

Formulasi dan Evaluasi Respons Immunologi terhadap Sediaan Ajuvan Liposom Berbasis PEI25k pada Mencit (*Mus musculus L.*) Galur BALB/c = Formulation and Evaluation of Immunological Responses to Liposome Adjuvant PEI25k-Based Preparations in Mice (*Mus musculus L.*) BALB/c Strain

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Abstrak

Ajuvan liposom penting dalam meningkatkan efektivitas kerja vaksin dan menjadi alternatif ajuvan yang menjanjikan karena memiliki kemampuan enkapsulasi tinggi dan induksi imun spesifik. Namun, rendahnya stabilitas struktur liposom menjadi tantangan utama dalam pembuatan ajuvan. Polimer polietilenimin PEI25k dapat meningkatkan dan memperkuat struktur liposom serta memiliki potensi sebagai ajuvan. Namun, PEI25k bersifat toksik. Oleh karena itu, kolesterol diformulasikan ke liposom untuk menekan tingkat toksisitas. Selain itu, lipid kationik dioleoylphosphatidylethanolamine (DOTAP) diketahui dapat meningkatkan efisiensi dan stabilitas struktur liposom. Penelitian ini bertujuan untuk mendapatkan formula serta evaluasi kemampuan kompleks liposom PEI25k/kolesterol, PEI25k/DOTAP dan PEI25k/DOTAP/kolesterol dengan DNA (0,5:1; 1:1; 2:1; 4:1) dalam mengkondensasi dan melindungi DNA dari degradasi; toksisitas dan sifat imunostimulan liposom terhadap sel RAW 264.7; mengetahui potensi formula dalam induksi respons imunologi secara *in vivo*. Liposom dipreparasi dengan metode injeksi etanol dan kompleks DNA-liposom dikarakterisasi melalui uji mobilitas dan uji stabilitas. Liposom juga dievaluasi secara *in vitro* melalui uji MTT dan uji pelepasan nitrit oksida (NO). Selain itu, uji analisis hematologi darah mencit BALB/c dilakukan untuk mengetahui respons imun lebih lanjut. Kompleks DNA liposom dengan perbandingan 1:0,5 sampai 1:4 mampu mengkondensasi dan melindungi DNA dari degradasi enzim DNase. Seluruh formula bersifat non-toksik pada konsentrasi 0,01 ppm-6,25 ppm dan bersifat imunostimulan pada konsentrasi 6,25 ppm dan 25 ppm. Formula PEI25k:DOTAP (1:1) memiliki tingkat viabilitas dan menginduksi pelepasan NO tertinggi. Analisis hematologi menunjukkan formula PEI25k:DOTAP:kolesterol (1:1:1) dan (2:1:1) memiliki potensi paling signifikan dalam induksi respons imun.

.....Adjuvant liposome has a crucial role in enhancing the vaccine's efficiency and has become a promising alternative due to its high encapsulation and specific immunity induction capability. However, the low stability of its structure has become the main obstacle in adjuvant manufacture. Polyethyleneimines PEI25k could increase and strengthen the structure of liposomes and also have adjuvant potential. Due to the high cytotoxicity of PEI25k, the liposomes are being formulated with cholesterol. Also, cationic lipids such as, *dioleoylphosphatidylethanolamine* (DOTAP) are known to enhance the efficiency and structure of liposomes. This research aims to obtain formulas and evaluate the ability of the liposome PEI25k/cholesterol, PEI25k/DOTAP, and PEI25k/DOTAP/cholesterol-DNA (0.5:1; 1:1; 2:1; 4:1) in condensing and protecting DNA from degradation; toxicity, and immunostimulant properties against RAW 264.7 cells; as well as *in vivo*. The liposomes are prepared by ethanol injection method. The DNA-liposome complexes are characterized by mobility and stability assays. Liposomes are also evaluated *in vitro* through MTT assay and nitric oxide release assay. In addition, a hematological analysis was performed to determine further immune response. The DNA-liposome complexes with a ratio of 1:0,5 to 1:4 are capable of

condensing and protecting DNA from degradation. The whole formula is non-toxic at concentrations of 0.01 ppm – 6.25 ppm and immunostimulant at concentrations of 6.25 and 25 ppm. The formula PEI25k:DOTAP (1:1) has the highest level of viability and NO released. Hematological analysis indicates that the formula of PEI25K:DOTAP:cholesterol (1:1:1) and (2:1:1) has significant potential to induce an immune responses.