Evaluasi Kitosan sebagai Agen Transfeksi Plasmid pCSII-EF-AcGFP (Gene Delivery Vehicle) terhadap Sel Peritoneal dan Sel Splenosit Mencit = Evaluation of Chitosan as a Plasmid Transfection Agent of pCSII-EF-AcGFP (Gene Delivery Vehicle) to Peritoneal Cells and Splenocyte Cells of Mice

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Abstrak

Transfeksi merupakan metode penghantaran gen menggunakan vektor nonviral dalam terapi genetika. Kitosan merupakan senyawa alami polimer kationik yang dapat membentuk ikatan ionik dengan molekul DNA yang merupakan polimer anionik sehingga dapat digunakan sebagai penghantar materi genetik. Pada penelitian ini kitosan digunakan sebagai agen penghantar gen pengkode Green Fluorescence Protein (GFP) dari plasmid pCSII-EF-AcGFP yang ditransfeksikan pada sel primer peritoneal, sel splenosit, serta galur sel HEK293T. Tujuan penelitian ini untuk mengetahui kemampuan kitosan dalam mengompleks dan melindungi DNA terhadap degradasi enzim DNAse. Selain itu, uji transfeksi dan uji sitotoksisitas kitosan dilakukan untuk mengetahui sifat dan kemampuan kitosan sebagai agen penghantar pada sel peritoneal, sel splenosit, dan galur sel HEK293T. Hasil penelitian menunjukkan bahwa kitosan mampu mengompleks dan melindungi DNA terhadap degradasi enzim DNAse. Hasil uji sitotoksik menggunakan MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) terhadap sel peritoneal, sel splenosit, dan galur sel HEK293T relatif tidak toksik, pada konsentrasi kitosan 8 g/L menunjukkan viabilitas sel berturut-turut sebesar 64% (sel peritoneal), 80% (sel splenosit), dan 70% (galur sel HEK293T). Kitosan secara kualitatif dapat memfasilitasi ekspresi gen pengkode Green Fluorescent Protein (GFP) pada galur sel HEK293T, meskipun intensitas ekspresi gen GFP tidak lebih terang daripada agen transfeksi komersil Lipofectamine® 3000. Akan tetapi, pembanding Lipofectamine maupun kitosan belum berhasil memfasilitasi ekspresi gen GFP yang ditunjukkan dengan tidak adanya pendaran cahaya pada sel peritoneal dan sel splenosit meskipun rasio perbandingan massa kitosan dan Lipofectamine telah ditingkatkan dari 1:2 menjadi 1:4 selama inkubasi 2x24 jam. Modifikasi struktur atau formulasi kitosan yang tepat diperlukan untuk meningkatkan kemampuan senyawa kitosan dalam memfasilitasi ekspresi gen GFP pengkode plasmid pCSII-EF-AcGFP ke dalam sel peritoneal dan splenosit.

.....Transfection is a gene delivery method using nonviral vectors in genetic therapy. Chitosan is a natural cationic polymer compound that can form ionic bonds with DNA molecules which are anionic polymers so that it can be used as a conductor of genetic material. In this study, chitosan was used as a delivery agent for the gene encoding Green Fluorescence Protein (GFP) from the pCSII-EF-AcGFP plasmid which was transfected on peritoneal primary cells, splenocytes, and HEK293T cell lines. The purpose of this study was to determine the ability of chitosan to form complexes and protect DNA against DNAse enzyme degradation. In addition, chitosan transfection and cytotoxicity tests were carried out to determine the nature and ability of chitosan as a conducting agent in peritoneal cells, splenocytes, and HEK293T cell lines. The results showed that chitosan was able to complex and protect DNA against DNAse enzyme degradation. The results of the cytotoxic test using MTT (3[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) against peritoneal cells, splenocytes, and HEK293T cell lines were relatively non-toxic, at a concentration of

8 g/L chitosan showed cell viability was 64% (peritoneal cells), 80% (splenocyte cells), and 70% (HEK293T cell line). Chitosan can qualitatively facilitate the expression of the gene encoding Green Fluorescent Protein (GFP) in the HEK293T cell line, although the intensity of GFP not brighter than that of the commercial transfection agent Lipofectamine® 3000. However, neither Lipofectamine nor chitosan comparisons have been GFP showing the absence of light luminescence in peritoneal cells and splenocytes cells although the ratio of mass ratio of chitosan and Lipofectamine has been increased from 1:2 to 1:4 during 2x24 hours incubation. Modification of the structure or proper formulation of chitosan is needed to increase the ability of chitosan compounds to facilitate the expression of the GFP encoding the plasmid pCSII-EF-AcGFP into peritoneal cells and splenocytes.