International Journal of Pharmaceutics 483 (2015) 19–25

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

Pharmaceutical nanotechnology

Polyethylene imine-6-phosphogluconic acid nanoparticles – a novel zeta potential changing system

Sonja Bonengel, Felix Prüfert, Glen Perera, Johanna Schauer, Andreas Bernkop-Schnürch *

Center for Chemistry and Biomedicine, Center for Molecular Biosciences, Department of Pharmaceutical Technology, Institute of Pharmacy, University of Innsbruck, Innrain 80/82, 6020 Innsbruck, Austria

ARTICLE INFO

Article history:
Received 22 October 2014
Received in revised form 15 January 2015
Accepted 21 January 2015
Available online 23 January 2015

Keywords:
Nanoparticles
Mucus barrier
Zeta potential
Zeta potential changing systems
6-Phosphogluconic acid
Intestinal alkaline phosphatase

ABSTRACT

The aim of the study was to develop nanoparticles with the ability to change their zeta potential. By covalent attachment of 6-phosphogluconic acid to polyethylene imine, a charged, enzymatically removable moiety was introduced into the polymer. The novel conjugate displayed 400 μmol phosphate per gram polymer, as determined by malachite green assay. Studies evaluating the cleavage by intestinal alkaline phosphatase revealed that 69 % of the coupled phosphate could be released from the polymer. Furthermore, nanoparticles generated by polyelectrolyte complexation technique using carboxymethyl cellulose as negatively charged component exhibited a zeta potential of −6 mV and an average particle size of 300 nm. Enzymatic cleavage of the phosphate ester moiety by isolated intestinal alkaline phosphatase on these nanoparticles caused shift of the zeta potential from negative to positive value of +3 mV whereby 58 % of the total amount of phosphate were released. Studies on Caco-2 cells revealed the capability of a living system to hydrolyze the phosphate ester in the novel conjugate as well as on the nanoparticles via their intestinal alkaline phosphatase. Based on these results, polymeric nanoparticles comprising an enzymatically degradable phosphate ester moiety can provide a promising strategy for zeta potential changing systems.

© 2015 Elsevier B.V. All rights reserved.

1. Introduction

Mucus gel layer lines most of the cavities in the body especially the respiratory tract and gastrointestinal compartments, with the main aim to protect the underlying epithelia from the external environment. Xenobiotic substances like bacteria or irritants can be rapidly removed, due to entrapment by covalent and non-covalent bonds with mucus components and due to continuous mucus turnover (Cone, 2009).

Despite these essential protective functions, the mucus barrier in the gastro-intestinal tract emerged to be an outstanding hindrance for orally administered drug delivery systems, as drugs and drug vehicles are removed before reaching the absorption membrane (Bernkop-Schnürch, 2013; Ponchel and Irache, 1998). In the field of nanoparticulate carrier systems, however, immobilization of mucolytic enzymes, such as papain (Müller et al., 2014) and PEG-coating (Lai et al., 2011) emerged to be promising strategies to overcome this stumbling block. While degradation of the mucus network is the driving force in the first case, the latter technique for enhanced permeability is based on reduced ionic interactions between particles and the mucus gel layer. By a slightly negative or almost neutral zeta potential ionic interactions with negatively charged sialic acid and sulfonic acid moieties of the mucus gel layer can be reduced resulting in enhanced mucus penetration (Lai et al., 2011), whereas particle mobility is suppressed in case of particles exhibiting a high surface charge (Lieleg et al., 2010). On the contrary, further desirable properties for an efficient drug delivery system, such as permeation enhancing effects (Ranaldi et al., 2002), efficient endocytosis (Alexis et al., 2008) and immobilization close to the epithelium to prevent back-diffusion within the mucus layer, would be mainly fulfilled by positively charged particles. Although seeming incongruous, these advantageous features of a positive zeta potential and mucus permeability (Aljayyoussi et al., 2012; Cone, 2009) could be combined in zeta potential changing systems. Such systems are equipped with a charged moiety, which can be removed enzymatically or chemically leading to an alteration in the zeta potential.

Therefore, it was the aim of the study to evaluate polymeric nanoparticles comprising an enzymatically degradable phosphate ester moiety as a strategy for zeta potential changing system.

* Corresponding author. Tel.: +43 512 507 58601; fax: +43 512 507 58699.
E-mail address: andreas.bernkop@uibk.ac.at (A. Bernkop-Schnürch).

http://dx.doi.org/10.1016/j.ijpharm.2015.01.041
0378-5173/© 2015 Elsevier B.V. All rights reserved.

Table 1
Composition of the reaction mixture for the synthesis of PEI-6PGA and control polymer.

	PEI (g)	6-PGA (g)	EDAC (mM)	NHS (mM)	Total amount of phosphate (μmol/g)	Amount of unbound phosphate (μmo/g)
PEI-6PGA	1.0	0.2	50	20	407.87	5.02
Control	1.0	0.2	–	–	10.01	8.74

Displaying a slightly negative zeta potential in the beginning, these particles should provide sufficient permeability within the mucus gel layer (Aljayyoussi et al., 2012). While penetrating the mucus layer, nanoparticles should be dephosphorylated by the brush border membrane-bound enzyme, intestinal alkaline phosphatase (IAP). This 140 kDa enzyme catalyzes the hydrolysis of a broad variety of phosphate esters (Fosset et al., 1974; Holtz et al., 1999; Lallès, 2010) and in case of the mentioned nanoparticles this would cause a shift in the zeta potential to positive values.

Hence, 6-phosphogluconic acid was covalently linked to the polymeric backbone of polyethylene imine. In a second step, nanoparticles were formed out of poly-ion complexes with carboxymethyl cellulose, which was chosen as anionic polymer, due to its biodegradability and therefore, good biocompatibility (Shelanski and Clark, 1948).

The novel synthesized polymer and the nanoparticles were investigated for their phosphate releasing properties after enzymatic cleavage via isolated alkaline intestinal phosphatase as well as on Caco-2 cell monolayer. Furthermore, nanoparticles were examined in terms of size distribution, toxicity and zeta potential changing properties throughout this study.

2. Materials and method

2.1. Materials

Polyethylene imine (PEI), branched (Mwt 50,000–100,000 Da), 30% (w/w) aqueous solution was purchased from Alfa Aesar GmbH & Co., KG, Karlsruhe, Germany. 6-Phosphogluconic acid (6-PGA), alkaline phosphatase from bovine intestinal mucosa (IAP; 10 kU), *N*-hydroxysuccinimide (NHS), malachite green oxalate salt, carboxymethyl cellulose (CMC; Mwt. 90,000 Da), Triton X-100 and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) were obtained from Sigma–Aldrich, Austria.

2.2. Methods

2.2.1. Synthesis of polyethyleneimine-6-phosphogluconic acid

Polyethyleneimine-6-phosphogluconic acid (PEI-6PGA) was synthesized by covalent attachment of 6-PGA to the polymeric backbone of PEI via a carbodiimide reaction. The exact amounts of the utilized reagents for the synthesized conjugates is provided in Table 1. In detail, 6-PGA was dissolved in 40 ml of distilled water and the carboxylic acid moieties were activated using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDAC). Thereafter, NHS and PEI were added to the mixture and the volume was filled to 100 ml. The reaction mixture was stirred over night at 50 °C. The purified PEI-6PGA was obtained after dialyzing eight times against distilled water and lyophilization of frozen polymer solution under reduced pressure (Christ Gamma 1-16 LSC freeze dryer). A control polymer was prepared in exactly the same way, but EDAC and NHS were omitted in the reaction.

2.2.2. Phosphate assay with malachite green

Within this study, phosphate was quantified spectrophotometrically using malachite green assay according to a previously described method with slight modifications (Feng et al., 2011). In this assay, a complex formed between malachite green, ammonium molybdate and inorganic phosphate – the phosphomolybdatemalachite green complex – led to a change of absorbance at 620–650 nm, whereby only free phosphate released from the conjugate PEI-6PGA was measured in contrast to organic phosphate bound to PEI-6PGA showing no increase in absorption at all. In detail, malachite green reagent solution was prepared by addition of 0.4 ml of an aqueous Triton X-100 solution (1.1% w/v) and 6 ml of ammonium molybdate solution (8% w/v) to 10 ml of a 0.15% malachite green solution in 3.6 M sulfuric acid under constant stirring. Thereafter, 100 μl of this colorimetric reagent was added to 50 μl of sample and absorbance of the formed complex between malachite green, molybdate and phosphate was measured at 630 nm. The amount of phosphate in the analyzed sample was determined using a standard curve with increasing amounts of KH_2PO_4. All enzymatic reactions were carried out in 100 mM HEPES buffer pH 7 containing 5 mM magnesium chloride and 0.2 mM zinc chloride.

2.2.3. Enzymatic cleavage of 6-PGA

The utilized ligand 6-phosphogluconic acid was evaluated for enzymatic degradability using isolated intestinal alkaline phosphatase and free phosphate was analyzed by malachite green method as described above. In brief, 1.0 mg of 6-PGA was dissolved in 9.9 ml of 100 mM HEPES buffer pH 7 containing 5 mM magnesium chloride and 0.2 mM zinc chloride and spiked with 100 μl of an aqueous IAP solution (10 U/ml). After incubation for 3 h at 37 °C, samples were analyzed regarding phosphate release.

2.2.4. Characterization of PEI-6-PGA

2.2.4.1. Determination of the coupled amount of 6-PGA. The amount of 6-PGA attached to the polymer was determined via enzymatic cleavage of the phosphate in 6-PGA. Therefore, 1.0 mg of polymer was dissolved in 9.9 ml of 100 mM HEPES buffer pH 7 containing 5 mM magnesium chloride and 0.2 mM zinc chloride and 100 μl of an aqueous IAP solution (10 U/ml) were added afterwards. Test solutions were incubated for 150 min under constant shaking (37 °C, 500 rpm) and the released amount of phosphate was

Table 2
Overview of prepared CMC/PEI-6PGA nanoparticles and blank particles. Nanoparticles were characterized in terms of mean particle size and initial zeta potential as well as zeta potential after incubation with isolated alkaline phosphatase.

	Amount CMC (%)	Amount PEI-6PGA/PEI (%)	Mean particle size (nm)	Polydispersity index (PI)	Zeta potential (mV)	Zeta potential (mV) after IAP
Particles 1	0.015	0.0105	210	0.076	−9.15	−0.61
Particles 2	0.015	0.0108	227	0.074	−7.53	−0.08
Particles 3	0.015	0.0111	299	0.095	−6.44	+2.8
Blank particles	0.015	0.0111	203	0.078	−5.74	−5.48

Download English Version:

https://daneshyari.com/en/article/2501493

Download Persian Version:

https://daneshyari.com/article/2501493

Daneshyari.com