Effect of rubella vaccine to plasmodium-infected mice parasitemia levels

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Abstract. Malaria, an infectious disease causes by Plasmodium, contribute to 300-900 million morbidities and 1-3 million mortalities, annually. This study aimed to determine the Rubella vaccine potency to inhibit *Plasmodium berghei* merozoite invasion to erythrocyte which evaluated from parasitemia levels, mortality and mice clinical condition. This study conducted in mice injected Rubella vaccine prior injected with *P. berghei*. There were four groups: A, B and C injected with 500 µl, 2 µl, and 1 µl rubella vaccine respectively. Group D injected with 500 µl sterile aqua, as control group. After 28 days of vaccination all mice injected with 0.2 ml P. berghei. Furthermore, we observed to parasitemia levels, death, and clinical condition of mice to assess Rubella vaccine effectiveness to inhibit merozoite invasion. The results shown Rubella vaccine did not provide significant effect on parasitemia levels. It might Rubella vaccine dose we used under effective dose and less effective of target site. However, our study shown Rubella vaccine provided significant effect on mice mortality ($p<0.05$). It is probably due to Rubella antigen (213-239 amino acid sequence) and malaria antigen merozoite surface protein (MSP)-1_{19} (sequence amino acid 238) has similar structure, thus it was likely generated cross-immunity. In conclusion, 28 days Rubella vaccination did not provide significant effect on parasitemia levels, however it provided significant effect on mice mortality with infected by *P. berghei.*
Keywords: Malaria, rubella vaccine, *Plasmodium berghei*, malaria vaccine.

Introduction

Malaria, an infectious disease causes by Plasmodium, causes 300-900 million morbidities and 1-3 million mortalities annually (WHO, 2005). Effective malaria vaccine to reduce malaria incidence is still unavailable. Recently, malaria vaccine, SPF-66, was proved to be efficacious because it did not increase natural antibody to malaria and decrease interferon (IFN)-γ levels, thus SPF-66 seem uncorrelated with protection against malaria (Wang *et al*., 2010). It was allegedly because SPF-66 has ineffective target sites. There are three sporozoite surface proteins, 83, 55, and 35 kDa (Graves & Gelband, 2009). Therefore, it is necessary to find out new alternative vaccine which has a potential target site to increase immunity against malaria. Previous study showed that merozoite surface protein (MSP)-1_{19} was potential target for malaria vaccine, because MSP-1_{19} has essential role in merozoite expansion to erythrocyte (Dluwzeski *et al*., 2008). MSP-1_{19} has two epidermal growth factors (EGFs). MSP-1_{19} has an N-glycosylation area on the overall length of MSP-1 and MSP-1_{19} that may come through N-glycosylation when expressed in mammalian cells (Langhorne, 2005). Study by Li *et al*. (2008) shown MSP-1_{19} antigen is a peptide protein antigen which has 238 amino acid sequences. Rubella proteins have three structures, envelop 1 (E1), envelop 2 (E2), and capsid (C). E1 and E2, membrane glycoproteins, are part of the rubella virus that consist cysteine-rich glycoprotein (Abernathy *et al*., 2009). The role of these proteins is associated with membrane fusion process (Liu *et al*., 2009; Zhou *et al*., 2009).

Several studies confirmed E1 is a peptide protein antigen which has 213-239 amino acid sequences (Mitchell *et al*., 1994; Ovsyannikova *et al*., 2004; Ovsyannikova *et al*., 2006). Based on amino acid sequence similarity between E1 Rubella and MSP-1_{19} Plasmodium, we predicted that there was host cross-immune development which protect host against Plasmodium if we treat with Rubella vaccine prior infect with Plasmodium parasite. Therefore, present study to determine Rubella vaccine potency to inhibit *Plasmodium berghei* merozoite invasion to erythrocytes which evaluated from parasitemia levels, mortality and mice clinical condition.

Materials and Methods

Research design

Post test-only control group design was used in this study. Mice divided in 4 groups (A, B, C and D), and each group consist 5 mice. Group A, B and C injected with 500 µl, 2 µl, and 1 µl Rubella vaccine per subcutaneously respectively. Group D injected with 500 µl sterile aqua, as control group. After 28 days of vaccination all mice injected with 0.2 ml *P. berghei*.

Rubella vaccine preparation

Rubella vaccine used was mmr II, purchased from Kimia Farma, Surabaya, Indonesia. Rubella vaccine dose preparation, 500µl, 1µl and 2µl based on manufacture instruction.

P. berghei preparation

P. berghei preparation was conducted at Parasitology and Biomedical Laboratory, School of Medicine Brawijaya University, Malang, using standard procedures of *P. berghei* thawing process which described previously (Doolan, 2002).

Microscopic examination

Malaria microscopic preparation and parasitemia level determination carried out according to standard procedures (Doolan, 2002). Briefly, parasitemia levels are accessed on 5th day after *P. berghei* infection; we counted the number of Plasmodium-infected leukocyte per 200 leukocytes or per 1000 erythrocytes.

Statistical analysis

Statistical analysis in this study was ANOVA one way using Statistics Calculators version 2.0 with $p<0.05$.

Results and Discussion

The results of parasitemia can be seen in Fig. 1A-C, while the results the mice mortality can be seen in Fig. 1D. Furthermore, these parasitemia datas used to calculate malaria invasion inhibition. Fig. 1A-C showed that group A had the lowest average parasitemia and had the highest inhibition value. Data analysis on parasitemia level and parasite number per µl of blood found there were no statistically significant, $p=0.2$, $p=0.09$ respectively. In term of mice mortality (See Fig. 1D). Group A had the lowest number of mice mortality. In addition, group A also showed a good clinical condition. The result of variance analysis of the mice mortality obtained F-value of the 6th day of death was 1.333 [F table 3.24] and F-value of overall mortality was 5.867 [F table 3.24]. It indicated that Rubella vaccine effected mice infected *P. berghei* mortality significantly ($p<0.05$).

Parasitemia, parasite quantities in blood, is indicator to determine malaria growth or inhibition (Bisoffi *et al*., 2010). Based on parasitemia levels in this study we found there was no significant difference among groups. It might caused by Rubella vaccine doses used and Rubella vaccine target site effectiveness. The highest Rubella vaccine dose, 500 µl, inhibited 49% of parasitemia level. To obtain a higher inhibition effect may need to give a higher dose. However, Rubella vaccine toxicity at higher dose needs to be considered.

Theoretically, target site of Rubella vaccine in this study was MSP-1_{19}. Merozoite invasion to erythrocyte is a complex process. It involves several merozoite surface proteins MSP such as erythrocyte binding protein (EBP), MSP-1_{19}, MSP-7, apical membrane antigen (AMA), acid basic repeat antigen (ABRA), and serine-repeat antigen (SERA) (Rollinson *et al*., 2009). Inhibition to MSP-1_{19} indicates partial inhibition only to merozoite invasion to erythrocyte. Thus, this seems lead less effective inhibition.

Based on data analysis shown Rubella vaccine effected on total mice mortality significantly ($p<0.05$). However, Rubella vaccine not significantly affected parasitemia levels and number of parasites, $p=0.2$ and $p=0.09$ respectively. The result showed group A has lowest in term of parasitemia levels, parasite number per µl of blood, and the number of deaths. Dose of group A was based on the product information whereas the dose of group B and C were based on weight conversion. Based on the results of this study indicated that the effectiveness of the best protection in this study was in group A. This protection had a 49% growth inhibition or the highest than other groups.

Rubella vaccine protection against malaria might caused by amino acid sequence similarity between E1 Rubella virus and MSP-1_{19} Plasmodium. E1 has 213-239 amino acid sequences and MSP-1_{19} has 238 amino acid sequences (Prendergast & Mann, 1978; Mitchell *et*

al., 1996). Because of these structural similarities (between 5 or 10 amino acid sequences), it was predicted to lead specific immunity (Frank, 2002). The possibility pathway it can cause cross immunity reaction by inhibiting MSP-1_{19}. This immunity mechanism possibility involves human leukocyte antigen (HLA), IFN-γ, interleukin (IL)-10 and immunoglobulin (Ig)-G (Ovsyannikova *et al*., 2007; Aschermann *et al*., 2010). Furthermore this mechanism result inhibition mechanism and phagocytosis, caused a lower parasitemia level and mortality rate in group A.

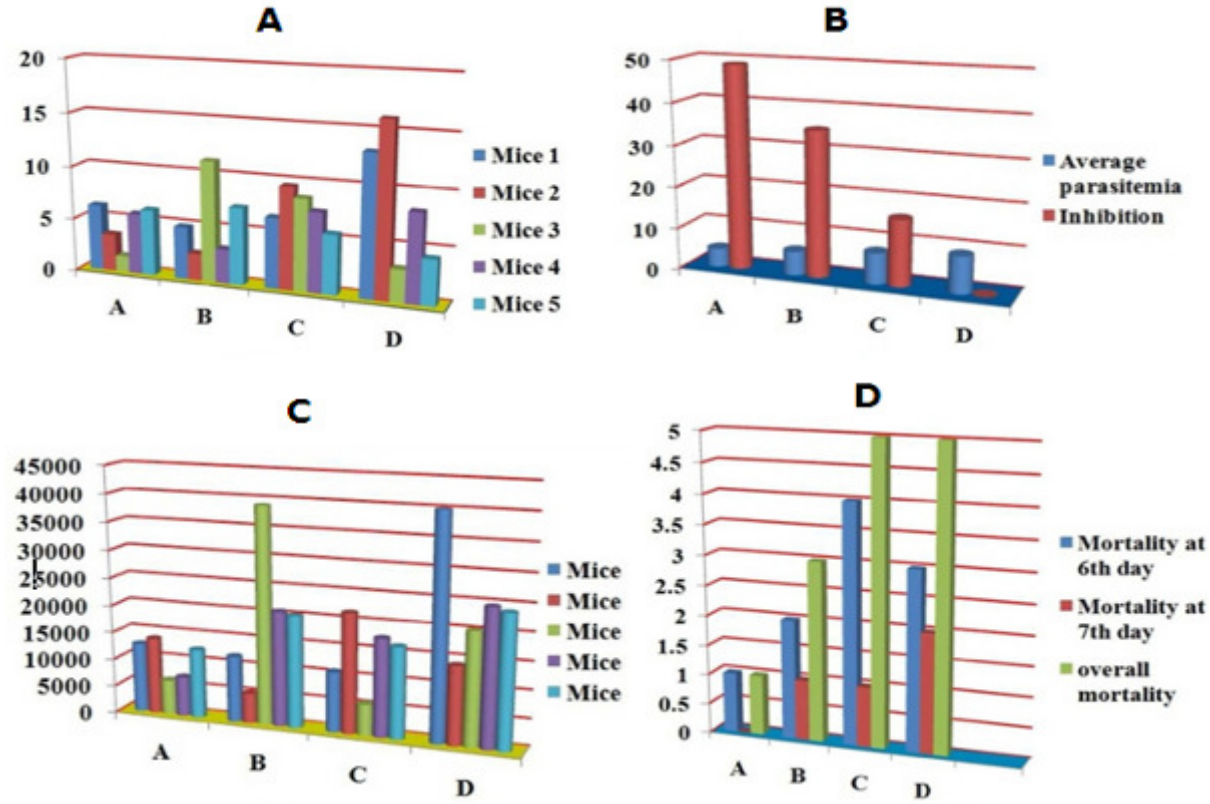

Figure 1. A. Total parasitemia percentage on 5th day after *P. berghei* infection. **B.** Average of parasitaemia percentage (group A=4,62%; group B= 5,82%; group C=7,58%; and group D=9%) and percentage of Rubella vaccine inhibition (group A=49%; group B=35%; and group C=16%). **C.** The number of parasites per µl of blood. The average of group A=10477; group B=19559; group C=14528; and group D=24701. **D.** Mice mortality after infected with *P. berghei* (total mortality of group A=1; group B=3; group C=5; and group D=5).

Parasitemia level has a clear correlation with clinical condition and mortality rate (Doolan, 2002). Merozoite invasion into erythrocyte is responsible to malaria clinical manifestations. This study shown good clinical conditions in group A, contrary in group B, C, and D which shown poor clinical conditions. Therefore, these results are consistent with another study confirmed that some virus vaccines could prevent malaria mortality (Hutchings *et al*., 2007). But advance research that to confirm the role of Rubella vaccine inhibition against malaria is need.

Conclusions

This study indicated that rubella vaccination did not provide significant effect on total parasitemia level of mice infected *P. berghei.* However, rubella vaccination provided significant effect on *P. berghei* infected-mice mortality ($p<0.05$) and it seem dose-dependent effect.

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