PHARMACEUTICAL NANOTECHNOLOGY

Synthesis and Characterization of Surface-Modified PBLG Nanoparticles for Bone Targeting: *In vitro* and *In Vivo* Evaluations

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ABSTRACT: In this study, poly(γ-benzyl-l-glutamate) (PBLG) polypeptide derivatives were synthesized by ring-opening polymerization of amino acid N-carboxyanhydride using selected amine-terminated initiators. Alendronate, a targeting moiety that has a strong affinity for bone, was conjugated to PBLG. Monomethoxy polyethylene glycol (PEG) was used for a hydrophilic layer on the surface of the nanoparticles (NPs) to avoid reticuloendothelial system uptake. NPs were prepared by nanoprecipitation technique not only for PBLG or PBLG–PEG but also for composite polymers with different ratios. Fluorescein isothiocyanate would be attached to the NPs as a labeling agent. The size and morphology of NPs were evaluated by dynamic laser light scattering and transmission electron microscopy, and were found to be in a useful range (less than 80 nm) for bone-targeted drug delivery. In addition, the PEGylation of NPs was supported by isothermal titration calorimetry analysis. The bone-targeting potential of NPs was evaluated *in vitro* by calcium binding and hydroxyapatite affinity assays, and *in vivo* by fluorescent imaging experiments on rats. The targeted NPs showed bright fluorescent labeling in femur tissue. These results demonstrated the possibility of optimized NPs prepared with new PBLG derivatives to accumulate in bone successfully. © 2011 Wiley-Liss, Inc. and the American Pharmacists Association J Pharm Sci 100:4877–4887, 2011

Keywords: poly(γ-benzyl-l-glutamate); nanoparticles; alendronate; bone targeting; PEGylation

INTRODUCTION

Targeted drug delivery is the most promising way to reduce side effects of a specific drug. In terms of "active targeting," to deliver nanoparticles (NPs) to a desired site, site specificity is based on the affinities between targeting moiety and desired organ. The attachment of a specific moiety onto the surfaces of NPs can improve the targeting efficiency.[1] Many researchers have shown specific and efficient cellular uptake of particles that had been modified with a functional ligand with a high affinity for target cells.[2,3]

For a successful bone targeting, the selection of targeting moieties with affinity to bone plays a key role. When NPs reach the bone tissues, they should release the loaded drug without altering osteoblasts. The advantages of a bone-targeted drug delivery system for the treatment of bone-related diseases are obvious. Such a system could easily impart osteotropicity to a variety of bone drugs and improve their therapeutic efficacy. It is expected that the system not only increases patient comfort and compliance but also minimizes side effects of drug, especially for cancer agents. Skeletally targeted therapies have significant opportunity in the areas of osteoporosis prevention, cartilage repair, cancer treatment, fracture repair, and tissue engineering.[4–6]

Bisphosphonates (BPs) are widely used to treat diseases characterized by osteolysis and have an exceptional affinity to hydroxyapatite (HA), the mineral phase of bone which is not present in other tissues, their rapid binding at sites of osteoclastic activity, and their ability to inhibit bone resorption.[7] BPs

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display a common backbone structure of P–C–P, where C is carbon and each P is a phosphonate group. The two-phosphonate groups are essential both for binding to HA and for the biochemical mechanism of action. This peculiarity of BPs led to explore their utility as carriers of pharmacological agents for targeting bone tissues.[8] Among the BPs, the most important one is alendronate (ALD), which is well documented bone-targeting compound with strong bone affinity and has a primary amine useful for conjugation with our polymer.[9,10]

Polypeptides or poly(amino acid)s are very versatile synthetic materials that fulfil many important roles in natural systems. Poly(γ-benzyl-l-glutamate) (PBLG), a synthetic polypeptide, has attracted attention for biomedical applications because of the presence of a degradable amide bond in the polymer backbone and various chemical moieties can be quite easily introduced in the structure of PBLG to form various copolymers. The functional side group of -COOH in glutamic acid units can be modified by chemical reactions to form new molecular structures of the polypeptide copolymers. Nevertheless, the peptide bonds are actually biodegraded *in vivo* by peptidases. Consequently, due to their low immunogenicity, good biocompatibility, adjustable biodegradability, and excellent mechanical properties, polypeptide-derived copolymers have drawn tremendous attention for their potential biomedical applications (carriers of drug delivery, surgical sutures, implants for bone fixation, temporary matrices, or scaffolds in tissue engineering).[11]

Parenteral drug delivery systems based on polymeric NPs have been intensively investigated during the last decades covering a wide variety of drugs. NPs could modify the distribution of an active substance *in vivo* and increase its concentration in the target tissue, thereby improve its efficacy and reduce the toxicity.[12]

Nanoparticles are very rapidly opsonized in the bloodstream by phagocytic cells, following intravenous (i.v.) administration. To avoid the reticuloendothelial system (RES) uptake, there are two most often-mentioned criteria: the formation of a hydrophilic surface using polyethylene glycol (PEG) and obtaining particle size under 100 nm.[13] PEGylation simply refers to the decoration of a particle surface by the covalent grafting, entrapping, or adsorbing of PEG, which is widely used for the preparation of diblock and triblock polypeptide-based copolymers as biomedical materials for its good solubility in aqueous solution, lack of toxicity, immunogenicity, and ease of excretion from living organisms.[14] PEGylated nanoparticulate drug carriers made of polylactide homopolymers or poly(lactide-co-glycolide) heteropolymers copolymer with long-circulating properties present great potential for drug targeting and enhanced circulation times.[15]

The primary objective of our study was to synthesize novel PBLG derivatives to prepare surface-modified NPs with ALD and PEG for providing a bone-targeting drug delivery system. This study is the first report on the bone-targeting capacity of NPs prepared with these PBLG derivatives. Fluorescein isothiocyanate (FITC) was attached to the NPs as a model drug and it was confirmed visually by confocal laser scanning microscopy (CLSM). The presence of PEG onto NP surface was confirmed by isothermal titration calorimetry (ITC) experiments. The targeting capacity of NPs was also evaluated using an *in vitro* (calcium binding and HA affinity assays) and an *in vivo* (excised rat femur) approach.

MATERIALS AND METHODS

Materials

N,*N*-Dimethylformamide (DMF; Acros, 99%, Geel, Belgium) and benzylamine (Janssen Chimica, Beerse, Belgium) were distilled under reduced pressure over barium oxide and potassium hydroxide (KOH), respectively, and stored under argon atmosphere. γ-Benzyl-l-glutamate–N-carboxyanhydride (BLG–NCA) monomer, from ISOCHEM-SNPE (Paris, France), was used as received. Methoxy PEG amine (mPEG–NH_2), molecular weight (MW) = 5000 g/mol from Shearwater Corporation (USA) was dried separately under vacuum over P_2O_5 at 30°C for 24 h. FITC, HA, and commercially available PBLG (PBLG-com), MW = 37,000 g/mol^{-1} as determined by viscosity, were purchased from Sigma –Aldrich (Schnelldorf, Germany). ALD was supplied from CHEMOS GmbH (Regenstauf, Germany). α,ω-Disuccinimidyl ester PEG (Su-OOC-PEG-COO-Su), MW_{PEG} = 6000 Da, was obtained from IRIS Biotech (Marktredwitz, Germany). Purified water by reverse osmosis was used (MilliQ®; Millipore, USA). All other solvents and chemicals were of analytical grade.

Synthesis and Characterization of PBLG Derivatives

Poly(γ-benzyl-l-glutamate) derivatives (PBLG–Bnz, PBLG–PEG, and PBLG–FITC) were obtained by anionic ring-opening polymerization of BLG–NCA initiated by Bnz, mPEG–NH_2, or terminated by addition of FITC, respectively, in DMF, using a slightly modified method described in the literatures.[16,17]

All initiator solutions were prepared under argon atmosphere and used immediately. (Bnz solution: A 0.1 mol/L solution was prepared by diluting freshly distilled benzylamine in freshly distilled DMF. mPEG–NH_2 solution: Dried mPEG–NH_2 was dissolved, at 30°C, into freshly distilled DMF to prepare