjugated with paclitaxel (pac) via a glutaric anhydride (glu) linker, followed by labeling with FITC. Biological evaluation of the dendrimer and conjugates was conducted using the human colon adenocarcinoma cell line (Caco-2) and primary cultured porcine brain endothelial cells (PBECs). LDH assay was used to evaluate the cytotoxicity of the dendrimer and conjugates. Cytotoxicity studies showed that the conjugation of lauryl chains and paclitaxel on G3 dendrimer significantly ($p < 0.05$) increased the cytotoxicity against both cell types. Permeability studies of dendrimer–drug conjugates demonstrated an increase in the apparent permeability coefficient (P_{app}) in both apical to basolateral A → B and basolateral to apical B → A directions across both cell monolayers compared to unmodified G3 and free drug. The B → A P_{app} of paclitaxel was significantly ($p < 0.05$) higher than the A → B P_{app}, indicating active function of P-gp efflux transporter system in both cell models. L6-G3-glu-pac conjugate had approximately 12-fold greater permeability across both cell monolayers than that of paclitaxel alone.

1. Introduction

Over the years, numerous attempts have been made to devise therapeutic drug carrier systems able to cross cellular barriers (e.g. intestinal barrier and the blood–brain barrier) for efficient drug delivery. Challenges encountered during drug delivery are normally associated with low solubility and permeability of therapeutic drugs. Efflux transporter systems (e.g. P-gp efflux transporter) actively function at cellular barriers and limit drugs that are substrates from permeation across the barrier. Chemical modification is one of the strategies to enhance permeability and solubility of drugs for more efficient delivery. Addition of lipophilic components to drugs and conjugation of drug molecules to a more soluble carrier which can bypass efflux transporters have been proven to enhance the permeation of drugs across cellular barriers (Abbott and Romero, 1996; Najlah and D'Emanuele, 2006).

In this study, paclitaxel was selected as a P-gp substrate with low water solubility (<1 μM). It represents a class of anti-microtubule anticancer drugs which have been shown experimentally to have antitumor activity (Rowinsky et al., 1990) by promoting microtubule polymerization, a process which disrupts the normal tubule dynamics essential in cellular division, and leads to cell death by apoptosis (Guillemard and Saragovi, 2001). Paclitaxel has been reported to demonstrate remarkable efficacy against ovarian and breast cancer and more recently, against malignant gliomas and brain metastases (Fellner et al., 2002). Despite its clinical efficacy, pharmaceutical applications of paclitaxel are limited by its poor solubility as well as low permeability due to exclusion by the P-gp efflux transport system present in cellular barriers, e.g. the intestinal and the blood–brain barriers.

Polyamidoamine (PAMAM) dendrimers have shown great potential as drug carriers in pharmaceutical applications due to their well-defined architecture (D'Emanuele et al., 2006; Tomalia et al., 1985). They are highly branched polymers with a high degree of uniformity and monodispersity, and amendable surface groups for specific functionality (D'Emanuele and Attwood, 2005; Tomalia et al., 1990). Their unique properties and characteristics have been of great interest for encapsulation/solubilization of drugs,

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0378-5173/$ – see front matter © 2012 Published by Elsevier B.V.
http://dx.doi.org/10.1016/j.ijpharm.2012.10.024

and conjugation of drugs for transepithelial transport (D'Emanuele et al., 2004). Dendrimers have been reported to cross cellular barriers via both paracellular and transcellular pathways (El-Sayed et al., 2002, 2003; Jevprasesphant et al., 2003a; Kitchens et al., 2005, 2007; Saovapakhiran et al., 2009). Goldberg et al. (2010) reported that the internalization of G3.5 PAMAM dendrimers via endocytosis promoted transient tight junction opening, indicating that transcellular and paracellular pathways are interconnected for dendrimer cellular transport. For targeting to tumors, conjugation of paclitaxel to G4-OH PAMAM dendrimers resulted in a 10-fold increase in cytotoxicity in A2780 human ovarian carcinoma cells compared to free drug (Khandare et al., 2006). Partially acetylated G5 PAMAM dendrimer conjugated with paclitaxel, folic acid and FITC demonstrated significant cytotoxicity against KB cells (human epidermoid carcinoma cells that over-express the folate receptor) in cellular uptake and specific delivery studies (Majoros et al., 2006). Previous work in our research group has shown the ability of PAMAM dendrimer conjugates to enhance drug solubility and bypass P-glycoprotein (P-gp) efflux transporters, therefore increasing drug bioavailability (D'Emanuele et al., 2004; Najlah et al., 2007a,b). G3 PAMAM dendrimer was reported as a potential drug carrier for propranolol, a P-gp substrate drug with low water solubility. Enhanced permeability and ability to bypass the P-gp efflux transporter through Caco-2 cell monolayers were observed when propranolol was conjugated to surface-modified G3 PAMAM dendrimer (D'Emanuele et al., 2004). Surface-engineered PAMAM dendrimers with lauryl chains demonstrated higher permeability and lower cytotoxicity compared to unmodified dendrimers (Jevprasesphant et al., 2003a,b). PAMAM dendrimers conjugated to drugs via biodegradable linkers were assessed by Najlah et al. (2006, 2007a,b). Diethylene glycol (deg) and succinic acid (suc) were used as the linkers to attach drugs to PAMAM dendrimers. Enhanced solubility and permeability were obtained when naproxen was conjugated to G0 PAMAM dendrimer via a deg linker (Najlah et al., 2007b). Further studies were conducted with the conjugates of terfenadine (a water-insoluble P-gp substrate drug) with lauryl-modified G1 PAMAM dendrimers via a double linker (suc-deg) (Najlah et al., 2007a). The dendrimer prodrugs demonstrated enhanced permeability and solubility, and ability to bypass the P-gp efflux transport system.

There are relatively few studies of permeation of PAMAM dendrimer across the blood–brain barrier (BBB). PEGylated G5 PAMAM dendrimer conjugated with brain-targeting ligands transferrin (Tf) and lactoferrin (Lf), demonstrated increased brain uptake, transfection efficacy and brain gene expression in gene delivery studies. These findings offer a promising non-viral approach for gene delivery to the brain via non-invasive administration (Huang et al., 2007, 2008).

In the present study, dendrimer-based drug delivery systems consisting of lauryl-modified G3 PAMAM dendrimer conjugated with paclitaxel were synthesized and characterized. The synthesis of paclitaxel–dendrimer prodrugs has been reported in which a double ester linkage was employed to attach the drug to dendrimer surface using succinic anhydride/acid linkers (Khandare et al., 2006; Majoros et al., 2006). It has been previously shown that the chemical and enzymatic stability of dendrimer conjugates is related to the type of the linkage (Najlah et al., 2006). The primary ester bond was found to be more labile than the amide bond under the influence of pH and in the presence of esterases. In this study, a glutaric anhydride linker was selected to attach to paclitaxel via an ester bond and then conjugated with PAMAM dendrimer via an amide bond. This ensures that the dendrimer conjugates are stable during transit yet able to release the drug once delivered to the target. The transport and cytotoxicity of dendrimer carriers were investigated using Caco-2 cells and the cytotoxicity and permeability of the dendrimer prodrugs were assessed. Furthermore, the ability of the paclitaxel–dendrimer prodrugs to overcome the blood–brain barrier was investigated using a model based on primary porcine brain endothelial cells (PBECs) (Abbott et al., 2008, 2010; Skinner et al., 2009).

2. Experimental

2.1. Materials

Third-generation PAMAM dendrimer (G3) with an ethylene diamine core in methanol (20%, w/w) was purchased from Dendritech Inc. (Michigan, USA). Paclitaxel was purchased from Advance Tech. & Ind. Co., Ltd. (Kln, Hong Kong). Silica gel for flash chromatography was purchased from BDH Laboratory Supplies (Lutterworth, UK). Diphenyl phosphoryl chloride (DPC), *N*-hydroxysuccinimide (NHS), fluorescein isothiocyanate (FITC) 98%, Sephadex LH-20, fibronectin, Dulbecco's Modified Eagle's Medium (DMEM) low glucose, 10 KU/ml penicillin and 10 mg/ml streptomycin, L-glutamine, 100 KU Heparin, puromycin, 8-(4-Chlorophenylthio)adenosine 3′,5′-cyclic monophosphate sodium salt (CPT-cAMP), hydrocortisone, Hank's Balanced Salt Solution (HBSS) without calcium ions and magnesium ions, trypsin–EDTA, Corning Transwell® polycarbonate membrane inserts (pore size 3.0 μm, membrane diameter 12 mm), Costar Transwell® clear 12-well tissue culture-treated sterile polyester membrane inserts (pore size 0.4 μM, membrane diameter 12 mm), and Corning Costar® 96-well flat bottom cell culture plates were purchased from Sigma–Aldrich Co. Ltd. (Gillingham, Dorset, UK). Human plasma (SeraChem® control level 1) was provided by Instrumentation Laboratory UK Ltd. Cytotoxicity detection kit (lactate dehydrogenase, LDH) was purchased from Roche Applied Science (Mannheim, Germany). 4-(3-Butoxy-4-methoxybenzyl)-2-imidazolidinone (RO-20-1724) was purchased from Merck-Calbiochem Chemicals Ltd. (Beeston, Nottingham, UK). BD type 1 rat tail collagen was purchased from Scientific Laboratory Supplies Ltd. (Wilford, Nottingham, UK). Bovine plasma derived serum was purchased from First Link Ltd. (Birmingham, UK). All other cell culture materials were purchased from Invitrogen Life Technologies (Paisley, Scotland). Caco-2 cells were kindly provided by Dr. Jeff Penny at The University of Manchester. Primary porcine brain endothelial cells (PBECs) were acquired from Prof. N. Joan Abbott, Blood-Brain Barrier Group, King's College London.

2.2. General synthesis procedures

The synthesis of dendrimer–paclitaxel conjugates is illustrated in the reaction scheme in Fig. 1. Lauryl chains were attached covalently to the surface of G3 PAMAM dendrimers as described by Najlah et al. (2007a). The lauryl chain was activated to form lauryl 4-nitrophenyl carbonate, and then reacted with the surface amine groups of G3 PAMAM dendrimers at the appropriate molar ratio to obtain G3L3 (1) and G3L6 (2). Paclitaxel was conjugated to G3 and lauryl-G3 PAMAM dendrimers through a glutaric anhydride linker using the NHS method (Majoros et al., 2005). Firstly, paclitaxel was reacted with glutaric anhydride to yield 2′-glutaryl-paclitaxel (pac-glu). The drug-linker was converted to pac-glu-NHS ester, followed by equimolar conjugation with G3 or lauryl-G3 PAMAM dendrimers to yield G3-glu-pac (3), L3-G3-Glu-pac (4) and L6-G3-glu-pac (5). Unmodified G3 PAMAM dendrimer and all the dendrimer conjugates ((1)–(5)) were labeled with fluorescein isothiocyanate (FITC) for quantitative detection by spectrofluorimetry in permeability studies. The dendrimer–paclitaxel conjugates were characterized by proton (^{1}H) and carbon (^{13}C) nuclear magnetic resonance spectroscopy (NMR). Pac and pac-glu were also characterized by electrospray ionization mass spectrometry (ESI-MS). Analysis of particle size distribution of FITC-labeled PAMAM

Please cite this article in press as: Teow, H.M., et al., Delivery of paclitaxel across cellular barriers using a dendrimer-based nanocarrier. Int J Pharmaceut (2012), http://dx.doi.org/10.1016/j.ijpharm.2012.10.024