Intisari

Mukosa bukal dipertimbangkan sebagai rute pemberian vaksin karena banyak mengandung sel dendritik yang berperan sebagai *antigen presenting cell*. Vaksinasi melalui mukosa bukal membutuhkan desain dan adjuvan yang tepat. Karbonat apatit (CHA) dipertimbangkan sebagai kandidat adjuvan karena mempunyai sifat biokompatibel serta mempunyai afinitas yang tinggi terhadap protein dan mampu meningkatkan stabilitas dari protein yang dibawa. Membran mukoadhesif dengan *impermeable backing layer* dapat dipertimbangkan untuk vaksinasi melalui mukosa bukal karena dapat melepaskan substansi aktif hanya satu arah serta dapat melindungi agen aktif/protein dari aliran saliva dan proses digesti enzimatik. Pada penelitian ini, kompleks CHA-OVA diformulasikan ke dalam membran mukodhesif dan dilakukan vaksinasi melalui mukosa bukal.

Protein OVA ditambahkan ke dalam CHA dan dilakukan uji karakterisasi yang meliputi uji PSA, FTIR dan TEM. Membran mukoadhesif dibuat dengan cara mencampurkan hidrogel kitosan dan gelatin kemudian ditambahkan kompleks CHA-OVA. *Backing layer* dibuat dengan cara mencampurkan etil selulosa dengan PEG400. Uji karakteristik fisik membran mukoadhesif meliputi *swelling index*, pH permukaan, keseragaman bobot, ketebalan dan ketahanan lipat kemudian dilakukan uji pelepasan protein *in vitro* dan *ex vivo*. Uji *in vivo* dilakukan dengan melakukan vaksinasi melalui mukosa bukal kelinci.

Hasil uji PSA menunjukkan ukuran partikel kompleks CHA-OVA paling kecil adalah 1122,1 nm. Ukuran partikel CHA akan secara signifikan meningkat dengan adanya penambahan protein ovalbumin. Hal ini disebabkan karena protein akan cenderung untuk agregasi pada larutan dengan pH yang mendekati *isoelectric point*. Hasil uji FTIR pada kompleks CHA-OVA menunjukkan adanya ikatan antara gugus karboksil protein OVA dengan ion kalsium CHA pada pita 1553 cm^{-1}. Membran mukodhesif didapatkan dengan perbandingan campuran hidrogel kitosan dan gelatin 3:7; 4:6; 5:5. Hasil pengujian karakteristik fisik menunjukkan semua formula sesuai untuk aplikasi di membran bukal, yaitu *swellling index* yang baik, pH permukaan 6,7–6,8, ketebalan 70-110 µm, bobot 0,25-0,40 gr dan ketahanan lipat yang adekuat. Hasil uji pelepasan protein *in vitro* dan *ex vivo* menunjukkan profil pelepasan protein yang bertahap tanpa adanya pelepasan besar (*burst release*) dalam sekali waktu. Hasil uji *in vivo* menunjukkan tidak terdapat perbedaan signifikan titer IgG spesifik ovalbumin. Akan tetapi, terdapat perbedaan signifikan titer IgA spesifik ovalbumin, yang ditunjukkan dengan titer IgA spesifik ovalbumin lebih tinggi pada pemberian vaksinasi melalui membran mukoadhesif CHA-OVA dibandingkan membran OVA tanpa CHA dan pemberian melalui injeksi intramuskular.

Kata kunci: adjuvan, mukosa bukal, karbonat apatit, membran mukoadhesif

Abstract

Buccal mucosae is considered as a site for vaccine delivery since it relatively abundant of antigen-presenting dendritic cells, mainly Langerhans cells. Moreover, it offers other advantages such as needle free vaccination, higher patient compliance, easier and safer than parenteral administration. Vaccination through buccal mucosae need proper design and adjuvant. Biomimetic carbonate apatite (CHA) is now considered as candidate for adjuvant because it has high affinity to protein and increase protein stability. Unidirectional release mucoadhesive membrane with impermeable backing layer can be considered as proper design because it can control the drug release, protect antigen from salivary clearance and enzymatic digestive. In this study, we formulated CHA-OVA complexes into bilayer mucoadhesive membrane and vaccinated via buccal mucosae.

We investigated the effect of the concentration of CHA and protein OVA on particle size of of CHA-OVA complexes. Particle size distribution was determined using PSA. The morphology of CHA-OVA complexes was examined with TEM. The characterizations of CHA-OVA complexes was studied using FTIR. Bilayer mucoadhesive membrane were prepared using chitosan hydrogel mixed with gelatin then CHA-OVA complexes were added. Ethylcellulose blend with PEG400 were used as impermeable backing layer. We examined physical properties of mucoadhesive membrane contain CHA-OVA complexes, i.e. swelling index, surface pH, weight uniformity, thickness and folding endurance. We also conducted in vitro and ex vivo release study. For in vivo study, we vaccinated through rabbit buccal mucosae using mucoadhesive membrane and examined specific antibody against ovalbumin.

Particle size of CHA significantly increase because the addition of protein OVA resulted in micro-sized particle of CHA-OVA complexes. It was as expected because protein is tend to aggregate around the isoelectric point. TEM photograph of CHA-OVA complex showed that particles are spherical and uniform. Characterization of CHA-OVA complex using FTIR showed that ions calcium from CHA binds into COO- groups of protein. Mucoadhesive membrane were prepared by mixture ratio of gelatin and chitosan hydrogel 3:7; 4:6 and 5:5 respectively. Physical properties of all formula mucoadhesive membrane was found suitable for mucosal application, i.e. good swelling index, surface pH at 6,7–6,8, thickness 70-110 µm, weight 0,25-0,40 gr, and adequate folding endurance. In vitro and ex vivo release study showed that there are no burst release for all formula. In this study, we found that there are no significant difference on the level of IgG specific ovalbumin. Otherwise, significance difference detected on the level of IgA specific ovalbumin, higher level found on CHA-OVA mucoadhesive membrane group compared to control group. The present study demonstrate the induction of mucosal specific antibody in vivo by CHA loaded into mucoadhesive membrane.

Keywords: adjuvant, buccal vaccination, carbonate apatite, mucodhesive membrane