UNIVERSITI PUTRA MALAYSIA

ORAL IMMUNIZATION WITH CHOLERA DNA VACCINE EMPLOYING MICROENCAPSULATION DELIVERY SYSTEM

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By

NADINE HAYUDINI NOGRALES

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

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To

~My Beloved Family~

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

ORAL IMMUNIZATION WITH CHOLERA DNA VACCINE EMPLOYING MICROENCAPSULATION DELIVERY SYSTEM

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Chairman: Professor Rozita Rosli, PhD

Faculty: Medicine and Health Sciences

Cholera disease remains to be a major public health problem in areas with poor sanitation, unsafe water supplies or in cases of epidemic outbreaks. Current oral whole-cell cholera vaccines are unable to elicit long-term protection against cholera and require maintenance of cold-chain during transport. Another vaccination strategy includes delivery of genetic components of the infectious agent. Plasmids carrying gene-coding sequences for immunostimulatory antigens such as the cholera toxin B subunit (*ctxB*) and/or the toxin coregulated pilin A (*tcpA*) genes were explored in this study as DNA vaccines for cholera. Advantages of DNA vaccines include relatively low cost and chemical stability during storage.

A non-viral delivery system described as microencapsulation into alginate material, a natural polysaccharide, was utilized in this study as an oral DNA delivery vehicle. The efficiency of DNA encapsulation was evaluated *in vitro* through characterization of the physical and behavioral properties of the microspheres. Subsequently, comparative evaluation of the *in vivo* immunoglobulin production after vaccination with encapsulated DNA vaccine was performed.

Alginate microspheres were produced through water-in-oil (w/o) emulsification. The average size of alginate microspheres was about 46.88 ± 3.07 µm in diameter whereby significant size reduction (p=0.028) was attributed through utilization of 1.0% Span80 in the preparation. Plasmid DNA (pDNA) was encapsulated within microspheres with encapsulation efficiencies ranging from 72.9 to 74.4% and a maximum pDNA load of 6 µg for each preparation. In simulated gastrointestinal conditions, alginate microspheres demonstrated shrinkage and minimal release of pDNA in pH 1.2 while exhibiting swelling properties in pH 9.0 with consequent pDNA release about twice the pDNA amount released in acidic environment (p<0.01).

As proof-of-concept studies *in vivo*, orally administered pDNA–loaded alginate microspheres comprising the mammalian expression vector (pVAX), designed for DNA vaccine development, carrying the GFP reporter gene was performed. pVAX–GFP loaded–alginate microspheres, at doses of 50 µg, 100 µg and 150 µg pDNA, were delivered through oral feeding needle to BALB/c mice. Tissue biodistribution, investigated through flow cytometric analysis, demonstrated GFP positive intestinal cells (<1.0%) for the 100 µg dose, a 1.3-fold higher expression as compared to 50 µg dose. Succeeding experiments were conducted with DNA vaccines using the same approach.

Oral delivery of DNA vaccine loaded–microspheres were performed on BALB/c mice and New Zealand White (NZW) rabbits. Test animals and control groups were immunized with pVAX-*ctxB* and/or pVAX-*tcpA* encapsulated within alginate microspheres. The production of antibodies observed among the test animals were

compared with the control groups. Stool sIgA levels or the mucosal sIgA among vaccinated mice groups (100 µg dose) showed an increase as compared to controls with an average 6.3-fold, 1.6-fold and 1.9-fold increase at day 7, 14 and 21, respectively. The response in the rabbits were lower (on average only 29% or 159% higher than controls), observed at day 21. These results demonstrate local immune responses at mucosal surfaces of the intestinal tract among vaccinated mice and rabbits.

Therefore, the alginate microspheres used in this study have shown to be potential carriers for cholera DNA vaccine into the intestinal mucosa surfaces; although further improvements are needed. The overall strategy of alginate microencapsulation which served as oral DNA vaccine therapy in this study may also serve as a delivery system for other forms of antigens or biological substances through the oral route.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PELALIAN ORAL DENGAN VAKSIN DNA TAUN MENGGUNAKAN SISTEM PENYAMPAIAN PEMIKROKAPSULAN

Oleh

NADINE HAYUDINI NOGRALES

Januari 2013

Pengerusi: Profesor Rozita Rosli, PhD

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Taun masih kekal sebagai masalah kesihatan awam yang besar di kawasan yang kurang bersih atau tidak dilengkapi bekalan air bersih atau dalam kes wabak jangkitan. Vaksin taun seluruh sel oral semasa tidak dapat mengaktifkan perlindungan jangka masa panjang terhadap taun serta memerlukan penyenggaraan simpanan sejuk berangkai sepanjang pengangkutan. Strategi pemvaksinan lain merangkumi penyampaian komponen genetik agen berjangkit. Plasmid yang membawa jujukan pengekod gen bagi antigen perangsang imun seperti gen-gen subunit B toksin taun (*ctxB*) dan/atau pilin A diregulasi bersama toksin (*tcpA*) dikaji dalam penyelidikan ini sebagai vaksin DNA bagi penyakit taun. Antara kelebihan vaksin DNA termasuklah kos yang kurang serta kestabilan kimia semasa penyimpanan.

Salah satu sistem bukan berantarakan virus yang dihuraikan sebagai pemikrokapsulan ke dalam bahan alginat, sejenis polisakarida semula jadi, digunakan sebagai penyampai oral DNA dalam kajian ini. Keberkesanan pengkapsulan DNA dinilai secara *in vitro* menerusi pencirian sifat fizikal dan

perlakuan mikrosfera. Berikutan itu, penilaian secara perbandingan penghasilan *in vivo* imunoglobulin setelah pemvaksinan menggunakan vaksin DNA yang dikapsulkan dijalankan.

Mikrosfera alginat dihasilkan melalui pengemulsian air-dalam-minyak. Saiz purata mikrosfera alginat ialah saiz diameter 46.88 ± 3.07 µm, di mana pengurangan signifikan saiz (p=0.028) berlaku melalui penambahan 1.0% Span80 semasa penyediaan mikrosfera. DNA plasmid dikapsulkan dalam mikrosfera dengan keberkesanan pengkapsulan berjulat daripada 72.9 sehingga 74.4% serta muatan plasmid maksimum pada 6 µg bagi setiap penyediaan. Dalam simulasi keadaan gastrousus, mikrosfera alginat menunjukkan pengecutan dan pelepasan minimum plasmid pada pH 1.2 serta menunjukkan ciri pengembangan pada pH 9.0 dengan pelepasan plasmid yang berikutan pada kadar dua kali ganda berbanding dengan jumlah yang terlepas dalam keadaan berasid (p<0.01).

Sebagai kajian pembuktian konsep *in vivo*, mikrosfera alginat bermuatkan plasmid diberikan secara oral terdiri daripada vektor DNA plasmid ekspresi mamalia (pVAX), yang direka bagi penghasilan vaksin DNA dan membawa gen pelapor GFP. Mikrosfera alginat dengan muatan pVAX-GFP disampaikan pada dos 50 µg, 100 µg dan 150 µg, melalui jarum pembekal makanan kepada mencit BALB/c. Taburan bio tisu yang dikaji melalui analisis sitometri aliran, menunjukkan sel usus yang positif GFP (<1.0%) pada tahap 1.3 kali ganda lebih tinggi bagi dos 100 µg berbanding dengan dos 50 µg. Eksperimen yang berikutnya dijalankan dengan vaksin DNA menggunakan pendekatan yang sama.

Penyampaian oral mikrosfera dengan muatan vaksin DNA dilakukan terhadap mencit BALB/c dan arnab *New Zealand White* (NZW). Kumpulan haiwan yang diuji dan kawalan dilalikan dengan pVAX-*ctxB* dan/atau pVAX-*tcpA* yang dikapsulkan di dalam mikrosfera alginat. Aras antibodi yang dihasilkan dalam kalangan haiwan yang diuji ditentukan dan dibandingkan dengan kawalan . Aras sIgA tinja atau sIgA mukosa dalam kalangan mencit (dos 100 μg) menunjukkan peningkatan berbanding dengan kumpulan kawalan dengan purata peningkatan 6.3 kali ganda, 1.6 kali ganda dan 1.9 kali ganda, masing-masing pada hari ke-7, ke-14 dan ke-21. Tindak balas dalam kalangan arnab adalah lebih rendah (secara purata hanya 29% atau 159% lebih tinggi daripada kawalan), diperhatikan pada hari ke-21. Hasil keputusan ini menunjukkan tindak balas lokal di permukaan mukosa saluran usus dalam kalangan mencit dan arnab yang dilalikan.

Oleh itu, mikrosfera alginat yang digunakan dalam kajian ini telah didapati menunjukkan potensi sebagai pembawa vaksin DNA taun ke permukaan mukosa usus; walau bagaimanapun penambahbaikan lebih lanjut diperlukan. Strategi keseluruhan bagi pemikrokapsulan alginat yang bertindak sebagai terapi oral vaksin DNA taun dalam kajian ini mungkin boleh juga digunakan sebagai sistem penyampai antigen atau bahan biologi lain melalui laluan oral.

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I certify that an Examination Committee has met on (date) to conduct the final examination of Nadine Hayudini Nograles on his Doctor of Philosophy thesis entitled "Oral Immunization by Cholera DNA Vaccine Employing Microencapsulation Delivery Systems" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the student be awarded the Doctor of Philosophy.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or currently submitted for any other degree at Universiti Putra Malaysia or other institutions.

NADINE H. NOGRALES

Date: 17 January 2013

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