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PLA-PEG particles as nasal protein carriers: the influence of the particle size

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Abstract

Previous studies have shown that PLA-PEG nanoparticles (NP) are able to enhance the transport of the encapsulated model protein, tetanus toxoid (TT), across the rat nasal mucosa. The aim of this work was to study if the size of PLA-PEG particles affects the nasal transport of the encapsulated protein and, also, the potential contribution of blank nanoparticles to the transport of the free protein. To achieve this purpose, ^{125}I-TT was encapsulated into PLA-PEG particles of different sizes (200 nm, 1.5, 5 and 10 μm) prepared by the water-in-oil-in-water solvent evaporation technique. Firstly, in order to investigate the carrier role of the particles, two series of either conscious or anaesthetized rats were nasally treated with ^{125}I-TT-loaded NP, free ^{125}I-TT, and a physical mixture of blank NP and free ^{125}I-TT. Secondly, the influence of the particle size on the nasal transport of TT encapsulated into PLA-PEG particles was evaluated in conscious rats. The amount of radioactivity recovered in the blood compartment, lymph nodes and other relevant tissues was monitored for up to 24 h. Finally, the nasal bioavailability of ^{125}I-TT-loaded PLA-PEG NP was calculated. The results indicated that the use of anaesthesia enhances the transport of ^{125}I-TT and that the physical presence of PLA-PEG NP does not affect the transport of the toxoid. In contrast, when TT was encapsulated into the particles its transport across the nasal mucosa of conscious rats was significantly enhanced. Furthermore, the efficacy of this transport was related to the particle size, reaching the most important transport for the smallest particle size. The intensity of this transport was also illustrated by the high nasal bioavailability of TT encapsulated into nanoparticles (200 nm) (F = 70–80%). These results led us to conclude that PLA-PEG NP can be accepted as nasal protein carriers for nasal administration.

Keywords: PLA-PEG; Microparticles (MP); Nanoparticles (NP); Nasal administration; Protein delivery; Vaccine delivery

1. Introduction

At the beginning of the nineties, Gref et al. (1994) presented PLA-PEG nanoparticles (NP) as long

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circulating systems for intravenous administration. This initial work has been followed by a number of reports that have shown the potential of these new carriers as controlled release systems for parenteral administration (Peracchia et al., 1999; Verrechia et al., 1995). Over the last years, we have attempted to explore the potential of these systems as transmucosal carriers for proteins (Tobío et al., 1998, 2000). With this idea in mind, we encapsulated radiolabelled TT into PLA and PLA-PEG NP and evaluated the TT absorption following nasal and oral administration to rats. The results showed that, irrespective of the administration route, the TT levels in the blood stream and lymph nodes were significantly higher for PLA-PEG NP than for PLA NP. Consequently, these initial studies suggested the potential of PLA-PEG NP as transmucosal protein delivery systems. More recent studies aimed at evaluating the performance of these NP for immunization provided additional evidence of their potential for nasal protein delivery (Vila et al., 2002a). More specifically, the anti-tetanus IgG levels elicited following nasal administration of TT-loaded NP were significantly higher than those corresponding to the fluid vaccine. Nevertheless, despite these positive results, the mechanism of action of these NP at the nasal mucosa level and the specific role of the PEG coating has not been clearly identified yet. In this sense, we should keep in mind some interesting features of PEG, with respect to its application for mucosal drug administration. These are related to its mucoadhesion promoting effect (Ascentiis et al., 1995; Peppas, 1998) and its ability to increase the stability of drugs on the nasal mucosa (Bechgaard et al., 1999; Lindhardt et al., 2000).

Besides the potential benefit of the PEG coating, the idea of using nanoparticles as nasal drug carriers was supported by previous work that showed the transport of model polystyrene particles across the nasal mucosa. Indeed, Almeida et al. (1993) investigated the transport of fluorescently labelled, carboxylated polystyrene NP from the nasal cavity into the systemic circulation. The authors observed that the NP crossed the nasal barrier and reached the blood stream. Some years later, Huang and Donovan (1996) reported the effect of size on the particulate uptake through a rabbit nasal mucosa mounted in a diffusion chamber. Following these initial reports and also the results of our own work (Tobío et al., 1998, 2000), other authors have investigated further the influence of the size on the nasal transport of nanoparticles. For example, Brooking et al. (2001) studied the transport of ^{125}I-radiolabelled latex NP across the nasal mucosa of rats using a range of particle sizes (20, 100, 500 and 1000 nm). They found a relationship between the intensity of the transport and the particle size. In addition to these transport studies using model polystyrene particles, other authors observed that the size of PLA particles influences the immune responses to nanoencapsulated antigens following nasal administration. Indeed, Somavarapu et al. (1998) showed that the immune response to encapsulated ovalbumin administered intranasally was significantly greater for PLA NP than for PLA microparticles (MP). More recently, Jung et al. (2001) investigated the influence of the size of sulphobutylated poly(vinyl alcohol)-graf-poly(lactide-co-glycolide) particles on the immune response to TT adsorbed onto the particles, administered by the nasal route. The authors found that the induction of antibody responses was influenced by the size of the particles, being the response most important for the antigen associated to the smallest particles.

Taking this previous information on the nasal transport of nanoparticles into account, as well as the results of our own work on the efficiency of PLA-PEG particles as transmucosal antigen carriers, the main purposes of the present work were: first, to study the potential effect of blank PLA-PEG nanoparticles in the nasal absorption of the free toxoid, and, second, to evaluate the influence of the PLA-PEG particles size on their ability to transport the encapsulated TT. Additionally, given the sensitivity of the nasal mucosa to external agents we studied if the use of anaesthesia in the in vivo experiments affects the transport of TT across the rat mucosa.

2. Materials and methods

2.1. Chemicals and animals

For the polymer synthesis, D,L-lactide was purchased from Aldrich (Milwakee, USA), monomethoxypolyethylenglycol (MW: 5000 Da) and stannous octoate were obtained from Sigma Chemical (St. Louis, USA). Purified tetanus toxoid (MW: 150,000 Da, 85–95% monomeric) dissolved in phosphate buffer saline, pH 7.4, was kindly donated by the