Less Phagocytosis of Biotinylated Polymeric Prodrug Micelles Using CD47-Streptavidin Fusion Protein

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Statement of Purpose: The success of therapeutic carriers is reliant upon the mode of administration. Each route of administration plays a marked role in the synthesis of a therapeutic carrier. While each route of delivery has its own pros and cons, intravascular injection due to its precise and almost immediate onset of action is widely employed for drug delivery. However, intravenous injection must overcome the barriers set by the mononuclear phagocyte system (MPS) if prolonged blood circulation is desired. To this end, biomimetic strategies are being exploited in my group to camouflage drug carriers in order to bypass immune surveillance, thereby prolonging the circulation time in bloodstream and leading to effective delivery of therapeutic agents. CD47 protein, known as a self-marker on red blood cells (RBCs), regulates phagocytosis by its interaction with signal regulatory protein alpha (SIRPα) on macrophages. Through CD47 ligation with SIRPα, Fcγ and complement receptor mediated phagocytosis is repressed. If the inhibitory signals generated by CD47-SIRPα association are sufficient to counteract the phagocytic signals mediated through Fcγ and the complement receptors, ingestion of particles (e.g., RBCs or CD47-tagged drug carriers) is reduced or inhibited.

Methods: The extracellular domain cDNA of mouse CD47 was inserted into a plasmid containing the coding region of core streptavidin to obtain pMA006 plasmid encoded with the CD47-streptavidin (CD47-SA) gene sequence. The BL21CodonPlus bacterial strain was transformed with pMA006 plasmid. Expression of CD47-SA fusion protein was achieved in BL21(DE3) host cells following induction with 1 mM IPTG for 1 hr. The CD47-SA fusion protein was purified from bacterial lysate by biotin-agarose affinity chromatography. Polymeric prodrug micelles for delivery of acyclovir (ACV) were synthesized. First, ACV was used directly to initiate ring-opening polymerization of ε-caprolactone to form ACV-polycaprolactone (ACV-PCL). Through conjugation of hydrophobic ACV-PCL with hydrophilic polyethylenimine (PEI), polymeric micelles for drug delivery were formed. ^{1}H NMR, FTIR, and gel permeation chromatography were employed to show successful conjugation of PEI to hydrophobic ACV-PCL. Through dynamic light scattering, zeta potential analysis, transmission electron microscopy, and critical micelle concentration (CMC), the synthesized ACV-tagged polymeric micelles were characterized. The formed polymeric prodrug micelles were biotinylated via the interaction of biotin-NHS with the amines on cationic polymers. CD47 was bound to biotinylated polymeric prodrug micelles (pre-labeled with FITC) via biotin-SA affinity.

Results: It was found that the average size of the polymeric micelles was under 200 nm and the CMCs of ACV-PCL-MPEG and ACV-PCL-chitosan were 2.0 mg/L and 6.6 mg/L, respectively. As shown in Fig 1, the prepared CD47-polymeric prodrug micelles (labeled with FITC) interacted with J774A-1 macrophages for 6 hours, and revealed significant less phagocytosis compared to non-CD47-tagged polymeric prodrug micelles which reveals strong green fluorescence. This was due to a sufficient density of CD47 bound onto the polymeric micellar particle surface via biotin-streptavidin affinity.

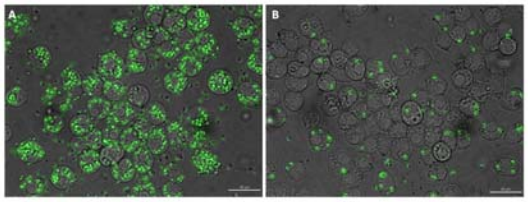

Fig 1. Antiphagocytic potency of zymosan microparticles bound with CD47 ligand and FITC fluorescent dye. The CD47-tagged polymeric prodrug micelles were homogenously layered on top of murine J774A.1 macrophages. For the distinction between internalized and surface-bound FITC-labeled polymeric micelles, trypan blue was used to quench surface-bound fluorescence. The microscopic images of macrophages treated respectively with (A) biotinylated polymeric prodrug micelles and (B) CD47-tagged polymeric prodrug micelles for 6 hours (scale bar = 40 μm).

Conclusions: Here we report a biomimetic approach of functionalizing synthetic particulates with CD47 self-marker proteins to endow drug delivery carriers with phagocytosis-resistant features. CD47-streptavidin fusion protein was constructed and attached to the biotinylated nanoparticles. Our results revealed that CD47 could reduce macrophage-mediated clearance of particles via its interaction with signal regulatory protein alpha on macrophages.