

Contents

	Page
Abstract (Thai).....	iv
Abstract (English).....	v
Acknowledgements.....	vi
Contents.....	vii
List of Figures.....	x
List of Abbreviations.....	xii
 Chapter	
I. Introduction.....	1
Background and Rational.....	1
Research Questions.....	2
Objectives.....	2
Hypothesis.....	2
Conceptual Framework.....	3
Assumption.....	4
Keywords.....	4
Operation Definition.....	4
Research Design.....	4
Research methodology.....	5
Benefits of Study.....	7
Obstacles and Strategies to Solve the Problem.....	7

Chapter	Page
II. Review Literature.....	8
General background.....	8
Epidemiology.....	8
Clinical manifestation.....	10
Transmission cycle.....	10
Treatment.....	10
Classification and molecular biology.....	10
Life cycle and replication.....	14
Microglia and JEV infection.....	16
Autophagy.....	18
Molecular events of autophagy.....	18
Detection of autophagy.....	23
Autophagy and virus infection.....	25
III. Materials and Methods.....	27
Source of Materials.....	27
Cell culture.....	29
Determination of cell viability.....	30
JEV propagation in C6/36 cells.....	30
Virus titration by standard Plaque Assay.....	30
Viral infection of human microglial cells.....	31
Growth curve analysis of JEV-infected CHME-5 cells.....	31
Determination of the percentage of JEV infectivity.....	31
Determination of autophagic cell death.....	32
Detection of autophagic marker by indirect immunofluorescence.....	33

Chapter	Page
Chemical treatment and JEV infection in CHME-5 cells.....	34
IV Results.....	35
Propagation of JEV and virus titration.....	35
Growth curve analysis of JEV-infected CHME-5 cells.....	35
Determination of the percentage of infectivity.....	38
Autophagy in JEV-infected human microglial cells.....	44
Determination of autophagy by transmission electron microscope.....	44
Determination of autophagy by indirect immunofluorescence staining.....	44
Determination of autophagy by western blotting.....	49
Effect of autophagy modulation to JEV production.....	55
V Discussion and Conclusion.....	55
References.....	58
Appendix.....	63
Biography.....	70

List of Figures

Figure	Page
2.1 The global distribution of JEV.....	9
2.2 Facial Grimacing in a Vietnamese Boy with Japanese Encephalitis.....	11
2.3 Transmission Cycle of Japanese Encephalitis Virus.....	12
2.4 The classification of Flavivirus.....	13
2.5 Schematic of Japanese encephalitis virus structure.....	15
2.6 Morphology of microglia.....	17
2.7 The autophagy pathway.....	19
2.8 The autophagic process.....	22
2.9 The electron micrograph of autophagosome and autolysosome in starved mouse embryonic fibroblast cells.....	24
3.1 Standard plaque assay of JEV on LLC-MK-2.....	36
3.2 Growth curve of mock-infected and JEV-infected CHME-5 cells (MOI of 10).....	37
3.3 Growth curve of mock-infected and JEV-infected CHME-5 cells (MOI of 100).....	39
3.4 Morphological alterations of JEV-infected CHME-5 cells.....	40
3.5 JEV infection of CHME-5 cells.....	42
3.6 Immunocytochemistry of JEV-infected CHME-5 cells.....	43
3.7 Histogram statistics of percent infectivity determined by immunocytochemistry in JEV-infected CHME-5 cells.....	44
3.8 Electron micrographs of mock-infected CHME-5 cells.....	46
3.9 Electron micrographs of JEV-infected CHME-5 cells.....	47
3.10 Autophagosome in JEV-infected CHME-5 cells.....	48

Figure	Page
3.11 Induction of autophagy in JEV-infected CHME-5 cells.....	49
3.12 Co-localization of LC3 and JEV E-protein in JEV-infected CHME-5 cells.....	50
3.13 Western blotting of LC3 expression in CHME-5 cells	52
3.14 Histogram statistics of western blotting analysis of LC3-II / Actin expression in CHME-5 cells.....	53
3.15 Viral production in JEV-infected CHME-5 cells in presence of autophagy inhibitor.....	54

List of Abbreviations

%	Percent
µg	Microgram
µl	Microlitre
µm	Micrometre
°C	Degree Celsius
CaCl ₂	Calcium Chloride
DAB	3,3-Diaminobenzidine
DAPI	4',6-Diamidino-2-Phenylindole
DMEM	Dulbecco's Modified Eagle's Medium
EDTA	Ethylenediaminetetraacetic acid
E protein	Envelope Protein
EBSS	Earle's Balanced Salt Solutions
FBS	Fetal Bovine Serum
FITC	Fluorescein isothiocyanate
g	Gram
H ₂ O	Water
hr(s)	Hour(s)
HRP	Horse radish peroxidase
IgG	Immunoglobulin G
JE	Japanese Encephalitis
JEV	Japanese Encephalitis Virus
KCl	Potassium Chloride
kDa	Kilodalton
LAMP-1	Lysosomal-associated membrane protein 1
LC3	Microtubule-associated 1 light chain 3

M	Molar
3-MA	3-Methyladenine
mA	miliampere
MEM- α	α -modified Minimal Essential Medium
mg	Milligram (s)
MgSO ₄	Magnesium Sulphate
min	Minute (s)
ml	Milliliter
MOI	Multiplicity of Infection
MW	Molecular Weight
NaCl	Sodium Chloride
NaHCO ₃	Sodium Hydrogen Carbonate
nm	nanometre
NS	Nonstructural Protein
NS1	Nonstructural Protein 1
NS2A	Nonstructural Protein 2A
NS2B	Nonstructural Protein 2B
NS3	Nonstructural Protein 3
NS4A	Nonstructural Protein 4A
NS4B	Nonstructural Protein 4B
NS5	Nonstructural Protein 5
p.f.u.	Plaque-Forming Unit
PBS	Phosphate Buffered Saline
rpm	Revolution per Minute
SDS	Sodium Dodecyl Sulfate
Seakem LE agarose	Seakem Low Electroendosmosis Agarose
TEM	Transmission electron microscope

Tris base	2-Amino-2-(Hydroxymethyl)-1,3-Propanediol
TRITC	Tetramethylrhodamine-5-(and 6)-isothiocyanate
V	Volt
YE-LAH	Yeast Extract-Lactalbumin Hydrolysate