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Targeting fibroblast-like synovial cells at sites of inflammation with peptide targeted liposomes results in inhibition of experimental arthritis

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Abstract In this study we examined a synovium-specific targeted liposomal drug delivery system for its ability to localize and release its drug cargo to inflamed joints. Targeted liposomes were tested in vitro for binding to synovial fibroblast like (FLS) and endothelial cells using flow cytometry and in vivo for localization to joints using a rat model of adjuvant induced arthritis (AIA). Targeted liposomes were then loaded with anti-arthritic medications and examined for clinical efficacy in AIA. Targeted liposomes specifically bound to rabbit FLS and human FLS and showed a 7–10 fold increase in vivo localization in affected joints compared to unaffected joints. Histological sections from rats treated with prednisone and a new immunosuppressive peptide CP showed minimal inflammation. This report substantiates the ability of the novel FLS sequence to target liposomal drug delivery and offers an alternative therapeutic approach for the treatment of arthritis.

1. Introduction

Rheumatoid arthritis (RA) is a systemic autoimmune disease that is characterized by recruitment of inflammatory cells into the synovium leading to synovial hyperplasia, neovascularization and erosion of cartilage and bone with subsequent joint

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destruction and deformity. Although the pathogenesis of RA has not yet been fully elucidated, the focus of inflammation is the synovium lining the diarthrodial joints. The two predominant cell types within the synovial membrane consists of macrophage-derived type A synoviocytes and fibroblast-derived type B synoviocytes (FLS) characterized by the expression of UDP-glucose-6-dehydrogenase, an enzyme required for the production of hyaluronic acid, and of CD55 known as complement decay-accelerating factor [1]. The latter, type B synoviocytes play a pivotal role in the maintenance of joint fluid volume and secretion of hyaluronan into the joint space for lubrication [2,3] and have been receiving increasing recent research interest as key players in the initiation and perpetuation of destructive joint disease [1,3–5].

Based on our current understanding of the pathogenesis of RA the goal of treatment is to prevent inflammation and thereby prevent joint damage and bone destruction. Ideally direct targeting of FLS or other joint markers with localizing nanoparticles loaded with drugs aimed at modifying the disease course would provide specificity and decrease systemic side effects. Investigating new strategies to identify synovial biomarkers and improve drug delivery and bioavailability to inflamed joints has been an important research goal in the treatment of RA and has drawn recent attention of many investigators [6]. A considerable volume of experimental data already exists in the cancer literature examining ways to reduce tumor growth by targeting angiogenic endothelial cells overexpressing $\alpha_v\beta_3$ integrins at sites of tumor neovascularization. In particular, anti-angiogenic drugs have been directly attached to an integrin binding peptide RGD [7] or the RGD peptide anchored to liposomes bearing anti-cancer chemotherapy drugs and assessed for eradication of primary cancer cells or prevention of their metastasis [8]. The neovascularization seen in the rheumatoid pannus is not too dissimilar to the rapidly growing tissue in tumors forming the basis for a similar investigative approach to RA. In this respect, liposomes conjugated with cyclic RGD and loaded with prednisolone have already been investigated and shown to successfully target the neovasculature of inflamed joints thereby alleviating arthritis in animal models of the disease [9,10]. Although the cyclic peptide ligand attached to PEGylated liposomes successfully targets the neovasculature in inflamed joints, it is not synovium specific.

Mi et al. [11] identified a peptide, which they termed HAP-1 (SFHQFARATLAS) that demonstrated specificity for FLS. Our group using chimeric peptide constructs have confirmed these findings and shown that linkage of synovial homing peptide HAP-1 to an immunosuppressive peptide (core peptide, CP) provides greater drug localization and bioavailability to inflamed joints [12]. CP is a short nine amino acid peptide (GLRILLLKV) corresponding to the T-cell antigen receptor (TCR)-alpha transmembrane region known to be an effective immunosuppressant able to decrease inflammation in the adjuvant induced arthritis (AIA) rat model [13,14]. Given the therapeutic efficacy of CP in the treatment of rats with AIA and our interest in targeted delivery strategies the efficacy of prednisolone- and/or CP-loaded and joint-targeted long circulating PEGylated liposomes in arthritic rats was examined.

This study describes conjugation of targeting peptides [RGD, HAP-1 and a scrambled HAP-1 peptide (ALSQAFRHAFTS; sc-HAP-1)] to the surface of long circulating PEGylated liposomes. Localization of these targeted liposomes was monitored using near-infrared fluorescence (NIRF) imaging. Arthritic rats given targeted liposomes loaded with prednisolone phosphate or CP were monitored clinically and at the end of the experiment histologically. The conclusion from these experiments were: the development of new and specific drug delivery system for targeting FLS cells which may provide interesting therapeutic and investigational opportunities for OA and RA; the verification of efficacy of anti-arthritic peptide CP as an adjunct or alternate therapy for RA; and the therapeutic potential of nanoparticles in the treatment of inflammatory joint disease.

2. Methods

Peptides and liposomes used in these experiments were constructed, purified and characterized in the Rheumatology Dept., Westmead Hospital, Sydney. Peptide sequences attached to PEG on targeting liposomes included an additional cysteine on the N-terminal of the sequence, Cys-SFHQFARATLAS (Cys-HAP-1) and Cys-ALSQAFRHAFTS (Cys-scHAP-1).

2.1. Preparation of liposomes

Liposomes composed of the lipids 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC), cholesterol (Chol), and 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-N [methoxy (polyethylene glycol)-2000] (DSPE-PEG; Avanti Polar Lipids Alabama, USA) in a molar ratio of 1.85:1.0:0.15 were prepared. For the preparation of targeted liposomes 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-N-[maleimide(polyethylene glycol)-2000] (DSPE-PEG-maleimide) (Avanti Polar Lipids Alabama, USA) was added to enable the attachment of a ligand. The molar ratio of lipids here used was DPPC:Chol:DSPE-PEG and DSPE-PEG-Mal 1.85:1:0.075:0.075. For flow cytometry and in vivo localization studies the fluorescent marker1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate (DiL; Invitrogen, Melbourne, Australia) was incorporated into liposomes at a molar ratio of 0.075. All preparations were carried out in the dark. Liposomes were sized by sequential extrusion (at least 11×) with a Mini-Extruder (Avanti Polar Lipids, Alabama, USA), using polycarbonate membrane filters (Avanti Polar Lipids, Alabama, USA) with gradually decreasing pore size of pore diameters of 400, 200 and 100 nm. The morphology, mean diameter, size distribution and zeta potential were characterized [15].

2.2. Preparation of targeted liposomes

Synovial homing peptides were attached to the distal end of the DSPE-PEG-maleimide moiety by the formation of a thio-ether bond between the maleimide derivatized PEG and a terminal cysteine on the peptide ligands. Reaction conditions were first optimized in solution by following the reaction between maleimide functionalized lipid and the cysteine–peptide by TLC. Using these conditions the homing peptide was attached to the liposomal surface using a chosen