Development of Bone-Targeting Nanoparticles for Bone Cancer Therapies

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Statement of Purpose: We hypothesize that bone-targeting nanocarriers can preferentially accumulate in the skeleton and locally release proteasome inhibitors (PI) to impair the capacity of myeloma cells to survive and grow in vivo, thereby reducing the formation and growth of tumor-induced lytic bone lesions. PIs are not selective to bone and their therapeutic-toxic window may be narrow when administered systemically. Targeted bone delivery has potential to reduce systemic exposure, increase efficacy in the bone environment, and the opportunity to reverse catastrophic disease processes. Site-specific targeting requires quantitatively distinct receptors. We selected the calcified matrix as our initial site for bone-targeting. We identified bone-binding ligands and selected two well-known for their predilection to bone surfaces, methylene bisphosphonate (MBP)[1] and an aspartic acid oligopeptide (Asp_n)[2]. We present work on the development and characterization of bone-targeting nanoparticles to be used in our preclinical studies.

Methods: Polylactide-co-glycolide (PLGA, 75/25, Brookwood) was used to prepare nanoparticles. Functional polyethylene glycols (PEG) were obtained from NOF Corp. Asp_4 was obtained from Sigma. PLGA-b-PEG was prepared by conjugating PLGA to an amine-terminated mPEG via DCC/NHS coupling. Maleimide-terminated PEG-b-PLA copolymer was prepared by ring-opening polymerization of lactide on to a hydroxyl-terminated bifunctional PEG. Maleimide was added to the PEG terminus by reaction with 3-maleimidopropionic acid NHS ester. Amino-MBP was synthesized by modified reported method [3]. Either aMBP or Asp_n was linked via sulfhydryl-amino conversion to the functionalized copolymer. Ligand conjugation was monitored by sulhydryl. All structures were confirmed by ^{1}H-NMR. Nanoparticles were prepared by either emulsification/solvent-loss or nanoprecipitation. Solvent was removed by evaporation. Particles were purified by ultracentrifugation or cross-flow filtration and lyophilized. Various lyoprotectants were studied. Particle size was determined by photon correlation spectroscopy and zeta-potential measured at pH 7.4. Preferential binding of ligand-containing nanoparticles to hydroxyapatite substrates was determined using radiolabeled nanoparticles. Model PIs were encapsulated and their release profiles determine in vitro.

Results/Discussion: In previous work, we prepared ligand-containing liposomes and showed these nanocapsules preferentially adhered to hydroxyapatite substrates in vitro [4]. However, encapsulating hydrophobic actives in liposomes is problematic. Therefore, we turned to polymer nanoparticles as an alternative. Nanoparticles were prepared from PLGA/PLA-PEG blends. Nanoparticles prepared by emulsification ranged in size from 150nm to about 200nm, depending on the PEG content, with particle size decreasing with increasing PEG content. Similarly, zeta-potential decreased with increasing PEG content, suggesting shielding of the PLGA surface by the surface PEG groups, although nanoparticle dispersion stability was not affected.

Nanoparticles prepared by nanoprecipitation ranged in size from about 75nm to about 150nm, depending on solvent choice, polymer concentration, and PEG content. Lyophilization of nanoparticle dispersions resulted in irreversible agglomeration. Traditiona lyoprotectants, such as oligosaccharides, reduced agglomeration, but only at concentrations of 0.5% or higher. Pluronic F68 provided excellent lyostability at considerably lower concentrations.

Structural analysis of the custom synthesized maleimide-PEG-b-PLA polymer indicated the PLA segment had a length of about 710 units, or a M_w of about 50k. Attachment of the maleimide group had a conversion of 100% and ligand attachment to the maleimide was similarly determined to occur with high conversion. Ligated polymers were incorporated into PLGA nanoparticles during preparation in compositions up to about 10% wt. The gamma emitter, ^{99m}Tc, was encapsulated into nanoparticles with efficiency typically of about 20% for radiolabeling. The preferential adherence of these ligand-containing, radiolabeled nanoparticles was evaluated with several hydroxyapatite powders. Attachment of ligand-containing nanoparticles was found to occur in a Langmuir-like behavior. Nanoparticles without targeting ligands had no significant affinity for the HAp substrates.

Proteasome inhibitors PS-1, PS-IX, and MG262 were encapsulated into nanoparticle formulations with encapsulation efficiencies up to about 50%, resulting in payload compositions of typically less than about 10% wt. Particles and payload remained stable through lyoprotection. In vitro release studies showed the actives to be completely released within 1 – 3 weeks.

Conclusions: We prepared and verified the structure of bone-targeting ligands conjugated to biodegradable polymers and their use in the formation of polymer nanoparticles. These nanoparticles could be made lyostable with Pluronic F68 being effective at lower, reasonable concentrations than oligosaccharides. We further showed that specifically formulated bone-targeting nanoparticles preferentially adhere to bone-like surfaces. Cancer drugs could be encapsulated in these nanoparticles with reasonable efficiencies and payloads, and further released under in vitro conditions.

References: 1. Davis, et al., Semin Nucl Med. 1976; 6:19-31. 2. Kasugai, et al., J Bone Miner Res. 2000; 15:936-943. 3. Kontoci, et al. Synth Comm. 1996;26:2037-2043. 4. Vail, et al., CRS, 2003.