Oral Presentation (PCS-6)

Preparation and Application of Nano Chitosan Particles as Adjuvan in Rabies Vaccination Based on Anti-Idiotype Antibody

Sayu Putu Yuni Paryati[1*], K. Hasan[2], S. Ramadhanti[3]

[1]Department of Microbiology, Faculty of Medicine Universitas Jenderal Achmad Yani, Cimahi;
[2]Department of Biochemistry, Faculty of Medicine Universitas Jenderal Achmad Yani, Cimahi;
[3]Faculty of Medicine Universitas Jenderal Achmad Yani, Cimahi
*yuni.paryati@lecture.unjani.ac.id

Keywords: adjuvant, antibody anti-idiotype vaccine, chitosan, nanoparticles, rabies.

INTRODUCTION

Rabies is a fatal disease to humans and animals, but can be controlled by prophylaxis administration before being exposed. One type of vaccine that can be utilized is an anti-idiotype antibody vaccine originating from IgY purification of chicken egg yolk. The use of vaccine additives in the form of adjuvants is very important to improve the effectiveness of vaccines [1].

Chitosan is non-toxic, easily synthesized, safe to use and able to induce an immune response by activating macrophages. Chitosan can modulate dendritic cell maturation so that it can induce interferon interactions and stimulate activity of T lymphocytes and B lymphocytes. Chitosan can be made in the form of chitosan nanoparticles that can be used to deliver drugs and vaccines through inhalation pathways, orally, intravenously and as non-viral gene delivery vectors. In the oral delivery of chitosan nanoparticles are able to overcome the problem of solubility, protect the drug from enzymatic degradation, controlled release, and extend the time of action in the bloodstream through ionic mechanisms with mucin. Inhalation of chitosan 0.5mg/ml with influenza vaccine produces a good response [2-4].

In this work, the preparation of chitosan nanostructures and their application as adjuvan in antibody anti-idiotype rabies vaccination were reviewed.

MATERIALS AND METHODS

Purification and characterization IgY Anti-Idiotype

Purification of IgY anti-idiotype antibody was done through precipitation of Precipitation Solution A and B and was followed by dialysis using PBS pH 7.4 for 24 hours at a temperature of 8^{o}C. The precipitate was then characterized using the SDS-PAGE method which produced various amounts of proteins however the ones that were IgY had a weight of 161 KDalton's [5].

Preparation of Chitosan Nanoparticles

Nano chitosan was used as an additive to increase the vaccine's effectivity and can be potentially used as an adjuvant.

Chitosan nanoparticles can be prepared by the interaction of oppositely charged macromolecules. Tripolyphosphate (TPP) has often been used to prepare chitosan nanoparticles because TPP is nontoxic, multivalent and able to form gels through ionic interactions. The interaction can be controlled by the charge density of TPP and chitosan, which is dependent on the pH of the solution [6].

Nanoparticles were prepared by adding drop wise a tripolyphosphate-pentasodium solution to chitosan solutions under stirring. Trehalose, mannitol and polyethylene-glycol as bioprotectants were used to prevent particle aggregation and to reduce mechanical stress during freezing and drying processes [6]. Furthermore, the nanoparticles were dissolved into physiological NaCl with a concentration of 0.5mg/ml, mixed with anti-idiotype antibodies with a ratio of 1:1 (v/v) [6].

Immunization

A total of 9 rats were immunized using anti-idiotype antibody vaccine with nano chitosan adjuvant additive, 9 rats were immunized with a commercial rabies vaccine as the positive control group, and 9 rats were immunized with physiological NaCl as the negative control group. The administration of anti-idiotype antibody vaccines was prepared by injecting mice subcutaneously in loose skin tissue areas such as neck skin areas on days 0, 7, and 14. Each group of mice was injected with 0.5 ml injection volume according to treatment groups [1].

Examination of serum levels were done for 4 weeks in order to know the level of the rabies antibody by using the AGPT methods. Rat blood collection was carried out a week after the last vaccination and carried out every week for 4 weeks.

AGPT is made with a composition of 0.8 grams of agarose, 2.4 grams of PEG, 40 ml of aquadest, and 40 ml of PBS with a pH of 7.4. The ingredients are mixed and heated to a boil and allowed to stand for 5-10 minutes, then the AGPT media formed is poured 3 ml in a petri dish with a diameter of 6 cm to become solid. By using a media perforator, AGPT is perforated with 7 holes to form a certain pattern. In the hole that is in the middle of the rabies antigen is inserted, while in the other 6 holes the serum is inserted according to the dilution titre. In the study, this serum was diluted serially in mikroplate. Serum is diluted using PBS with a pH of 7.4. The diluted serum is then poured into each well that has been numbered. AGPT media containing serum and rabies antigen were then given a damp cotton pad and incubated at 37°C for 24-48 hours then observed. The positive results of this examination are illustrated by the formation of white precipitation lines between antigen and serum holes.

Data analysis was performed by Saphiro Wilk's normality test to determine the normality of data distribution. After obtaining normal distribution data, continued with one way Anova to find out the differences between treatment groups in experimental animals.

RESULT AND DISCUSSION

Purification of IgY from freeze dried egg yolks was carried out using Precipitaion Solution A and Solution B kits, obtained with white protein deposits. Analysis of IgY protein bands purified from egg yolks was carried out with Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) and stained with Fast Coomassie ® Stain. It can be shown that pure IgY has one major protein band with a large molecular weight (185 kD) (Fig. 1). This is in line with Narat (2003) which states, that IgY has a greater molecular weight than IgG, which is about 180,000 dalton or greater [7].

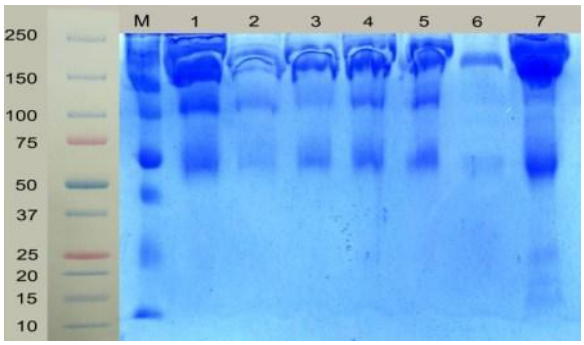

Fig.1. Protein band profiles purified by affinity chromatography (column 6) show protein bands with a molecular weight of 180 and 60 kDa compared to marker (M).

In addition, protein is also often obtained with a molecular weight of 95,000 dalton and 49,000 dalton which is thought to be Fc and Fab fragments from IgY that have not yet been assembled into intact immunoglobulins. Sun et al. (2001) states that IgY degradation will produce Fc and Fab fragments, where the molecular weight of Fab is around 45,000 dalton.

The preparation by titration method produced chitosan nanoparticles shaped like white gel, felt soft, easily dissolved in physiological NaCl and did not settle. When mixed with the anti-idiotype antibody vaccine shows the results of mixing well. Next, 0.5% concentration will be used as an adjuvant in the vaccination process.

Observations of the three groups of rats immunized showed that all rats looked healthy, appetite did not change with normal behavior until 4 weeks after vaccination. Rat body weight experienced a normal increase. In group II which was immunized with anti-idiotype antibodies with the addition of chitosan nanoparticle adjuvants, there were no changes in rat skin tissue at the site of vaccine injection (Fig.2).

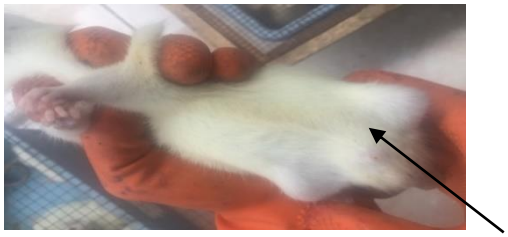

Fig. 2. The location of vaccine injections subcutaneously, there was no change in rat skin tissue

Blood collection is done through retro-orbital veins while the measurement of antibody titers is done by the AGPT method. Positive reactions are indicated by the formation of a line of precipitation between antigens and antibodies as in Fig. 3.

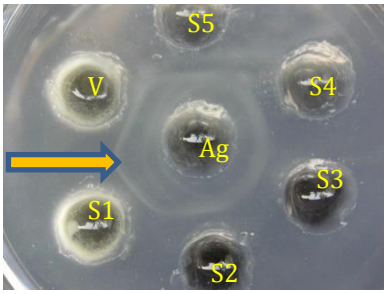

Fig. 3. Precipitation lines (arrows) in the immunodifusion test (AGPT) indicate a homologous reaction between antigen (Ag) (Ab1) and protein (IgY) and rabies virus (V).

Rabies antibody preparation in rats produced good antibody titers in the idiotype group with the addition of chitosan nanoparticles from week 2-4 after vaccination. In the second week, the precipitation line appears to be less clear.

The precipitation line continues to appear until the 4th week. Whereas in the positive control group antibodies were detected at week 4 and the negative control group did not form rabies antibodies.

Anti-idiotype antibody vaccines can mimic the structure of the antigen so that it can be used as an immunogen. The antigen will be phagocytosed by macrophages but the process of antigen destruction in the macrophages is slowed due to the addition of adjuvants so that the antigen is gradually released thereby extending antigen exposure with an immune response and has the capacity to intervene in a selective immune system such as T cells and B cells, antigen that have been phagocyted by macrophages will be presented to T lymphocytes with MHC II presenting molecules. The introduction of antigens by the presenting cells causes activated T lymphocytes to activate, thereby triggering the process of active proliferation and differentiation of other T lymphocytes in lymphoid tissue [8].

With the addition of nano chitosan adjuvant can increase the stimulation of antibody formation by B lymphocytes so that antibody levels can increase rapidly in the body.

Analysis with Mann Whitney test showed significantly different results between rat serum antibody titers in the positive control group immunized with the commercial rabies vaccine with the treatment group immunized with anti-idiotype antibody vaccine with the addition of chitosan adjuvant nanoparticles (Table 1).

Table 1 *Mann Whitney Test*

	Positv e	Negativ e	Idiotype and nanoparticl e
Positive		0,011*	
Negative			0,028*
Idiotype and Nanoparticl e	0,923		

*p-value <0,05 there are meaningful differences between groups

Based on the Kruskal Wallis test, it was found that the negative control group had the lowest mean rank compared to the idiotype and chitosan nanoparticles, which was 9.50 while the highest mean rank was found in the positive control group with a value of 16.39. These results show that the idiotype and nano chitosan and positive controls have an effect on the increase in rat antibody titers.

CONCLUSION

Vaccination of anti-idiotype antibodies using adjuvant chitosan nanoparticles can induce rabies antibody formation in rats. In the positive control antibodies were formed starting from week 4, whereas in vaccination with anti-idiotype antibodies with the addition of nano chitosan formed from week 2 to week 4 after the last vaccination and there were significant differences between negative control antibodies with positive controls and anti-idiotype antibodies with addition of adjuvants.

ACKNOWLEDGMENTS

Thanks to the Universitas Jenderal Achmad Yani Research and Community Service for the support of research costs through the Unjani competitive grant in 2018. Thanks also to the Ministry of Research and Technology for research funding through Higher Education Applied Research in 2018.

REFERENCES

[1] World Health Organization (WHO). WHO*: Guide for Rabies Pre and Post Exposure Prophylaxis in Human.*

[2] Kim SK. Chitin and chitosan derivates advances in drug discovery and developments [e-book, CD ROM]. CRC press; 2014.

[3] Dzung NA. Chitosan and chitosan derivates as potential adjuvants for influenza vaccine. http://reasearchgate.net/publication/262183686

[4] Lawson BL, Elizabeth BN, John DC. Defending the mucosa adjuvant and carier formulation for mucosa immunity. http://www.sciencedirect.com

[5] Paryati SPY. Antibodi anti idiotipe sebagai kandidat vaksin rabies. Sekolah pasca sarjana Institut Pertanian Bogor.2006.

[6] Sivakami MS, Thandapani,Hee-Seok Jeong. Preparation and characterization of nano kitosan for treatment wastwater:International Journal of Biological Macromolekules. http://www.elsivier.com/localate/ijbiomac.

[7] Narat M. 2003. Production of Antibodies in Chickens. *Food Technol. Biotechnol.* 41(3):259-267.

[8] Abbas AK, Andrew H, Shiv P. Basic Imunnulogy [e-book, CD ROM]. edisi 5. 2016