

ADJUVANT PROPERTIES OF GANTREZ® AN NANOPARTICLES

Sara Gómez Martínez¹, Carlos Gamazo de la Rasilla², Beatriz San Román Aberasturi¹, Juan Manuel Irujo Garreta¹

¹Pharmacy and Pharmaceutical Technology Department. ² Microbiology Department. University of Navarra. Pamplona. Spain.

Abstract

The purpose of this work was to optimise the preparation of Gantrez® AN [poly(methyl vinyl ether-co-maleic anhydride)] nanoparticles coated with ovalbumin (OVA) and to evaluate their potential as adjuvants in immunotherapy.

Introduction

Gantrez® AN is a copolymer of methyl vinyl ether and maleic anhydride, and it is a cheap excipient widely used in both cosmetic and pharmaceutical industry. The aim of this work was to study the ability of Gantrez® AN nanoparticles to modulate the immune response in order to be applied in immunotherapy strategies, as the treatment of allergic disorders.

Allergy is a pathology due to an imbalance in the Th1/Th2 pattern which is characterised by a shift toward Th2 response. This fact has turned the attention to the use of many immunological **adjuvants**, which are able to increase and/or modulate the immune response against the co-administrated antigens. Nowadays, the most applied immunological adjuvants for human use are the aluminium salts. However, other recent studies have demonstrated the potent adjuvant capacity of many non-biological vectors, such as microparticles, nanoparticles and liposomes¹⁻³.

Materials and methods

1. Materials

Gantrez® AN 119 (MW 200,000) was kindly gifted by ISP (Barcelona, Spain). OVA (grade V), 1,3 - diaminopropane (DP), 2,2' - Azino - bis (3 - ethylbenzo - thiazoline - 6 - sulfonic acid) diammonium salt (ABTS) and alhydrogel, were purchased from Sigma-Aldrich Chemie (Germany). The MicroBCA protein assay was supplied by Pierce (USA). The peroxidase immunoconjugates (GAM/IgG1/PO and GAM/IgG2a/PO) were obtained from Nordic Immunology (Tilburg, The Netherlands). All other chemicals used were of reagent grade and obtained from Merck (Madrid, Spain).

2. Preparation of the nanoparticles

The nanoparticles were prepared by a method previously described⁴. Briefly, 100 mg of Gantrez® AN were dissolved in 5 mL acetone. Then, the nanoparticles were formed by the addition of 20 mL ethanol:water (1:1 vol). After the evaporation of the organic solvents, the nanoparticles were incubated with different amounts of OVA (5, 10, 50 and 100 mg: NP 5, NP 10, NP 50 and NP 100 respectively) for 1 h at room temperature. Finally, the resulting nanoparticles were purified by centrifugation and freeze-dried using sucrose as cryoprotector.

3. Characterisation of the nanoparticles

The particle size and the zeta potential of nanoparticles were determined by photon correlation spectroscopy (PCS) and electrophoretic laser doppler anemometry, respectively, using a Zetamaster analyser system (Malvern Instruments, UK). The samples were diluted with distilled water and measured at room temperature with a scattering angle of 90°. All measurements were performed in triplicate.

The amount of associated OVA to nanoparticles was determined using the microbichinchoninic acid (Micro BCA) protein assay. In addition, the quantification of the OVA content was confirmed by size exclusion chromatography with fluorescent detection.

The release of OVA from PVM/MA formulations was studied by incubating 8 mg nanoparticles (NP 100) in 1 mL PBS (Phosphate Buffer Saline, pH 7.4) in eppendorf tubes. The samples were incubated in rotating vials at 37°C and at predetermined time intervals, the samples were centrifuged at 26,500 x g for 20 minutes and the supernatants removed for later analysis. The released OVA was determined using the microbichinchoninic acid (Micro BCA) protein assay.

4. Immunisation studies

The adjuvant properties of Gantrez® AN nanoparticles were evaluated in BALB/c mice after administration of the formulations either by the intradermal and oral routes.

When administrated by intradermal route, mice were divided in 5 groups of 5 animals each. The first one was treated with 50 µL PBS containing 10 µg OVA /mice (negative control). The second one was treated with 50 µL PBS containing 10 µg OVA adsorbed onto alhydrogel (OVA-ALUM). This formulation was used as positive control for a Th2 response⁵. Another group was administrated with empty nanoparticles (NP) and the rest of groups served to evaluate the ability of Gantrez® AN nanoparticles (NP 10 and NP 100) to modulate the Th1/Th2 pattern.

By the oral route, mice were divided in 3 groups of 5 animals each. The first one was treated with empty nanoparticles (NP), the second group was treated with PBS containing 50 µg of OVA /mice (negative control) and the third batch of animals was treated with 50 µg of OVA loaded in Gantrez® AN nanoparticles (NP 10).

In all cases blood samples were collected from the retroorbital plexus at different times (7, 14, 28 and 35 days after the administration). The samples were centrifuged and the sera were mixed for each group. Finally, this pool was analysed by quantification of anti- OVA specific anti-bodies (IgG1 and IgG2a) by ELISA.

Results and discussion

1. Characterisation of the nanoparticles

Figure 1 shows the influence of the bulk OVA on its association to Gantrez® AN nanoparticles. As we can see, by increasing the amount of protein used to coat nanoparticles, the OVA loaded-rate also increased. On the other hand, the two highest adsorption efficiencies were presented when the bulk OVA was 10 and 50 mg. In both cases this efficiency was close to 50%.

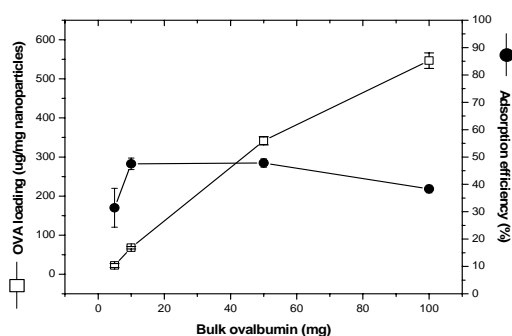


Figure 1: Influence of the bulk OVA (mg) (X axis) on its loading (µg/mg nanoparticles) (Y₁ axis), and the OVA adsorption efficiency (%) (Y₂ axis).

NP 10 and NP 100 were selected for the immunisation studies. Table 1 summarises the main physicochemical properties of these formulations. The size of the OVA-loaded

nanoparticles ranged from 200 to 300 nm. Interestingly, in all cases, the yield of the process was always close to 70% of the copolymer transformed into nanoparticles. On the other hand, the protein content clearly increased by increasing the bulk OVA.

Table 1: Physicochemical characteristics of nanoparticles. (mean \pm SD; n=10)

	Size (nm)	Zeta Potential (mV)	OVA content ($\mu\text{g}/\text{mg}$)
NP	178 \pm 4	-45.1 \pm 0.5	-
NP 10	300 \pm 3	-61.3 \pm 4.5	67.8 \pm 3.0
NP 100	222 \pm 44	-68.5 \pm 4.4	546.6 \pm 20.1

Figure 2 shows the cumulative release of OVA from NP 100 in PBS at 37°C. From this curve we can observe a latency period of about 6 h. Then, an important release of about 40% of the adsorbed protein was observed followed by a third non release period.

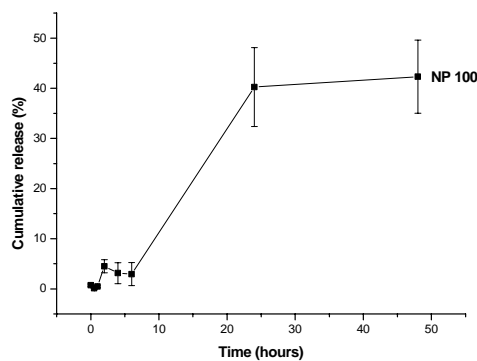


Figure 2: Cumulative release of ovalbumin (%) from the nanoparticles (NP 100).

2. Immunisation studies

Balb/c mice were immunised with different intradermal treatments: i) 10 μg of OVA in PBS solution (OVA); ii) 10 μg of OVA in PBS, adsorbed in alhydrogel (OVA-Alum); iii) empty nanoparticles (NP); iv) 10 μg of OVA loaded in NP 10; and v) 10 μg of OVA loaded in NP 100.

From these results, it was clear that Gantrez® AN nanoparticles were able to elicit an immune response against OVA. In addition, the effect induced by these carriers was found to be stronger and more rapid than the control formulation (OVA-Alum).

On the other hand, the Th1/Th2 pattern was quite similar to both nanoparticulate formulations. However, the adjuvant potential appeared to be dependent on the copolymer dose. In fact, NP 10, which Gantrez® AN/OVA ratio was ten times higher than for NP 100, displayed higher levels of both IgG1 and IgG2a isotypes.

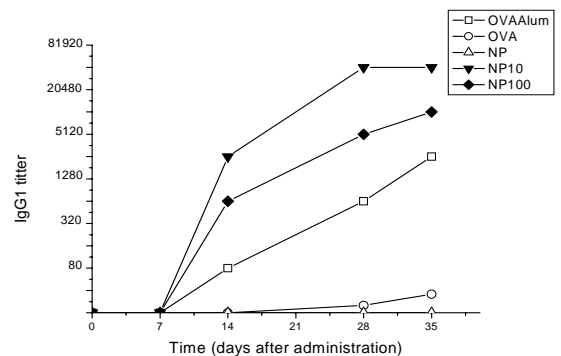
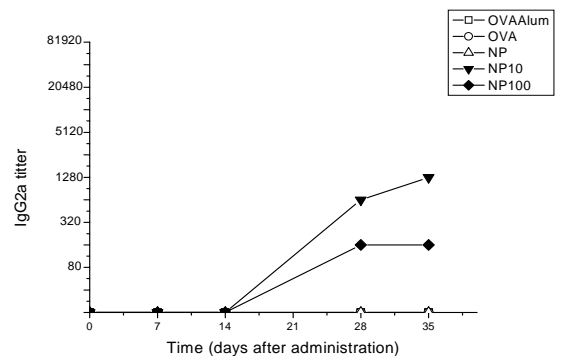


Figure 3: IgG1 and IgG2a titers after intradermal administration with OVA-Alum, OVA solution, empty nanoparticles, NP 10 and NP 100.

By the oral route NP 10 did not induce a significant immunological response (data not shown). This fact could be explained by a rapid enzymatic degradation of the adsorbed OVA in the gastrointestinal tract.

Finally, according to the optimisation of the preparation of the OVA loaded nanoparticles, the best formulation was NP 10 because of its higher adsorption efficiency. On the other hand, the immunisation study demonstrated that the amount of the copolymer has an important influence on the adjuvant capacity of the OVA loaded nanoparticles.

Institución: Universidad de Navarra
Dirección: Irunlarrea s.n.
Ciudad: Pamplona
Tel.: 948425600
Fax: 948425649

References

1. Murillo M., Grilló M.J., Reñé J., Marín C.M., Barberán M., Goñi M.M., Blasco J.M., Irache J.M., Gamazo C., A Brucella ovis antigenic complex bearing poly-ε-caprolactone microparticles confer protection against experimental brucellosis in mice. *Vaccine*, 19, 4099 (2001).
2. Eldridge J.H., Staas J.K., Meulbroek J.A., Tice T.R., Gilley R.M., Biodegradable and biocompatible poly(DL-lactide-co-glycolide) microspheres as an adjuvant for staphylococcal enterotoxin B toxoid which enhances the level of toxin-neutralizing antibodies. *Infect Immun*, 59, 2978 (1991).
3. O'Hagan D.T., Ugozzoli M., Barackman J., Singh M., Kazzaz J., Higgins K., Vancott T.C., Ott G., Microparticles in MF59, a potent adjuvant combination for a recombinant protein vaccine against HIV-1. *Vaccine*, 18, 1793-1801 (2000).
4. Arbós P., Wirth M., Arangoa M.A., Gabor F., Irache J.M., Gantrez AN as a new polymer for the preparation of ligand-nanoparticles conjugates. *J Control Release*, 83 321 (2002).
5. Faquim-Mauro E.L., Macedo M.S., Induction of IL-4-dependent, anaphylactic-type and IL-4-independent, non-anaphylactic-type IgG1 antibodies is modulated by adjuvants. *Int Immunol*, 12, 1733 (2000).

Acknowledgements:

- Asociación de Amigos de la Universidad de Navarra
- Consejería de Educación del Gobierno de La Rioja
- Fundación de Roviralta
- Ministerio de Ciencia y Tecnología (Project SAF2001-0690-C03-01)

Autor de contacto:

Nombre y apellidos: Sara Gómez Martínez

e-mail: sgommar@alumni.unav.es