CHAPTER I

INTRODUCTION

1. Background and Rationale

Rabies is a zoonotic viral disease caused by rabies virus in the family Rhavdoviridae and in the genus *Lyssavirus.* Wide range of mammals including human, wildlife and domestic animals can be infected although with different susceptibility level.

It is transmitted to other mammals including humans through contamination of broken skin or mucous membrane with rabies virus containing saliva from infected animals via bites, scratches, licks on broken skin and mucous membranes. Once symptoms of the disease develop in man, it is considered universally fatal.

Rabies has been one of the most feared diseases and human rabies remains one of the most common causes of death among viral encephalitides in developing countries where canine rabies is inadequately controlled. These cases are usually attributable by dog bites. Only a few human cases associated with other animals than dogs have been reported in Thailand. Human rabies death statistics in the years 2001, 2002, 2003 and 2004 were 38, 30, 21, and 19 cases respectively (MOPH report). Despite an increasingly diminished number of rabies deaths, more than 400,000 individuals required rabies postexposure prophylaxis (PEP) in 2004. It has been estimated that the total costs for rabies biologics and budget spent for controlling vectors and dog population control and immunization have been over 1 billion baht per year.

Classical clinical features in human rabies can be distinctively classified into 2 separate types, encephalitic or furious and paralytic or dumb rabies.(1) Another form "nonclassic or atypical rabies" lacks the usual rabies characteristic symptoms and signs thus, make diagnosis extremely difficult. This latter form has been associated with bat rabies variant of genotypes 1, 5, 6 and 7 and recently (since 1997) with some of the

canine variants in Thailand. Diagnosis during life can be aided by the use of neuroimaging and molecular techniques. Computerized axial tomography (CAT) scan of the brain is not helpful and the result is unremarkable.(2,3,4) Although hypodense cortical lesions(5) and nonenhancing basal ganglia hypodensities(6) have been described, these may be due to hypoxic insult. Magnetic resonance imaging (MRI) studies of the brain yields characteristic findings which can differentiate rabies from other viral encephalitis. Previous MRI reports showed diverse findings which might be readily explained by differences in techniques and equipments used as well as uncertainties at which stage of the disease the studies were performed. Moreover, this may also be complicated by the fact that there might have been another concomitant illnesses such as hypotension and severe hypoxia during the time that these studies were performed.(3,6,7,8) Recent report by Laothamatas et al, included MRI study of rabies patients at various stages, the results of which definitely showed that rabies of both forms (furious and paralytic) share a similar MRI pattern. Nonenhancing, ill-defined, mind hyperintensity changes in the brain stem, hippocampi, hypothalami, deep and subcortical white matter and deep and cortical gray matter were demonstrated on T2-weighted images. Enhancement appeared only at the time when the patients lapsed into coma. Such pattern and the lack of enhancement in noncomatose patient with suspected encephalitis may differentiate rabies from other viral encephalitides.(9) These changes in the brain can also be demonstrated even at the time when patient had only local neuropathic pain at the bitten arm but was fully conscious and rational and did not show any symptoms and signs indicative of encephalitis. It remains to be determined the responsible mechanism(s) in causing white matter changes in the brain, areas where virus cannot replicate itself.

For laboratory studies, direct fluorescent antibody test (DFA) is the gold standard due to its practicality and reliability when performed in brain impression smear. This test requires brain tissue from humans or animals suspected of rabies. The test can be performed with post-mortem or biopsy brain tissue, although the latter is

not convenient and brain biopsy may be judged too invasive. Other tests for diagnostic and research purposes, such as electron microscopy (EM), histologic examination, immunohistochemistry (IHC), reverse transcriptase polymerase chain reaction (RT-PCR) and nucleic acid sequence based amplification (NASBA) and isolation in cell culture are useful for studying the virus structure, histopathology, molecular typing and when combined with animal pathogenicity study will add more information on virulence of rabies variants.

In vivo, rabies virus can spread from cells to cells and can migrate from periphery along peripheral nerve axon to reach central nervous system (CNS). This is possible by the interaction between rabies virus phosphoprotein (P protein of residues 138 to 172) and motor protein dynein light chain 8 (LC8), a component in actin- and microtubule-based transport, in the retrograde movement of viral ribonucleoprotein and phosphoprotein within the peripheral nerve and also in the CNS. Nevertheless, rabies virus can also be transported in its entire virion or in subviral fragment within the peripheral nerve in a vesicular cargo. The enveloped glycoprotein (G protein) can be the ligand through the vesicular transport by the P75 neurotrophin receptor (P75 NTR). This latter mechanism has been suggested by Mazarakis et al. based on the demonstration of a rabies virus glycoprotein-pseudotyped lentivirus vector. Once rabies virus reached dorsal root ganglion and neuronal cells in the spinal cord, it replicates and rapidly spread in neuronal cells of the CNS, through intraaxonal or trans-synaptic transport in a microtubule network-dependent process. For histologic examination of human rabies brain tissue, excessive accumulation of ribonucleoprotein in the cytoplasm of infected neuron results in the formation of inclusion body, called Negri body, previously considered pathognomonic in rabies(10,11), however, this has been proven otherwise. Even in the absence of Negri body, rabies virus antigens or proteins and RNA can be demonstrated by IHC and *in situ* hybridization technique as well as Western Blot.

In adult mice infected intracerebrally with CVS, morphologic changes of apoptosis were observed in neurons, particularly in pyramidal neurons of the hippocampus and cortical neurons, where positive Terminal deoxynucleotidyl transferase-mediated dUTP-digoxigenin nick end labeling (TUNEL) staining could be demonstrated. Double labeling studies indicated that not only infected neurons underwent apoptosis, noninfected-neurons at distant locations also displayed apoptotic pictures. This supports the theory of differential response and ideas of indirect effect which follows direct neuronal infection process. Increased expression of the Bax protein was observed in neurons in areas in where apoptosis was prominent.(12)

Analysis of regional distribution of rabies virus antigen in the CNS of human rabies patients of both forms revealed similar pattern. Rabies virus antigen preferentially localizes in the spinal cord and brainstem and midline structures of thalamus, basal ganglia if the survival period is less than 7 days. This is also similar in rabies infected dogs when the survival period is less than 9 days. Electrophysiologic studies of the peripheral nerve in human rabies revealed a subclinical evidence of anterior horn cell dysfunction in the spinal cord of furious rabies patients. These patients did not exhibit any demonstrable weakness of the arms and legs. In paralytic rabies patients limb weakness is explained by peripheral nerve and not the anterior horn cell dysfunction. This is also confirmed by prominent inflammation in the peripheral nerve in the case of paralytic rabies. It is only at the time when rabies patients become comatose that weakness of all limb musculatures can be demonstrated. It has also been previously demonstrated that spinal cord motoneuron resist to cytolysis and apoptosis in spinal cord and anterior horn cell culture system with rabies virus infection. Along with this "resistance phenomenon" of rabies virus infected spinal cord, it is also intrigued that all rabies patients do not have depressed consciousness during the most entire clinical course despite an enormous amount of rabies virus in the brainstem (and spinal cord) since the early stage. Brainstem

structures are crucial in maintaining alertness and form an integral part of reticular activating system.

These findings raised important questions why rabies patients remain alert and why clinical weakness due to spinal cord AHC does not develop in all forms of rabies patients. It is difficult to explain the nature of weakness during the preterminal phase when these patients are already in coma since motor system control at various levels of neuraxis may be impaired. Postmortem studies in human CNS tissue agree to a similar conclusion that necrotic process is usually lacking in the CNS. Apoptosis can be induced by multiple insults and then operates via extrinsic and intrinsic pathways in which cytochrome appears to be a major inducer of the entire cascade though the activation of caspase-9 and -3. Whether these resistant areas of brainstem and spinal cord are lacking of either apoptosis or evidence of mitochondrial outer membrane permeabilization or both may explain this resistance phenomenon.

2. Research Question

Major question

Are spinal cord and brainstem vulnerable to apoptosis based on the evidence of TUNEL staining and mitochondrial outer membrane permeabilization (MOMP).

Minor question

Are there any changes in white matter structures (cytoskeleton and myelin) of the brain and spinal cord in human rabies patients of both forms?

If there is any, it has to be determined whether there is any correlation between the degree of apoptosis or MOMP and such changes.

Is there any difference in the degree of apoptosis and MOMP and cytoskeleton and white matter changes in furious and paralytic rabies?

3. Objective

The purposes of this study are as follows:

3.1 To determine the degree of apoptosis and MOMP at various CNS regions in rabies patients of both forms.

3.2 To determine whether there are cytoskeleton and myelin changes at any CNS regions and whether there is correlation of such changes and apoptosis and MOMP at the same region.

3.3 To determine the effect of survival period and the degree of apoptosis and MOMP at various CNS regions in rabies patients of both forms.

3.4 To determine whether it is necessary that apoptosis and MOMP co-exist in each CNS region.

4. Hypothesis

4.1 Spinal cord and brainstem can survive in condition of rabies virus infection.

4.2 Cytoskeleton and myelin, the structure of white matter change in both forms of human rabies patients.

4.3 Degree of apoptosis or MOMP has correlation with white matter structure changing.

4.4 No different degree of apoptosis, MOMP and white matter structural change in furious and dump rabies.

5. Key words

Rabies virus

Apoptosis

Spinal cord

Brainstem

Cytoskeleton

Myelin