Journal of Controlled Release 206 (2015) 75–90

Contents lists available at ScienceDirect

Journal of Controlled Release

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

Improved intracellular delivery of peptide- and lipid-nanoplexes by natural glycosides

Alexander Weng [a,d,*], Maria D.I. Manunta [a], Mayank Thakur [b], Roger Gilabert-Oriol [d], Aristides D. Tagalakis [a], Ayad Eddaoudi [a], Mustafa M. Munye [a], Conrad A. Vink [a], Burkhard Wiesner [c], Jenny Eichhorst [c], Matthias F. Melzig [d], Stephen L. Hart [a,**]

[a] Wolfson Centre for Gene Therapy of Childhood Disease, UCL Institute of Child Health, University College London, 30 Guilford Street, London WC1N 1EH, United Kingdom
[b] Institut für Laboratoriumsmedizin, Klinische Chemie und Pathobiochemie, Charité — Universitätsmedizin Berlin, Augustenburger Platz 1, D-13353 Berlin, Germany
[c] Leibniz-Institut für Molekulare Pharmakologie (FMP), Robert-Rössle-Str. 10, 13125 Berlin, Germany
[d] Institute of Pharmacy, Free University Berlin, Königin-Luise-Str. 2+4, D-14195 Berlin, Germany

ARTICLE INFO

Article history:
Received 27 November 2014
Received in revised form 5 March 2015
Accepted 6 March 2015
Available online 7 March 2015

Keywords:
Gene delivery
Endosomal escape
Natural glycoside

ABSTRACT

Targeted nanocarriers undergo endocytosis upon binding to their membrane receptors and are transported into cellular compartments such as late endosomes and lysosomes. In gene delivery the genetic material has to escape from the cellular compartments into the cytosol. The process of endosomal escape is one of the most critical steps for successful gene delivery. For this reason synthetic lipids with fusogenic properties such as 2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE) are integrated into the nanocarriers.

In this study we show that a natural, plant derived glycoside (SO1861) from *Saponaria officinalis* L. greatly improves the efficacy of lipid based as well as non-lipid based targeted nanoplexes consisting of a targeted K_{16} peptide with a nucleic acid binding domain and plasmid-DNA, minicircle-DNA or small interfering RNA (siRNA). By confocal live cell imaging and single cell analyses, we demonstrate that SO1861 augments the escape of the genetic cargo out of the intracellular compartments into the cytosol. Co-localisation experiments with fluorescence labelled dextran and transferrin indicate that SO1861 induces the release of the genetic cargo out of endosomes and lysosomes. However, the transduction efficacy of a lentivirus based gene delivery system was not augmented.

In order to design receptor-targeted nanoplexes (LPD) with improved functional properties, SO1861 was integrated into the lipid matrix of the LPD. The SO1861 sensitized LPD (LPDS) were characterized by dynamic light scattering and transmission electron microscopy. Compared to their LPD counterparts the LPDS-nanoplexes showed a greatly improved gene delivery. As shown by differential scanning calorimetry SO1861 can be easily integrated into the lipid bilayer of glycerophospholipid model membranes. This underlines the great potential of SO1861 as a new transfection multiplier for non-viral gene delivery systems.

1. Introduction

Nanocarriers for nucleic acid (DNA) and small interfering ribonucleic acid (siRNA) delivery are transport vehicles in the nanoscale range, which protect the genetic material against degradation and increase their bioavailability. Typically these nanocarriers consist of two motifs: A positively charged carrier component to facilitate complex formation with siRNA/DNA and a targeting ligand to facilitate cell-specific binding of the nanocarrier [1]. A nanoparticulate formulation of siRNA, termed CALAA-01 is currently under investigation in clinical phase I and consists of a cyclodextrin derivative (cationic polymer), PEG (polyethylene glycol) and transferrin as a targeting ligand [2]. Successful targeting of siRNA containing nanocarriers has been reported by conjugation of targeting ligands such as transferrin, folic acid [3] or RGD peptides [4] to the carriers.

An ideal sequence of events hypothesized in all these strategies is a) binding of the nanocarrier to the cell surface, b) endocytosis of the carrier and delivery of the cargo into intracellular compartments such as early endosomes and c) release of the genetic cargo (siRNA/DNA) out of the intracellular compartments into the cytosol and in the case of DNA transport into the nucleus. The lumen of early endosomes is acidified by V-ATPases [5] to pH ~ 6 and further decreases to pH ~ 5–6 in late endosomes and pH ~ 4–5 in lysosomes. In lysosomes siRNA/DNA are degraded by nucleases [6]. The RISC (RNA-Induced-Silencing Complex) is located in the cytosol. Thus, to exert a silencing effect, siRNA has to escape from the endosomes/lysosomes into the cytosol. The endosomal escape is also a critical step for successful DNA delivery.

* Correspondence to: A. Weng, Institute of Pharmacy, Free University Berlin, Königin-Luise-Str. 2+4, D-14195 Berlin, Germany.
** Corresponding author.
E-mail addresses: weng@zedat.fu-berlin.de (A. Weng), s.hart@ucl.ac.uk (S.L. Hart).

http://dx.doi.org/10.1016/j.jconrel.2015.03.007
0168-3659/

However to get access to the cellular transcription machinery the nucleic acid has to overcome the nuclear membrane as well. If the endo-/lysosomal escape of the genetic cargo does not occur the siRNA/DNA is degraded within the lysosome thus leading to a therapeutic failure. The limited release of siRNA/DNA into the cytosol is one of the major obstacles for efficient siRNA/DNA delivery [7]. The development of innovative strategies to augment the endo-/lysosomal escape of siRNA/DNA and the delivery of a minimal amount of siRNA/DNA are desired for an efficient therapeutic response. It is therefore essential to develop methods and tools to increase the endo-/lysosomal escape of siRNA/DNA containing nanocarriers.

Different reagents have been investigated in order to promote the endosomal escape of siRNA/DNA. Amine modified polymers [8] have been used as additives for an improved endosomal escape. These polymers exhibit a high buffering capacity in the pH range 5–7 leading to a reduced acidification of endosomes/lysosomes. This leads to an increased accumulation of chloride (Cl^-) within the endo-/lysosomal compartments [9]. The osmotic pressure finally results in the rupture of the endo-/lysosomal membranes followed by the release of captured material into the cytosol. This principle was coined as the proton-sponge effect. In contrast to these mechanisms, cell penetrating peptides (CPPs) [10] and viroporins [11], which interact directly with cellular membranes, have also been utilized as enhancers for endosomal/lysosomal escape of siRNA [12]. Photochemical internalization (PCI) is another mechanism, which has been highlighted to improve the endosomal/lysosomal escape of siRNA and DNA [13,14].

One of the most commonly used nanocarriers for siRNA/DNA delivery are lipoplexes. Lipoplexes are formed by mixing siRNA or DNA with cationic liposomes, consisting of lipids such as 1,2-di-O-octadecenyl-3-trimethylammonium propane (DOTMA) [15] or 2,3-dioleyloxy-N-[2-(sperminecarboxamido)ethyl]-N,N-dimethyl-1-propanaminium trifluoroacetate (DOSPA) [16–19]. To facilitate receptor specific internalization of lipoplexes different targeting ligands are attached to lipoplexes [17]. Since an inefficient endosomal escape is a problem of lipoplexes, lipids such as 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE) are added to the lipoplex formulations [20]. The ability of fusogenic lipids to facilitate the endo-/lysosomal escape is generally determined by their hydrophobic tails [21]. Cationic lipids are used to augment the transfection efficiency of oligopeptides consisting of a 16-fold lysine domain (K_{16}) and integrin binding motifs such as Arg(R)-Gly(G)-Asp(D) [22]. Pathogens such as *Yersinia pseudotuberculosis* efficiently exploit the integrin-mediated endocytosis for cell entry [23]. Via short linker sequences the K_{16} nucleic acid binding domain is fused to the integrin-binding motif resulting in a targeted nucleic acid binding peptide. By mixing these peptides with DNA or small interfering RNA, nanoplexes (PD-nanoplexes) are formed. The main purpose of the peptides is to bind and condense the DNA and to target the condensed siRNA/DNA via the integrin-targeting domain. To increase the transfection efficiency of the PD-nanoplexes fusogenic lipids such as DOPE are incorporated.

Recently we have shown that a particular plant secondary metabolite (SO1861) triggers the endo-/lysosomal escape of a 30 kDa protein toxin (saporin) [24] into the cytosol of cells that have endocytosed saporin (coined as synergistic principle). Both components (SO1861/saporin) are biosynthesized by the plant *Saponaria officinalis* L., which is growing in Europe, Northern America and Australia. Developed by evolution the synergistic principle is a native, very efficient toxin delivery system. SO1861 (Fig. 1) consists of a hydrophobic triterpene core and two branched carbohydrate side chains attached to the triterpene backbone. Due to the presence of one glucuronic acid (one negative charge) SO1861 is moving within the electric field.

In this study we aimed to show the potential of SO1861 as a new transfection multiplier for DNA delivery as well as for siRNA-mediated gene silencing. For this purpose we generated different lipid and non-lipid based nanoplexes and investigated, if SO1861 triggers and/or augments the delivery of plasmid-DNA, minicircle-DNA and siRNA into target cells. In order to design nanoplexes with improved functional properties (SO1861 sensitized receptor-targeted nanoplexes) we aimed to integrate SO1861 into the matrix of targeted nanoplexes.

2. Materials and methods

2.1. Isolation of SO1861

The plant material was purchased from *Wilhelm-Lindig-Käuterparadies*, Munich, Germany. Dried roots (150 g) of *S. officinalis* L. were grinded and a glycoside raw fraction was obtained by methanol extraction (1 L methanol, 90%). The methanol was removed by rotary evaporation and 100 mL ice-cold acetone was added to the aqueous solution. The solution was kept overnight at 2–8 °C. A glycoside precipitate of 1.5 g was collected by centrifugation. The precipitate was dissolved in 20 mL *Milli-Q*-water and extensively dialysed against *Milli-Q*-water (MWCO 1000). The solution was lyophilized and 350 mg SO1861 was

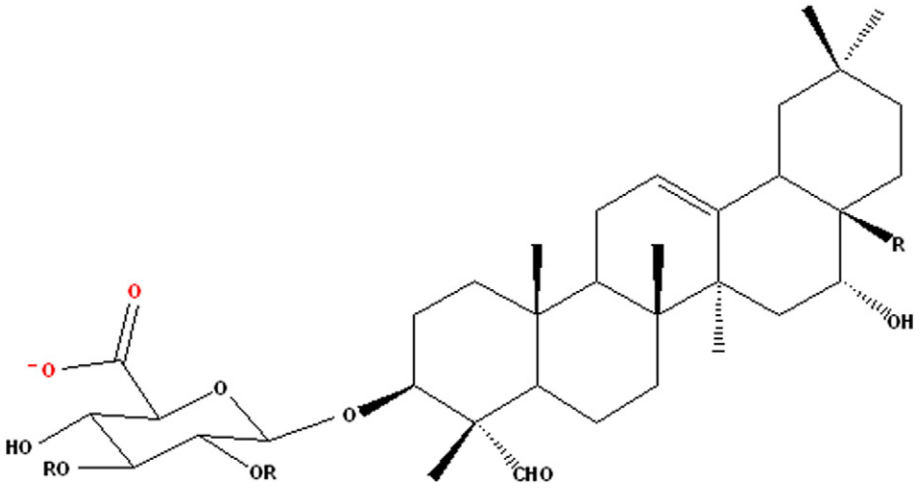

Fig. 1. Core structure of SO1861. SO1861 consists of a hydrophobic triterpene backbone and branched carbohydrate chains (R). The carbonic acid (marked in red) contributes with one negative charge.