Studi efek imunostimulasi CpG ODN 2006x3_PD-Nanopartikel Kitosan sebagai kandidat anti alergi intra nasal: Studi in vitro dan in vivo pada mencit = Study of immunostimulation effects of CpG ODN 2006x3_PD-Chitosan Nanoparticles as intranasal anti-allergic candidates: In vitro and in vivo studies in mice.

Febriana Catur Iswanti, author

Abstrak

Latar belakang: Saat ini prevalens alergi semakin meningkat. Terapi alergi lebih terfokus untuk mengatasi gejala simptomatik. Pendekatan lain adalah terapi imunomodulasi dengan agonis toll like receptor (TLR) 9 yang dapat mengalihkan respon imun sel TCD4+ Th2 ke arah Th1. CpG ODN 2006x3_PD adalah oligonukleotida sintetik kelas B merupakan agonis TLR9 yang berpotensi aman karena mengandung fosfodiester. Pada penelitian ini CpG ODN 2006x3_PD diuji kemampuan untuk mengatasi alergi dengan menggunakan penghantar nanopartikel kitosan, melalui uji in vitro pada sel mononukleus darah tepi dan in vivo pada hewan coba mencit alergi. Metode: Preparasi nanopartikel kitosan dilakukan dengan metode gelasi ion, melalui reaksi ikat silang kitosan dan tripolifosfat. Nanopartikel dikarakterisasi dengan Dynamic light scattering (DLS), zeta sizer dan transmission electron microscope (TEM). Uji pengikatan dilakukan dengan elektroforesis pada gel agarosa 12%, uji toksisitas dan kemampuan aktivasi NF-κB dilakukan pada sel RAW Blue, dengan menggunakan cel counting kit 8 dan kit Quanti blue. Sel mononukleus darah tepi yang distimulasi selama 7 hari pada uji in vitro dan plasma hewan coba mencit Balb/c pada uji in vivo diukur konsentrasi IFNγ, IL-10, IL-13 dan IgE dengan metode ELISA.
Hasil: Diperoleh nanopartikel dengan ukuran <300 nm, muatan permukaan positif, bentuk sferis, tidak toksik dan dapat mengaktivasi NF-κB. Uji in vitro pada sel mononukleus darah tepi menunjukkan CpG ODN 2006x3_PD yang dihantarkan nanopartikel kitosan dapat menstimulasi sitokin tipe Th1 IFNγ dan T reg IL-10, menurunkan sitokin tipe Th2 IL-13 namun belum dapat menurunkan secara bermakna produksi IgE total pada sel mononukleus darah tepi individu sehat dan alergi Aplikasi intra nasal 10 ug/kali, 3 kali seminggu selama 3 minggu CpG ODN 2006x3_PD dan CpG ODN 2006x3_PD yang dihantarkan nanopartikel kitosan pada mencit Balb/c yang diinduksi alergi dengan ovalbumin dapat menstimulasi sitokin tipe Th1 IFNγ dan Treg IL-10, namun belum dapat menurunkan secara bermakna sitokin tipe Th2 IL-13 pada plasma mencit. Aplikasi CpG ODN 2006x3_PD dapat menurunkan produksi IgE spesifik anti ovalbumin pada plasma mencit meskipun belum dapat menurunkan produksi IgE total.
Simpulan: CpG ODN 2006x_PD dapat menjadi kandidat imunostimulator yang potensial pada alergi.
<hr>
Background: Currently, prevalence of allergy has increased in worldwide. Allergy treatment is mainly focused to reduce clinical symptoms. Another approach is immunomodulation therapy which utilizes toll like receptor (TLR) 9 agonist that may redirect pro-allergenic Th2 biased CD4+ T cell response toward Th1. CpG ODN 2006x3_PD which is classified as synthetic oligonucleotides B has potential as a safe TLR9 agonist due to its natural backbone. In this study, CpG ODN 2006x3_PD was examined about its ability in overcoming allergies by using chitosan nanoparticles delivery, through in vitro tests on peripheral blood

mononuclear cells and in vivo through allergic mice animal model.
Methods: Chitosan nanoparticles was prepared by ionic gelation method, through crosslinking reaction of chitosan and tripolyphosphate. Nanoparticles are characterized by Dynamic light scattering (DLS), zeta sizer and transmission electron microscope (TEM). The binding test was carried out with electrophoresis on 12% agarose gel, toxicity test and NF-κB activation ability performed on RAW Blue cells, using cel counting kit 8 and Quanti blue kit. IFNγ, IL-10, IL-13 and IgE level of in vitro tests of peripheral blood mononuclear cells after 7 days stimulation and Balb/c mice plasma of in vivo study were measured by ELISA method.
Results: Less than 300 nm, positive surface charge, spherical shape and nontoxic chitosan nanoparticles were obtained. These nanoparticles could deliver CpG ODN to activate NF-κB of mouse RAW-Blue cells effectively. In vitro assays of peripheral blood mononuclear cells showed that CpG ODN 2006x3_PD delivered by chitosan nanoparticles may stimulate Th1 IFNγ and T reg type cytokines IL-10, also decrease the Th2-type cytokine IL-13 but it couldn't inhibit total IgE production in peripheral blood mononuclear cells significantly. Intranasal application of 10 ug, 3 times per week for 3 weeks of CpG ODN 2006x3_PD and CpG ODN 2006x3_PD which were delivered by chitosan nanoparticles in allergen induced Balb/c mice could stimulate Th1 IFNγ and Treg type cytokines IL-10, but it couldn't significantly reduce the Th2-type cytokine IL-13 in mice plasma . The CpG ODN application decreased the specific IgE production of anti ovalbumin in mice plasma although it couldn't significantly reduce total IgE production.
Conclusions: CpG ODN 2006x_PD could be a potential candidate for allergic immunostimulator.