EUROPEAN JOURNAL OF PHARMACEUTICAL SCIENCES 36 (2009) 474–485

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Specific and non-specific phagocytosis of ligand-grafted PLGA microspheres by macrophages

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ARTICLE INFO

Article history:
Received 31 July 2008
Received in revised form
25 November 2008
Accepted 27 November 2008
Published on line 6 December 2008

Keywords:
PLGA microspheres
Functionalization
Ligands
Macrophages
Phagocytosis

ABSTRACT

We evaluated the influence of ligand grafting on the rate and intensity of uptake of poly(D,L-lactide-co-glycolide) microparticles by alveolar macrophages. Microspheres with a mean diameter of 2.5 μm were obtained by spray drying. Three ligands (WGA, an RGD containing peptide and mannose-PEG_3-NH_2) and a cationic molecule (PLL) were covalently grafted on the particle surface using the carbodiimide method. Their grafting efficiency was quantified, and WGA grafting was characterized by confocal laser scanning microscopy (CLSM) and by atomic force microscopy (AFM). The uptake by macrophages of surface-modified microspheres was quantified by CLSM. This work showed that the uptake of negatively charged ligand-grafted microspheres (−26 to −51 mV) was increased up to two to four times according to the ligand compared to ungrafted microspheres (−81 mV) and displayed saturation as opposed to the cationic PLL-grafted microspheres. Moreover, a specific receptor-mediated phagocytosis mechanism was suggested based on free ligand, cytochalasin D and +4 °C incubation that decreased the microparticle uptake. Furthermore, this work clearly showed that the relative contribution of specific and non-specific processes to the overall uptake varied greatly according to the ligands, and was dependent on the particle-to-cell ratio. In conclusion, this work showed that ligand grafting can enhance the uptake of microparticles, with a variable relative contribution of specific and non-specific uptake mechanism.

1. Introduction

Particulate systems in the micron and submicron level are increasingly used for biomedical applications as a result of their ability to allow a cellular drug targeting and/or a controlled drug release (Chavanpatil et al., 2006; Panyam and Labhasetwar, 2003; O'hagan and Singh, 2003). Nanoparticles are mainly used for their cellular targeting ability follow-

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doi:10.1016/j.ejps.2008.11.013

ing systemic administration, while microparticles have been used for many years as controlled release delivery systems for drugs and therapeutic proteins. Moreover microparticles offer the advantage of a higher payload especially for hydrophilic drugs in particles, and a better ability to provide a controlled release. This point is of interest for antigen delivery since it has been suggested that the release rate influence the response (Waeckerle-Men et al., 2006). Among the applications for microparticles, the delivery of recombinant proteins, plasmid DNA and peptides for antimicrobial vaccination is growing (Davis, 2006; O'hagan et al., 2006; Reddy et al., 2006; Niborski et al., 2006).

For vaccine application, administration via the mucosal route has been shown to provide an advantage over systemic administration (Mutwiri et al., 2005; Sedgmen et al., 2004; Vyas and Gupta, 2007), namely the production of a mucosal immunity. In addition, compared to systemic vaccination leading only to systemic Ig induction, mucosal vaccination induces both a systemic and a mucosal immunity with the production of IgAs at the epithelia a common site of pathogen entry. Among the mucosal routes, intranasal administration of antigen delivery systems has been largely studied (Byrd and Cassels, 2006; Jaganathan and Vyas, 2006; Stanley et al., 2004; Strindelius et al., 2004). The combination of alternative immunization routes and the use of appropriate antigen delivery systems appear to be a rational approach for providing an effective mucosal immunity. Microparticles based on poly(lactide-co-glycolic acids) (PLGAs) have been studied for this application because of their safety record, e.g. used as controlled delivery of drugs (Jain, 2000).

For an efficient mucosal vaccination using microparticles, it is necessary to maximize their uptake by the cells of interest, i.e. antigen-presenting cells (APCs), since a low level of particle uptake by cells is a limiting factor. Furthermore, diffusional barriers at the mucosal level impede the local trafficking of microparticles throughout the mucosa, especially at the gut-associated lymphoid tissue (GALT) level (Torche et al., 2006). Many parameters can influence cellular uptake efficiency of microparticles namely, diameter, nature and hydrophobicity of the polymer and the surface properties (Carr et al., 1996; Desai et al., 1997; Thiele et al., 2003, 2001; Torche et al., 2000). Among surface properties, the surface charge (i.e., zeta potential) has been focussed upon and, contrary to nanoparticles, little work has been undertaken on the influence of the presence of ligands at the surface. For several years, many studies performed on nanoparticles have suggested that different molecules such as lectins, invasins and peptides can be used as targeting molecules to increase the particles uptake by cells. Lectins are glycoproteins appropriate for targeting as wheat germ agglutinin (WGA) from *Triticum vulgare*, because they can bind to specific oligosaccharides present at the surface of different cell types, and many authors have used these molecules to evaluate these cytoadhesive properties (Russell-Jones et al., 1999; Weissenboeck et al., 2004). The use of invasin as a cellular ligand has been tested using latex and PLGA nanoparticles to demonstrate an increase of particulate uptake in caco-2, Hep2 2B and MDCK cells (Dawson and Halbert, 2000; Hussain and Florence, 1998).

Integrins constitute a family of transmembrane receptors involved in cell–cell and cell–matrix interactions and are central players in signal transduction pathways. This led many groups to design selective arginine–glycine–aspartate (RGD)-containing ligands to specifically target the integrins. The RGD serves as a model ligand for the specific interaction with integrin receptors, which are expressed on the surface of phagocytic cells. The C-type lectins family comprises a number of pathogen recognition receptors that bind to carbohydrate ligands. The targeting of C-type lectins receptors on APCs was identified as a promising strategy for immunomodulation, as documented by several studies showing that immune responses were enhanced or modified by coupling mannose type ligands (Cui et al., 2004; Jain and Vyas, 2006). The study of these three ligands (WGA, RGD and mannose) was considered as relevant approach. Indeed, the use of these ligands can lead to applications beyond macrophages targeting and vaccination.

To our knowledge, there is only one study showing an increase in the cellular uptake of ligand-grafted microparticles (Thiele et al., 2003), as opposed to nanoparticles for which this is highly documented. Indeed, ligand grafting onto microparticles may present advantages compared to nanoparticles since they allow a better controlled release of the drug and a higher payload. In the study of Thiele et al. (2003), phagocytosis of IgG coated carboxylated polystyrene microparticles was significantly enhanced in dendritic cells and macrophages. Another study with an RGD-motif onto 4.5 μm PLL-g-PEG carboxylated polystyrene microparticles led to ligand-specific phagocytosis by APCs, but did not specifically address the influence of ligand on uptake. Indeed, they showed that the grafting of RGD abolished the repellent nature of particles (Faraasen et al., 2003).

However, the uptake mechanisms of microspheres remain unclear, and eventhough a ligand is grafted onto microspheres, specific and non-specific uptake can simultaneously occur and their relative contribution to overall uptake should be studied. For that purpose, the comparison of different ligands in increasing the uptake should be carried out. Endocytosis can be divided into two categories, 'pinocytosis' or cell drinking (fluid and solutes) and 'phagocytosis' or cell eating (large particles) (Conner and Schmid, 2003). Pinocytosis is a well described mechanism of uptake of small particles or nanoparticles (Dawson and Halbert, 2000; Mo and Lim, 2004; Russell-Jones et al., 1999). Pinocytosis has four basic mechanisms of action: macropinocytosis, clathrin-mediated endocytosis (CME), caveolae-mediated endocytosis, and clathrin- and caveolae-independent endocytosis. The mechanism of uptake of WGA-conjugated PLGA nanoparticles by A549 cells has been described as an endocytosis by a receptor-mediated, caveolae-dependent pathway (Mo and Lim, 2004). Phagocytosis is a mechanism for uptake of large pathogens such as bacteria or yeast, and particles by specialized cells including macrophages. Phagocytosis may be specific (requiring contact with a receptor) or non-specific (Camner et al., 2002).

The aim of this study was to compare, for uptake of PLGA microparticles, the influence of different ligands on the rate and intensity of their phagocytosis by pig alveolar macrophages, and to estimate by mathematical analysis the relative contribution of the specific and non-specific mechanisms. We selected three ligands: a lectin wheat germ