 ISBN 978-602-60569-3-1

EFFECT OF PARTICLE SIZE AND SURFACE CHARGE ON THE UPTAKE AND IMMUNE RESPONSE OF OVALBUMIN-ALGINATE MICROSPHERES

Dewi Melani Hariyadi*, Pharmaceutics Department Faculty of Pharmacy, Universitas Airlangga, Surabaya, East Java-Indonesia, dewiffua96@yahoo.com **; Idha Kusumawati,** Pharmacognosy and Phytochemical Department, Faculty of Pharmacy, Universitas Airlangga, Surabaya, East Java-Indonesia**; Fauzia Azzahra,** Pharmaceutics Department Faculty of Pharmacy, Universitas Airlangga, Surabaya, East Java-Indonesia, rara.darodjatun@gmail.com

INTRODUCTION

Microspheres as one of drug and protein delivery system have some advantages such as producing small particles; protect extreme conditions and other benefits. Microspheres as one of particulate systems have benefits in vaccine delivery systems for enhancing immune response through several mechanisms. As vaccine delivery system, the particles can through phagocytosis deliver antigens to Antigen Presenting Cells (APC) (Foged et al, 2005). Particulate antigens might mediate the induction of both humoral and cellular immunity.

The particle size, surface charge and physicochemical properties are factors that affect the uptake of particles from the gut. Zeta potential may also influence the drug release profiles, stability and physicochemical properties.

Microparticles or nanoparticle surface is a very important consideration in drug delivery ystem, especially in targeting drug delivery. Surface modification of micro/nano-drug delivery systems is the most common strategy to controlling the opsonization process and thus sustains the systems for longer period in the blood stream. Zeta potential is one of important properties which contribute to the effectiveness. Zeta potential can be defined as the electrokinetic value associating a realistic magnitude of surface charge and its unit is usually millivolt.

There are some investigations that showed that the surface charge of nano/microparticles has an effect on the stimulation of the immune response. Some studies showed that antigen loaded cationically charged particles could be beneficial for gut up take (Honary S and Zahir F, 2013).

Peyer's patches (PP) is the main target of oral delivery systems in the small intestine as a place for the transport of pathogens to the lymphoid tissue. This function is carried out by M-cells which are located between epithelial cells, bringing antigens and microparticles measuring less than 10 µm (Lubben et al, 2001).

This study used alginate microspheres contain safe and biodegradable polymer and $CaCl_2$ non toxic crosslinking agent produced by gelation ionotropic technique by aerosolisation. This technique had the advantage of spherical shape, smooth with a small particle size that meets the requirements of particle size for oral delivery systems (Hariyadi et al, 2014). Maltodextrin lyoprotectant were found to stabilize microspheres (Hariyadi et al, 2015). Ovalbumin was used as model antigen.

The hemagglutination assay was used to evaluate the formation of antibody and ability to stimulate immune response. Uptake microspheres were studied using fluorescent label microscopically. In the present study we addressed the importance of particle size and surface charge for efficient interactions and effect on the immune response.

MATERIALS AND METHODS

Materials

Ovalbumin, Sodium alginate, protein quantification kit (Sigma Aldrich), $CaCl_2.2H_2O$ pharmaceutical grade (Solvay Chemicals Internationals), Sodium citrate p.g, maltodextrin (Bratachem Chemicals), Rhodamin B (E Merck), Optimal Cutting Temperature (O.C.T) Compound (Sakura), aquadest, red gout blood cell, and mus musculus strain Balb C from Pusat Veterenaria Farma (PUSVETMA) Surabaya..

Preparation of Ovalbumin-loaded alginate microspheres

Sodium alginate (2.5%) was dissolved in distilled water and ovalbumin (2.5%) was dissolved in it. This solution was then sprayed into solution of 1.5 M $CaCl_2$ solution at pressure of 40 psi. The mixture was stirred at 1000 rpm for 2 hours. Microspheres formed were collected and then separated by centrifugation at 2500 rpm for 6 min and washed twice. Microspheres were resuspended in lyoprotectant solution (1g/10mL) with concentration according to the formula. The

suspension was dried by freeze dryer at a temperature of -80 °C for 29 hours.

Particle size and zeta potential

Formulas of ovalbumin-alginate microspheres with and without lyoprotectant were characterized in terms of particle size using optical microscopy and software and zeta potential measured by zetasizer.

Hemagglutination test

Animals in vivo study has been approved by ethics committee and met National Ethic Standard by Faculty of Veterinary Universitas Airlangga. Mice were given orally ovalbumin-loaded alginate microspheres or control for five days for all groups of mice. At day 7, animals were injected intraperitonially using gout red blood cell suspension. At day 17, bloods were taken intracardially and were analysed for the serum or supernatant after centrifugation. Hemagglutination study was conducted to analyse immune response by measuring IgG titres.

Uptake of microspheres

Formulas of ovalbumin-alginate microspheres with and without lyoprotectant compared to ovalbumin and blank microspheres were used. Rhodamine B was a fluorochrome and was used to label all groups. All groups were dispersed into CMC Na in aqueous solution as control. Prior sacrificed, Mice were adapted for a week in a room with a temperature of 25 ºC ± 2 °C in a separated cage. Mice were then fasted for 16 hours followed by orally administered. Volume oral administration was 500 µL/ 25 gram body weight. To determine the uptake in intestinal mice, following after 6,7,8,9 and 10 hours after oral administration, mice were sacrificed. Mixture intestinal tissue histology was then observed with a fluorescent microscope using a red filter.

Data Analysis

The data are expressed as the mean ± SD from triplicates experiments.

RESULTS AND DISCUSSION

Particle size and surface charge

The particle size resulting of all formulas was below 5µm and zeta potential as surface charge showed the negative charge (Table 1). The optimal particle diameter for oral delivery through peyer's patches is below 10µm. Uptake of small particles was greatly enhanced when particles displayed negative surface charge.

Table 1. Particle size and zeta potential of formulas

FORMULA	PARTICLE SIZE (µm)	ZETA POTENTIAL (mV)
Blank Microspheres without lyoprotectant	4.11	-7.08 ± 0.86
Blank microspheres with 5% lyoprotectant	4.18	-6.03 ± 1.08
Ovalbumin-loaded alginate microspheres without lyoprotectant	4.20	-7.67 ± 0.53
Ovalbumin-loaded alginate microspheres with 5% maltodextrin	4.36	-8.44 ± 0.25

Small-sized and negatively charged particles might be contributed to the immune response as shown in further study. Moreover, the surface charge density of microparticles should be optimized for minimum toxicity and effective delivery of encapsulated drug or protein.

Hemagglutination study

Figure 1 showed the lyophilized ovalbumin-Ca alginate microspheres with 5% maltodextrin exhibited higher antibody titre compared to ovalbumin-Ca-alginate microspheres without lyoprotectant, whereas ovalbumin-alginate microspheres produced higher IgG than blank microspheres and ovalbumin control.

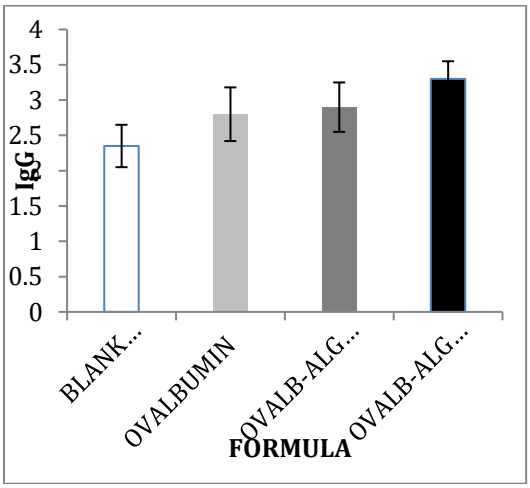

Figure 1. IgG titre of formulas

This indicated that ovalbumin antigen has arrived at the target site and ovalbumin microspheres was able to across the GI tract barrier in peyer's patches produced immune response and potential for oral vaccine delivery system. Maltodextrin lyoprotectant seemed stabilized microspheres due to hydrogen bonding between sugar or alcohol sugar and protein during freeze drying and avoid aggregation (Musumeci et al, 2006).

Uptake of microspheres using fluorescence microscope

Observations uptake of ovalbumin-loaded alginate microspheres on the ileum of mice was performed after several hours both of microspheres with and without lyoprotectant.

Uptake of ovalbumin-loaded alginate microspheres on the ileum of mice was performed on 6 to 10 hours after administration and showed that in the 6th and 7th hours after oral administration, red luminescence was seen in the intestinal villi. From previous research, ovalbumin administered orally produced lower IgG titre compared to ovalbumin entrapped in the microsphere delivery system; this may be because ovalbumin was degraded by stomach acid (Hariyadi et al, 2015).

Uptake of microparticles in the intestine was influenced by particle size (Tabata & Ikada, 1996), surface charge and hydrophobicity (Chen & Langer, 1998). In the study conducted by Tabata et al (1996), after the uptake in Peyer's Patches, the particle size of the particles was less than 5µm was transported to the lymph, which is a lymphoid tissue systemic, where the antigen contained would be released and produce an immune response, whereas particles with size larger than 5µm would stay in Peyer's Patches and released antigen. Some particles may stay longer in Peyer's Patches and delivered ovalbumin that can cause an immune response. Results of uptake of ovalbumin-alginate microspheres with 5% maltodextrin was seemed to be slower release of ovalbumin and longer stay in villi, this can be attributed the possibility that the microparticles released ovalbumin slowly and may stay longer in Peyer's Patches.

Small-sized, negatively charged particles might therefore interact with inside the villi or peyer patches. This result may be suggested that certain particle size may be taken up antigens to initiate an immune response (Kiama et al., 2001).

CONCLUSION

Hemaglutination test of ovalbumin-loaded alginate microspheres with lyoprotectant showed high antibody titres. In vivo uptake study of microspheres in mice's villi and Peyer's patches showed that ovalbumin-alginate microspheres with lyoprotectant were able to be taken up by villi after given orally and taken up further by Peyer's Patches. Small particle size and required zeta potential showed their contribution to the absorption into body membranes. The particle size and surface charge is potential to demonstrate the ability to target drug delivery systems at specific sites of the body especially in order to induce antibody response.

ACKNOWLEDGEMENT

This work was supported by grant DIKTI (Directorate of Higher Education). We would like to thank Faculty of Pharmacy Airlangga University (UNAIR) for supporting research facilities.

REFERENCES

1. Camilla Foged, Birger Brodin, Sven Frokjaer, Anne Sundblad., Particle size and surface charge affect particle uptake by human dendritic cells in an in vitro model, *Int. J. Pharm*. **298,** 315–322 (2005).
2. Chen H. & Langer R. Oral particulate delivery: status and future trends. *Adv. Drug Deliv.Rev*. **34**, 339-350 (1998)
3. Hariyadi DM, Hendradi E, Purwanti T, Fadil FDGP, Ramadani CN. Effect Of Cross Linking Agent And Polimer on The Characteristic of Ovalbumin Loaded Alginate Microsphere. *Int. J. Pharm. Pharm. Sci*. **6(4)**, 469-474 (2014)
4. Hariyadi DM, Purwanti T, Nirmala RN. Effect of Lactose and Maltodextrin on The Physical Characteristics of Ovalbumin-loaded Alginate Microspheres Produced By Aerosolization. DOI: 10.5176/2345-783X_PHARMA14.29
5. Hariyadi, DM, Purwanti T, Kusumawati I, Nirmala RN, Maindra HMC. Physical Characterization and In Vivo Study of Ovalbumin Encapsulated in Alginate Microspheres, *Int. J. Drug Deliv.Tech* **5(2)**: 48-53 (2015)
6. Kiama, S.G., Cochand, L., Karlsson, L., Nicod, L.P., Gehr, P., Evaluation of phagocytic activity in human monocyte-derived dendritic cells. *J. Aerosol. Med*. **14,** 289–299 (2001).
7. Lubben IM, Verhoef JC, Van Aelst AC, Borchard G, Junginger HE. Chitosan microparticles for oral vaccination: Preparation, characterization and preliminary in vivo uptake studies in murine Peyer's patches. *Biomaterials.* **22**, 687-694 (2001).
8. Musumeci T, Vicari L, Ventura CA, Gulisano M, Pignatello R, Puglisi G. Lyoprotected nanosphere formulations for paclitaxel controlled delivery. *J. Nanosci Nanotech*. **6,** 457-503 (2006).
9. Tabata, Y., Ikada, Y. Effect of the size and surface charge of polymer microspheres on their phagocytosis by macrophage. *Biomaterials* **9**, 356–362 (1988).
10. Tabata Y, Inoue Y, Ikada Y. Size effect on systemic and mucosal immune responses induced by oral administration of biodegradable microsphere. *Vaccine*, **14**, 1677-1685(1996).
11. Soheyla Honary and Foruhe Zahir, Effect of Zeta Potential on the Properties of Nano-Drug Delivery Systems - A Review (Part 1),

Tropical J.Pharm.Research; **12 (2)**, 255-264 (April 2013).
12. Soheyla Honary and Foruhe Zahir, Effect of Zeta Potential on the Properties of Nano-Drug Delivery Systems - A Review (Part 2), *Tropical J.Pharm.Research*; **12 (2)**, 265-273 (April 2013).