CHAPTER I

INTRODUCTION

Herpes simple viruses (HSVs) are unique members of the *Herpesviridae* family which has been classified into subfamily *alphaherpesvirinae* (1-3). They are divided into two types; Herpes simplex virus type-1 (HSV-1) and Herpes simplex virus type-2 (HSV-2). HSV is a linear double-stranded (ds) DNA viral genome and has icosahedral capsid surrounded with envelope (lipid bilayer) containing spikes that probably represent viral glycoproteins (3,4). Both HSV-1 and HSV-2, share extensive nucleic acid sequence homology fifty percent (5).

HSV is one of significant pathogens in public health worldwide. It causes many diseases such as herpes labialis, gingivostomatitis, herpes keratoconjunctivitis, herpes encephalitis, and genital HSV infection, etc (6). HSV-1 is usually transmitted from person to person by direct contact with saliva and respiratory secretion which associated with infections of the lips, mouth, and face (7). HSV-2 is usually transmitted by sexual contact which associated with infections of genital area. However, cross-infection of type-1 and -2 viruses may occur from oral to genital sexual contact (6,7). HSV is specifically characterized by its ability to establish and maintain latent infection that can be reactivated to become reinfection after receiving a number of stimuli: including sunlight, stress, febrile illnesses, menstruation or immunosuppressive drug (8,9). This is because after infection the virus is not cleared by the immune system. It hides in the nerves and is never completely removed from the body. Although primary infection induces HSV specific antibodies which can neutralize HSV-1 or HSV-2, the antibodies cannot protect recurrent infection. Recurrence may be related to factors which in some way decrease an individual's disease resistance (2,9,10). Normally, in immunocompetent patients, HSV infection is less severe and extensive but HSV causes a variety of infections with significant morbidity among immunocompromised persons (6,11,12).

Nowadays, many medications are available for treatment of HSV infection such as acyclovir, foscanet, ganciclovir, penciclovir, famciclovir, vidarabine, idoxuridine, trifluridine, etc (5,13). Among those, acyclovir (ACV) is the drug of choice for the

treatment of HSV infections in both immunocompetent and immunocompromised hosts (14-20). It has been proven to have a high efficacy in suppressing HSV replication and an excellent safety profile to host (2,21-23). It is also significantly reduce the severity, duration, and frequency of recurrences (21).

ACV is an acyclic analogue of guanosine. Virus-specified thymidine kinase (TK) phosphorylates ACV to its monophosphate derivation. ACV is further phosphorylated by cellular enzymes to its triphosphate derivative. ACV triphosphate binds viral DNA polymerase, active as a DNA chain terminator. This event does not occur to any significant extent in uninfected cells (21,24,25). Thus, ACV has good safety and efficacy to hosts (5). However, ACV is only effective against actively replicating viruses and does not affect viruses in persistent or latent state (14).

Recently, HSV infection, a number of reports has described HSV isolates that are resistant to ACV (26-28). Especially, it has emerged with the occurrence of chronic, progressive, debilitating disease in immunocompromised patients receiving prolonged courses of continuous or intermittent suppressive therapy. The more patients treated with ACV increase, the more ACV-resistant HSV (ACV^r HSV) strains are found. The development of viral resistance to ACV and the morbidity and mortality associated with these virus strains is a problem of concern (29-32).

The mechanism responsible for the majority of ACV resistant isolates is a lack of viral TK, the enzyme required for initial phosphorylation of ACV before it undergoes further phosphorylation by cellular kinases to its active triphosphate form (33). Occasionally, ACV resistance can be due to an alteration of the TK protein such that HSV developes resistance predominantly (95%) as a result of mutation in gene that code for TK (*TK* gene) (33-40) or more rarely, a mutation may occur in the viral DNA polymerase (*pol*) gene (41-46). In these cases, an alteration in antiviral therapy is necessary for successful outcome. Otherwise, infection with ACV resistant mutants can result in locally progressive mucocutaneous lesions in immunocompromised patients, becoming a source of severe pain and bacterial superinfection (47,48). Resistant to antiviral agents is likely to become an increasing therapeutic problem for clinicians (11).

Therefore, the aim of resistance monitoring is to provide the necessary information to enable the physician to prescribe the most optimal drug combination for

the individual patient. ACV^r HSV has been two distinct main principle methods to measuring resistance;

(i) genotypic assay: these tests detect specific genetic changes (mutations) in the viral genome that are known to be associated with resistance. These are expressed in terms of the amino acid that is coded for by a particular three-base sequence (codon), in the genome, rather than the change in the nucleotide itself. For example, genotyping methodologies were used such as hybridization assay and DNA sequencing (49).

Genotypic characterization of ACV^r HSV mutant has been studied. The genetic variation may compensate for the loss of TK (50). Changes in the *TK* gene can result in viruses producing no or partial amuonts of TK or with an altered substrate specificity (45,51). On the basis of nucleotide sequences of TK mutants displaying altered substrate interactions, Darby *et al* have proposed a preliminary model for the active center of HSV-1 TK enzyme including three distinct regions: an ATP-binding (amino acids 51 to 63), a nucleoside-binding site (amino acid 168 to 176) and the amino acid 336 (52). Indeed, single-point mutations in one of resistant HSV isolates have been reported (12,36,39,53-55). Furthermore, Sasadeusz *et al* have identified mutational hot spots consisting of frameshift mutations within homopolymer nucleotide stretches of G's and C's (55). Recent studies by Gaudreau *et al* (39) and Morfin *et al* (53) have demonstrated that about 50% of the clinical ACV-resistant strains contain an insertion or a deletion of one or two nucleotides in homopolymer runs of G's and C's, whereas the other half presents single-base substitutions in conserved or nonconserved regions of the *TK* gene. However, the main disadvantage is that the results are extremely difficult to interpret (49) and it is traditionally compared with phenotypic antiviral susceptibility testing, which has been the gold standard (56).

(ii) phenotypic assay: these tests measure the ability of virus taken from a patient to grow in the presence of a drug, in the laboratory. The phenotype is measured in terms of the IC_{50} for a particular drug i.e. the concentration of drug required to inhibit virus replication by 50%. This is compared to the IC_{50} for the drug using wild type virus-virus that has not been exposed to that drug and is fully sensitive.

One of the most commonly used types of antiviral susceptibility assay is the plaque reduction assay (PRA). In this assay, the inhibition of viral plaque formation (zones of viral cytopathic effect and lysis in the cell tissue culture monolayer) in the presence of varying concentrations of an antiviral agent is used to determine the effectiveness of the agent on the particular viral isolate.

The advantage of phenotyping over genotyping is that is a direct measure of any change in sensitivity resulting from all the mutations that have occurred, and any interactions between them. As such, it is the gold standard of resistance testing. The PRA, provided in the National Committee for Clinical Laboratory Standards (NCCLS), is the most commonly reported method for *in vitro* evaluations of the susceptibilities of clinical HSV isolates to ACV and the technique to which other methods are compared (56). Then, the PRA has been employed to estimate drug sensitivity and the phenotype of TK and DNA polymerase activity of HSV (34,48).

The phenotypic screening of the HSV has become increasingly important for choosing the appropriate therapy, especially in the case of patients who have a risk factor for ACV resistance viral infection, such as immunodeficiency and long duration of ACV treatment (27,57). However, the previous studies showed that a number of the prevalence of ACV^r HSV isolates was reported varying from 4 to 15% (47,58) in immunocompromised patients and 0-7% in immunocompetent host (57,59-61). Furthermore, Sarisky *et al* have reported the correlation of HSV type and the frequency of mutation that HSV-2 mutation frequency was approximately 30 times higher than that for HSV-1 isolates (62). It is therefore of interest to study about the prevalence of HSV type in clinical specimens from genital and non-genital lesions and the sensitivity of HSV isolates to ACV.

In this study, experiments were performed (i) to type all HSV isolates by using indirect immunofluorescent (IFA) method with a fluorescence isothiocyanate-conjugated (FITC) monoclonal (MAb) HSV type specific antibodies for estimate the prevalence of HSV type in clinical specimens from genital and non-genital lesion (ii) to determine the sensitivity of HSV isolates to ACV by PRA and estimate of the prevalence of ACV^r HSV in Thai patients, especially in the general immunocompetent patients (iii) characterization

of *TK*-mutation in ACV^r HSV by DNA sequencing method will be performed, if ACV^r HSV was determined.

The information of sensitivity assay against ACV in HSV isolates will be helpful in management of patients and, more importantly, can be used in the future as a baseline against which to assess changes prevalence. Finally, the survey was to provide information on the relative frequency of appearance of different types of ACV^r HSV in patients.