# CHAPTER I

## INTRODUCTION

Herpes simplex viruses (HSV) are large DNA viruses belonging to the herpesvirus family. In humans, there are two serotypes of HSV as herpes simplex virus type 1 (HSV-1) and herpes simplex virus type 2 (HSV-2). Both types share many common features with respect to pathogenesis,clinical manisfestation and epidemiology. HSV are ubiquitous and contribute, significantly in terms of mobidity and mortality in different human populations. In addition to acute illness associated with the initial infections, the viruses usually persist in a latent form and could periodically produce recurrent disease upon reactivation. Thus, the viruses are neither cleared completely from the infected individual nor do stimulate the immune-mechanisms completely, so the second and consequence episodes of disease could be prevented (1, 2). Recurrences of both oral labial and genital herpes simplex virus infections in humans occur frequently. More than 60% of patients with initial HSV-2 infection, the infection recurs within 6 months, and patients with recurrent genital disease have a median of five recurrences per year (3, 4). Moreover, overall incidence of genital herpes is significantly increasing in the past few years in all parts of the world (3,5). The disease is often painful, sometime debilitating, and causes considerable social and psychological stress.

Moreover, a number of clinical and epidemiological studies have shown a significant correlation between genital HSV-2 infections and a higher incidence of cervical carcinoma (3, 6, 7). Therefore, the benefits that would be derived from controlling the disease caused by herpes simplex virus have become increasingly apparent particularly in the study of antiviral agents that are specific for the inhibition of viral multiplication without affecting normal cell division.

Antiviral chemotherapy should be specific for the inhibition of viral multiplication without alteration the host cell functions. Among the antiviral compounds, acyclovir has been synthesized and proved to be a potential inhibitor against herpes simplex virus infection with extremely low toxicity to normal cells. Acyclovir is a guanosine analog and has been extensively studied on herpes simplex virus infection. A herpes virus-coded thymidine kinase was found to be essentially responsible for transforming acyclovir to acyclovir monophosphate, which in turn,was converted to the acyclovir di-and triphosphate, respectively by host cell enzymes. The acyclovir triphosphate was explicitly incorporated preferentially into viral DNA and the elongation of the viral DNA synthesis was terminated (8, 9, 10, 11, 12). In a series of controlled clinical trials, acyclovir has shown to be the compound with demonstrated efficacy in the treatment of herpes simplex virus infection (5, 13). Acyclovir is the first drug to offer some hope to herpes genitalis

sufferers. Whether administered topically, orally, or intravenously, acyclovir reduces the duration of virus shedding, local pain/itching, and lesion healing time, in primary herpes genitalis, though less persuasively in the recurrent attacks (13, 14, 15). Unfortunately, there appears to be no significant reduction in the establishment of latency nor in the frequency of subsequent recurrence (14).

In primary genital HSV-infection, patients treated with acyclovir showed that the seroconversion to major HSV - proteins are delayed (16). Analysis using immunoblotting have shown that the amounts of antibodies made to individual proteins are decreased when compared with those from placebo recipients (17, 18). These alternations in the antibody responses may be due to the decreased amounts of the HSV antigens initiating the immune response by acyclovir. In vitro the effect of acyclovir on viral protein synthesis has not been well documented. Since acyclovir had been reported to inhibit DNA synthesis thus the inhibition of DNA synthesis may be extented to the inhibition of protein synthesis. Therefore, it is interested to study the in vitro effect of acyclovir on HSV-2 specific antigens.

SDS-polyacrylamide gel electrophoresis has become a standard tool in laboratory in which proteins are analyzed and purified (19). Moreover, electrophoretic transfer of proteins to nitrocellulose paper and their localization with specific ligands, also known as "Western

blotting", is rapidly becoming an important tool in immunological and virological studies (20). The combination of seperation of a mixture of proteins by SDS-PAGE following by an electrotransferring of proteins in the gel to nitrocellulose membrane and detection by an immunological procedure will be the high-rise of assays used in this study.

The sensitivity of HSV in vitro to acyclovir is dependent on the strain of virus, the type of the host cell, and tissue culture condition used in the experiments (9, 21, 22). Thus, in this study, herpes simplex virus type 2 was chosen for study the inhibitory effects of acyclovir because 1. the incidence of genital herpes is increasing significantly in all parts of the world, 2. its infection associated with cervical cancer, and 3. it is easy to propogate in tissue-culture. Therefore, HSV-2 and HeLa cell were used throughout the experiments. The protocol of this study is following; 1. study the inhibitory effect of acyclovir on the viral yield of HSV-2 by plaque reduction assay, 2. study the inhibitory effect of acyclovir on the viral DNA synthesis of HSV-2 by $^{3}H$-thymidine incorporation, and 3. study the inhibitory effect of acylovir on the viral protein synthesis by Western blot analysis.

The main purpose of this investigation was to study the in vitro inhibitory effect of acyclovir on HSV-2 protein synthesis by SDS-PAGE and Western blotting. In

addition, the analysis in this point was extended to compare the inhibitory effect of pure chemical acyclovir with intravenous acyclovir.