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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

Massachusetts Biological Laboratories (MBL, Boston, USA). Cholic acid (sodium salt) was purchased from Sigma Chemical (Madrid, Spain) and the solvent ethyl acetate was obtained from Panreac (Madrid, Spain).

Male Sprague–Dawley rats (200–250 g) from the Central Animals House of the University of Santiago de Compostela (Spain) were used. They were kept in a 12 h light–dark cycle and at a temperature of 20 °C ± 2. The animals were allowed access to food and water ad libitum.

2.2. Polymer synthesis, preparation and physicochemical characterization of NP and MP

The diblock copolymer PLA-monomethoxy polyethylenglycol was synthesized by the ring-opening polymerization of dimer, as described by García et al. (1999). The molecular weight of the PLA-PEG polymer used in this study was determined by GPC (MW: 28 kDa; Mn: 17 kDa) and the copolymer composition was determined by nuclear magnetic resonance spectroscopy (LA:EG; 31:69).

PEG-PLA particles were prepared using the double emulsion technique, as described elsewhere (Tobío et al., 1998; Blanco and Alonso, 1998). Briefly, solutions of tetanus toxoid (500 μg TT, 100 μl) were emulsified in a 1 ml solution of PLA-PEG (50 mg) in ethyl acetate by sonication (Branson, Sonifier 250, UK) for 15 s (15 W). Then, 2 ml of an aqueous sodium cholate solution (1%, w/v) were added to this emulsion and the resulting (w/o)/w emulsion was formed by sonication (15 s, 15 W) for NP and by homogeneization (Heidolph DIAX 600, Heidolph, Germany) at 13,500, 9500, and 8000 rpm (15 s) for MP of 10 μm, 5 μm and 1 μm, respectively. Double emulsions were diluted in 100 ml sodium cholate solution (0.3%, w/v) and the solvent was rapidly eliminated by evaporation under vacuum (Rotavapor R-114, Buchi, Switzerland). Finally, particles were isolated by centrifugation (Avanti^{TM} 30, Beckman, Spain). NP at 22,000 × *g* for 30 min, MP of 10 μm, 5 μm and 1 μm at 8000 × *g* (20 min), 6000 × *g* (20 min), 4000 × *g* (20 min), respectively. Particles were washed three times with water.

The particle size of NP and MP were determined, by photon correlation spectroscopy (PCS) using a Zetasizer 3000-HS (Malvern Instruments, UK) and by optical microscopy. Three batches from three different lots were analyzed for each formulation (nine replicates).

The morphological examination of NP and MP were performed using a transmission electron microscope (TEM, CM12 Philips, The Netherlands) and a scanning electron microscope (SEM, JEOL JSM-6400, Tokyo, Japan).

2.3. Absorption and biodistribution of TT-loaded PLA-PEG particles after nasal administration

Tetanus toxoid was radiolabelled with ^{125}I (sodium salt) (Amersham Iberica, Madrid, Spain) using *N*-chloro-benzene sulphonamide (Iodo Beads®, Pierce, Rockford, IL) as iodination reagent. Briefly, 28 μl of 4.2 mg ml^{-1} TT solution was mixed with 500 μl of phosphate buffer (PB, pH 6.5) and 10 μl (1 mCi) of ^{125}I solution. Then, one Iodo-bead® was incorporated to allow the iodination process to occur. Radiolabelled protein was separated from free ^{125}I on D-salt Dextran Plastic Desalting column (Pierce, Rockford, IL) and the activity of the radiolabelled protein assessed in a Cobra II-Autogamma (Packard Instruments, CT).

PLA-PEG particles containing TT together with a trace of ^{125}I-iodinated TT were prepared according to the procedure indicated above, centrifuged and the supernatants were assessed for gamma emission. The amount of TT encapsulated into the NP was calculated by the difference between the total amount used to prepare the NP and the amount of TT present in the aqueous phase or the amount encapsulated into the isolated particles. The stability of particle labelling with ^{125}I-TT was investigated by measuring the radioactivity remaining associated with the isolated NP (by centrifugation) after incubation with serum for 24 h at 37 °C (particle concentration in serum: 0.2 μg/ml).

These in vivo studies were divided in three parts.

2.3.1. Evaluation of the effect of the physical presence of NP on the transport across the nasal mucosa of tetanus toxoid and the effect of the anaesthesia (ether)

Radiolabelled TT in three different forms (free, encapsulated and mixed with blank NP) was administered to rats by nasal instillation with and without anaesthesia (ether).

2.3.2. Evaluation of the effect of the size of the particles on the absorption and biodistribution of the encapsulated toxoid (without anaesthesia)

With the purpose of evaluating the effect of particle size on the nasal absorption, the radiolabelled TT was encapsulated into NP and MP using the w/o/w solvent evaporation technique. The size could be conveniently adjusted by modifying the emulsification energy. Thus, for the preparation of the NP the formation of the w/o and w/o/w emulsions were achieved by sonication (15 s; 15 W) and, in the case of the MP, the emulsification was performed first by sonication (15 s; 15 W; w/o emulsion) and by homogeneization (15 s; 13,500, 9000 or 8000 rpm depending on the size; w/o/w emulsion).

2.3.3. Evaluation of the nasal bioavailability of TT-loaded PLA-PEG NP

With the objective of studying the nasal bioavailability of TT-loaded PLA-PEG NP, the radiolabelled TT was encapsulated into NP and administered by nasal and i.v. routes. The area under the blood concentration–time curve (AUC) was calculated by trapezoidal method between 0 and 6 h, and between 0 and 24 h, and the nasal bioavailability (F) was calculated using the following equation:

$$F = \frac{\mathrm{AUC_{NASAL}} \times \mathrm{DOSE_{i.v.}}}{\mathrm{AUC_{i.v.}} \times \mathrm{DOSE_{NASAL}}}$$

2.3.3.1. Protocol of administration and dose of particles. The animals were dosed 20 mg of TT-loaded NP or MP nasally by instillating 80 μl (PBS) into each nostril (20 μl four times in 3-min time intervals), and the rats were dosed 5 mg of TT-loaded NP intravenously by injection of 200 μl (saline serum) in the tail vein (in the case of free TT was administered the same amount encapsulated into the particles).

2.3.3.2. Blood and tissue sampling. Samples of blood were collected from animals at 1, 2, 6 and 24 h ($n = 5$) post-administration by cardiac puncture. Furthermore, in the case of the i.v. administration of TT, an extra sample of blood was taken at 5 min post-administration. Twenty-four hours after administration, the animals from each group were sacrificed. The lymph nodes, lung, liver, spleen, stomach and small intestine were removed from the sacrificed animals. The aliquots of blood and tissues were weighed and the radioactivity was measured. Results of the amount of radioactivity recovered to the organs were represented as the percentage of the total amount of radioactivity administered.

2.3.3.3. Statistical methods. Results are presented as mean ± S.D. Statistical comparisons were made with ANOVA test at a 95% confidence level. Differences were considered statistically significant at $p < 0.05$.

3. Results and discussion

3.1. Preparation and physicochemical characterization of NP and MP

For the preparation of PLA-PEG particles (NP and MP) we chose the double emulsion technique. This is a versatile technique that allowed us to control the size of the particles while achieving significant protein loadings. The characteristics of the particles such as, size, encapsulation efficiency and final loading are shown in Table 1. The formulation parameters were conveniently adapted in order to produce particles of different critical sizes: 200 nm, 1.5, 5 and 10 μm. The encapsulation efficiency was related to the size, being it more important for the MP than for the NP. This could be easily explained by the fact that TT is a hydrosoluble protein and therefore, it has a tendency to migrate to the external aqueous phase. In the present study, no efforts were made attempting to improve the TT loading of the particles, because the ^{125}I-TT was simply used as a marker. In contrast, it was important for us to assess the absence of radioactivity released from the particles following their incubation in serum for 24 h at 37 °C. The results showed that the amount of radioactivity that remained associated to the particles was about 92% (90–94%). Consequently, these results led us to assume that ^{125}I-TT would remain associated to the particles following in vivo administration.

As shown in Fig. 1, independent of the size, TT-loaded PLA-PEG particles had a spherical shape and a rough surface. Similar micrographs were shown by other authors for PLA-PEG and PLGA-PEG MP (Delgado et al., 2000; Lavelle et al., 1999). The roughness of the surface could be attributed to the presence of hydrophobic (PLA) and hydrophilic (PEG) domains

Table 1
Size, encapsulation efficiency (%) and final loading of PLA-PEG nanoparticles (NP) and microparticles (MP)

	NP (nm)	1 μm-MP (μm)	5 μm-MP (μm)	10 μm-MP (μm)
Size	196 ± 20	1.48 ± 0.17	5.93 ± 1.72	10.34 ± 1.83
Encapsulation efficiency (%)	19.9 ± 1.1	23.1 ± 1.6	25.4 ± 1.0	32.2 ± 1.8
Loading (%)	0.248 ± 0.014	0.257 ± 0.029	0.282 ± 0.011	0.357 ± 0.020

Teorical loading 1%, initial amount of TT was 500 μg in 50 mg of polymer.

and their separate disposition following the drying process of the particles.

3.2. Absorption and biodistribution of TT-loaded PLA-PEG particles after nasal administration

3.2.1. Evaluation of the effect of the physical presence of NP on the transport across the nasal mucosa of tetanus toxoid and the effect of the anaesthesia (ether)

As indicated in the introduction, a major goal of this work was to investigate the mechanism by which PLA-PEG nanoparticles are able to increase the nasal transport of TT (Tobío et al., 1998; Vila et al., 2002b). For the interpretation of this mechanism we should take into account not only the previously demonstrated transport of model polystyrene (Almeida et al., 1993) nanoparticles, but also the possible role of PEG. Indeed, polyethylene glycols (PEGs) have been previously found to affect the nasal absorption of drugs and also the mucoadhesive properties of polymers. For example, PEGs were used as excipients in pharmaceutical formulations to increase the aqueous solubility and stability of drugs intended for nasal administration (Bechgaard et al., 1999; Lindhardt et al., 2000). In addition, other authors have reported the PEG mucoadhesion promoting effect (Ascentiis et al., 1995; Peppas, 1998). Therefore, taking this information into account, we have considered the possibility that the toxoid could be partially released from the particles at the mucosal surface, following intranasal instillation, and that the physical presence of PLA-PEG NP could affect the absorption of free TT. In other words, we attempted to investigate if the physical presence of PLA-PEG NP would be responsible for the enhanced TT transport. With this objective in mind, we administered 3 preparations: free antigen (TT), a physical mixture of blank NP and free TT (NP + TT), and TT encapsulated into NP [NP (TT)] to rats and determined the radiactivity levels in blood and other relevant tissues. On the other hand, we have also considered the possibility that the anaesthesia would affect the absorption process and, hence, we administered the particles to conscious (A) and anaesthetized (B) animals.

Fig. 2 shows the blood radioactivity levels at different times after nasal administration of free antigen (TT), a physical mixture of blank NP and free TT (NP + TT), and TT encapsulated into NP [NP (TT)] in conscious (A) and anaesthetized (B) animals. Similarly, Fig. 3 shows the tissue radioactivity levels at 24 h post-administration of the preparations in both groups of rats (A: conscious; B: anaesthetized). A general observation from these two figures is that the radioactivity levels obtained for the TT-loaded PLA-PEG NP were significantly higher than those observed for the physical mixture of NP and TT solution. The statistical analysis of the data indicated that, irrespective of the use of anaesthesia, the concentration of radioactive TT is significantly greater in animals treated with the antigen encapsulated into the NP than in those treated with the free antigen or the physical mixture. Consequently, these results led us to the conclusion that the physical presence of blank PLA-PEG NP does not enhance the transport of the TT and hence, that the particles must have a carrier capacity for the associated toxoid.

On the other hand, taking into account that TT has a high molecular weight (150 kDa) we decided to study if the presence of the particles could affect the absorption of smaller molecules i.e. peptides. With this idea in mind, we administered a physical mixture of blank NP with free bovine insulin (molecular weight 5.6 kDa) and determined the hypoglycaemic levels. These levels were similar to those obtained in rats after nasal administration of free insulin or PBS (data not shown), thus, corroborating the lack of penetration enhancement ability of the particles and supporting their role as protein carriers.

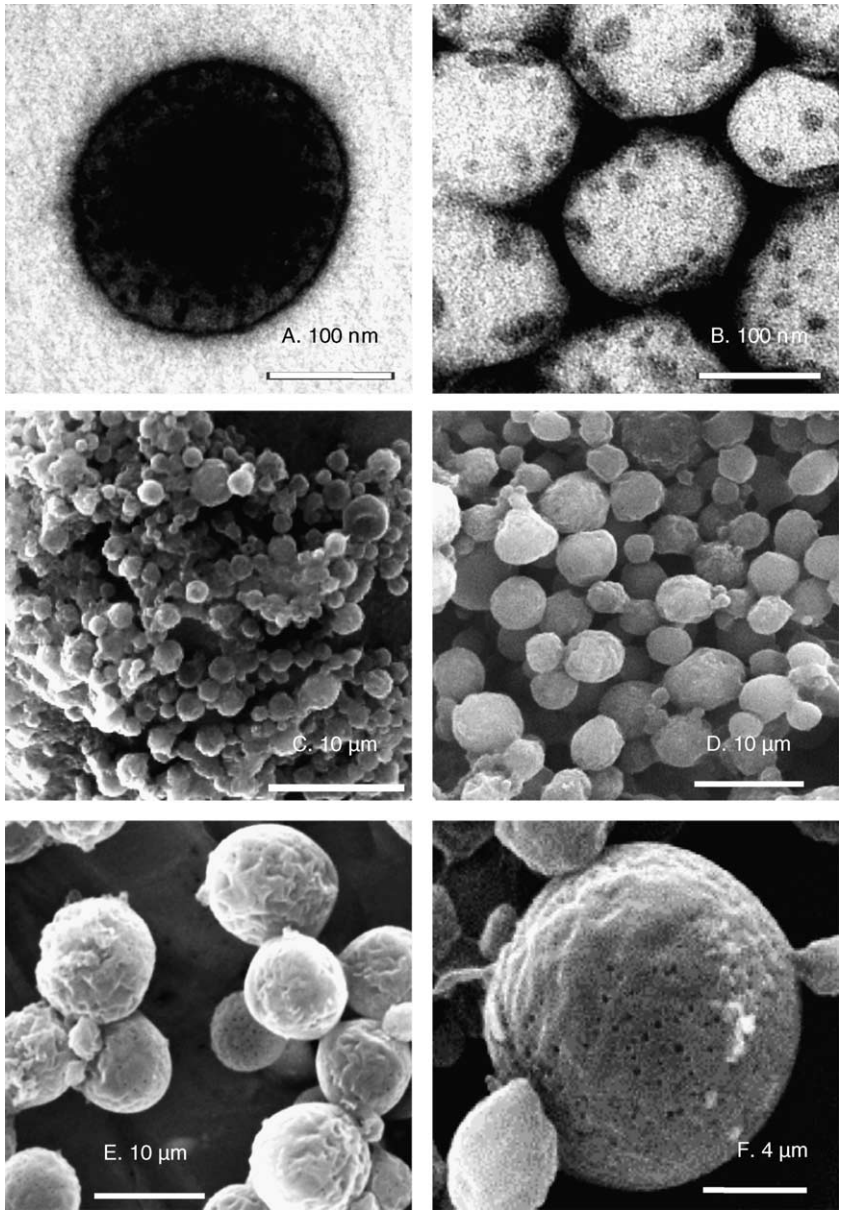

Fig. 1. Transmission electron microscope micrograph of NP (A and B), and scanning electron micrographs of MP with a size of 1.5 μm (C), 5 μm (D) and 10 μm (E and F). All formulations were prepared by the double emulsion technique.

With respect to the influence of the anaesthesia, the results in Figs. 2 and 3 showed that, indeed, this factor has an effect on the transport of the free or encapsulated TT. Over all, the concentration of the antigen in blood and tissues was slightly, but significantly, higher in anaesthetized than in conscious animals. This could be understood as an increase of the mucosal permeability due to the irritant properties of ether and its effect in the mucociliar clearance (Waynforth and Flecknell, 1992). A similar effect of the anaesthesia was detected by Haan et al. (1995a, 1995b) who observed that i.n. immunization of mice under light ether anaesthesia

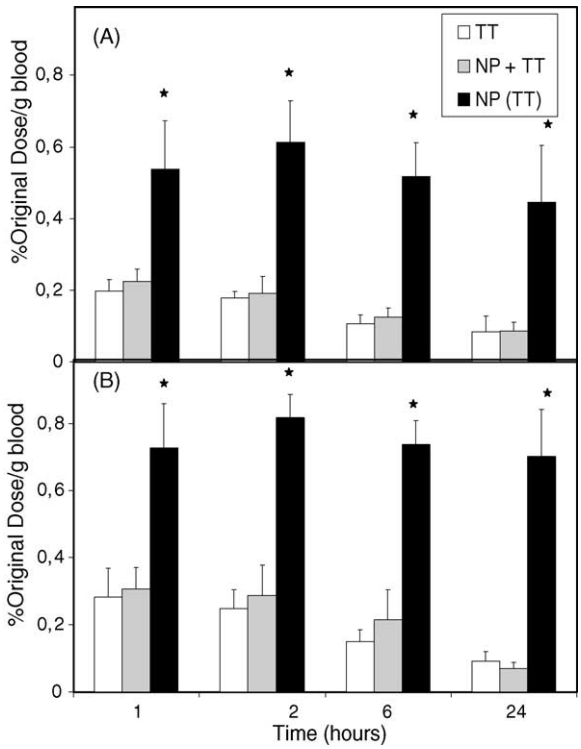

Fig. 2. ^{125}I-TT blood levels of following nasal administration of the free toxoid (TT), a physical mixture with the nanoparticles (NP + TT) and encapsulated TT (NP-TT), to conscious rats (A) and rats under light ether anaesthesia (B) ($p < 0.05$, * significant differences of NP (TT) respect to TT and NP + TT, in conscious and anaesthetized animals).

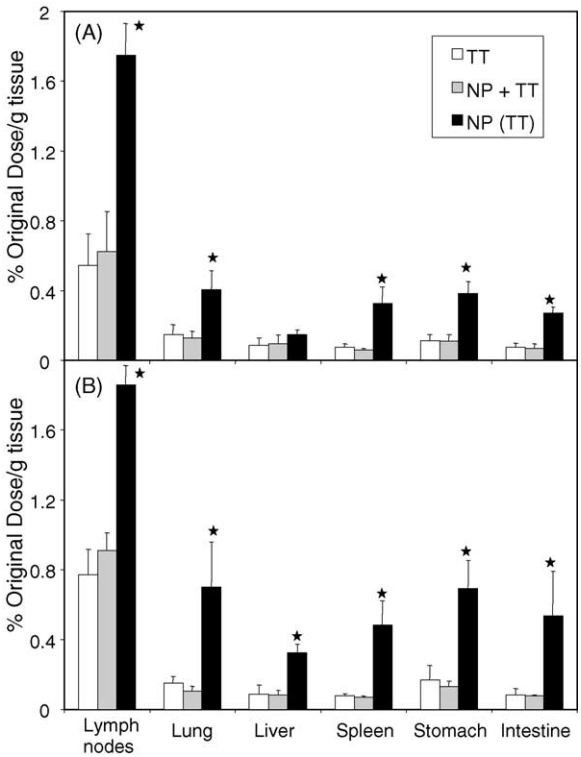

Fig. 3. ^{125}I-TT tissue levels of following nasal administration of the free toxoid (TT), a physical mixture with the nanoparticles (NP + TT) and encapsulated TT (NP-TT), to conscious rats (A) and rats under light ether anaesthesia (B) ($p < 0.05$, * significant differences of NP (TT) respect to TT and NP + TT, in conscious and anaesthetized animals).

with a free or liposomal antigen induced a detectable serum antibody response in mice, while no response was observed in the case of i.n. immunization of non-anaesthetized mice. These results highlight the sensitivity of the nasal mucosa and the importance of the experimental conditions used for studying nasal absorption. As a consequence, we decided to avoid the use of anaesthesia in further experiments.

3.2.2. Evaluation of the effect of the size of the particles on the absorption and biodistribution of the encapsulated toxoid (without anaesthesia)

As indicated in the introduction, the effect of the size on the nasal transport of polystyrene particles has been previously reported (Brooking et al., 2001). In addition, it has been shown that the particle size has an effect on the immune response to antigens associated to PLA (Somavarapu et al., 1998) and sulphobutylated poly(vinyl alcohol)-graf-poly(lactide-co-glycolide) particles (Jung et al., 2001). On the other hand, given the fact that not only the size but also the surface composition of the particles may affect their transport across mucosal surfaces, we have compared the transport of TT encapsulated either into PLA-PEG nanoparticles or into microparticles (30 μm). From this initial study it was clear that nanoparticles were preferable that microparticles in terms of enhancing the transport of TT (Tobío et al., 1998). In the present study, we aimed to explore in more detail the effect of the size of the nasal transport of TT and, thus, we compared the behavior of particles with sizes ranging between 200 nm and 10 μm.

Fig. 4 shows the blood radioactivity levels at different times following nasal administration of free TT or

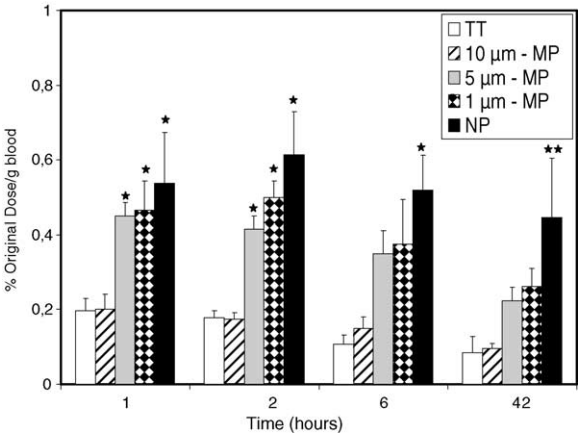

Fig. 4. ^{125}I-TT blood levels following nasal administration of free ^{125}I-TT or ^{125}I-TT encapsulated in PLA-PEG particles of different sizes (*significant differences ($p < 0.05$) with respect to TT and 10 μm-MP; ** significant differences with respect to TT, 10 μm-MP and 5 μm-MP).

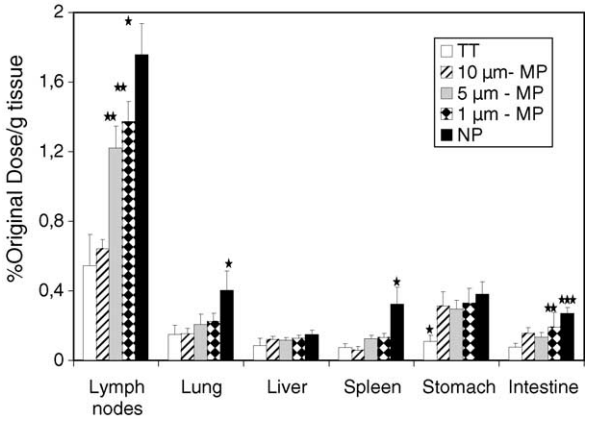

Fig. 5. ^{125}I-TT tissue levels following nasal administration of free ^{125}I-TT or ^{125}I-TT encapsulated in PLA-PEG particles of different size (* significant differences ($p < 0.05$) with respect to the other groups; ** significant differences respect to TT and 10 μm-MP; *** significant differences with respect to TT, 10 μm-MP and 5 μm-MP).

TT encapsulated into PLA-PEG particles of different sizes. The statistical analysis indicated that the blood radioactivity levels corresponding to the three smaller PLA-PEG particles formulations were significantly higher than those corresponding to the TT solution (TT) or to the 10 μm-MP, which showed similar radioactivity levels. Furthermore, the statistical analysis indicated that there is a ranking in the efficacy of these carriers depending on their size, the levels corresponding to the NP being higher than those corresponding to the MP. These results obtained for PLA-PEG particles are clearly in agreement with those previously reported for hydrophobic polystyrene (Brooking et al., 2001; Almeida et al., 1993) and PLA particles (Somavarapu et al., 1998). Consequently, it seems reasonable to conclude that irrespective of the surface composition the size of the particles is a critical parameter for enhancing the transport of the associated protein.

In Fig. 4, it can also be noted that the high levels attained at 60 min post-administration (first blood sample) remained constant during the experiment, irrespective of the size of the particles. These high and constant radiactivity levels may be taken as an evidence of the transport of the particles and their persistency in the blood stream due to their long circulating properties.

The radioactive tissue levels following nasal administration of free TT or TT encapsulated into PLA-PEG particles are shown in Fig. 5. The highest radioactivity levels were found in the lymph nodes. The statistical analysis indicated that the radioactivity levels in lymph nodes, lung, spleen and intestine, following administration of the NP, were significantly higher than those observed for the MP. On the other hand, the levels provided by the 1 μm-MP and 5 μm-MP in lymph nodes were significantly higher than those corresponding to the TT solution and 10 μm-MP. Nevertheless, no differences were found between levels corresponding to 1 μm-MP and 5 μm-MP.

These high levels of radioactivity, specially in blood and lymph nodes are in agreement with those previously reported (Tobío et al., 1998) and suggest that the PLA-PEG NP could be transported through the nasal mucosa and reach the subepithelial layer which is highly irrigated by lymph and capillary vessels. It could also be hypothesized that a quantity of particles will pass directly into the systemic circulation and, some would probably be taken up and delivered to the underlying lymphoid cells of the nasal-associated-lymphoid-tissue (NALT). In this regard, it is important to mention that current studies aimed at visualizing the transport of particles using confocal microscopy have, indeed, evidenced that the size has any effect of the ability of the particles for overcoming the nasal mucosa. Moreover, the transfer of the particles administered intranasally to the NALT was previously reported for polystyrene

particles (Eyles et al., 2000). This transfer would explain the important immune responses elicited following intranasal administration of TT-loaded PLA-PEG NP (Vila et al., 2002a). Besides the improved transport of PLA-PEG NP across the nasal mucosa, it could also be possible that the PEG coating facilitates their access to the lymph nodes. In this sense, it was recently reported that the PEG coating improved the drainage of particles administered subcutaneously and enhanced their lymph node localisation (Illum et al., 2001).

3.2.3. Evaluation of the nasal bioavailability of PLA-PEG NP

Blood concentration of TT encapsulated into the NP following nasal and i.v. administration to rats are shown in Fig. 6. The results showed that, after nasal administration of the radioactive antigen encapsulated into NP, there is an important accumulation of the toxoid in the blood stream and that the maximum concentration remains steady for, at least, 24 h. In contrast, as expected, following i.v. administration, there is an initial important decrease of radioactivity in blood due to the biodistribution of the carrier. The normalized area under the curve was 0.80 ± 0.19 between 0 and 6 h, indicating a bioavailability of 80%, and was 0.72 ± 0.14 between 0 and 24 h. These results should be taken cautiously since these types of systems should logically have a dose-dependence kinetics. However, they represent useful information about the ability of these nanocarriers to overcome the nasal mucosa. On the other hand, the similar values of bioavailability observed at 6 and 24 h suggest that the elimination rate of the toxoid from the blood stream is similar irrespective of the modality of administration. Consequently, these results provide additional evidence of the ability of the particles to cross the nasal mucosa. Finally, the long persistency of the particles in the blood stream could be attributed to their long-circulating properties.

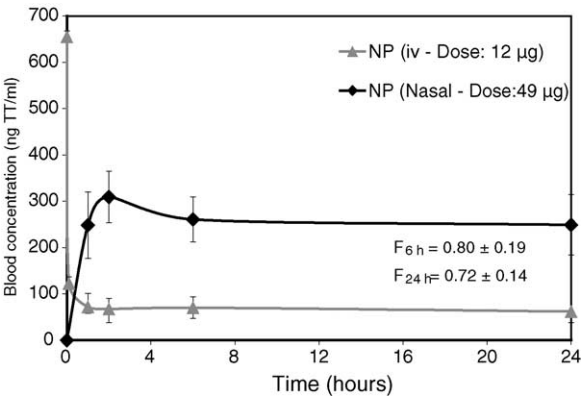

Fig. 6. TT blood concentration following nasal and i.v. administration of the TT encapsulated into NP.

4. Conclusions

In the light of these results, we can conclude that PLA-PEG NP work as carriers that are able to transport efficiently the encapsulated tetanus toxoid through the nasal mucosa. In addition, it can be concluded that the extent of the absorption of the toxoid encapsulated into the particles was dependent on the size of the particles, being more important for the NP than for the MP.

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