PROTECTION FROM ANAPHYLACTIC SHOCK BY GANTREZ AN NANOPARTICLES

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INTRODUCTION

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 liposomes.

Gantrez® AN, a copolymer of methyl vinyl ether and maleic anhydride, is widely used in both cosmetic and pharmaceutical industry. In the last years, this copolymer has been proposed as material to prepare bioadhesive nanoparticles[1]. On the other hand, this copolymer shows a high capability to be combined with other excipients and/or ligands, which can be of interest to modify the physico-chemical properties and biological behaviour of the resulting nanoparticles. 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.

METHODS

Materials

Gantrez® AN 119 (MW 200,000) was kindly gifted by ISP (Spain). Ovalbumin (Ova), 1,3 - diaminopropane (DP) and alhydrogel were purchased from Sigma *NanoBiotechnology/NanoMedicine* (Germany). All other chemicals used were of reagent grade and purchased from Merck (Madrid, Spain).

Preparation of the nanoparticles

Nanoparticles were prepared by a method previously described[2]. Briefly, 4 mg ovalbumin were dispersed in 1 mL acetone by ultrasonication for 1 min. The dispersion was then added to an acetone solution containing Gantrez AN and stirred for 30 min. Some batches were also incubated with rough lipopolysaccharide (LPS) dispersed in acetone. Then, the polymer was desolvated by addition of a hydroalcoholic phase. The organic solvents were eliminated and the resulting nanoparticles were then cross-linked. Finally nanoparticles were purified by centrifugation and freeze-dried.

Characterisation of the nanoparticles

The particle size and the zeta potential of nanoparticles were determined by photon correlation spectroscopy and electrophoretic laser doppler anemometry, respectively, using a Zetamaster analyser system. The amount of associated Ova to nanoparticles was determined using the microbichinchoninic acid (Micro BCA) protein assay and size exclusion chromatography with fluorescent detection.

Sensitization, vaccination and challenge studies

Female BALB/c mice were sensitized by i.p. injection of 50 µg Ova emulsified in 1 mg alum (alhydrogel) in a total volume of 150 µL on days 1 and 8. Once the animals were sensitized to Ova, they were divided into several groups depending on the formulation administered and the route of administration. Some groups received an i.d. injection with 3 µg of Ova on days 14, 17 and 20, and other groups received four oral administrations of 12.5 µg Ova each, on days 14, 17, 20 and 23. The formulations tested were: OvaNP and LPS-OvaNP. As controls Ova-Alum by intradermal route and Ova solution by oral route were used. Finally on day 35 the animals were challenged by an injection of 1 mg Ova by the i.p. route. The intensity of the anaphylaxis was evaluated determining mortality or symptomatology (anaphylatic symptoms score), and by quantification of the histamine blood level and decrease on body temperature. All of these parameters were measured 30 min after the challenge.

RESULTS AND DISCUSSION

Characterisation of the nanoparticles

Table 1 summarises the main physico-chemical properties of the different formulations tested. Gantrez® nanoparticles displayed a size of about 200 nm and were able to carry about 30 µg OVA/mg nanoparticle. For LPS-OvaNP, the presence of the LPS (13.8 µg/mg) did not influence the physicochemical characteristics, except for the zeta potential which was found to be significantly less negative than for OvaNP.

Sensitization, vaccination and challenge studies

In vivo, mice treated intradermally with nanoparticles containing innocuous *Brucella ovis* LPS (LPS-OvaNP) displayed the most favorable symptomatology. In fact, this group of mice showed a significantly lower capacity to rise the histamine blood level after the challenge (Figure 1).

Similarly, the decrease on the body temperature was quite low (Figure 2). In addition, all the treated animals were protected against death by anaphylactic shock whereas 80% of the control animals dead. By the oral route, OvaNP appeared to be the best formulation permiting to protect mice against anaphylaxis, while the control group showed a 60% mortality (See Table 2). Both groups, OvaNP and LPS-OvaNP, developed less anaphylactic symptoms than the control group.

In summary, intradermal LPS-OvaNP protect against the anaphylactic shock in experimentally sensitized mice with ovalbumin. However, by the oral route, OvaNP seems to be the best formulation. In fact, the presence of LPS did not add any significant improvement in the protective effect of nanoparticles. Additional work is conducted to evaluate their efficacy with "real" allergens.

ACKNOWLEDGEMENTS

We wish to thank "Consejería de Educación del Gobierno de La Rioja", "Fundación Universitaria de Navarra", "Fundación Roviralta" and "Ministerio de Ciencia y Tecnología (Project SAF2001-0690-C03-01)" for their financial support.

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Figures:

Table 1: Characterisation of the nanoparticles (mean ± sd, n=10).

	Size (nm)	Zeta Potential (mV)	Ova content (µg/mg)	LPS content (µg/mg)
NP	158±2	-45.1±0.5	-	-
OvaNP	239±3	-50.8±2.9	30.1±4.5	-
LPS-OvaNP	227±4	-34.1±3.4	26.5±0.3	13.8±3.0

Table 2: Symptoms score after oral immunisation and anaphylactic challenge.

	Piloerection	Mobility	Cyanosis	Mortality
Ova	+	Low	+++	60%
OvaNP	++	Normal	++	0%
LPS-OvaNP	+	Normal	++	10%

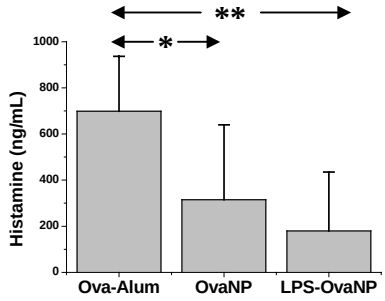

Figure 1: Increase on the histamine blood levels 30 minutes after the challenge.

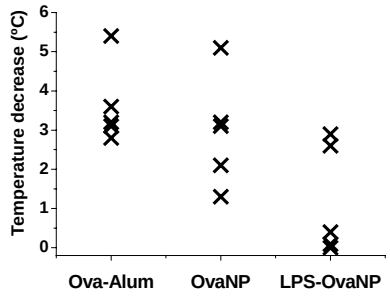

Figure 2: Decrease on the body temperature 30 minutes after the challenge.