Microparticles Prepared by Spray-drying as a Vaccine Delivery against Brucellosis

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Abstract

The antigenic extract Hot Saline from *Brucella ovis* was microencapsulated by the spray-drying technique with different polyesters (poly-lactide-co-glycolide 502H and blends with poly-ε-caprolactone) in order to obtain microparticles smaller than 5 μm. Microparticles were tested for encapsulation efficiencies, release studies, acidification of the *in vitro* release medium, and *in vitro* J744-macrophage experiments to determine the optimal formulation for vaccination purposes. Encapsulation efficiencies were similar in all formulations, however, antigen release was lower as the ratio of PEC increased. For the formulation based on PLGA 502 H, the pH of the medium during release dropped from 7.4 to 3.5 while the presence of PEC attenuated the pH drop. All formulations showed light toxicity by the MTT assay, but differences were observed in terms of phagocytosis, as particles prepared with PEC showed the higher uptake by J744-macrophages and cell respiratory burst, determined by oxygen peroxide release. All these characteristics suggest that the microparticulated antigenic formulation containing the higher ratio of PEC is susceptible to be used in animal vaccination studies

Keywords: brucellosis; poly-ε-caprolactone; PLGA; polymeric blend; spray-drying; vaccine

Brucellosis, is an infectious disease caused by the bacteria of the genus Brucella. It causes severe illness and death in livestock and humans, remaining a significant threat in most developing areas of the world. *Brucella* species may affect sheep, goats, cattle, pigs, dogs, and several other animals. Considered a zoonotic disease, humans become infected by coming in contact with animals or animal products that are contaminated with these bacteria. Vaccines are available but, adverse reactions to vaccination have been reported (Blasco, 1997; Blasco and Diaz, 1993), and even the use of those commercial vaccine interferes with diagnostic test, precluding eradication programs.

We have previously reported that the Hot Saline antigenic extract (HS) from *Brucella ovis* (Gamazo et al., 1989; Riezu-Boj et al., 1990) was effective against experimental brucellosis, however, as being subcellular, booster doses were needed (Blasco et al., 1993; Jiménez de Bagüés, 1994). Previously, we published the results obtained with HS microencapsulated in PEC (Murillo et al., 2001a) by the solvent evaporation technique, obtaining that in the mice model of infection was significantly effective against *B. ovis* and *B. abortus* (Murillo et al., 2001b). We report here the microencapsulation of HS-antigens with different polymers by the spray-drying technique, recently described by several authors as being appropriate for the encapsulation of antigens for vaccinal purposes (Baras et al., 2000a; Baras et al., 2000b; Baras et al., 2000c).

Poly-lactide-co-glycolide acid (PLGA) 50:50, Resomer® 502H, Boehringer Ingelheim (Ingelheim, Germany) and Poly-ε-caprolactone (PEC) (Sigma, St.Louis, USA) blends at different proportions were used. Briefly, the HS extract was dispersed in a 4% polymer solution in methylene chloride. The suspension was spray-dried, and the

resulting microparticles collected, washed and dried under vacuum (Blanco-Príeto et al., 1999).

Three selected preparations [*Batch 1:* 502H (100%); *Batch 2:* 502H:PCL (75:25) and *Batch 3:* 502H:PCL (50:50)] were studied. The size of microparticles was measured by laser diffractometry (Mastersizer-S®) obtaining an average diameter of microparticles below 5 μm.

Encapsulation efficiencies (EE) were determined after hydrolysis of the samples with NaOH 0.1 N and the amount of antigen was determined by BCA assay at 562 nm (Smith et al., 1985). Release studies after incubation of the particles in PBS at 37 ºC was determined by BCA assay, and the pH of the samples were measured during a the release studies, without changing the medium.

Macrophages from the cell line J774.2 were used to study the uptake of particles by optical microscopy. To study the cellular damage after microparticle uptake, the respiratory burst caused by the different preparations was measured by flow cytometry (FACScan®Becton Dickinson) in macrophages as the oxidation of dihidrorhodamine-123 to rhodamine-123 green fluorescent product (Busttest, ORPEGEN®Pharma, Heidelberg, Germany) by the hydrogen peroxide released. Toxicity of these formulations was determined by a colorimetric assay based on the use of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay (Sigma, St. Louis, USA), viable cells are able to reduce MTT to colored formazan serving as indirect measurement of viability (Hansen et al., 1989).

The nominal loading of all preparations was 1.7%. As shown in table 1, the EE ranged from 23.5 to 31.9%. The formulation containing no PCL showed the highest EE (31.9%). The *in vitro* release kinetics were characterized by a high burst effect after 1

hour of incubation (Table 1 and Figure 1) followed by a slow and continuos release, being slower in the case of 502H formulation (Batch 1). Burst effect may indicate that most of the HS extract could be adsorbed onto the surface of the particles (Figure 1). Similar *in vitro* release profiles were observed for all the formulations. As it was expected, pH drop was attenuated by increasing the ratio of PEC in the formulation, as its degradation does not confer an acidic medium (Figure 2).

Phagocytosis was studied by optical microscopy. The results were expressed as the percentage of cells capable of taking up one or more microparticles. Results shown in table 1 indicate that particles with the higher content of PEC showed the highest capture by macrophages due to the higher hydrophobicity of the polymer mixture (Torché et al., 2000). Accordingly, the formulation containing more PEC in the polymeric solution (*Batch* 3) induced the highest burst activation (H_2O_2 release).

Finally, toxicity studies by the MTT colorimetric assay showed a very reduced *in vitro* toxicity for all formulations (Table 1), being lower that the reported previously with HS-PEC by solvent evaporation (Murillo et al., 2001a).

Summing up, the results indicate that spray-drying is a suitable technique for microencapsulation of the HS antigenic extract from *B. ovis*, and that particles with higher content of PEC present the better characteristics for vaccine purposes.

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Figure Legends

Fig. 1. Cumulative *in vitro* release profile for spray-drying microparticles; 502H (○); 502H:PEC (75:25) (□) and 502H:PEC (50:50) (△). Expressed as the percentage of antigenic complex (HS from *Brucella ovis*) release over 1,200 hours (days?). Mean ± sd (n=3)

Fig. 2. pH drop during release for spray-drying microparticles; 502H (○); 502H:PEC (75:25) (□) and 502H:PEC (50:50) (△).

Tables

Table 1. Characterization of HS-microparticles.

	Batch 1	*Batch 2*	*Batch 3*
EE (%)	31.9 ± 6.5	23.5 ± 1.4	29.6 ± 1.4
Burst (%)	40.3 ± 2.7	52.4 ± 7.4	52.7 ± 0.8
%Phagocytic cells	14.6 ± 3.4	34.9 ± 5.9	43.7 ± 8.8
% Toxicity	24.4 ± 6.3	12.0 ± 4.9	18.1 ± 2.6
FLH-1 increase	295 ±140	110 ± 25	324 ± 152

EE: encapsulation efficiency (%); *Burst:* Burst release after first hour of incubation (% HS); *Phagocytic cells:* Percentage of phagocytic cells capable of taking up microparticles; *Toxicity:* Percentage of toxicity respect to 100% viability for cells without particulate material; *FLH-1:* Respiratory burst effect expressed as the increase in fluorescence respect to a basal value.

Figures

Figure 1.

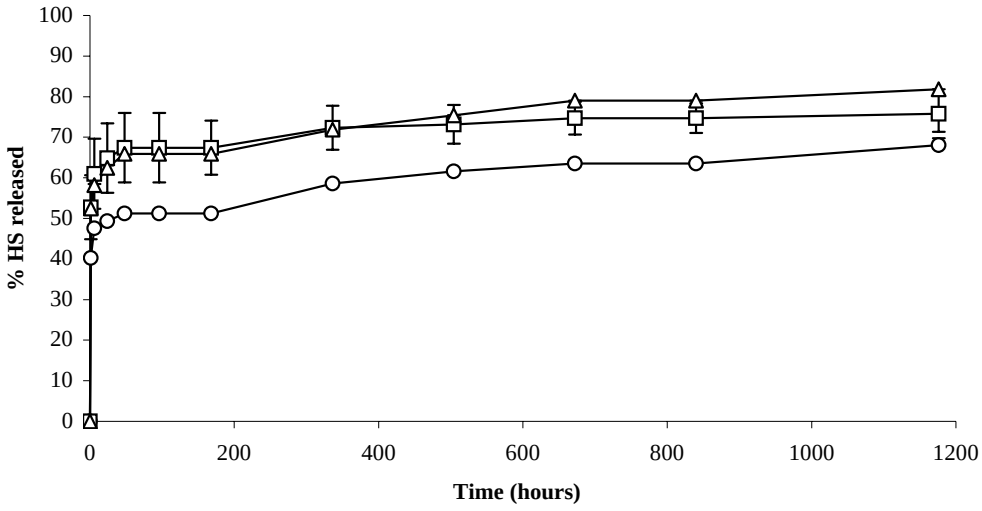

Figure 2.