CHAPTER I

INTRODUCTION

Herpes simplex viruses (HSV) are the first of human herpesviruses and remain one of the most common viral infections in humans. Infections of these viruses occur world wide and have been reported in both developed and developing countries. Consequently, the infection has been recognized as a public health concern (Corey *et al.*, 1983). In addition, HSV is an intense human pathogen and responsible for causing a widespread spectrum of mild to severe diseases. These include acute primary and recurrent mucocutaneous disorders recognized as herpes labialis, eczema herpeticum, and genital herpes through herpes keratitis, herpes meningitis, and life-threatening herpes encephalitis in the otherwise healthy adult. Moreover, HSV infection in immunocompromised patients and neonates are usually more severe than in the normal host (Whitley, 1995). These individuals, which compose of AIDs patients and immunocompromised patients resulted from pathogenic reasons or receiving immunosuppressive drugs, are also prone to increased frequency of secondary herpes episodes (Greenberg *et al.*, 1987), and the severity of herpes infection has been shown to correlate with the degree of immunosuppressive therapy used (Rand *et al.*, 1997). In addition, HSV-2 infection may be a risk factor for the transmission of human immunodeficiency virus (HIV) (Hook *et al.*, 1992).

The existence of two distinct antigenic types of HSV was revealed in the early 1960s, they are now designated as human herpesviruses 1 (HHV-1 or HSV-1) and human herpesviruses 2 (HHV-2 or HSV-2) by the International Conference for Taxonomy of Viruses (ICTV) (Ginsberg, 1980). HSV-1 and HSV-2 significantly differ in their pathogenic potential. HSV-1 infection is generally limited to the oropharynx and transmitted by direct contact of a susceptible individual with infected secretions. Thus, initial replication of HSV-1 normally occurs in oropharyngeal mucosa, and the trigeminal ganglion becomes colonized and harbors latent virus. Acquisition of HSV-2 infection is usually the consequence of transmission by genital contact. This virus replicates in the genital, perigenital, or anal skin sites with seeding of sacral ganglia for latent infection. However, changes in sexual behaviors have somewhat altered this common pattern: occasionally, HSV-2 viruses are isolated from oral lesions and HSV-1 from genital lesions (Ginsberg, 1980).

Among several populations, between 60% and more than 95% are infected with HSV-1, and between 6% and 50% with HSV-2 (Cunningham and Mikloska, 2001). Recurrences of both oral labial and genital HSV infections in human occur frequently. More than 60% of patients with initial HSV-2 infection develop recurrent infection within 6 months, and patients with recurrent genital disease have a median of 5 recurrences per year. The disease is often painful, sometime debilitating, and causes considerable social and psychological stress. Furthermore, a number of clinical and epidemiological studies have shown a significant correlation between HSV-2 infection and a higher incidence of cervical carcinoma. In Thailand, HSV infections have been frequently found in various populations. Virus Research Institute, Department of Medical Sciences, Ministry of Public Health reported that 10-17% of young people in Bangkok and Chinart provinces ever had symptomatic herpes. In this group, 5% of them were genital herpes and 0.35% had HSV infection in the vagina and cervix which increased in the past years. In Bangkok, almost upward 30-year old people had HSV-1 infections and had a little lower HSV-2 infection incidence. Meanwhile, in Chainart where the population density is lower than Bangkok, there were also lower incidences of HSV infection (ประเสริฐ, 2528). It was also reported that the frequencies of recovery from HSV diseases seemed to be higher in female than in male, particularly during the first episode of infection. Asymptomatic shedding of HSV-2 represented 98.4% of all isolates from female genitalias and the remaining isolates were HSV-1.

There are various antiviral drugs with clinically relevant activity against HSV infection such as idoxuridine, vidarabine, trifluridine, and foscarnet, however, these drugs have some undesirable effects such as they are potentially toxic, mutagenic, and teratogenic to the host (Coen, 1991). Acyclovir (ACV) is most commonly used for the treatment of HSV infections, followed by penciclovir or famciclovir. It has been reported that ACV in topical, oral, or intravenous forms was highly effective especially on the first episode of HSV infection (Leung and Sacks, 2000). However, a serious problem for the use of ACV is the increase of HSV strains that resist to drug treatment (Pottage and Kessler, 1995; Shin *et al.*, 2001) particularly in immunocompromised patients. Resistance to ACV and related nucleoside analogues can occur commonly following mutation in HSV thymidine kinase or rarely DNA polymerase (Khan *et al.*, 2005). Foscarnet, an antiviral drug inhibiting HSV DNA polymerase, is often used to treat ACV-resistant virus and recommended for only severe infection (Hasegawa and Kaeagushi, 1994). Nevertheless, foscarnet may induce mutation in viral DNA polymerase gene when used upon prolonged period, and

foscarnet-resistant viruses have been isolated (Birch *et al.*, 1990; Hwang *et al.*, 1992). Consequently, these mutants are often resistant to combination chemotherapy with existing compounds. Therefore, there is a worldwide interest in the development and identification of efficacious new anti-HSV agents with on adverse effects.

α-lipoic acid (LA) is a universal antioxidant that combines free radical scavenging and metal chelating properties with an ability to regenerate the levels of other nonenzymatic and enzymatic antioxidants such as glutathione, ascorbate, α-tocopherol, catalase, and peroxidase (Maitra *et al.*, 1995; Packer *et al.*, 1995). As a precursor of the lipoamide prosthetic group in α-keto-acid dehydrogenase complexes, LA has been shown to prevent diabetes, hyperglycemia-associated complications such as neuropathy, cataract formation, and radiation injury. It has been reported that LA interrupted HIV replication, while LA supplementation in AIDs patients resulted in increases in CD_4 and CD_4/CD_8 ratios (Lyn, 2000). Moreover, oral LA administration has been shown to be safe with no apparent side effects, no mutagenicity, and no genotoxicity (Cremer *et al.*, 2006).

Therefore, the purpose of this study was to investigate the antiviral activities of LA and lipoamide against HSV-1 strain KOS and HSV-2 strain Baylor 186. The antiviral activity was evaluated by inactivation, plaque reduction, and MTT reduction assay. To determine the anti-HSV activities of lipoic acid in different cell cultures, normal human dermal fibroblast cell line and cervix epithelium cell line were used instead of Vero cells and the MTT reduction assay was performed. Furthermore, preliminary mechanism studies of LA were performed through virucidal, post-binding, penetration, and virus growth inhibition assays to find its possible mode of antiviral activity.

The results from this study could provide preliminary information on the *in vitro* anti-HSV-1 and HSV-2 activities of LA and lipoamide. Moreover, LA and lipoamide would be interesting candidates for the anti-HSV-1 and HSV-2 drug development in the future.