CHAPTER I

INTRODUCTION

Background and rational

Japanese encephalitis (JE) is one of the most endemic viral encephalitis in East and South East Asia with an estimated 50,000 cases annually. The mortality rate is approximately 20-30% (1, 2). Japanese encephalitis virus (JEV), a positive single stranded RNA arbovirus, belongs to family of *Flaviviridae*. Clinical manifestations of JE patients are ranging from non-specific flu-like illness to acute and possibly fatal encephalitis. Most of the survivors have neurological sequelae such as mental retardation and epilepsy. The severity of the disease depends on the location and the degree of brain destruction. However, pathogenesis of Japanese encephalitis remains unclear (2-4).

During JEV infection, even though neuron performs as a primary target cell in central nervous system (CNS) but microglia, a brain residental macrophage, is activated by JEV infection(5). The JEV antigens have been detected in neuronal and glial cells (6, 7). The activation of microglia demonstrates, at microscopic level, a common histological feature in both of human autopsy and animal experimental models (8).The mechanism of neuronal cell death upon JEV infection is proposed in two ways. One is direct neuronal killing, the viral multiplications within neuronal cells cause cell death. The other is indirect mode of killing, whereas massive inflammatory response causes an up-regulation of reactive oxygen species and pro-inflammatory cytokines (9). It had been reported that many mediators like IFN-α, TNF-α,MIF, IL-8, IL-6, RANTES, IL-1ß and MCP-1 are elevated during JEV infection (9-11).

In recent study, microglial were suggested as a viral reservoir for CNS infection and JEV infection also triggered apoptosis (12). The overexpression of Bcl-2, antiapoptotic gene, can effectively delay JEV-induced cell death and consequently convert some target cells such as Baby hamster kidney (BHK-21) and Chinese hamster ovary (CHO) cells into persistently infected cells (13, 14). Infection of JEV triggers initiators caspase-8 and -9, probably through FADD-independent but mitochondrion-dependent path ways in mouse neuroblastoma (N18) cells (15).

Besides apoptotic cell death, autophagy can commonly be found in virus infected cells. The autophagy is the cellular degradation pathway for long-lived proteins, aggregated

proteins and damage organelles that involved delivery of cytoplasmic cargoes to lysosomes. Autophagy is induced during cell differentiation, starvation and intracellular stress. Several viruses trigger autophagy upon their infection such as Herpes simplex virus, Polio virus, Dengue virus (16, 17).

As there is currently no information about autophagy upon JEV infection in human microglial cell, therefore, this research aims to examine, whether Japanese encephalitis virus infection can trigger autophagic in human microglial cell death or not. The results from this study will provide basic knowledge for understanding JE pathogenesis.

Research Questions

Does Japanese encephalitis virus infection induce autophagic microglial cell death?

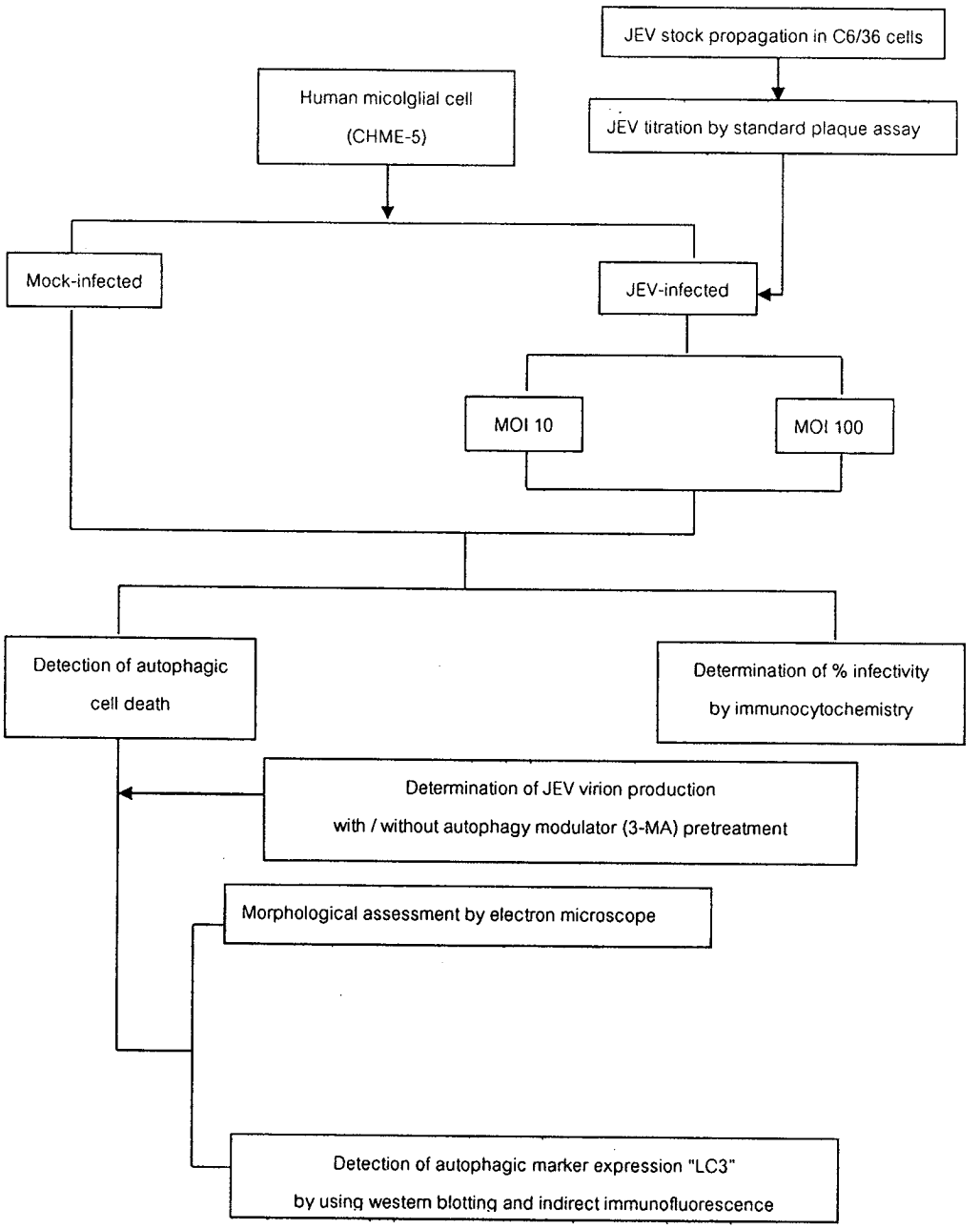
Objectives

1. To examine the autophagic cell death in human microglial cell in response to JEV infection.
2. To determine the percentage of infectivity of JEV in human microglial cell.

Hypothesis

Japanese encephalitis virus infection cause autophagic microglial cell death.

Conceptual Framework



Assumption

All Instruments were tested for the precision and accuracy according to the corresponding standardizations.

Keywords;

Japanese encephalitis virus

Microglia

Autophagy

Ultrastructure

Operational Definitions

Plaque assay is a method used to determine viral titration as plaque forming units (p.f.u.)/ml. A viral plaque is formed when a virus infects a cell. The infected cell would be seen as a white plaque for counting and calculating the virus titer, after crystal violet dye staining.

MOI stands for "Multiplicity of Infection". It is the ratio of virions to the number of cells being infected.

Percentage of the infectivity

$$\text{Percentage of the infectivity} = \frac{\text{Number of infected cells}}{\text{Total number of cells}} \times 100$$

Percentage of punctuate staining

$$\text{Percentage of punctuate staining} = \frac{\text{Number of positive punctuate staining cells}}{\text{Total number of cells}} \times 100$$

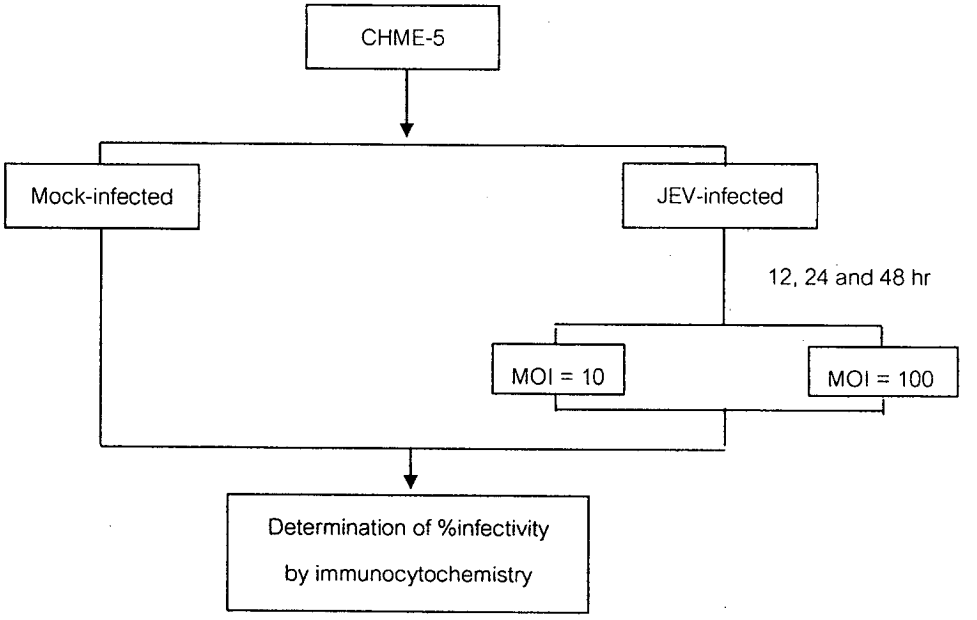
Research Design

Experimental Research

Research Methodology

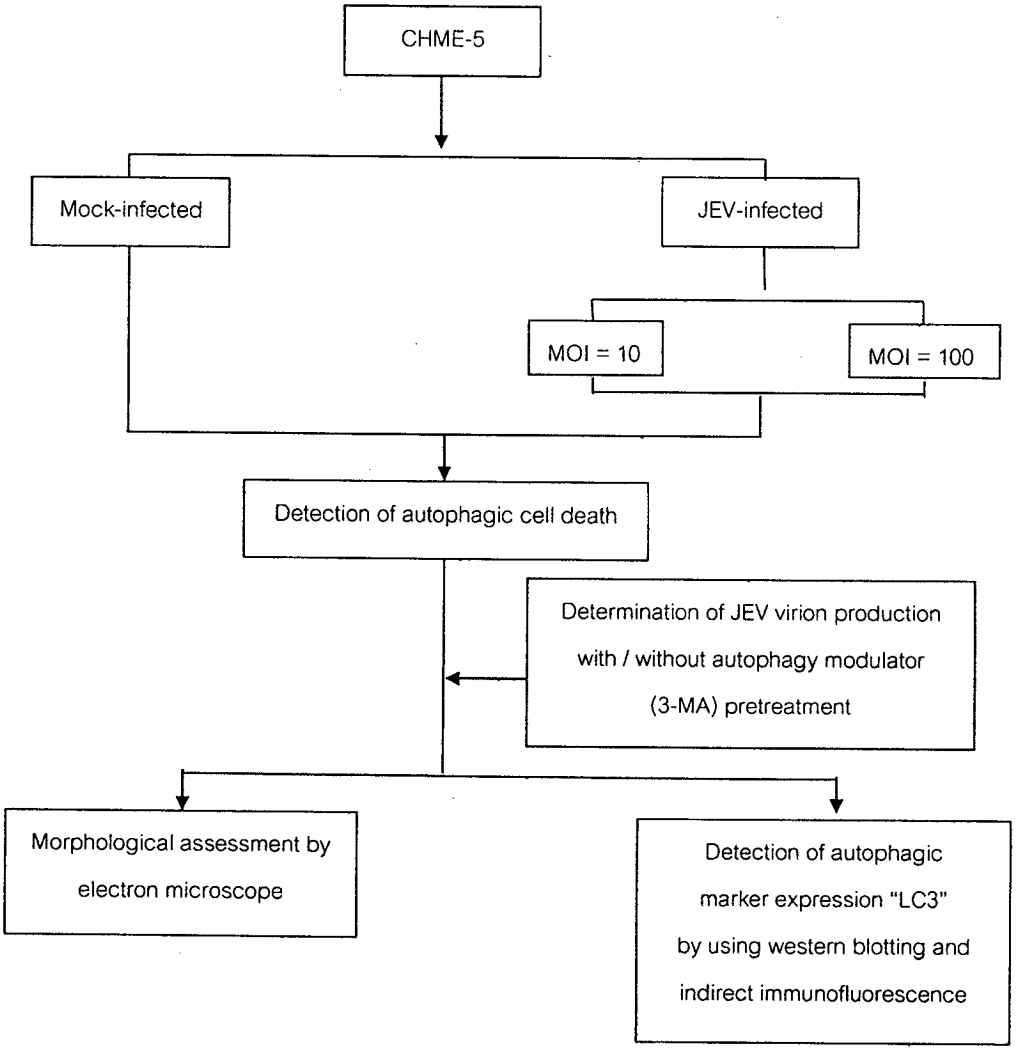
Experimental I: Determination of the percentage of infectivity in JEV-infected CHME-5 cells.

To determine the condition with, at least, an estimated of 70% infectivity for autophagic cell death examination, the cells were seeded onto glass cover slips and divided into two groups as follows: control and JEV- infected group. Control group were treated with normal culture media. In JEV infected group, cells were directly infected for 2 hr with JEV at an MOI of 10 and 100. Then, cells were cultured for 12, 24, 48 hr. Cells were later be processed for immunocytochemistry detection using anti-flavivirus as a primary antibody.



Experiment II: The autophagy assessment in JEV-infected CHME-5 cells.

CHME-5 cells were either mock-infected or JEV-infected for 2 hr with JEV at an MOI of 10 and 100. Then, cells were cultured for 48 hr and processed for morphological assessment by Transmission electron microscope. For detection of autophagic marker, LC3, cells were processed for indirect immunofluorescence and western blotting by using anti-LC3B as a primary antibody. To determine JEV viral production pretreats with/without autophagy modulator (3-MA), culture media were collected at 48 hr and measured virus titers by standard plaque assay.



Benefits of study

1. Better understanding of the Japanese encephalitis pathogenesis
2. To find out the role of autophagic cell death in microglia in which might be applied to other arboviral encephalitis
3. To provide basic knowledge for the development of Japanese encephalitis antiviral drugs

Obstacles and Strategies to Solve the Problem

1. Cell culture contamination: All kind of procedures involving cell culture require aseptic techniques.
2. Cell line cross-contamination: This research uses many cell types. Each cell line should be sub-cultured separately.