PREPARATION OF CHITOSAN AND TRIMETHYLCHITOSAN (TMC) PARTICLES FOR ORAL VACCINE DELIVERY

SUPAVADEE BOONTHA

A Thesis Submitted to the Graduate School of Naresuan University
in Partial Fulfillment of the Requirements
for the Doctor of Philosophy Degree
in Pharmaceutical Sciences (International Program)
May 2011

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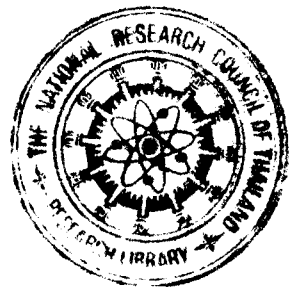

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This thesis entitled "Preparation of chitosan and trimethylchitosan (TMC) particles for oral vaccine delivery" submitted by Supavadee Boontha in partial fulfillment of the requirements for the Doctor of Philosophy in Pharmaceutical Sciences is hereby approved.

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Supavadee Boontha

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ABSTRACT

The principal aim of this thesis was to develop chitosan and trimethylchitosan (TMC) particles for oral Japanese encephalitis virus (JEV) delivery. In this study, three types of chitosan having different molecular weight (MW) and their respective TMC synthesized with a degree of quaternization (DQ) of 20%, 40% and 60% were used to prepare particles by ionic gelation method. The study started with preparation of chitosan and TMC particles having the potential for oral vaccine delivery using ovalbumin (OVA) as a model antigen. This step included the investigation of the effect of types of polymers (i.e. chitosan and TMC), the MW of chitosan and the DQ of TMC on the formation of particles and the characteristics of the particles obtained by optimizing the amount of tripolyphosphate (TPP) added. The chitosan and TMC particles prepared were tested for their potential to enhance immune response to OVA following oral immunization. Subsequently, the best formulations were selected and tested for the ability to induce immune response to JEV. The results showed that the amount of TPP added has an effect on the formation of chitosan and TMC particles. The formation of chitosan particles required higher amount of TPP than that of TMC particles. Using the same amount of TPP, the MW of chitosan did not have an effect on the formation of particles whereas the DQ of TMC did have. TMC particles with a DQ of 60% could not be prepared because of its high water solubility. Based on theirs

high loading capacity of OVA and slow release profile, particles prepared using chitosan with low and medium MW and theirs respective TMC with a DQ of 40% which was shown to be the most potent in the parallel study via nasal route were selected to test for the ability to enhance immune response to OVA. Chitosan was likely to enhance higher immune responses than TMC. Thus, only chitosans with three different MWs were selected to test for the ability to initiate immune response to JEV. It was found that JEV encapsulated in all types of chitosan particles could induce the JEV-neutralizing antibodies levels higher than the minimal requested level (>1:10) by World Health Organization (WHO) following oral immunization in Swiss albino mice. However, the JEV-neutralizing antibodies initiated by particles prepared using three types of chitosan were not significantly different. In conclusion, the results of this thesis demonstrate that chitosan particles as adjuvant have the feasibility for oral JEV immunization and the MW of chitosan appeared to have no effect on the ability to elicite protective neutralizing antibodies to JEV in Swiss albino mice after oral immunization. Interestingly, although the differences were not statistically significant, free JEV (i.e. JEV in sterile water for injection (SWFI)) seemed to be better in the initiation of immune responses to JEV compared with particles-encapsulated JEV. Thus, the further work on the enhanced of immune initiation against JEV encapsulated in any delivery systems is necessary to be carefully considered.

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ABBREVIATIONS

APCs	=	Antigen presenting cells
BSA	=	Bovine serum albumin
DCs	=	Dendritic cells
DD	=	Degree of deacetylation
DQ	=	Degree of quaternization
DT	=	Diphtheria toxoid
ELISA	=	Enzyme-Linked Immunosorbent Assay
FAE	=	Follicle-associated epithelium
FBS	=	Fetal bovine serum
g	=	Gram(s)
GALT	=	Gut-associated lymphoid tissue
GMT	=	Geometric mean titers
GPC	=	Gel permeation chromatography
GRAS	=	Generally Recognized As Safe
h	=	Hour(s)
HRP	=	Horseradish peroxidase
IgA	=	Immunoglobulin A
IgG	=	Immunoglobulin G
JEV	=	Japanese encephalitis virus
LC	=	Loading capacity
LE	=	Loading efficiency
M-cells	=	Microfold cells
MEM	=	Minimum essential medium
min	=	Minute(s)
MW	=	Molecular weight
NMP	=	N-methyl-2-pyrrolidone
NMR	=	Nuclear magnetic resonance

ABBREVIATIONS (CONT.)

OVA	=	Ovalbumin
PBS	=	Phosphate buffered saline solution
PBST	=	Tween 20 in phosphate buffered saline solution
PCS	=	Photon correlation spectroscopy
PDI	=	Polydispersity index
PRNT	=	Plaque reduction neutralization test
SCRs	=	Seroconversion rates
SEM	=	Scanning electron microscopy
sIgA	=	Secretory immunoglobulin A
SWFI	=	Sterile water for injection
T20	=	Tween 20
TPP	=	Sodium tripolyphosphate
TMC	=	Trimethylchitosan
TMB	=	3, 3', 5, 5' – tetramethylbenzidine
TT	=	Tetanus toxoid
WHO	=	World health organization
ZO-1	=	Zonula occludens 1