Journal of Controlled Release 253 (2017) 1–10

Contents lists available at ScienceDirect

Journal of Controlled Release

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

Potential of surfactant-coated nanoparticles to improve brain delivery of arylsulfatase A

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ARTICLE INFO

Article history:
Received 22 November 2016
Received in revised form 10 February 2017
Accepted 15 February 2017
Available online 16 February 2017

Keywords:
Metachromatic leukodystrophy
Lysosomal storage disease
Enzyme replacement therapy
Blood-brain barrier
Polymeric nanoparticles
Surfactant coating

ABSTRACT

The lysosomal storage disorder (LSD) metachromatic leukodystrophy (MLD) is caused by a deficiency of the soluble, lysosomal hydrolase arylsulfatase A (ASA). The disease is characterized by accumulation of 3-O-sulfogalactosylceramide (sulfatide), progressive demyelination of the nervous system and premature death. Enzyme replacement therapy (ERT), based on regular intravenous injections of recombinant functional enzyme, is in clinical use for several LSDs. For MLD and other LSDs with central nervous system (CNS) involvement, however, ERT is limited by the blood-brain barrier (BBB) restricting transport of therapeutic enzymes from the blood to the brain. In the present study, the potential of different types of surfactant-coated biodegradable nanoparticles to increase brain delivery of ASA was evaluated. Three different strategies to bind ASA to nanoparticle surfaces were compared: (1) adsorption, (2) high-affinity binding via the streptavidin-biotin system, and (3) covalent binding. Adsorption allowed binding of high amounts of active ASA. However, in presence of phosphate-buffered saline or serum rapid and complete desorption occurred, rendering this strategy ineffective for in vivo applications. In contrast, stable immobilization with negligible dissociation was achieved by high-affinity and covalent binding. Consequently, we analyzed the brain targeting of two stably nanoparticle-bound ASA formulations in $ASA^{-/-}$ mice, an animal model of MLD. Compared to free ASA, injected as a control, the biodistribution of nanoparticle-bound ASA was altered in peripheral organs, but no increase of brain levels was detectable. The failure to improve brain delivery suggests that the ASA glycoprotein interferes with processes required to target surfactant-coated nanoparticles to brain capillary endothelial cells.

1. Introduction

Lysosomal storage disorders (LSDs) are a group of >50 inherited diseases characterized by intralysosomal accumulation of undegradable macromolecules. LSDs have a combined incidence of about 1:8000, and most of the diseases affect the CNS [1]. Traditionally, LSDs are categorized according to the nature of the storage material. In the sphingolipidosis metachromatic leukodystrophy (MLD), the sulfated glycosphingolipid 3-O-sulfogalactosylceramide (sulfatide), a main lipid component of myelin, accumulates [2]. This eventually results in the demyelination of the CNS, progressive neurological symptoms and premature death of patients [3]. MLD is caused by a genetic defect resulting in reduced activity of the lysosomal enzyme arylsulfatase A (ASA), which is indispensable for the degradation of sulfatide [4].

Among the different treatment approaches proposed for LSDs, enzyme replacement therapy (ERT) is believed to have the most favorable benefit-risk ratio. It therefore represents the most promising option for the clinical management of the diseases to date [5]. Accordingly, ERTs for eight different LSDs have been approved for clinical use and several other ERTs are in clinical trials [6,7]. ERT aims at supplying recombinantly produced, active enzyme to the blood circulation of patients in order to reverse lysosomal substrate accumulation of cells and to ultimately ameliorate disease symptoms. On a molecular basis, most ERTs rely on the presence of mannose 6-phosphate (M6P) residues on the therapeutic enzyme. This molecular marker is attached to N-glycans during the de novo biosynthesis and maturation of lysosomal enzymes in the endoplasmic reticulum and Golgi apparatus. In the trans

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http://dx.doi.org/10.1016/j.jconrel.2017.02.016
0168-3659/

Golgi network the M6P-residues are recognized by M6P-receptors, which sequester their ligands from the secretory route and deliver them to a pre-lysosomal compartment. One of the two known M6P-receptors, the MPR300, also cycles between the plasma membrane and late endosomes (reviewed in [8]). Therefore, exogenously supplied lysosomal enzymes are also delivered to the lysosome. This endocytic pathway is the rationale for ERT.

Although ERT is a promising treatment option for a number of LSDs, the blood-brain barrier (BBB) limits its use for diseases with CNS pathology [9]. Indeed, in mouse models of MLD only repeated intravenous injections with high doses of ≥20 mg ASA/kg resulted in a reduction of sulfatide storage in the CNS and improvement of behavioral parameters [10,11]. Translated to the clinical situation, such a high-dose treatment would entail enormous and lifelong costs. A further drawback is the risk of dose-dependent adverse effects, including the expression of neutralizing antibodies and development of life-threatening anaphylactic reactions [5,12,13]. An option to reduce costs and side effects alike is to optimize transport of therapeutics to the CNS using specialized drug delivery systems.

Nanoparticles represent such a specialized drug delivery system. However, the nanoparticle field is complex, involving a large variety of core nanoparticles, drug loading strategies, and surface modifications, all impacting brain delivery [14]. Polymeric nanoparticles, for instance, have been successfully used as vehicles to deliver a number of small molecules [15,16] and proteins [17,18] across the BBB. The key to brain delivery of these nanoparticles seems to be their surfactant coating with polysorbate 80 or poloxamer 188 [16,19]. The mechanism underlying the transendothelial transport is still subject of scientific debate. However, increasing evidence suggests that surfactant coating mediates recruitment of endogenous apolipoproteins from the blood to the nanoparticle surface [20,21]. Subsequently, these apolipoproteins supposedly bind to members of the low density lipoprotein (LDL) receptor family expressed on the apical surface of brain capillary endothelial cells mediating transcytosis of the entire nanoparticle complex [18,22,23].

The potential of nanoparticles to increase efficacies of ERTs for LSDs with CNS pathology has received increasing attention in the last years [9,24–26]. So far, only one group has analyzed nanoparticle-mediated brain delivery of a recombinant human lysosomal enzyme in vivo [27,28]. However, the nanoparticles used were not biodegradable. To our knowledge, surfactant-coated, biodegradable nanoparticles have not been exploited to enhance brain delivery of a lysosomal enzyme. Recently, we analyzed the adsorption and desorption behavior of ASA and arylsulfatase B (ASB) to poly(butyl cyanoacrylate) (PBCA) nanoparticles in vitro [29,30]. Whereas stable adsorption of ASB could be achieved, results for ASA were discouraging as rapid desorption occurred in presence of serum. Here, we analyze the potential of several other nanoparticulate systems for ERT of MLD. To that end, we first optimized loading of ASA onto the nanoparticles comparing three binding strategies. Then, we analyzed the pharmacokinetics of two stably bound ASA-nanoparticle formulations after intravenous injections into $ASA^{-/-}$ mice.

2. Materials and methods

2.1. Materials

Recombinant human ASA was supplied by Zymenex (Hillerød, Dänemark). *N*-butyl- (2)-cyanoacrylate (Sicomet®) was purchased from Sichel-Werke, Hannover, Germany. The poly(lactic-co-glycolic acid) (PLGA) polymer (Resomer® 502H, molar ratio D,L-lactide:glycolide = 48:52 to 52:48, MM 7–17 kDa) was purchased from Evonik Röhm GmbH (Germany); polylactic acid (PLA, $\eta = 0.34$, MM ~39 kDa) was from LACTEL Absorbable Polymers (Birmingham, AL, USA). Amine-modified PLGA nanoparticles were purchased from Phosphorex (Hopkinton, MA, USA). Human serum albumin (HSA, fraction V, purity 96–99%), poloxamer 188, polysorbate 80, cystamine hydrochloride, and 4-nitrocatechol sulfate were purchased from Sigma-Aldrich (Schnelldorf, Germany). Biotin-PEG_2-maleimide, 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC), maleimide-activated neutravidin, and high-capacity streptavidin agarose were purchased from Pierce (Rockford, IL, USA). NHS-PEG_{12}-maleimide and 2-iminothiolane hydrochloride were purchased from Thermo (Waltham, MA, USA). Malhex-NH-PEG-O-C_3H_6-CONHS (5000 Da) was purchased from RAPP Polymere (Tübingen, Germany). Other, non-listed reagents were of analytical grade.

2.2. Preparation of nanoparticles

Nanoparticles made of the following four types of polymers were used: (i) PBCA, (ii) PLA, (iii) PLGA, and (iv) crosslinked HSA:

(i) PBCA nanoparticles were prepared by anionic polymerization, as described previously [29–31]. Briefly, 1% of *n*-butyl-(2)-cyanoacrylate was added to a 1% dextran 70000 solution in 0.01 M HCl whilst constantly stirring. After 2.5 h of stirring, the solution was neutralized using 0.1 M NaOH to stop the polymerization process. The mixture was filtered through a G2 sintered glass filter (Schott, Mainz, Germany) with a pore size of 40–100 μm and freeze-dried after addition of 3% of mannitol used as a cryoprotectant.

(ii, iii) PLA and PLGA nanoparticles were prepared as described before [16]. The polymer (250 mg) was dissolved in 5 mL of dichloromethane. The obtained solution was added to a 1% (w/v) aqueous solution of HSA (25 mL) and emulsified using an UltraTurrax disperser. Subsequently this pre-emulsion was homogenized at 1000 bar using a high-pressure homogenizer (Emulsiflex C5, Avestin, Canada). After having repeated this step three times, the organic solvent was removed using a rotary evaporator. The resulting nanosuspension was filtered through a glass filter with a pore size of 100–160 μm and freeze-dried after addition of 1% of mannitol used as a cryoprotector.

(iv) HSA nanoparticles were prepared as described before [32]. Briefly, 100 mg of HSA were dissolved in 1 mL of a 10 mM NaCl solution. Desolvation was achieved by adding 4 mL of absolute ethanol at a rate of 1 mL/min at pH 8 under constant stirring. Thus generated nanoparticles were stabilized by adding 118 mL of an 8% (w/v) glutaraldehyde solution. Nanoparticles were washed three times with water by centrifugation (16,100 × *g*, 8 min) and subsequent resuspension. HSA nanoparticles were stored in solution and used promptly.

All nanoparticle preparations were characterized regarding their size and surface charge using a nanosizer (Nano ZS, Malvern, UK).

2.3. Production of ASA_T365C variant

The known three-dimensional structure of human ASA [33] was exploited to introduce a unique crosslinkable sulfhydryl group onto the molecule's surface. For this purpose threonine 365 was exchanged by a cysteine residue. In vitro mutagenesis was carried out as described before [34] using the oligonucleotide 5′-CTGCTGCTGGGCTGTGGGAAGAGCCCTCGG-3′. After site-directed mutagenesis the mutated ASA cDNA was cloned into the expression vector pMPSVEH [35] harboring a hygromycin resistance cassette. Transfection, culture, harvesting, and purification were performed as described before for other recombinant ASA mutants [36]. Briefly, *Chinese Hamster Ovary Suspension* (CHO-S) cells were transfected by electroporation and single clones resistant to hygromycin (300 μg/mL) were screened for ASA-activity. A single clone with high activity was cultured in miniPERM bioreactors (Sarstedt) in serum-free ExCell 302 medium supplemented with HT supplement (both Sigma), 2 mM L-glutamine, 100 units/mL penicillin, and 100 g/mL streptomycin (all from Life Technologies). ASA_T365C was purified from the conditioned medium by immunoaffinity chromatography as described previously [37]. The activity of purified ASA_T365C was measured with the artificial substrate 4-nitrocatechol sulfate [38]. The concentration was determined by SDS-