ELSEVIER

International Journal of Pharmaceutics 242 (2002) 341–344

international journal of pharmaceutics

www.elsevier.com/locate/ijpharm

Note

Development of microparticles prepared by spray-drying as a vaccine delivery system against brucellosis

M. Murillo [a], C. Gamazo [b], M.M. Goñi [a], J.M. Irache [a], M.J. Blanco-Príeto [a,*]

[a] *Department of Pharmacy and Pharmaceutical Technology, University of Navarra, Pamplona 31080, Spain*
[b] *Department of Microbiology. University of Navarra, Pamplona 31080, Spain*

Received 27 December 2001; received in revised form 18 January 2002; accepted 18 January 2002

Abstract

The antigenic extract Hot Saline from *Brucella ovis* was microencapsulated by the spray-drying technique with different polyesters (poly-lactide-co-glycolide RG502H [PLGA], and blends with poly-ε-caprolactone [PEC]) in order to obtain microparticles smaller than 5 μm. Microparticles were tested for encapsulation efficiency, release studies, acidification of the in vitro release medium, and in vitro J744-macrophage experiments (phagocytosis and toxicity of the preparations) to determine the optimal formulation for vaccination purposes. Formulation containing no PCL showed the highest encapsulation efficiency, although the differences were not significant. The in vitro release kinetics were characterized by a high burst effect after 1 h of incubation, followed by a slow and continuos release. For the formulation based on PLGA, 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), and even the use of those commercial vaccines interferes with diagnostic test, precluding eradication programs.

We have previously reported that the Hot Saline antigenic extract (HS) from *Brucella ovis*

* Corresponding author. Tel.: +34-948-425600; fax: +34-948-425649
E-mail address: mjblanco@unav.es (M.J. Blanco-Príeto).

0378-5173/02/$ - see front matter
PII: S0378-5173(02)00212-0

(Gamazo et al., 1989) was effective against experimental brucellosis, however, as being subcellular, booster doses were needed (Blasco et al., 1993). Previously, we published the results obtained with HS microencapsulated in PEC by the solvent evaporation technique (Murillo et al., 2002), obtaining that in the mice model of infection was significantly effective against *B. ovis* and *B. abortus* (Murillo et al., 2001). We report here the microencapsulation of HS-antigens with different polymers by the spray-drying technique, recently described as being appropriate for the encapsulation of antigens for vaccinal purposes (Baras et al., 2000).

Poly-lactide-co-glycolide acid (PLGA) 50:50, Resomer® RG502H, 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: RG502H (100%); Batch 2: RG502H:PEC (75:25) and Batch 3: RG502H:PEC (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 °C 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. 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. Citotoxicity 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 formulation containing no PEC showed the highest EE, although the differences were not significant. The in vitro release kinetics were characterized by a high burst effect after 1 h of incubation (Table 1 and Fig. 1) followed by a slow and continuos release, being slower for the microparticles prepared with PLGA 502H (Batch 1) (Fig. 1). Burst effect may indicate that most of the HS extract could be adsorbed onto the surface of the particles. For the formulation based on PLGA RG502H, the pH of the release medium dropped from 7.40 to 3.54, however, 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 (Fig. 2).

Table 1
Characterization of HS-microparticles

	Batch 1 PLGA 502H (100%)	Batch 2 PLGA 502H:PEC (75:25)	Batch 3 PLGA 502H:PEC (50:50)
EE (%)	31.9 ± 6.5	23.5 ± 1.4	29.6 ± 1.4
Burst effect (%)	40.3 ± 2.7	52.4 ± 7.4	52.7 ± 0.8
% Phagocytosis	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
Respiratoty burst	295 ± 140	110 ± 25	324 ± 152

EE, encapsulation efficiency (%); Burst effect, Burst release after the first hour of incubation (% HS); Phagocytosis, Percentage of phagocytic cells capable of taking up microparticles; Toxicity, Percentage of toxicity respect to 100% viability for cells without particles; Respiratory burst, Hydrogen peroxide released, increase of fluorescence respect to a basal value.

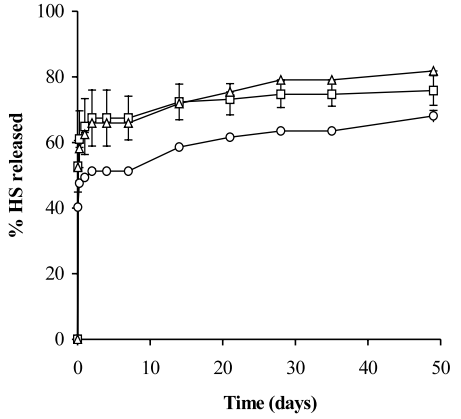

Fig. 1. Cumulative in vitro release profile of antigenic complex HS from spray-dried microparticles, expressed as the percentage of HS release over 50 days. Mean ± S.D. ($n = 3$); PLGA RG502H (○); PLGA RG502H:PEC (75:25) (□) and PLGA RG502H:PEC (50:50) (△).

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

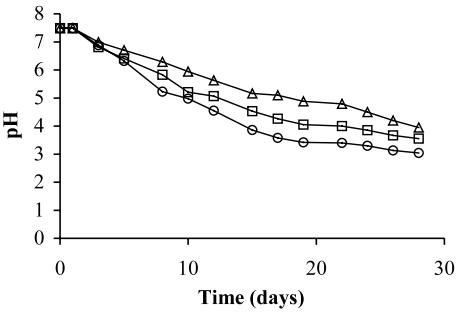

Fig. 2. pH drop during release for spray-dried microparticles; PLGA RG502H (○);PLGA RG502H:PEC (75:25) (□) and PLGA RG502H:PEC (50:50) (△).

the reported previously with HS-PEC by solvent evaporation (Murillo et al., 2002).

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.

Acknowledgements

This work was supported by grants from the 'Ministerio de Educación y Cultura de España, CICYT' (Grants BIO097-0777-C03 and AGL2000-0299-C03).

References

Baras, B., Benoit, M.-A., Poulain-Godefroy, O., Schacht, A.-M., Capron, A., Gillard, J., Riveau, G., 2000. Vaccine properties of antigens entrapped in microparticles produced by spray-drying technique and using various polyester polymers. Vaccine 18, 1495–1505.

Blanco-Príeto, M.J., Besseghir, K., Orsolini, P., Heimgartner, F., Deuschel, C., Merkle, H.P., Nam-Trân, H., Gander, B., 1999. Importance of the test medium for the release kinetics of a somatostatin analogue from poly(D,L-lactide-co-glycolide) microspheres. Int. J. Pharm. 194, 243–250.

Blasco, J.M., 1997. A review of the use of *B. melitensis* Rev 1 vaccine in adult sheep and goats. Prev. Vet. Med. 31, 275–283.

Blasco, J.M., Gamazo, C., Winter, A.J., Marín, C., Barberán, M., Alonso-Urmeneta, B., Jiménez de Bagüés, M.P., Moriyón, I., Díaz, R., 1993. Evaluation of whole cell and subcellular vaccines against *Brucella ovis* in rams. Vet. Immunol. Immunopathol. 37, 257–270.

Gamazo, C., Winter, A.J., Moriyón, I., Riezu-Boj, J.I., Blasco, J.M., Díaz, R., 1989. Comparative analysis of proteins extracted by hot saline or released spontaneously into outer membrane blebs from field strains of *Brucella ovis* and *Brucella melitensis*. Infect. Immun. 57, 1419–1426.

Hansen, M.B., Nielsen, S.E., Berg, K., 1989. Re-examination and further development of a precise and rapid dye method for measuring cell growth/cell kill. J. Immunol. Methods 119, 203–210.

Murillo, M., Grilló, M.J., Reñé, J., Marín, C.M., Barberán, M., Goñi, M.M., Blasco, J.M., Irache, J.M., Gamazo, C., 2001. A Brucella ovis antigenic complex bearing poly-ε-caprolactone microparticles confer protection against experimental brucellosis in mice. Vaccine 19, 4099–4106.

Murillo, M., Gamazo, C., Irache, J.M., Goñi, M.M., 2002. Polyester microparticles as a vaccine delivery system for

brucellosis: influence of the polymer on release, phagocytosis and toxicity. J. Drug Target. 10, 211–219.

Smith, P.K., Krohn, R.I., Hermanson, G.T., Mallia, A.K., Gartner, F.H., Provenzano, M.D., Fujimoto, E.K., Olson, B.J., Klenk, D.C., 1985. Measurement of protein using bicinchoninic acid. Anal. Biochem. 150, 76–85.

Torché, A.-M., Le Corre, P., Albina, E., Jestin, A., Le Verge, R., 2000. PLGA microspheres phagocytosis by pig alveolar macrophages: influence of poly(vinyl alcohol) concentration, nature of loaded-protein and copolymer nature. J. Drug Target 7, 343–354.