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Biomaterials 26 (2005) 661–670
Biomaterials
www.elsevier.com/locate/biomaterials

Characterization of biodegradable drug delivery vehicles with the adhesive properties of leukocytes II: effect of degradation on targeting activity

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Received 19 December 2003; accepted 13 March 2004

Abstract

The site-specific expression of selectins (P- and E-selectin) on endothelial cells of blood vessels during inflammation provides an opportunity for the targeted delivery of anti-inflammatory drugs to sites of chronic inflammation. It is well documented that the selectins mediate the initial interaction (rolling) of leukocytes in an inflamed vessel by binding to carbohydrate-presenting counter-receptors displayed on leukocytes. Previous work in our laboratory has shown that artificial capsules with the adhesive properties of leukocytes can be made by attaching leukocyte adhesive ligands to polymer microspheres (Biomaterials 23(10) (2002) 2167). Specifically, we showed that drug-loaded poly (lactic-*co*-glycolic-acid) (PLGA) microspheres coated with biotinylated-Sialyl Lewis^X (sLe^X), a carbohydrate that serves as a ligand to selectins, mimic the adhesive behavior of leukocytes on selectins in flow chambers, displaying slow rolling under flow, suggesting that these drug-loaded particles can potentially target inflammatory sites in vivo. Since the effectiveness of this delivery system might depend on the degradation of polymer microspheres as well as the degradation of sLe^X molecules, we measured the effect of polymer and ligand degradation on the adhesiveness of microspheres over time. We show that degrading sLe^X microspheres maintain the ability to recognize selectin surfaces under flow for at least 2 weeks and that the ability to sustain recognition depends upon the extent at which microspheres are loaded. We also show that microsphere rolling velocity increases as microsphere degrade and that this increase is due to a combination of increase in average microsphere size and loss of sLe^X molecules on microsphere surface—a result of microsphere degradation confirmed by flow cytometry. Control experiments show that microsphere, not sLe^X, degradation limits the lifetime of our targeted delivery system; therefore, factors affecting degradation such as type of polymer, type of drug, extent of drug loading and microsphere size, provide an opportunity for engineering the time-scale of activity for the delivery system.

Keywords: Selectins; Drug targeting; Poly (lactic-*co*-glycolic acid) (PLGA); Inflammation; Leukocytes; Degradation

1. Introduction

Targeting therapeutics to specific sites in the body is attractive because it potentially allows for the administration of potent therapeutic agents to diseased cells/tissues without affecting normal ones, thereby enhancing drug efficacy without generating deleterious side effects. Several avenues exist for drug targeting depending on the intended site of delivery and the targeted therapeutic. Some of the avenues that have been exploited include targeting via monoclonal drug-antibodies hybrids, immuno-liposomes, cellular carriers and antibody/receptor-linked biodegradable particles [1–3]. Drug carriers such as liposomes and polymer microspheres are often preferred over molecular conjugates and protein carriers because these carriers protect drug from degradation or digestion. However, biodegradable polymer carriers have advantages over lipid vesicles, such as stability and sustained release, and are increasingly finding application in several human diseases because they offer flexibility in design, allowing for high control over drug loading and the dynamics of release [4].

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doi:10.1016/j.biomaterials.2004.03.003

Much has been written regarding the preparation and use of biodegradable polymer microspheres, such as poly(lactic-*co*-glycolic acid) (PLGA), for targeted delivery of therapeutics in several diseases [3,5–7]. However, most work has focused on the fabrication and/or characterization of the physical properties of drug-loaded particle. Some work has focused on the conjugation of targeting ligands to the surface of biodegradable carriers and tests of functionality of targeted system. To our knowledge, no work exists describing the lifetime of a targeting system. We expect that the effectiveness of targeted, biodegradable delivery system over time will depend on the rate of degradation, since the targeting (adhesive) ligand is often linked to the biodegradable polymer chain.

We recently described a novel approach for targeting therapeutic agent to the vascular wall in inflammatory diseases using biodegradable polymer microspheres made from PLGA, to which we attached selectin ligand, sLe^X [8]. Ligand attachment to microspheres was achieved by covalently linking the protein NeutraAvidin, which strongly binds biotin, to the carboxylic acid end-group on the terminal lactic acid chain thereby allowing the attachment of biotinylated sLe^X molecules. We showed that these sLe^X-coated carriers transiently adhere to (roll on) P-selectin surfaces, similar to how leukocytes roll on P-selectin under flow, and that the adhesion can be tuned by changing the density of sLe^X on the microsphere surface, thus setting the rolling velocity in blood vessels [8]. Targeted delivery to the endothelium in inflammatory diseases via the selectins (E- and P-selectin) is attractive because of their localized and carefully regulated expression patterns in response to inflammatory mediators, such as histamine and tumor necrosis factor (TNF) [9,10].

Since the PLGA drug carriers are degradable, we expect that the lifetime of selectin-recognition of targeted sLe^X-particles will depend on the rate and mechanism of degradation of the polymer PLGA. Whether the bulk of adhesion is lost early during degradation depends on whether the surface or the bulk of the particle erodes first. Langer's lab has shown that PLGA particles degrade via bulk erosion [9,11], suggesting our particles will be active for an extended period of time. Here, we present the results obtained from in vitro assays designed to characterize the possible effect of polymer and ligand degradation on the selectin-recognition ability of this targeted delivery system. We show that degrading sLe^X microspheres maintain the ability to recognize selectin surfaces (roll) under flow for up to 3 weeks in solution and that the time-scale of recognition is directly related to the time-scale of degradation. Overall, understanding the relationship between the properties of degradable polymer carriers that prescribe the rate of degradation and the lifetime of targeting will help develop sophisticated targeted, degradable drug carriers with tunable life-times and activities.

2. Experimental methods

2.1. Materials

50,000 MW, acid-end, 50/50 PLGA was purchased from Alkermes Inc. (Cincinnati, OH). Methylene chloride, NeutraAvidin, diclofenac sodium salt (DSS), bovine serum albumin (BSA), polyvinyl alcohol (MW ~ 70,000), Dulbecco's phosphate buffered saline (DPBS), and Tris base were purchased from Sigma (St. Louis, MO). Biotinylated, multivalent sialyl $Lewis^X$ (sLe^X) carbohydrate was purchased from Glycotech (Rockville, MD). Soluble P-selectin (sP) was a generous gift from Drs. Raymond T. Camphausen and Gray Shaw, Wyeth (Cambridge, MA). Anti-P-Selectin, mAbs 9E1 was purchased from R&D systems (Minneapolis, MN). Anti-sLe^X mAb (HECA-452), FITC-labeled anti-rat IgM, and FITC-labeled anti-mouse IgG_1 were purchased from Pharmingen (San Diego, CA). Quantum 26 calibration beads were obtained from Flow Cytometry Standards Corporation (San Juan, PR). Rhodamine 6G (R6G) was purchased from J.T. Baker (Philipsburg, NJ). 10-μm diameter streptavidin-coated beads were purchased from Bangs laboratories (Fishers, IN).

2.2. Microsphere fabrication

Microsphere fabrication was as previously described [8] with minor modifications. Briefly, 50-mg PLGA was completely dissolved in 10-ml of R6G in methylene chloride (2.5% or 8% of polymer weight) or 10-ml of plain methylene chloride (oil phase). The oil phase was injected into 100-ml of 0.3% PVA and stirred at 1800 rpm using a Lightnin' mixer with a glass propeller (Chagrin Falls, OH) for an hour. After solvent evaporation, solidified microspheres were recovered by centrifugation, and recovered microspheres were frozen and placed on a Freezone 4.5 lyophilizer (Labconco, Kansas City, MO) overnight to dry. The resulting powder was stored at -20°C. Drug-loaded (Diclofenac Sodium Salt, DSS) microspheres were prepared according to the co-solvent method described by Tunçay et al. [12] with methanol as the co-solvent, and a full account of this method can be found in [8].

2.3. Microsphere preparation

Carbodiimide chemistry was used to covalently link amino groups of NeutrAvidin protein to activated carboxylic acid groups on the surface of PLGA microspheres. Details of experimental protocol is described in