CHAPTER III

LITERATURE REVIEW

Herpes simplex virus

History

Herpes simplex virus (HSV) infections of humans have been documented since ancient Greek times (21,22). The word "herpes", means creep or crawl, to describe the spreading nature of skin lesions (22,23). The Roman scholars Herodotus associated mouth ulcers and lip vesicles with fever and defined this association as "herpes febrilis" (24).

As noted by Wildy (22), Shakespeare was probably cognizant of recurrent HSV labial lesions. In Romeo and Juliet, Queen Mab the midwife of the fairies, stated: "O'er ladies lips, who straight on kisses dream, which oft the angry Mab with blisters plaques, because their breaths with sweetmeats tainted are."

In the 18th century, Astruc, physician to the King of France, drew the appropriate correlation between herpetic lesions and genital infection.

In 1893 Vidal specifically recognized person-to-person transmission of HSV infections (21). By the beginning of the 20th century, histopathologic studies described multinucleated giant cells associated with HSV infection and HSV was established by transmission of virus from human to the cornea of the rabbit, resulting in keratitis (26). The transmission of HSV could occur not only to the cornea rabbit, but also could lead to infections of skin or the central nervous system (CNS) (27).

Properties of herpes simplex virus

HSV are a group of viruses belong to the family *Herpesviridae*. More than 100 known members of this family that infect many different animal species, share the structure of their virions, general features of their reproductive cycle, and the capacity to remain latent. There are eight herpesviruses that commonly infect human; herpes simplex virus type 1 and 2, varicella zoster virus, cytomegalovirus, Epstein-Barr virus, and human herpesviruses 6, 7 and 8. However, they differ in many aspects, and have been classified into three subfamilies (alpha, beta, and gamma) with respect to the detail of their replication, the cell which they remain latent, gene content, and gene organization. HSV belong to the subfamily *alphaherpesvirinae* : neurotropic viruses. In this subfamily consists HSV and varicella- zoster virus (VZV). HSV divided into two types: HSV-1 and HSV-2. Their genomes are similar in organization and exhibit substantial sequence homology. The two viruses cross-react serologically, but some unique proteins exist for each type (28). They differ in their mode of transmission, clinical features of human infections and biologic characteristics (Table 1).

Morphology of HSV virion

The herpes simplex virion consists of four components : core, capsid, tegument, and envelope (Figure 1).

The Core

An electron dense core of linear double-stranded DNA ,in the form of a toriod, 50-75 nm in diameter. The torus appears are suspended by DNA embedded around the protein. The viral DNA genome has a molecular weight of approximately 100 million daltons and 152 kb with G + C content of 68 moles percent for HSV-1 and 69 moles percent for HSV-2 (28). The HSV-1 and HSV-2 DNA each encode at least 81 different polypeptides and consist of two stretches of unique sequences, U_L and U_S, each franked by relatively large inverted repeat DNA sequences. U_L and U_S can invert relative to each other to yield four populations of DNA molecules differing solely in the relative

orientation of these DNA sequences. Genome structure of herpes simplex virus compared with other herpesviruses were showed by Figure 2.

The Capsid

An icosahedral symmetry capsid consists 162 capsomeres, 95-105 nm in diameter. The hexameric capsomeres are 9.5 X 12.5 nm in longitudinal section; a channel 4 nm in diameter runs from the surface along the long axis (29).

The Tegument

An amorphous layer proteins surrounds the nucleocapsid are designated tegument. The thickness of tegument determines the size variation of HSV virion.

The Envelope

An envelope surroundings the capsid and tegument. The thin sections of electron-microscopic studies have shown the envelope that has a typical trilaminar appearance; it appears to be derived from patches of altered cellular membranes (30). Viral envelope presents the lipids which sensitizes to lipid solvent and detergent (31), and its surface contains numerous protrusions of spikes, which are approximately 8 nm long (29,30). The envelope contains at least 11 glycoproteins and nonglycosylated viral proteins, lipids and polyamines. The glycoproteins mediate attachment of the virus to cells and elicit host responses to the virus (32). The eleven glycoproteins were designated as gB, gC, gD, gE, gG, gH, gI, gJ, gK, gL and gM. The biologic properties of some of these glycoproteins have been identified (32). Glycoprotein D (gD) is likely related to viral infectivity and is the most potent inducer of neutralizing antibodies. Glycoprotein B (gB) is required for infection. Glycoprotein C (gC) binds to the C3b component of complement, whereas gE binds to the Fc portion of IgG. Glycoprotein G (gG) provides antigenic specificity to HSV and, therefore, results in an antibody response that allows for the distinction between HSV-1 (gG-1) and HSV-2 (gG-2). Glycoprotein I (gI) has biologic properties that are thought to be involved with gE at the Fc receptor. The role of gJ, gK, gL, and gM are not well appreciated (33).

Table 1. Characteristics of herpes simplex virus type 1 and type 2.

Characteristics	HSV-1	HSV-2
Biochemical		
Viral DNA base composition (guanine-plus-cytosine)	68%	69%
Buoyant density of DNA (g/cm^3)	1.726	1.728
Buoyant density of virions (g/cm^3)	1.271	1.267
Homology between viral DNAs	~50%	~50%
Biologic		
Animal vectors or reservoirs	None	None
Site of latentcy	Trigeminal ganglia	Sacral ganglia
Epidemiologic		
Age of primary infection	Young children	Young adult
Transmission	Contact (often saliva)	Sexual
Clinical		
Primary infection:		
Gingivostomatitis	+	-
Pharyngotonsillitis	+	-
Keratoconjunctivitis	+	-
Neonatal infection	±	+
Recurrent infection:		
Cold sores, fever blisters	+	-
Keratitis	+	-
Primary or recurrent infection:		
Cutaneous herpes		
Skin above the waist	+	-
Skin below the waist	-	+
Hands or arms	+	+
Herpetic whitlow	+	+
Eczema herpeticum	+	-
Genital herpes	±	+
Herpes encephalitis	+	-
Herpes meningitis	±	+

Modified from Oxman MN: Herpes stomatitis. pp. 752-772 in: Infectious Diseases and Medical Microbiology, 2nd ed. Braude AI, Davis CE, Fierer J (eds.). Saunders, 1986

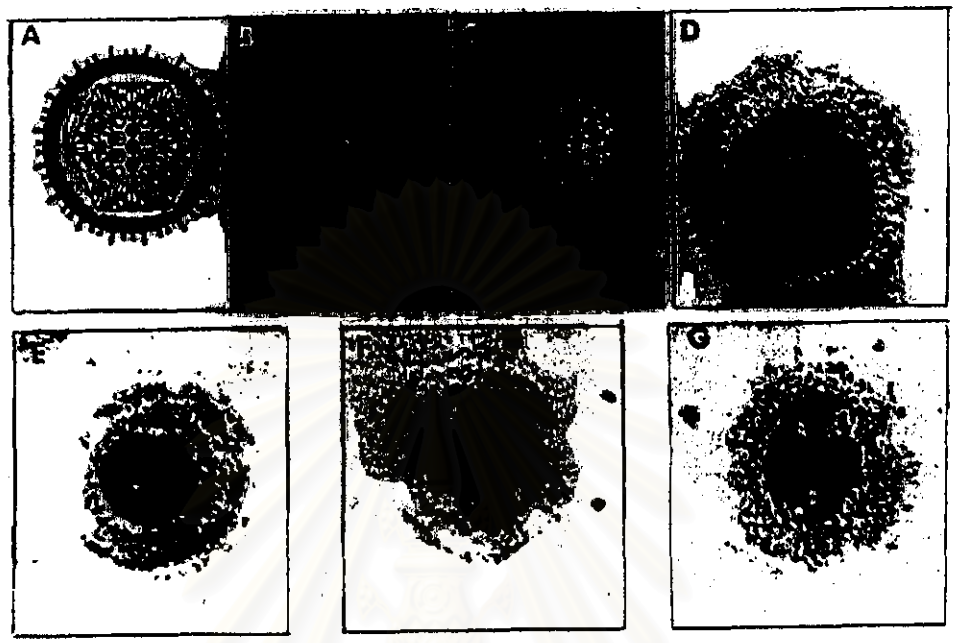


Figure 1. Morphology of herpesviruses

A: Schematic representation of the herpes virion seen through a cross section of the envelope with spikes projecting from the surface. The sides of icosadeltahedron forming the capsid show twofold symmetry. The irregular inner perimeter of the envelope is meant to represent occasional asymmetric arrangement of the tegument. B: An intact negatively stained HSV-1 virion. The intact envelope is not permeable to negative stain. The diameter of the virion is approximateely 120 nm. C: An HSV-1 capsid exposed to negative stain and showing twofold symmetry matching the diagrammatic representation of the capsid in A. D: HSV-1 capsid containing DNA permeated with uranyl acetate. The electron micrograph shows the presence of the threadlike structures 4-5 nm wide on the surface of the core. E-G: Electron micrographs of thin sections of HSV-1 virions showing the core cut at the different angles. The preparation was stained with uranyl acetate and counterstained with lead citrate. The DNA core preferentially takes up the stain appears as a toriod with an outer diameter of 70 nm and an inner diameter of 18 nm. The toriod appears to be suspended by a fibrous cylindrical structure. The micrographs show the toriod seen looking down the hole (E), in cross section (F), or from the side (G). (From Roizman B: Herpesviridae. pp 1787-1793 in: Virology, 2nd ed. Fields B.N. et al. (eds.) Raven press, 1990)

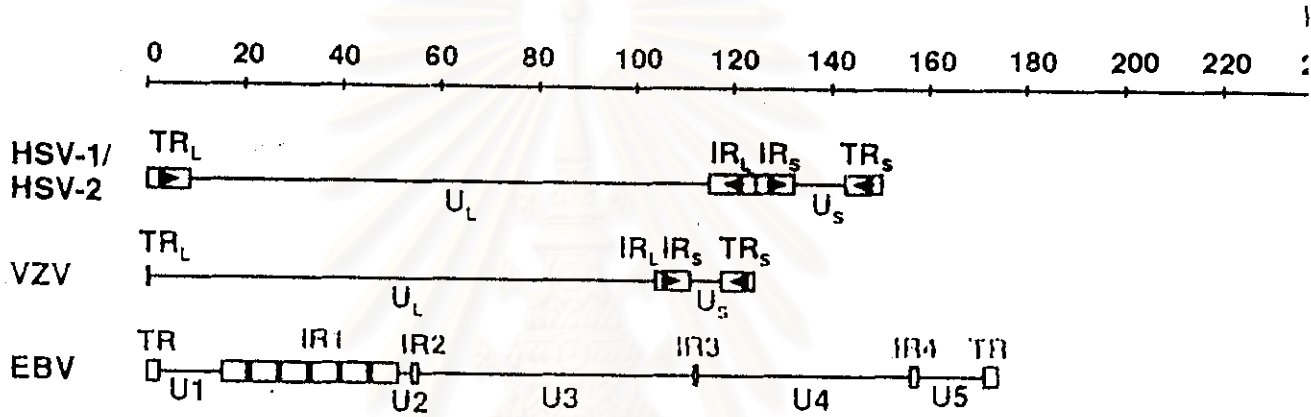


Figure 2 Genomes structure of human herpesviruses

(From Roungsakulrach, B.: Mechanism of latent herpesviruses infection in biology molecular. pp 2.2 In: Human herpesvirues, Puttawattana, P. and Yoosook,C. (eds.) Aksomsamai, 1995)

Viral Replication

To initiate infection, HSV must attach to cell-surface receptors, fuse its envelope to the plasma membrane, and transport to the nuclear pores with the de-enveloped capsid. At the nuclear core, the DNA is released into the nucleus, where transcription, replication of viral DNA and assembly take place (Figure 3).

The transcription of HSV DNA is characterized by the expression of three genes classes: alpha, beta, and gamma genes (immediate-early, early, and late, respectively) although there can be some overlap between each of these classes. These genes are expressed coordinately and temporally in a regulated sequences (2,34,35).

The synthesis of viral products - RNA and proteins- takes place in sequential three sets (2,35). The first set are made, consists six proteins known as α or immediate-early. The synthesis of α-RNA is induced by a structural protein of the virus brought into the cells during infection, of this set, five regulate the expression of the rest of viral genes and in effect regulate the reproductive cycle of the virus, whereas one blocks the presentation of antigenic peptides on infected cell surfaces. This set of proteins is essential for the synthesis of the second set; β or early mRNA and proteins.

Most of the β proteins concern with viral nucleic acid metabolism. At least seven proteins synthesize viral DNA by a rolling circle mechanism that yields endless, head-to tail concatemers. Other viral proteins increase the pool of deoxyribonucleotides, repair viral DNA, etc. The β proteins which are post translation, alter viral proteins primarily to modify their other functions (2). Members of this group of proteins are the main target of antiviral chemotherapy. Thus, viral thymidine kinase selectively phosphorylates most antiviral nucleotide analogs and other antiviral compounds are effectively used to block or reduce the capacity of the viral DNA polymerase to synthesize viral DNA.

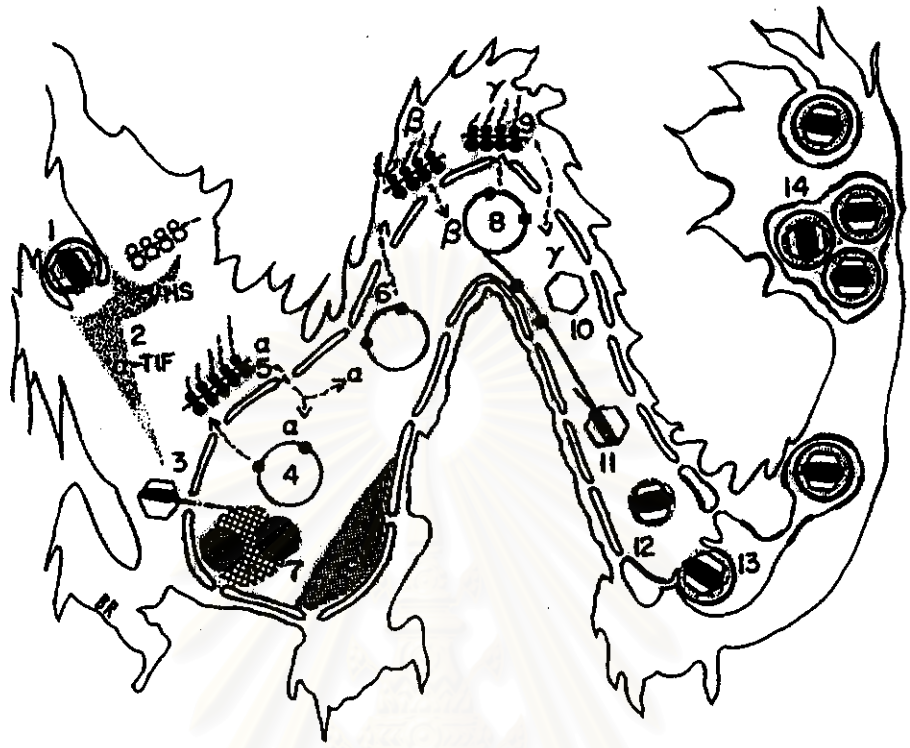


Figure 3. The replication of herpes simplex viruses in susceptible cells.

1.the virus initiates infection by the fusion of the viral envelope with the plasma membrane following attachment to the cell surface. 2, Fusion of the membranes releases two proteins from the virion. VHS shut off protein synthesis (broken RNA in open polyribosomes). α-TIF (the α gene trans- inducing factor) is transported to the nucleus. 3, The capsid is transported to the nuclear pore where viral DNA is released into the nucleus and immediately circularizes. 4, The transcription of α genes by cellular enzymes is induces by α-TIF. 5, The αmRNAs are transported into the cytoplasm and translated (filled polyribosome); the proteins are transported into the nucleus. 6, A new round of transcription results in the synthesis of β proteins. 7, At this stage in the infection the chromatin (C) is degraded and displace toward the nuclear membrane whereas the nucleoli (round hatched structures) become disaggregated. 8, Viral DNA is replicated by a rolling circle mechanism that yields head-to tail concatemers of unit length viral DNA. 9, A new round of transcription/translation yields the γ proteins consisting primarily of structural proteins of the virus. 10, The capsid proteins consisting primarily of structural proteins of the virus. 10, The capsid proteins form empty capsids. 11, Unit length viral DNA is cleaved from concatemers and packaged into the preformed capsids. 12, Capsids containing viral DNA acquire a new protein. 13, Viral glycoproteins and tegument proteins accumulate and form patches in cellular membranes. The capsids containing DNA and the additional protein attach to the underside of the membrane patches containing viral proteins and enveloped. 14, The enveloped proteins accumulate in the endoplasmic reticulum and are transported into the extracellular space.

(From Roizmam, B., Sear, A.E.: Herpes simplex virus and their replication. pp. 2231-2240 In: Fields of Virology, Fields, B.N., Knipe, D.M., Howley, P.M., et al. (eds.) Lippincott-Raven, 1996)

The third set of proteins,γ proteins, assemble to form the capsid, the tegument, and become incorporated into nuclear membranes for eventual envelopment of virions (2). Assembly takes place in steps. First, a procapsid is formed from scaffolding and capsid proteins. The scaffolding proteins consist of protease and its substrate. During capsid assembly, the protease cleaves the substrate. Next, the newly synthesized DNA is cleaved into unit length molecules and packaged into the virions. In the process, the scaffolding protein is extruded from the capsid. Generally, envelopment favors capsids containing DNA that attach the nuclear surface of the inner nuclear membrane and are rapidly enveloped and released into the space between the inner and outer nuclear membranes. Here, the virions were in transported vesicles and are transported through modified Golgi vesicles to the extracellular space. The process takes approximately 18 hours (2).

Pathology and Pathogenesis

Pathology

The pathologic chances induced by the replication of HSV are alike both primary and recurrent infection but vary in the quantitative of extent of cytopathology. These chances show a combination of virally mediated cellular death and associated inflammatory response. Changes induced by viral infection enclose ballooning of infected cells and the appearance of the condensed chromatin within the nuclei of cells, followed by subsequent degeneration of the cellular nuclei, generally within parabasal and intermediate cells of the epithelium. Cells lose intact plasma membranes and form multinucleated giant cells (Figure 4). With cell lysis, a clear (referred to as vesicular) fluid containing large quantities of virus appears in vesicular fluid between the epithelium and dermal layer. In dermal substructures, there is an intense inflammatory response, usually in the corium of the skin, more primary infection than recurrent infection. With healing, the vesicular fluid becomes pustular with the recruitment of inflammatory cells and, then scabs. Because of common shallow ulcers the vesicles rapidly rupture as the result of the very thin comified epithelium, scaring is uncommon but was noted in the

lesion patients. The histopathologic changes become particularly prominent when organs of the body other than skin are involved, as is encountered with HSV encephalitis or disseminated neonatal HSV infections, such as, widespread areas of hemorrhagic necrosis, mirroring the area of infection become most prominent. The intensity of the inflammatory response is significantly less with recurrent disease. Developing of host defenses, an influx of mononuclear cells can be detected in infected tissue (33).

Human Infection

The pathogenesis of human disease is dependent on intimate, personal contact of a susceptible individual (namely, one who seronegative) with someone excreting HSV. Virus must come with mucosal surfaces scraped skin for infection to be initiated. When virus replicates at the site of infection, viral nucleocapsid is transported by neurons to dorsal root ganglia, where virus is in a latent stage. Although the replication can sometimes lead to disease and can infrequently result in life-threatening CNS infection, the host-virus interection leading to latency predominates. After latency is established, a proper stimulus will reactivate virus evident at mucocutaneous sites, appearing as skin vesicles or mucosal ulcers. The pathogenesis of HSV infections appear in Figure 5.

Primary infection

HSV is transmitted by contact of susceptible person with an individual excreting virus. The virus must encounter mucosal surfaces or broken skin for an infection. HSV-1 infections are usually restricted to the oropharynx, and virus is spread by respiratory droplets or direct contact with infected saliva. HSV-2 is usually transmitted by genital routes. Viral replication occurs first at the site of infection, then invades local nerve endings and is transported by retrograde axonal flow to dorsal root ganglia, where , after further replication, latency is established. Oropharyngeal HSV-1 infections are latent infection in the trigeminal ganglia, whereas genital HSV-2 infections lead to latently infected sacral ganglia (33).

Figure 4. Multinucleated giant cell from Paopanicolaou stain, arrow indicates inclusion body which surrounded with halo cell.

(From Puttawattana, P. pp 13.2 In: Human herpesviruses, Puttawattana, P. and Yoosook, C. (eds.) Aksornsamai, 1995)

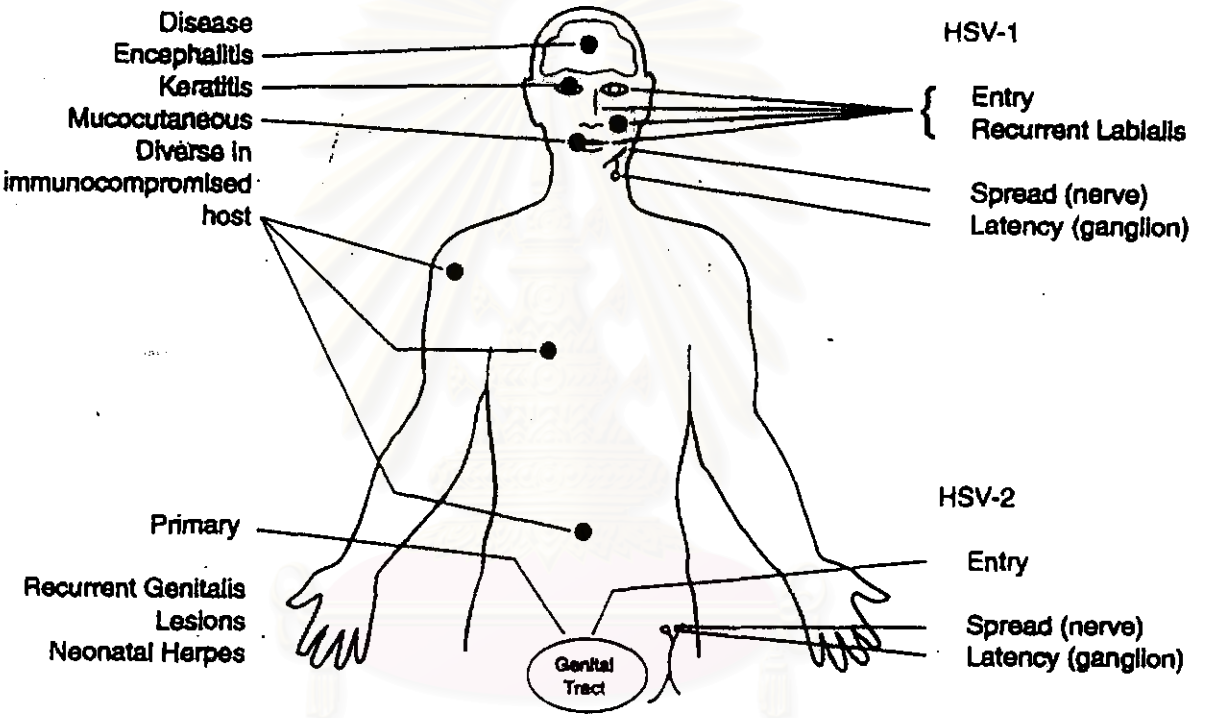


Figure 5. Pathogenesis of HSV infection and diseases.

(Modified from Whitley RJ: Herpes Simplex Virus. pp.2301 In: Fields of Virology, Fields, B.N., Knipe, D.M., Howley, P.M., et al. (eds.) Lippincott-Raven, 1996)

Primary HSV infections are usually mild and most are asymptomatic. Rarely develop to systemic disease. Widespread organ involvement can occur in immunocompromised host that is not able to limit viral replication and viremia ensues.

Latent infection

Virus rests in latently infected ganglia in nonreplicating state; only a few viral genes are expressed. Viruses can persist in latently infected ganglia for the lifetime of the host. Proper provocative stimuli can reactivate virus from the latent stage; virus follows axons back to the peripheral site and replicates at the skin or mucous membranes. In spite of HSV specific humoral and cellular immunity in the host, spontaneous reactivations can occur. However, this immunity limits local viral replication, so that recurrent infections are mild and less extensive. Many recurrences are asymptomatic, reflected only by viral shedding in secretions. When recurrences are symptomatic, episodes of recurrent HSV-1 infection usually manifested as cold sores (fever blister) near the lip. The molecular basis of reactivation is known; effective inducing stimuli include physical or emotional stress, fever, exposure to ultra violet light, tissue damage, and immune suppression (36-38). More than 80% of the human population provides HSV-1 in a latent form, but a small portion experience recurrences. It is not known the reason why some individuals suffer reactivations and others is not.

Clinical manifestations of HSV-2 disease

HSV-2 may cause many clinical manifestations of the diseases and the infections may be primary or recurrent. Primary infections occur in persons without antibodies and are usually asymptomatic, but result in antibody production (27).

In primary infections, clinical appearances occur or not, virus replicates in the site of infection and follows axon in order to latents in sensory ganglia. Recurrent lesions are common.

Genital herpes

Primary/Initial/Recurrent Genital disease

Genital disease is usually caused by HSV-2, although HSV-2 can also cause clinical episodes of genital herpes. After acquisition of HSV infection at a grazed site, macules, and papules, followed by vesicles, pustules, and ulcers, will appear. Genital herpes is characterized by vesiculoulcerative lesions of the penis of the male or of the cervix, vulva, vagina, and perineum of the female. The lesions are very painful and may be associated with fever malaise, dysuria, and inguinal lymphadenopathy. The duration of lesions averages 3 weeks. There are both similarities and differences in the clinical symptoms between men and women (39).

Primary genital herpes infections can be severe and has a larger quantities of virus replicating in the genital tract and a period of viral excretion may persist for an average of 3 weeks (39). Systemic complications in the male are relatively uncommon; however, aseptic meningitis can develop (33). The severity of primary genital herpes infections and its complications are statistically higher in women than men with unknown reasons (39).

In women with primary infection, lesions appear on the valva and are usually bilateral, involving cervix.

In men the vesicular lesions usually appear on the glans penis or the penile shaft. The total number of lesions can vary significantly from 6 to 10 to many more. Extragenital lesions of the thigh, buttocks, and perineum can occur.

Complications after primary herpetic infection have included sacral radioculomyelitis, which can lead to urinary retention, neuralgias, and meningoencephalitis (40). Primary perianal and anal HSV-2 infections, as well as associated proctitis, are becoming increasingly in male homosexuals (41).

An initial genital infections in a person already immune with preexisting antibody, is less severe and heals more quickly. The duration of the infection is closer to 2 weeks. The number of lesions, severity of pain, and likelihood of complications is significantly decreased. Preexisting antibodies to HSV-1 have an ameliorative effect on disease severity of HSV-2 (42).

Recurrent genital herpes is the mildest disease. The number of lesions usually limit 3 to 5, will appear on the shaft of the penis of the male or as simply a vulvar irritation in the female (43). The duration of recurrent genital herpetic infection is approximately 7-10 days. Virus is shed for an average of only 2-5 days and at lower concentrations (approximately 10^2 to 10^3 viral particles per 0.2 ml of inoculum in the tissue culture systems).

The major problem with recurrent genital HSV infection is the frequency of recurrences, which varies from one individual to the next. All patients are estimated to have recurrences in 3 - 7 per year, one third will have 3 per year, and the remaining third will have 4 to 7 per year (39). Some recurrences are asymptomatic. Whether a recurrence is symptomic or asymptomatic, a person shedding virus can transmit the infection to sexual partners (39). It has been showed that susceptible females are at high risk for contracting HSV infection from infected males. The most episodes of recurrent disease are due to reactivation of virus latent in sacral ganglia, a recurrence may occasionally represent a sexually acquired infection with a new HSV-2 strain.

Neonatal HSV infection

Neonatal herpes is estimated to occur about one in 5000 deliveries per year (44). HSV infection of the new born may be acquired in the utero, during birth, or after birth. The new born infant seems to be unable to limit the replication and spread of HSV and has a propensity to develop severe disease.

In utero infection can occur as a consequence of either transplacental or ascending infection (45).

The most of transmission (approximately 75% to 80%) to the new born during birth by contact with herpetic lesions in the birth canal (46). To avoid infection, delivery by cesarean section has been used in pregnant women with genital herpes lesions. To be effective, cesarean section must be performed before rupture of the membranes (46). However, many fewer cases of neonatal HSV infection occur than cases of recurrent genital herpes, even when virus is present at term.

Neonatal herpes can be acquired postnatally by exposure to either HSV-1 or HSV-2. Sources of infection include family members and hospital personal who are shedding virus (47,48).

About 75% of neonatal herpes infections are caused by HSV-2. They do not appear to be different between the nature and severity of neonatal herpes in premature or full-term infants, in the infections caused by HSV-1 or HSV-2, or in disease when virus is acquired during delivery or postpartum.

Most of neonatal herpes infections are always symptomatic. The overall mortality rate of untreated disease is 50%. So, classification of newborns with herpes infection is mandatory for prognostic and therapeutic diseases. Babies with congenital infection should be identified with in 48 hours after birth. Babies with neonatal herpes exhibit three categories of disease: (a) lesions localized to the skin, eye, and mouth; (b) encephalitis with or without localized skin involvement; and (c) disseminated diseases involving multiple organs, including CNS, lung, liver, adrenals, skin, eye and/or mouth (49). The worst prognosis (mortality rate about 80%) applies to infants with disseminated infection, many of them develop encephalitis. The cause of death of babies is usually viral pneumonitis or intravascular coagulopathy.

Transplacental infection of the fetus with HSV may cause congenital malformations, but this phenomenon is very rare. On occasions, the fetus may be spontaneously aborted.

Epidemiology

Genital HSV Infections

Genital HSV-2 infections are usually acquired through sexual contact, antibodies to this virus are rarely found before the age of onset of sexual activity (50). Genital HSV infections are caused by HSV-1 because of sexual behavior: oral sexual contact (51). The distinction in virus type is not insignificant, because genital HSV-1 infections are usually both less severe clinically and less prone to recur (51).

The number of new cases of genital HSV infections have been conservatively estimated to be approximately 500000 individuals annually (52). Prevalence of HSV-2 infections are vary in many parts of the world. The detection of prior HSV-2 infection by newer serologic methods predict Americans are infected with HSV-2 at a range of 40 to 60 million (53). Seroprevalence of HSV-2 increases from 6.9% at 15 to 29 years of age to 23.4% by the age of 60. When the populations were analyzed according to race, these same percentages were 4.6% and 19.7% for Caucasians and 21.8% and 64.7% for African Americans, respectively. Factors found to influence acquisition of HSV-2 include sex (women greater than men), race (blacks more than whites), marital status (divorced versus single or married), place of residence (city greater than suburb) (54). The highest prevalence of antibodies to HSV-2 in the United States is female prostitutes (75%), as same as in Tokyo (54). Homosexual men have seroprevalence rates to HSV-2 varying from 83.1% in San Francisco, 50% in Amsterdam, Netherlands and 21.6% in Seville, Spain (1985 - 1986). Thus, multiple sexual partners, has found to correlate directly with acquisition of HSV-2 infection (54). Asymptomatic shedding is the major risk of susceptible persons for contracting HSV-2 infections.For women with established genital HSV-2 infection, Asymptomatic shedding was detected on 1% of all days cultures were

obtained. The incidence of HSV-2 infection during pregnancy is about 0.58% - 2.5% per gestation (55).

In Thailand, prevalence data on genital HSV infections is not known exactly, because genital HSV infections are not reportable diseases in Thailand. However, genital HSV infections are usually found in the genital ulcers both men and women (56).

Diagnosis

Isolation by Tissue Culture

The appropriate use of laboratory tools is essential, if a diagnosis of HSV infections is to be achieved. Virus isolation remains the definitive diagnostic method. If skin lesions are present, a scraping of skin vesicles should be made and transferred in appropriate virus transport media to a diagnostic virology laboratory. Clinical specimens should be shipped on ice for inoculation into cell culture systems (e.g., foreskin fibroblasts, Vero cells, etc.), which are susceptible for the demonstration of the cytopathic effects characteristic of HSV replication. Cytopathic effects tends to develop within 24 to 48 hours after inoculation of specimens containing infectious virus. The shipping and processing of specimens should be expedited. In addition to skin vesicles, other sites from which may be isolated include the CSF, stool, urine, throat, nasopharynx, and conjunctivae. Duodenal aspirates from infants with hepatitis or other gastrointestinal abnormalities are useful for HSV isolation. Typing of an HSV isolate may be accomplished by one of several techniques, which may not be routinely available. Because outcome with treatment does not appear to be related to the virus type, identification is only of epidemiologic and pathogenetic importance, therefore, is not usually necessary.

Every effort should be made to confirm infection by virus isolation. The sensitivity of cytologic examination of cells from the maternal cervix or from the infant skin, mouth, conjunctivae, or corneal lesions is low, being approximately 60% to 70% (57). Cellular material obtained by scraping the periphery of the base of lesions should be smeared on

on the glass slide and promptly fixed in cold ethanol. The slide can be stained according to the methods of Papanicolaou, Giemsa, or Wright before examination by trained cytologist. Giemsa or Tzanck smears likely will not demonstrate the presence of intranuclear inclusions. The presence of intranuclear inclusions and multinucleated giant cells are indicative, but not diagnostic, of HSV infection. Electron microscopic assays are available but impractical (58).

Serologic Assessment

Serologic diagnosis of HSV infection is of little clinical value. Therapeutic decisions cannot wait the results of serologic studies. Commercially available serologic assay to distinguish between antibodies of HSV type 1 and 2 are limited to research laboratories as well as to denote the presence of transplacentally acquired maternal IgG, as opposed to endogenously produce antibodies, makes the assessment of the neonate's antibody status difficult during acute infection. Serial antibody assessment may be useful if a mother without a prior history of HSV infection has a primary infection late in gestation and transfer a little or no antibody to the fetus. The most commonly used tests for measurement of HSV antibodies are complement fixation, passive hemagglutination, neutralization, immunofluorescence, and the enzyme - linked immunosorbent (ELISA) assay. The more recent development of type specific antibody assays will likely replace many of the older system.

Polymerase Chain Reaction

An ever- increasing experience with the PCR indicates that it is the diagnostic method of choice for HSV infections of the CNS (59). Primer from the HSV DNA sequence that are common to both HSV-1 and HSV-2 (either the glycoprotein B domain or HSV DNA polymerase), identify HSV DNA in the CSF. The evaluation of CSF specimens obtained from patients with biopsy - proven herpes simplex encephalitis and those with proven other diseases indicates a sensitivity more than 95% at the time of clinical presentation and a specificity that approaches 100% (60). False - negative

assessment can be found when there is contamination of hemoglobin in the CSF or the presence of inhibitors, such as heparin. PCR analyses of CSF specimens will likely redefine the spectrum of HSV infections of the CNS. Importantly, PCR evaluation of CSF can be utilized to follow therapeutic outcome in patients with HSV encephalitis. A recent publication (61) identified persistence of HSV DNA in the CSF of the newborns with suspect herpes simplex encephalitis.

PCR has also been used to detect HSV-DNA in skin lesions. Sensitivity and specificity must be justified careful evaluation (62). In addition, it is not a cost-efficient diagnostic method.

Control of HSV infections

Two avenues exist for the control of HSV infections; one approach is that of antiviral chemotherapy and the alternative approach is the prevention.

Genital HSV Infections

Because of the growing awareness of the increasing incidence of genital herpes and neonatal herpes and its association with an increased risk of acquisition of HIV, every effort should be made to prevent HSV infections. Until a vaccine is proven effective, educational efforts must be developed for adolescents and adults at greatest risk. The use of condoms should be promoted.

Neonatal HSV infections

Surgical abdominal delivery will decrease transmission of infection when membranes are ruptured less than 4 hours, but cesarean section has not been proven efficacious when membranes are ruptured for longer periods of time. Nevertheless, it is recommended when membranes are ruptured up to 24 hours in the presence of active lesions. Although the recommendation seems logical, no data exist from adequately performed clinical trials to fully support it.

For women with a past history of genital HSV infection, a careful vaginal examination at presentation to the delivery suite is a greatest importance. Although visualization of the cervix is often difficult, speculum examination for documentation of recurrent lesions is extremely important and should be attempted in all women. HSV culture at the time of delivery is the great importance in establishing whether excretion can lead to transmission of infection to the fetus.

Clearly, identifying women who excrete HSV at delivery and then optimizing either prophylaxis with safe and acceptable antivirals or cesarean section remains the optimal management of genital infection at delivery. The detection of type-specific antibodies to gG-2 will identify those women at greatest risk. Documentation of discordant serologic status between sexual partners may assist in prevention of maternal primary infection. Clearly, education of male partners who are seropositive should be educated regarding HSV transmission.

Nosocomial Transmission

A policy of requiring transfer or provision of medical leave for nursery personnel who have labial HSV infection is impractical and causes an excessive burden in those attempting to provide adequate care. Temporary removal of personnel who have cold sores has been suggested. As noted previously, individuals with herpetic withlow shed virus. These individuals should be removed from the care of newborns at risk for acquiring neonatal HSV infection because even gloves may not prevent transmission of infection. Education regarding the risks of transmission of virus and the importance of handwashing when lesions are present should be repeatedly emphasized to health-care workers. In addition, hospital personnel should wear masks when active lesions are present.

Vaccine Development

Vaccination remains the ideal method for prevention of HSV infection; however, prevention of HSV infections introduces unique problems because of recurrences in the presence of humoral immunity. Nevertheless, protection from life-threatening disease can be achieved in animal models with avirulent, inactivated, or even subunit glycoprotein vaccines.

Wild-type virus vaccines were demonstrated by an inoculation of autologous virus, virus from another infected individual or virus recovered from an experimentally infected rabbit. The inoculation of virus led to infection at the site of injection in 40% to 80% of patients. In some cases, inoculation led to recurrence of latent infection (63). This approach is considered unacceptable.

Inactivated (killed) virus vaccines have been used as vaccines in a variety of animal model systems (64 - 65). When these vaccines were administered to patients with pre-existing HSV infection in order to alleviate recurrences, most of studies failed to include an appropriate control group. The results were introduced to decrease in the frequency of recurrent lesions when compared with placebo (66). The inactivated vaccines were made from phenol-treat, formalin, ultraviolet light, or heat. The conclusion of results utilizing inactivated vaccines were that there may be some initial benefit could not be established.

Subunit vaccines evolved from attempts to remove viral DNA to eliminate the potential for cellular transformation, to enhance antigenic concentration, induce stronger immune responses, and to exclude any possibility of contamination with residual live virus. Subunit vaccines were prepared using a variety of methods for antigen expression or extraction from infected cell lysates by detergent and subsequent purification. The immunogenicity of envelope glycoproteins, free of viral DNA, was demonstrated in animals (67). Envelope glycoproteins do not appear to convey protection in uninfected sexual partners of individuals with genital infection but results are preliminary. However, subunit vaccines were produced by cloning specific glycoproteins in yeast, Chinese

hamster ovary cells or other methods; for example gD-2 and gB-2 vaccine (68). In a study of the Merck Sharpe & Dohme glycoprotein envelope subunit vaccine, carried out in sexual partners known to have genital herpes, the number of individuals developing herpetic infection was nearly the same for both placebo and vaccine recipients, indicating that the vaccine was not effective (68). But a controlled trial of the Biocine/Chiron gD-2 vaccine (bound to alum) significantly decreased the number of culture-positive episodes and total number of episodes by approximately one-third compared to the placebo recipients (69). These vaccines are being confirmed in large phase III clinical trial that is being conducted in parallel with primary prevention study.

Genetically engineered HSV was developed by Roizman and colleagues to construct recombinant HSV as a prototype of herpes vaccines (70). The genome of the HSV-1 (F) strain was deleted in the domain of the viral thymidine kinase (TK) gene and in the junction region of the U_L and U_S segments in order to excise some of the genetic loci responsible for neurovirulence as well as to create convenient sites and space within the genome for insertion of other genes. Finally, a type 2 HSV DNA fragment encoding the HSV-2 gD, gG, and gI were inserted in place of the internal inverted repeat. This virus expresses TK; thus, it is susceptible to antiviral chemotherapy with acyclovir. When these vaccines were evaluated in rodent models, the contracts were attenuated in pathogenicity and ability to establish latency and were capable of inducing protective immunity. However, in humans, low-level immune responses were elicited (71). Unfortunately, production difficulties precluded the administration of higher dosages of vaccine.

Treatment

All the antiviral agents active against human herpes act by specific inhibition of viral DNA polymerase. Except for phosphonoformate, all the other anti-herpesvirus agents (nucleoside analogues) need to be phosphorylated intracellularly before they interact as competitive inhibitor/alternative substrates with respect to the natural substrates for the DNA polymerase reaction. At the DNA polymerase level some of the

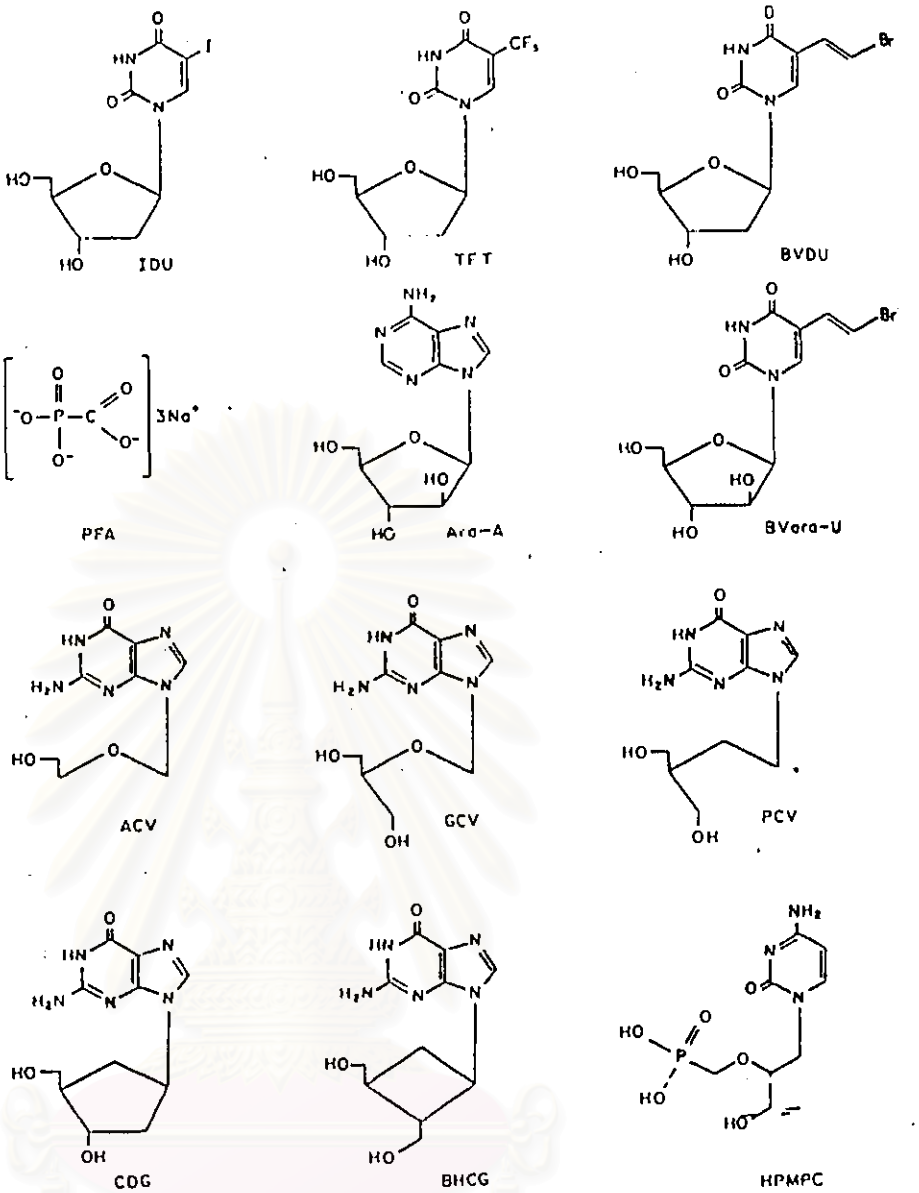
compounds (e.g. acyclovir) interact as chain terminator, for their incorporation into the DNA does not allow further chain elongation. The selective antiviral action of the anti-herpes agent is due to preferential phosphorylation of the compounds by the virus-encoded thymidine kinase (TK), as has been demonstrated for acyclovir, brivudin and brovavir, and/or higher affinity of the viral DNA polymerase (as compared to the cellular DNA polymerases) for the active forms: triphosphate, as has been demonstrated with virtually all nucleoside analogues. The formulae of compounds are presented in Figure 6. Some of these compounds have already been licensed for clinical use, while other are still under development. 1-(3-hydroxy -2-phosphonylmethoxypropyl)cytosine (HPMPC) seems to be superior to the established anti-herpetic drugs (i.e. acyclovir, penciclovir) in the treatment of both HSV and CMV infections (72).

Licensed antiviral drugs

The following antiviral drugs have been approved for clinical use; idoxuridine, trifluridine, vidarabine and acyclovir for the topical treatment of HSV keratitis; vidarabine for the systemic (intravenous) treatment of HSV encephalitis, neonatal herpes and Varicella-Zoster virus (VZV) infections in immunosuppressed patients; acyclovir for the systemic (oral or intravenous) treatment for genital herpes, HSV encephalitis, neonatal herpes, and HSV or VZV infections in immunosuppressed patients; brivudin for the systemic (oral treatment of HSV-1 and VZV infections in immunosuppressed patients; and foscarnet and ganciclovir for the systemic (intravenous) treatment of cytomegalovirus (CMV) retinitis in AIDS patients (72). The compounds have been specifically licensed, listed as follows: brivudin, in the topical treatment of herpetic keratitis that is clinically resistant to idoxuridine, trifluridine, vidarabine, or acyclovir ; foscarnet, in the systemic (intravenous) treatment of acyclovir-resistant, thymidine kinase-deficient (TK^-) HSV infections (73); and both acyclovir and gancyclovir in the prophylaxis of CMV in bone marrow or kidney transplant recipients (72).

Mechanism of action

Anti-herpesvirus agents all act on viral DNA polymerase but compounds differ from one another in the way they are metabolized to their active forms and in the precise mechanism by which the latter interact with the viral DNA polymerase (Figure 7). Foscarnet (PFA) does not require any previous metabolism to interact, as a pyrophosphate antagonist, with the viral DNA polymerase. It preferentially inhibits DNA polymerase of vial origin over cellular DNA polymerase. Acyclovir and brivudin are specifically recognized as substrates by the HSV-encoded and VZV-encoded thymidine kinases (TK), which convert acyclovir and brivudine to their monophosphate derivatives, and BVDU 5'-monophosphate to its 5'-diphosphate derivatives (since the HSV-1-encoded and VZV-encoded TK is also endowed with a concomitant dTMP kinase activity). After ACVMP and BVDUDP have been phosphorylated by cellular kinases to their triphosphate forms, the latter can interact with the viral DNA polymerase. ACVTP acts as an obligate chain terminator, as it does not contain the 3'-hydroxyl function required for further chain elongation. However, BVDUTP can be incorporated interactly, via an internucleotide linkage, and this incorporation may lead to breakage of the DNA strains, as has been demonstrated with the 5-(2-iodovinyl)-substituted counterpart of BVDUTP. Brovavir follows a similar metabolic pathway to BVDU in that it is also preferentially phosphorylated by the HSV-1-encoded and VZV-encoded TK; but, at the DNA polymerase level, Bvara-UTP primarily acts as a competitive inhibitor with respect to the natural substrates dTTP, although it could also be incorporated at the 3'- end and thus act as a chain terminator. Ganciclovir is assumed to acquire its anti-CMV specificity from a facilitated phosphorylation in the virus-infected cell, followed by a greater affinity of its triphosphate form (GCVTP) for the viral DNA polymerase than for the cellular DNA polymerase. At the DNA polymerase level, GCVTP could act as either a competitive inhibitor; with respect to dGDP, or an alternative substrate, and thus be incorporated either 3'- terminally or internally, as GCV contains an hydroxyl function that is equivalent to the 3'- hydroxyl group of the nucleotides (72).



IDU: Idoxuridine, 5-iodo-2'-deoxyuridine.
TFT: Trifluridine, 5-trifluoromethyl-2'-deoxyuridine.
BVDU: Brivudin, (*E*)-5-(2-bromovinyl)-2'-deoxyuridine (Helpin).
PFA: Foscarnet, phosphonoformic acid (Foscavir).
Ara-A: Vidarabine, 9-β-D-arabinofuranosyladenine (Vira-A).
BVaraU: Brovavir, 1-β-D-arabinofuranosyl-(*E*)-5-(2-bromovinyl)uracil.
ACV: Acyclovir, aciclovir, 9-(2-hydroxyethoxymethyl)guanine (Zovirax).
GCV: Ganciclovir, 9-(1,3-dihydroxy-2-propoxymethyl)guanine, DHPG (Cymevene, Cytovene).
PCV: Penciclovir, 9-[4-hydroxy-3-(hydroxymethyl)but-1-yl]guanine.
CDG: Carbocyclic 2'-deoxyguanosine.
BHCG: (*R*)-9-[2,3-Bis(hydroxymethyl)cyclobutyl]guanine.
HPMPC: (*S*)-1-(3-Hydroxy-2-phosphonylmethoxypropyl)cytosine.

Figure 6. Formulae and abbreviations for anti-herpesvirus agents

(From De Clercq, E : Antiviral of the treatment of herpesvirus infections.

J Antimicrob Chemother 1993; 32, Suppl.A: 121-123.)

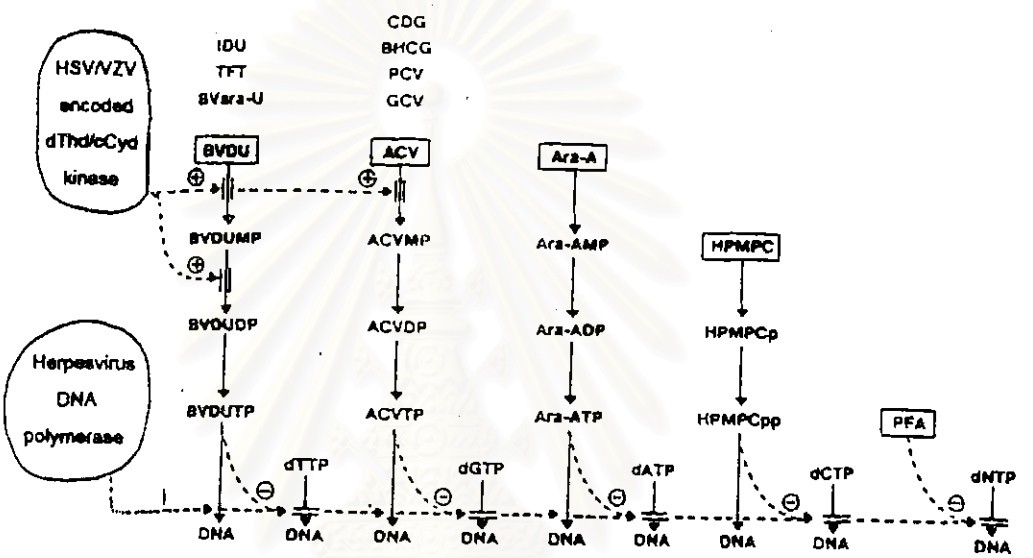


Figure 7. Mechanism of action of anti-herpesvirus agents.

The target enzyme is the viral DNA polymerase. To interact with this target enzyme, as either competitive inhibitor or alternative substrate, the compounds must first be phosphorylated to their triphosphate forms (or diphosphate form for HPMPC), except for PFA which interacts with the DNA polymerase. The initial phosphorylation of BVDU and ACV is catalyzed by the virus-induced dThd/dCyd kinase. The latter is also endowed with dTMP kinase activity which phosphorylates BVDUMP to BVDUDP. Abbreviations: MP, DP, and TP stand for mono-, di- and triphosphate, respectively; drugs, see legend to Figure 6.

(From De Clercq, E: Antiviral of the treatment of herpesvirus infections J Antimicrob Chemother 1993; 32, Suppl.A: 121-123.)

Treatment of Genital Herpes

Acyclovir (9-(2-hydroxyethoxymethyl) guanine), a synthetic acyclic purine nucleoside analog, has become the standard of the therapy of HSV infections. It is the most wildly prescribed and clinically effective antiviral drug available to date. The prodrugs valacyclovir and famciclovir have recently been licensed and provide enhanced oral bioavailability compared to acyclovir. Valacyclovir is a prodrug of acyclovir, when administered at 1 every 8 hours, resulting plasma levels are similar to 5 mg/kg given intravenously. Famciclovir is a prodrug of penciclovir.

Initial genital HSV infection can be treated with topical, oral, or intravenous acyclovir. Topical application of acyclovir reduces the duration of viral shedding and the length of time before all lesions become crusted, but it is less effective than oral or intravenous therapy (74). Intravenous acyclovir is the most effective treatment for first-episode genital herpes, and results in a significant reduction in the median duration of viral shedding, pain, and length of time to complete healing (8 versus 14 days) (75). Because intravenous acyclovir therapy usually requires hospitalization, it should be reserved for patients with severe local disease or systemic complications. Oral therapy (200 mg five times daily) is nearly as effective as intravenous acyclovir for initial genital herpes, and has become the standard treatment (Table 2). Neither intravenous nor oral acyclovir treatment of acute HSV infection reduces the frequency of recurrences (76).

Recurrent genital herpes is less severe and resolves more rapidly than primary infection; thus, there is less time to introduce antiviral chemotherapy successfully. Oral acyclovir therapy shortens both the duration of viral shedding and the length of time to healing (6 versus 7 days) when initial early (within 24 hours of onset), but the duration of symptoms and length of time to recurrence are not affected (77). Valacyclovir and famciclovir likely provide little added benefit.

Long-term oral administration of acyclovir effectively suppresses genital herpes in patients who have frequent recurrences. Daily administration of acyclovir reduces the frequency of the recurrences by up to 80%, and 25% to 30% of patients have no further

recurrences while taking acyclovir (78). Titration of the dose of acyclovir (400 mg twice daily or 200 mg two to five times daily) may be required to establish the minimal dose that is the most effective and economical. The emergence of acyclovir-resistant strains of HSV appears to be infrequent in immunologically normal individuals. Importantly, asymptomatic shedding of virus can continue despite clinically effective suppression with acyclovir, so that the possibility of person-to-person transmission persists (79).

Table 2. Indications for Acyclovir Therapy

Type of infection	Route and Dosage[a]	Comments
Genital HSV		
Initial episode	200 mg orally 5 times/day for 10 days	preferred rout in the normal host
	5 mg/kg IV every 8 hr for 5 days	Reserved for severe cases
	5% ointment topically 6 hr 7 days	Less effective than oral therapy
Recurrent episode	200 mg orally 5 times/ day for 5 days	Limit clinical benefit
Suppression	400 mg orally twice daily	Titrate dose as required
Mucocutaneous HSV in an immunocompromised patient	200-400 mg orally 5 times/ day for 10days	
	5 mg/kg IV every 8 hr for 7-10 days[b]	
	5% ointment topically every 6 hr for 7 days	For minor lesions only
HSV encphalitis	10 mg/kg IV every 8 hr for 10-14 day[c]	Alternative therapy; vidarabine, valacyclovir, famciclovir
Neonatal HSV[d]	10 mg/kg IV every 8 hr for 10-14 days[e]	Alternative therapy; vidarabine, valacyclovir, famciclovir

[a] The doses are for adults with normal renal function unless ortherwise notesd.

[b] A does of 250 mg per square meter of body-surface area should be given to children under 12 years of age.

[c] A does of 500 mg per square meter of body-surface area should be given to children under 12 years of age.

[d] Acyclovir has not been approved by the Food and Drugs Administration for this indication.

[e] Alternative regimens are being tested.

Thai medicinal plants in this research

Cerbera odollam Gaertn.

Family : APOCYNACEAE

Common Names : Pong Pong

Local Names (80) : ตีนเป็ดน้ำ Teenpet num, ตีนเป็ดทะเล Teenpet Thale (Central), ตูม Tum (Kanchanaburi), สั่งลา Sung laa (Krabi)

Botanical Description : The plant is round form, ever green tree, crown size, up to 15 meter high and light brown trunk. Leaves are obovate-lanceolate to oblong-lanceolate shape,12 - 31 cm by 4.5-7 cm, glossy green, spirally arranged, crowded at the tops of the glabrous branchlets, thin nerves inarching quite near the margin, turning black in drying. Flowers are white, acrid smelling, all-year round blooming period. Flowers in terminal peduncled cymes, 5-merous; bracts large; segments linear, large, pale, overlapping to the left; filaments very short. Corolla with a yellow eye; tube widened upwards from just above the middle 1.5 - 2 cm long. Fruits are round shiny green, turn red when ripe (81).

Ecology and Distribution : The plant grows in any soil, prefers water - edge environment or low and flooded areas, can be grown near the seaside, toxic resin, not recommended for play area (82).

Chemical Composition : The seeds contain cardiac glycosides: cerberin, odollin and cerbeside (83).

Ethanomedical Uses : The surface of a bark is used to treat fever, parasite and flu-cold (84). Seed- oil is used to treat hair (83) .

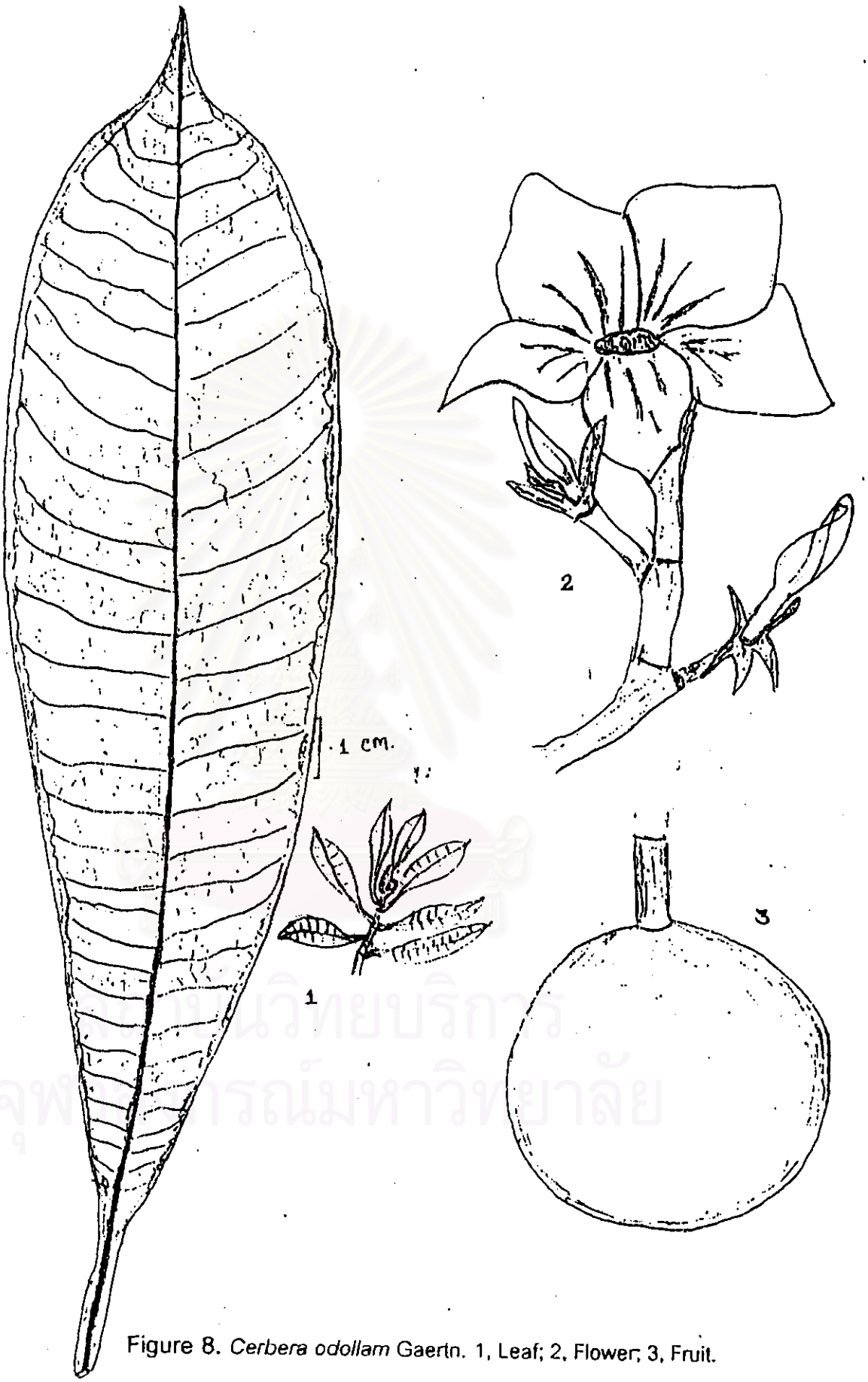


Figure 8. *Cerbera odollam* Gaertn. 1, Leaf; 2, Flower; 3, Fruit.

Clausena excavata Burm. F.

Family : RUTACEAE

Common Names : -

Local Names (80) : ขี้ผึ้ง Khee phueng , แสนโสก Saen sok, ชะมัด Chamut (Udonthani), เพี้ยฟาน Phiafaan, หญ้าสาบฮิ้น Yaa saap hin, หมี Mee (Northern), มะหลุย Ma Lui (Peninsular), ยม Yom (Chumphon), รุ้ย Rui (Kanchanaburi), สีสม Seesom, หมอน้อย Mo noi (Central), สมัดใบใหญ่ Samut baiyai, หัศคุณโศก Hussakhun khok (Petchabun), สามเสือ Saam Suea (Chonburi), สามโสก Saam sok (Chunthaburi), สำรุย Sam rui (Yala), หวดหม่อน Huat mon (Central, Northern), หัสคุณ Hassakhun, อ้อยช้าง Oichaang (Saraburi)

Botanical Description : The plant is shrubby tree, vase shape form. Leaves spirally arranged, at the top of the branches often densely crowded, odd-pinnate; leaflets15-30, distichous, oblong or falcate, often with an oblique base, leaf-rachis 20-50 cm. Flowers have short - hairy panicles, 4-5-merous; petals oval-oblong, acute, pale green or yellowish white, 3.5-5 mm long; stamens 7-10; filament 3.5 mm abruptly much broadened towards their base; ovary long - hairy, persistent; stigma not broadened (81).

Ecology and Distribution : The plant grows in any soil, ranges from India and South China through South-East Asia, cultivated by seeds.

Ethanomedical Uses : It is a good for remedy for stomach trouble. A decoction of the root is sudorific; the leaves are

insecticide. The plant is bitter , tonic , astringent and emmenagogue. A poultice of the leaves is applied to treat paralysis; an infusion of the stem may be taken against colic. The pounded root is a poultice for sores; the leaves , for headache, ulcerated nose; for the latter, a fumigation from burning leaves and bark is another treatment; a decoction of the leaves is administered post partum. The juice pressed or pounded out of the leaves is both a medication for fever, and a vermifuge; it may be given to lying- in women (85).

Figure 9. *Clausena excavata* Burm. F.

Coleus amboinicus Lour.

Family : LABIATAE

Common Names (85-87) : Country borage, Indian borage

Local Names (80) : เนียมหูเสือ Niam huu suea (Central), หอมด่วนหลวง Hom duan luang, หอมด่วนหูเสือ Hom duan huu suea (Northern)

Botanical Description : The plant is much branched, fleshy, highly aromatic herb, 20 - 50 cm high. Lower part of the stem woody. Leaves are simple, opposite, thick, pale - green succulent long-petioled, lamina broadly ovate or orbicular - obtuse, crenate - serrate on margin, to 9 cm across, fleshy, bullate above. Flowers are small , purple, very rarely, indense whorls at intervals on terminal racemose axis; calyx 2-lipped, tube decurved; corolla 2-lipped; stamens 4, didynamous. Fruit is 4 orbicular nutlets (81,87).

Ecology and Distribution : Species cultivated as a culinary herb and medicinal plant. Flowering period is March - May.

Chemical Composition : The whole plant contains an essential oil consisting of carvacrol.

Ethanomedical Uses : The fresh leaves possess antibacteral, antifebrile and antitusssive properties. They are used in treating coryza , influenza , hyperthermia , diaphoretic pyrexia, cough, asthma, haemoptysis, sore throat, laryngitis, hoarseness, haematemesis and epistaxis. They are prescribed in daily dose

of 10 to 16 gram in the form of a decoction, extracted juice, inhalation of vapour from a boiling decoction, or per-lingual administration of pounded leaves together with a little salt. For infants, the pounded fresh leaves are mixed with honey and steamed (86). Sometimes are used in treating insect string (centipedes and scorpions), also to relieve pain in the region of the stomach and heart. Juice or a decoction of leaves is carminative (85).

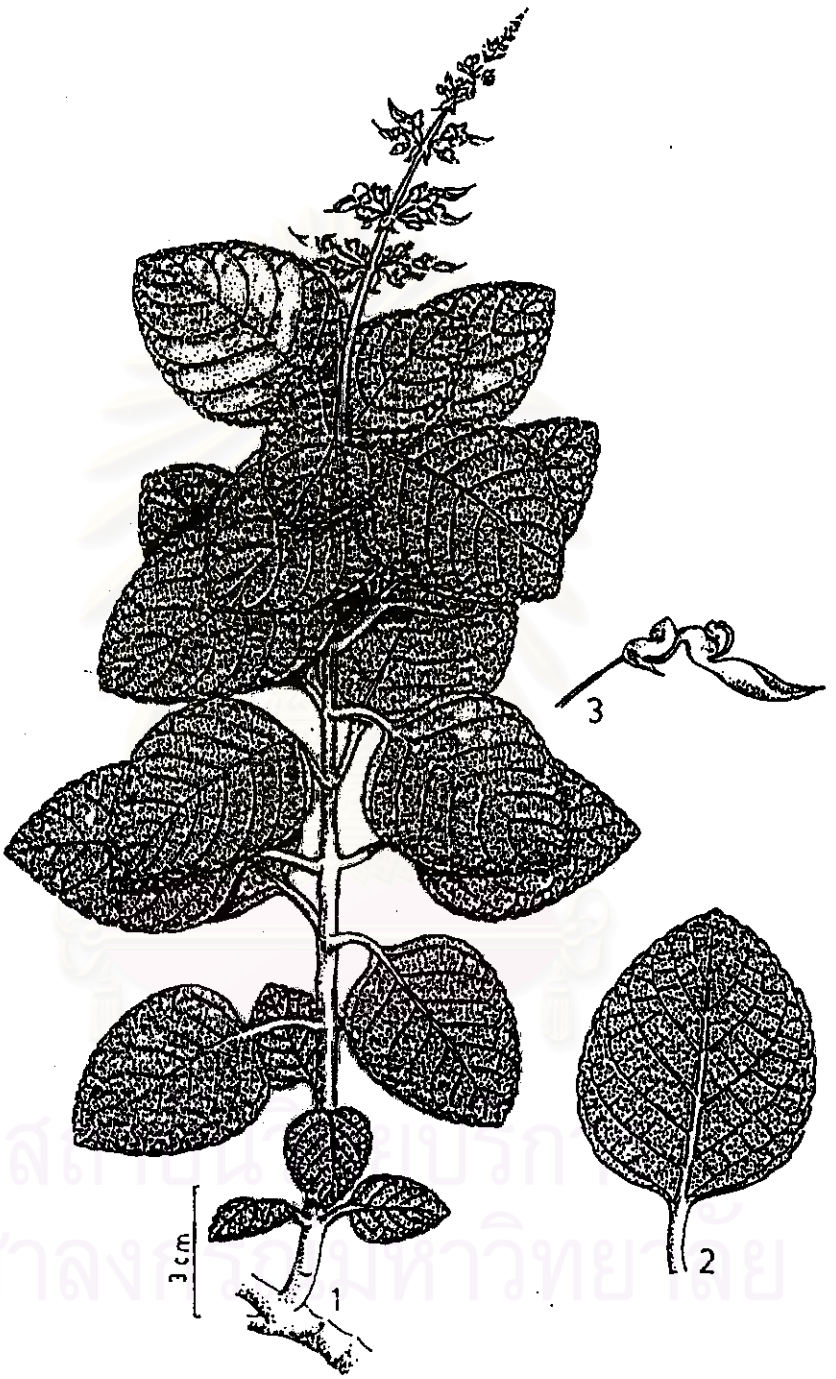


Figure 10. *Coleus amboinicus* Lour. 1, Flowering twig; 2, Leaf; 3, Flower.

Phyla nodiflora (L.) Greene.

Family : VERBENACEAE

Common Names : Yaa klet plaa

Local Names : หญ้าเกล็ดปลา Yaa klet plaa (central) (80), เหงือกปลาหมอ นา Hngueak plaa mo naa, ฟันกระต่าย Fun kra taai (88)

Botanical Description : The plant is a herb, creeping , copiously rooting, much - branched. Stem is angular. Leaves are opposite , obovate or subcuneate, from a cuneate , often decurrent base, rounded, coarsely serrate-dentate above the entire base, 8-50 mm by 4-25 mm; petiole 0.5-7 mm. Flower is small, in the axil of the bract; calyx thinly membranous, yellowish white or pellucid, laterally compressed, deeply bipartite; segments navicular. It has 4 stemens and stigma is thick. Fruit is encoded by the calyx (80,87).

Ecology and Distribution : The plant can grow in very high moisture places with fast growth rate. So, it may be found along the street, glass-garden, and the moisture areas (88).

Ethanomedical Uses : All parts are used in treating gonorrhoea, fever and burn-ulcer. Juice or a decoction of leaves and apex is carminative (88).

Figure 11. *Phyla nodiflora* (L.) Greene; 1, Leaf; 2, Flower; 3, Root.

Thevetia peruviana Schum.

Family : APOCYNACEAE

Common Names : yellow oleander, bastard oleander, lucky nut-tree, exile tree, exile oil plant, Be-Still tree, Tiger apple tree. (82,83)

Local Names (80) : กระบอก Krabok, กะทอก Ka thok, บานบุรี Baan buree, ยี่โถฝรั่ง Yeetho farang (Bangkok), แซน่าวา Sae naa waa, แซะศาลา Sae saa laa, ร่ำเพย Ram phoei (Central), Trumpet flower.

Botanical Description : The plant is small, shrubby, evergreen, vase shaped plant: 2-3 m in height with milky juice. Stem scaled with scars of fallen leaves. Leaves are bright glossy green alternate, opposite, linear- oblong, entire, only main nerve conspicuous. Inflorescence in axillary cyme of 2-3 flowers. Flower colors are white, yellow and orange, delicately fragrant, all- year round blooming period.; flower terminal and leaf-opposed, cymose, 5- merous; calyx deeply divided, on the inside with many basal glands ; segment lanceolate; corolla much widened above the narrow base with 5 narrow long-hair scales; segments in bud overlapping to the left. Fruit is green, ripe fruit is brown. The dried fruit is broken up to obtain 1-2 brown seeds (81-83,86).

Ecology and Distribution : The plant grows in sandy soil, low moisture, cultivated for its elegant foliage and handsome flowers.

Chemical Composition : The seeds contain cardiotonic glucosides, thevetin (A,B), 2'-O-acetyl cerberoside, neriifolin, cerberin, peruvoside, thevenenin, and peruvosidic acid (83,86).

Ethanomedical Uses : The purified glucoside thevetin, extracted from the seeds, is prescribed as a cardiotonic drug in a 0.1% solution for oral use in a dose of 1 to 2 milliliter ampoules for parenteral use, each ampoule containing 1 milligram; 1 to 2 ampoules are given per day. The crushed seeds can be used as an insecticide. Caution is needed because of their high toxicity (85,86).

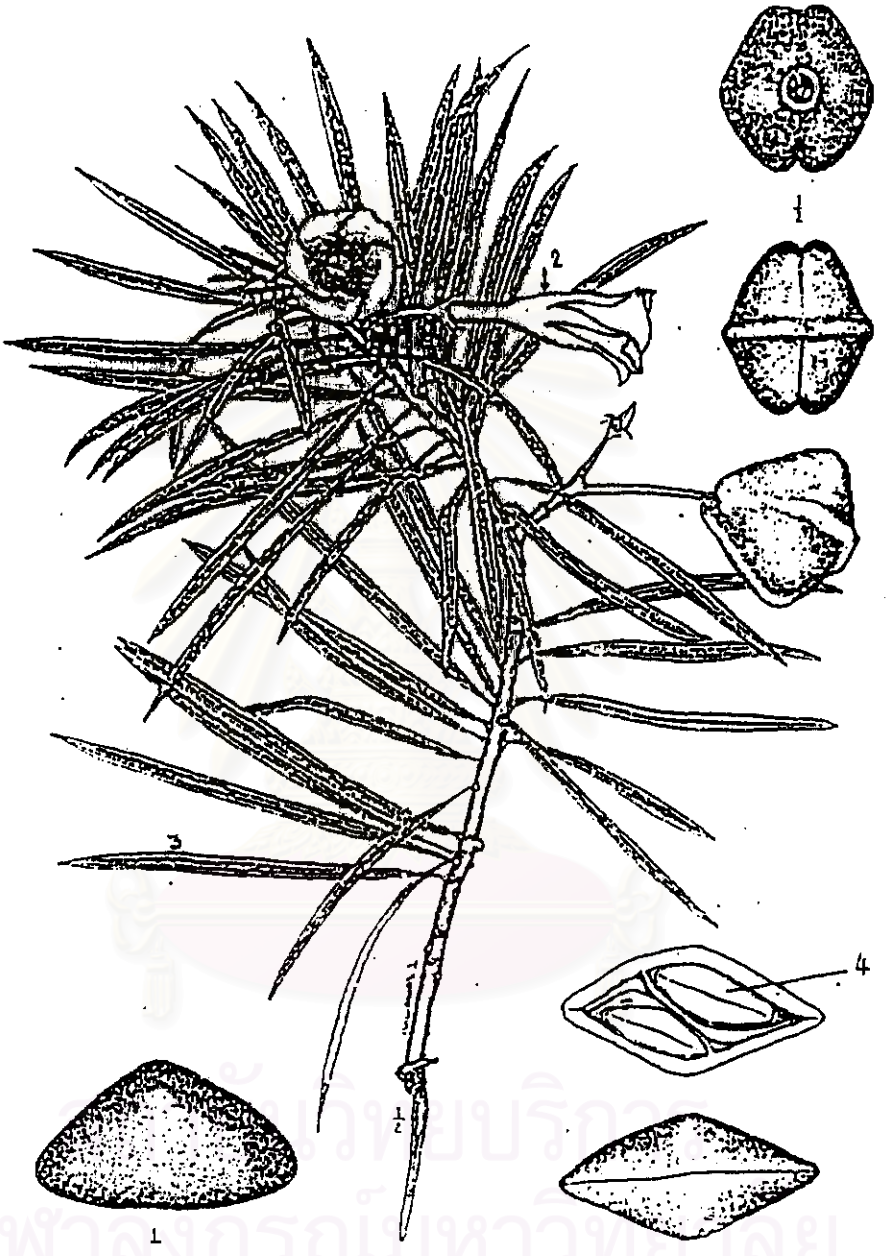


Figure 12. *Thevetia peruviana* Schum. 1, Fruit; 2, Flower; 3, Leave; 4, Seed.