CHAPTER II

LITERATURE REVIEW

1. History of Herpes Simplex Virus

HSV infections of humans have been documented since ancient Greek times. Records of human HSV infections began with descriptions of cutaneous spreading lesions thought to be of herpetic etiology, particularly in the writings of Hippocrates (Nahmias ans Dowdle, 1968). Scholars of Greek civilization define the word "herpes" to mean creep or crawl, in reference to the spreading nature of the visualized skin lesions (Beswick, 1962).

Several observations made at the beginning of the 20th century brought an end to the early imprecise descriptive era of HSV infections. Several points substantiate this conclusion. First, histopathologic studies described multinucleated giant cells associated with herpesvirus infections (Unna, 1886). Second, the unequivocally infectious nature of HSV was recognized by Lowenstein (1919).

The major laboratory advances of the past 25 years have provided a foundation for the recent application of molecular biology to the study of human disease. One significant advance was the detection of antigenic differences between HSV types. Although suggested by Lipschitz (1921) on clinical grounds more than 60 years ago and by others from laboratory observations (Plummer, 1964), in 1968 Nahmias and Dowdle demonstrated antigenic and biologic differences between HSV-1 and HSV-2. These investigators demonstrated that HSV-1 was more frequently associated with nongenital infection, whereas HSV-2 was associated with genital disease. This observation was pivotal for many of the clinical, serologic, immunologic, and epidemiologic studies.

Obviously, other critical advances made over the past decade have contributed to our understanding of the natural history of HSV infections. These include

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the following, among others. First, successful antiviral therapy was established unequivocally for HSV encephalitis (Whitley, 1977) and, subsequently, for genital HSV infections (Bryson et al., 1983) and HSV infections in the immunocompromised host (Meyers et al., 1980). Second, differences between strains of HSV were demonstrated by restriction endonuclease technology, which has become an important molecular epidemiologic tool (Buchman et al., 1978). Third, the use of type-specific antigens for seroepidemiologic surveys has advanced our understanding of the clinical epidemiology of infection (Roizman et al., 1984). Fourth, much work has focused on the replication of HSV and the resultant gene products. A principal goal of these efforts is to define the biologic properties of these gene products, a goal that is in the very early stages of accomplishment. Fifth, the engineering of HSV and the expression of specific genes will provide technology for new vaccines (Roizman et al., 1985), as well as for the use of such viruses for gene therapy. Finally, extensive effort was devoted to the study of latency with incremental advances (Stevens et al., 1987; Roizman and Sears, 1993).

2. General Characteristics of Herpes Simplex Virus

Herpes simplex viruses (HSV) are members of the subfamily *alphaherpesvirinae*. The members of this subfamily are classified on the basis of variable host range, relatively short reproductive cycle, rapid spread in culture, efficient destruction of infected cells, and capacity to establish latent infections primarily but not exclusively in sensory ganglia (Roizman, 1996).

The size of herpesvirions has been reported to vary from 120 to nearly 300 nm. The precise number of polypeptide species contained in the virions is not known and may vary from one virus to another. The estimates generally range from 30 to 35 polypeptides. The virions consist of four elements: (i) an electron-opaque core, (ii) an icosadeltahedral capsid surrounding the core, (iii) an amorphous tegument surrounding the capsid, and (iv) an outer envelope exhibiting spikes on its surface (Figure 1).The core of the mature virion contains the viral DNA in the form of a torus. The precise arrangement of the DNA in the toroid is not known. The capsid is approximately 100 nm in diameter and the number of capsomers is 162. The pentameric capsomeres at the

vertices have not been well characterized. 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. The tegument, a term introduced by Roizman and Furlong to describe the structures between the capsid and envelope, has no distinctive features in thin sections but may appear to be fibrous on negative staining. The thickness of tegument may vary, depending on the location of the virion within the infected cell. When the amount is variable, there is more of it in virions accumulating in cytoplasmic vacuoles than in those accumulating in the perinuclear space. The available evidence suggests the amount of tegument is more likely to be determined by the virus than by the host. The tegument is frequently distributed asymmetrically. The envelope of the virus has a typical trilaminar appearance; it appears to be derived from patches of altered cellular membranes. The presence of lipids was demonstrated by analyses of virions and by the sensitivity of the virions to lipid solvents and detergents. The herpesvirus envelope contains numerous protrusions of spikes, which are more numerous and shorter than those appearing on the surface of many other enveloped viruses. Wildy and Watson estimates that the spikes on HSV virions are approximately 8 nm long. The spikes consist of glycoproteins. The number and relative amounts of viral glycoproteins vary; HSV specifies at least 11 glycoproteins (Roizman, 1996)

The bulk of packaged HSV DNA is linear and double stranded. In the virion, HSV DNA is packaged in the form of a toroid. The ends of the genome are probably held together or are in close proximity inasmuch as a small fraction of the packaged DNA appears to be circular and a large fraction of the linear DNA circularizes rapidly in the absence of protein synthesis after it enters the nuclei of infected cells. DNA extracted from virions contains ribonucleotides, nicks, and gaps.

The HSV genome is approximately 150 kbp, with a G+C content of 68% for HSV-1 and 69% for HSV-2. (Morse, et al., 1978). The homology between the two type of HSV genome is about 50%. The HSV genome codes for approximately 100 proteins (Roizman, et al., 1975). Most of the polypeptides specified by one virus type are antigenically related to the polypeptides of the other type (Corey and Spear, 1986). They

are designated as either infected cell specific polypeptides ($ICSP_S$) or infected cell polypeptides (ICP_S). The three groups of HSV proteins, α, β and γ, are synthesized in a sequential order. There are five α-proteins namely ICP 0, 4, 22, 27 and 47. The synthesis of α-polypeptides reaches peak rate at approximately 2-4 hours post infection but some α-proteins continue to be produced throughout the period of infection (Honess and Roizman 1974). The β-proteins comprise two groups; β_1 and β_2. They reach peak rates of synthesis at about 5-7 hours post infection. The β_1 protein is synthesized earlier, its peak synthesis is overlapping slightly with α-class. β_1 proteins include the major DNA-binding protein and the large component of the viral ribonucleotide reductase (Huszar and Bacchetti, 1981). β_2 proteins are synthesized later, they include the viral thymidine kinase and DNA polymerase. All of these β-proteins involve in viral nucleic acid metabolism. The γ-proteins are primarily structural polypeptides and include the viral glycoprotein, capsid, and some tegument components. They are divided into two classes, γ_1 and γ_2. γ_1 proteins can be synthesized early in the absence of viral DNA replication. In contrast, the γ_2 protein are expressed late in the infection and absolutely require viral DNA replication (Silver and Roizman, 1985). These γ-proteins also act as a major target for host immune response.

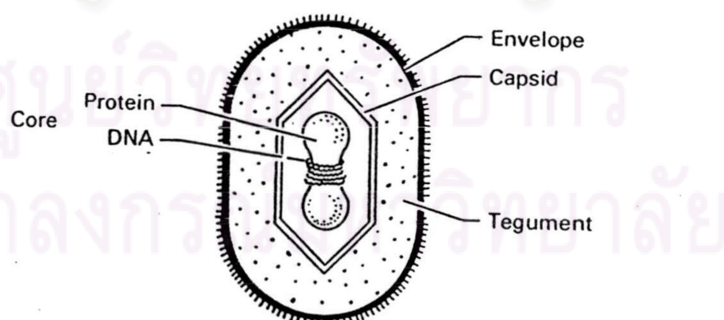

Figure 1. Diagram of a virion of herpes simplex virus

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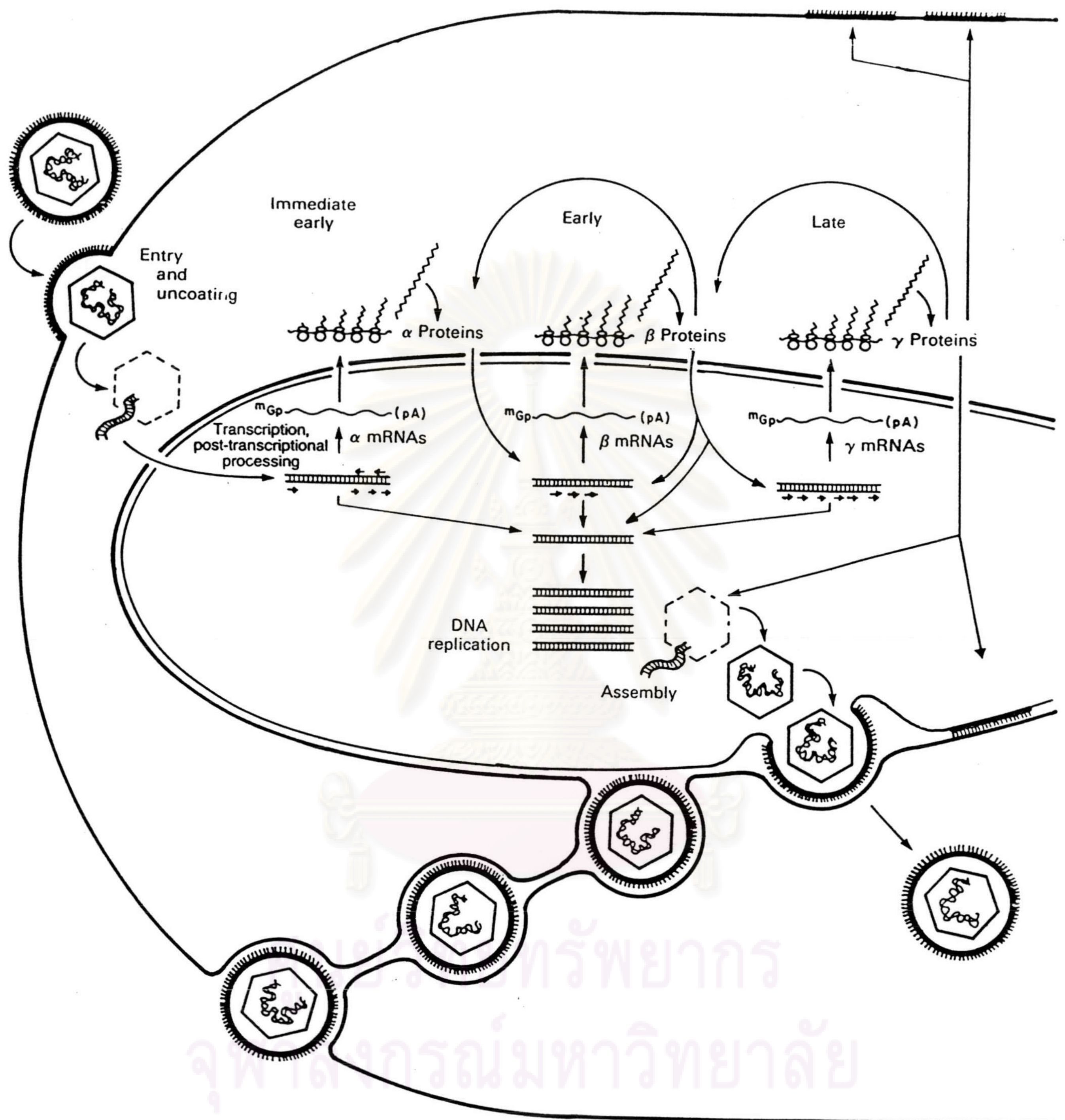

Figure 2 Sequence of events in the multiplication of herpes simplex virus

3. HSV Multiplication

The glycoproteins of the viral envelope provide the normal attachment of the virions to susceptible cells. Following attachment, the viral envelope glycoprotein B (gp B) induces its fusion with the cellular plasma membrane, permitting the nucleocapsid to enter directly into the cytoplasm. Intact virions may also enter via endocytosis, from which they are released into the cytoplasm by similar viral envelope-membrane fusion. In the cytoplasm the capsid migrates to a nuclear pore, where the viral DNA is released into the nucleus and initiates viral multiplication. The eclipse period is 5 to 6 hours in monolayer cell cultures, and virus increases exponentially until approximately 17 hours after infection; each cell has then made 10^4 to 10^5 physical particles, of which about 100 are infectious. Virions are released by slow leakage from infected cells.

As with other DNA-containing viruses, the biochemical events are sequentially regulated, presenting a cascadelike effect. Thus, after the viral DNA enters the nucleoplasm, even in the absence of protein synthesis, the host cell RNA polymerase II transcribes noncontiguous, restricted regions of the viral genome to produce five immediate early (α) mRNAs (Figure 2). If translation is blocked, only these α mRNAs accumulate in the cytoplasm, and the larger, unprocessed transcripts remain in the nucleus. If protein synthesis is permitted, α proteins are made, leading to transcription of other regions of the genome and production of delayed early (β) mRNAs. The β proteins block further synthesis of α proteins and lead to transcription of a third set of RNAs and their processing into late γ mRNAs. Thus, the synthesis and translation of the mRNAs are coordinately regulated: formation of the α proteins is necessary for synthesis of the β proteins, and both of these nonstructural and minor structural proteins are necessary for synthesis of the late major structural γ proteins. It is noteworthy that synthesis of all the γ proteins is not dependent on viral DNA replication: γ1 proteins such as gp B and the major capsid protein VP5 are made in the absence of viral DNA synthesis, although they are synthesized in relatively low abundance; but γ2 proteins (e.g., glycoprotein C) strictly require amplification of viral DNA. The three sets of mRNAs produce an aggregate of about 50 virus-encoded proteins.

Viral DNA replication is carried out by both viral α and β proteins, which include a DNA polymerase and DNA binding protein, and host cellular enzymes. The reactions are not yet precisely understood, but they appear to involve complex replicative intermediates, including "head-to-tail" concatemeric circular and linear-circular forms generated by the reiterated nucleotide sequences. Three origins of DNA replication have been described: two in the terminal *c* reiterated sequences of the S segment and one in the middle of the L segment near the genes encoding the DNA polymerase and DNA-binding protein. The concatemeric viral DNA is cleaved at a terminal reiterated *a* sequence, and it is packaged in preformed capsids. These particles are noninfectious and unstable until they acquire an envelope. Envelopment is initiated at sites on the inner nuclear membrane into which viral glycoproteins have been inserted. The nuclear membrane reduplicates to permit egress of viral particles into the cytoplasm, where it associates with the endoplasmic reticulum, and the viral particle appears either to complete envelopment or to become enveloped anew. Mature, infectious virus is slowly liberated from infected cells through the endoplasmic reticulum, but occasionally it also escapes by a process akin to reverse phagocytosis. Unlike the process with other enveloped viruses, envelopment and release of viral particles does not occur by budding from the plasma membrane. Rather, the plasma membrane is changed morphologically and contains virus-specific glycoproteins; this consequently makes the membrane a target for immunologic attack.

The movement of viral particles from the nucleus into the cytoplasm is accompanied by the transport of soluble antigens into the cytoplasm; concomitantly the originally basophilic intranuclear inclusion body is converted into an eosinophilic, Feulgen-negative mass. Thus, the eosinophilic inclusion body that is usually observed in infected cells does not contain viral particles or specific viral antigens (detectable by immunofluorescence) but actually is the burnt-out remnant of a viral factory (Ginsberg, 1980).

4. Pathology

Because HSV causes cytolytic infections, pathologic changes are due to necrosis of infected cells together with the inflammatory response. Lesions induced in the skin and mucous membranes by HSV-1 and HSV-2 are the same and resemble those of varicella-zoster virus. Changess induced by HSV are similar for primary and recurrent infections but vary in degree, reflecting the extent of viral cytopathology.

Characteristic histopathologic changes include ballooning of infected cells, production of Cowdry type A intranuclear inclusion bodies, margination of chromatin, and formation of multinucleated giant cells. The early inclusions virtually fill the nucleus but later condense and are separated by a halo from the chromatin at the nuclear margin. Cell fusion provides an efficient method for cell-to-cell spread for HSV, even in the presence of neutralizing antibody (Brooks, Butel, and Morse, 2001).

In primary Infection, HSV is transmitted by contact of a susceptible person with an individual excreting virus. The virus must encounter mucosal surfaces or broken skin in order for an infection to be initiated (unbroken skin is resistant). HSV-1 infections are usually limited to the oropharynx, and virus is spread by respiratory droplets or by direct contact with infected saliva. HSV-2 is usually transmitted by genital routes. Viral replication occurs first at the site of infection. Virus 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 result in latent infections in the trigeminal ganglia, whereas genital HSV-2 infections lead to latenly infected sacral ganglia.

Primary HSV infections are usually mild; in fact, most are asymptomatic. Only rarely does systemic disease develop. Widespread organ involvement can result when an immunocompromised host is not able to limit viral replication and viremia ensues (Brooks, Butel, and Morse, 2001).

In latent Infection, Virus resides in latently infected ganglia in a nonreplicating state; only a very few viral genes are expressed. Viral persistence in latently infected

ganglia lasts for the lifetime of the host. No virus can be recovered between recurrences at or near the usual site of recurrent lesions. Provocative stimuli can reactivate virus from the latent state, including axonal injury, fever, physical or emotional stress, and exposure to ultraviolet light. The virus follows axons back to the peripheral site; and replication proceeds at the skin or mucous membranes. Spontaneous reactivations occur in spite of HSV-specific humoral and cellular immunity in the host. However, this immunity limits local viral replication, so that recurrent infections are less extensive and less severe. Many recurrences are asymptomatic, reflected only by viral shedding in secretions. When symptomatic, episodes of recurrent HSV-1 infection are usually manifested as cold sores (fever blisters) near the lip. More than 80% of the human population harbors HSV-1 in a latent form, but only a small portion experience recurrences. It is not known why some individuals suffer reactivations and others do not.

Many newborns acquire passively transferred maternal antibodies. These antibodies are lost during the first 6 months of life, and the period of greatest susceptibility to primary herpes infection occurs between ages 6 months and 2 years. Transplacentally acquired antibodies from the mother are not totally protective against infection of newborns, but they seem to ameliorate infection if not prevent it. HSV-1 antibodies begin to appear in the population in early childhood; by adolescence, they are present in most persons. Antibodies to HSV-2 rise during the age of adolescence and sexual activity.

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During primary infections, IgM antibodies appear transiently and are followed by IgG and IgA antibodies that persist for long periods. The more severe the primary infection or the more frequent the recurrences, the greater the level of antibody response. However, the pattern of antibody response has not correlated with the frequency of disease recurrence. Cell-mediated immunity and nonspecific host factors (natural killer cells, interferon) are important in controlling both primary infection and recurrent HSV infections .

After recovery from a primary infection (inapparent, mild, or severe), the virus is carried in a latent state in the presence of antibodies. These antibodies do not prevent

reinfection or reactivation of latent virus but may modify subsequent disease (Brooks, Butel, and Morse, 2001).

5. Epidemiology

Herpes simplex viruses are worldwide in distribution. No animal reservoirs or vectors are involved with the human viruses. Transmission is by contact with infected secretions. The epidemiology of type 1 and type 2 herpes simplex virus differs.

HSV-1 is probably more constantly present in humans than any other virus. Primary infection occurs early in life and is usually asymptomatic; occasionally, it produces oropharyngeal disease (gingivostomatitis in young children, pharyngitis in young adults). Antibodies develop, but the virus is not eliminated from the body; a carrier state is established that lasts throughout life and is punctuated by transient recurrent attacks of herpes.

The highest incidence of HSV-1 infection occurs among children 6 months to 3 years of age. By adulthood, 70-90% of persons have type 1 antibodies. Middle-class individuals in developed countries acquire antibodies later in life than those in lower socioeconomic populations. Presumably this reflects more crowded living conditions and poorer hygiene among the latter. The virus is spread by direct contact with infected saliva or through utensils contaminated with the saliva of a virus shedder. The source of infection for children is usually an adult with a symptomatic viral shedding in saliva.

The frequency of recurrent HSV-1 infections varies widely among individuals. At any given time, 1-5% of normal adults will be excreting virus, often in the absence of clinical symptoms.

HSV-2 is usually acquired as a sexually transmitted disease, so antibodies to this virus are seldom found before puberty. It is estimated that there are about 45 million infected individuals in the USA. Antibody prevalence studies have been complicated by the cross-reactivity between HSV types 1 and 2. Surveys using type-specific glycoprotein antigens recently determined that 20% of adults in the USA possess HSV-2

antibodies, with seroprevalence higher among women than men and higher among blacks than whites.

Recurrent genital infections may be symptomatic or asymptomatic. Either situation provides a reservoir of virus for transmission to susceptible persons. HSV-2 tends to recur more often than HSV-1, irrespective of the site of infection.

Maternal genital HSV infections pose risks to both mother and fetus. Rarely, pregnant women may develop disseminated disease after primary infection, with a high mortality rate. Primary infection before 20 weeks of gestation has been associated with spontaneous abortion. The fetus may acquire infection as a result of viral shedding from recurrent lesions in the mother's birth canal at the time of delivery. Estimates of the frequency of cervical shedding of virus among pregnant women vary widely. However, the majority of infants (≈ 70%) who develop neonatal disease are born to women who do not have a history of genital herpes and are asymptomatic at the time of delivery (Brooks, Butel, and Morse, 2001).

6. *In vitro* antiviral test

As the methodology used in the determination of the antiviral activity as well as the interpretation of the results have been virtually specific to each laboratory and are consequently not comparable to one another, simple procedures and guidelines for evaluating antiviral and/or virucidal activities of single compounds are urgently needed. This is even more true for the antiviral testing of crude extracts, containing a complicated mixture of different compounds present in several proportions.

Various cell culture-based assays are currently available and can be successfully applied for the antiviral or virucidal determination of single substances or mixtures of compounds e.g. plant extracts. Antiviral agents interfere with one or more dynamic processes during virus biosynthesis and are consequently candidates as clinically useful antiviral drugs, whereas virucidal substances inactivate virus infectivity

extracellularly and are rather candidates as antiseptics, exhibiting a broad spectrum of germicidal activities.

The method commonly used for evaluation of *in vitro* antiviral activities are based on the different abilities of viruses to replicate in cultured cells. Some viruses can cause cytopathic effects (CPE) or form plaques. Others are capable of producing specialized functions or cell transformation. Virus replications in cell cultures may also be monitored by the detection of viral products, i.e. viral DNA, RNA or polypeptides. Thus, the antiviral test selected may be based on inhibition of CPE, reduction or inhibition of plaque formation and reducing virus yield or other viral functions (Vlietinck and Vanden Berghe, 1991).

A survey of the various *in vitro* antiviral tests and their possible suitability for the antiviral and/or virucidal screening of plant extracts and plant products is presented in Table 2.

It should be emphasized that the toxic effects of an antiviral agent on the host cells must be considered since a substance may exhibit an apparent antiviral activity by virtue of its toxic effects on the cells. The cytotoxicity assay on cell cultures is usually done by the cell viability assay and the cell growth rate test, although other parameters such as destruction of cell morphology under microscopic examination or measurement of cellular DNA synthesis have been used as indicators of compound toxicity.

After the antiviral potency of a test substance together with its cytotoxicity is determined, the therapeutic index of the antiviral compound in a given virus-cell system can be calculated. Taking into account that the cell growth rate test has been claimed to be the most stringent method for measuring cytotoxicity, the therapeutic index (TI_x) can be defined as the ratio of the maximum drug concentration at which 50% of the growth of normal cells is inhibited (CD_{50}) to the minimum drug concentration at which x% (50%, 90% or 99%) of the virus is inhibited (ED_x).

It should be noted that the calculation of the therapeutic index of a mixture of compounds e.g. crude extracts is irrelevant since cytotoxicity and antiviral activity of the mixture are not necessarily due to the same components of the mixture. On the contrary, without the cytotoxicity data reports of antiviral activity of a single compound even at very low concentrations are of limited value. In addition, the relative potency of a new antiviral product should also be compared with existing approved drugs.

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Table 2 *In vitro* antiviral screening assays (Vlietinck and Vanden Berghe, 1991)

Determination of the viral infectivity in cultured cells during virus multiplication in the presence of a single compound (A-S) or a mixture of compounds e.g. plant extracts (A-M) or after extracellular incubation with a single compound (V-S) or a mixture of compounds (V-M).
(1) *Plaque inhibition assay* Only for viruses which form plaques in suitable cell systems. Titration of a limited number of viruses in the presence of a non-toxic dose of the test substance. Applicability: A-S.
(2) *Plaque reduction assay* Only for viruses which form plaques in suitable cell systems. Titration of residual virus infectivity after extracellular action of test substance(s). Cytotoxicity should be eliminated e.g. by dilution, filtration etc. before the titration. Applicability: V-S; V-M.
(3) *Inhibition of virus-induced cytopathic effect (CPE)* For viruses that induce CPE but do not readily from plaques in cell cultures. Determination of virus-induced CPE in monolayers, cultures in liquid medium, infected with a limited dose if virus and treated with a non-toxic dose of the test substance(s). Applicability: A-S; A-M.
(4) *Virus yield reduction assay* Determination of the virus yield in tissue cultures, infected with a given amount of virus and treated with a non-toxic dose of the test substance(s). Virus titration is carried out after virus multiplication by the plaque test (PT) or the 50% tissue culture dose end point test (TCD50). Applicability: A-S; A-M.
(5) *End point titration technique (EPTT)* Determination of virus titer reduction in the presence of two-fold dilutions of test compound(s). Applicability: A-S; A-M. This method has been especially designed for the antiviral screening of crude extracts.
(6) *Assays based on measurement of specialized functions and viral products* For viruses that do not induce CPE or form plaques in cell cultures. Determination of virus specific parameters e.g. hemagglutination and hemadsorption tests (myxoviruses), inhibition of cell transformation (EBV), immunological tests detecting antiviral antigens in cell cultures (EBV, HIV, HSV and CMV). Reduction or inhibition of the synthesis of virus specific polypeptides in infected cell cultures e.g. viral nucleic acids, determination of the uptake of radioactive isotope labeled precursors or viral genome copy numbers. Applicability: A-S; A-M; V-S; V-M.

A: antiviral; V: virucidal; S: single substances; M: mixtures of compounds

Plaque reduction/inhibition assay is generally considered as a reference or standard for antiviral assay (Ellis, et al. 1987). This assay uses a constant number of viral particles and varying the non-toxic concentrations of test substance (Abou-Karam and Shier, 1990). Typically, a monolayer of cultured cells is allowed to bind virus and then overlaid with a layer of semi-solid medium which prevents spreading of virus from the area of originally infected cells. The test substance can be added into cell monolayers before or after virus adsorption is accomplished. The infected cultures are incubated further for an appropriate period of time, then they are fixed, stained with dye, and plaques (areas of infected cells) are counted. By reference to the number of plaques observed in virus control (untreated culture), the effective concentration or dose of test compound which inhibited plaque number by 50% (EC_{50}, ED_{50}, IC_{50} or ID_{50}) is calculated and expressed as antiviral activity.

Tebas, Stabell, and Olivo (1995) developed histochemical staining for plaque assay using a cell line, Vero ICP6LacZ#7, that expresses β-galactosidase activity. Antiviral compound was added into HSV-infected cell monolayers, followed by pooled human immunoglobulin which limits the spread of virus to surrounding cells. Two days later, the cell monolayers were stained for β-galactosidase activity. The plaques appeared blue against a clear background of unstained, uninfected cells. The EC_{50} determined by this method correlated with that determined by the plaque inhibition assay.

The procedure of the CPE inhibition assay is similar to the plaque inhibition assay except the semi-solid substance is not included in the culture medium. The infectivity of virus could be revealed through microscopic observations of characteristics of viral CPE (Yip et al., 1991). or determined by the dye uptake assay (Marchetti, et al 1996). Treated and untreated infected cells are stained with neutral red 2 days post-infection. The uptake dye is then extracted and the optical density at 550 nm measured. Then, the concentration required to inhibit CPE by 50% is calculated.

For virus yield reduction assay, cell monolayers are infected with virus and serial dilution of test compound are added after virus adsorption. Following a cycle of

virus replication, the harvested cultures are disrupted by freezing and thawing, supernatants are kept, and virus yields are determined by plaque assay. The drug concentrations required to reduce 90% (1 log_{10} reduction) are determined for antiviral activity in this assay. The plaque inhibition and CPE inhibition methods have limitations from the low or limited amount of virus input so virus yield reduction assay have been used instead. The multiplicity of infection (MOI) of virus in yield reduction assay is usually carried out with one or more PFU per cell. Thus, progeny virus could be recovered at a much greater range, i.e., 1-10^6 PFU/ml in yield reduction compared to 1-10^2 PFU in plaque reduction/inhibition assay (Prichard, et al 1990). However, yield reduction assay is not routinely utilized due to its labor-intensive nature and time consuming.

The antiviral assays based on measurement of specialized functions and viral products have been studied using methods such as nucleic acid hybridization, enzyme-linked immunosorbent assay (ELISA) and flow cytometric analysis.

7. Antiherpetic agents

Three major categories of antiherpesvirus agents can be discerned (Andrei et al., 1995). The first category consists of some pyrophosphate analogs such as phosphonoacetic acid (PAA) and phosphonoformic acid (PFA; foscarnet). These drugs inhibit a viral DNA polymerase directly by binding to the site involved in releasing the pyrophosphate product of DNA synthesis. The second category comprises a variety of nucleoside analogs including acyclovir (ACV), ganciclovir (GCV), brivudin (BVDU), and penciclovir (PCV). These agents depend on virus-induced thymidine kinase (TK) to effect their antiviral action. Formation of monophosphate (for BVDU, also further phosphorylation to the diphosphate) forms of these nucleoside analogs is catalyzed by viral TK. Following further phosphorylation to their triphosphate forms by cellular enzymes, they inhibit the DNA polymerase reaction. The third class consists of those drugs that are independent of viral TK for their activation. This class includes vidarabine (araA) and the acyclic nucleoside phosphonates, i.e., phosphonylmethoxyethyl (PME)

and 3-hydroxy-2-phosphonylmethoxypropyl (HPMP) derivatives of purines (i.e., adenine [PMEA and HPMPA] and 2,6-diaminopurine [PMEDAP]) and pyrimidines (i.e., cytosine [HPMPC]). AraA is converted to araA triphosphate by cellular enzymes and then inhibits HSV DNA polymerase. Phosphorylation of acyclic nucleoside phosphonates to their mono- and diphosphoryl derivatives is also carried out by cellular enzymes, and then the resulting diphosphoryl derivatives interact at the DNA polymerase level (Balzarini and De Clercq, 1991). Also, the novel N-7 isomeric acyclic nucleoside 2-amino-7-(1,3-dihydroxy-2-propoxymethyl)purine (S2242) has proved to be a potent and selective inhibitor of HSV replication, acting independently of virus-induced TK (Neyts et al., 1994).

Acyclovir (aciclovir), an acyclic guanosine analogue, is a selective inhibitor of the replication of HSV types 1 and 2 and varicella-zoster virus. In virally- infected cells, it is initially monophosphorylated by HSV-specific viral thymidine kinase (TK), then converted to its di- and triphosphate forms by cellular enzymes. The active form is acyclovir triphosphate , which lacks the 3'-hydroxyl group required to elongate the DNA chain. Acyclovir triphosphate competes with deoxyguanosine triphosphate as a substrate for viral DNA polymerase, thereby terminating viral DNA replication. This results in inactivation of viral DNA polymerase (Figure 3). Acyclovir is a highly selective inhibitor of HSV replication. The concentration required for 50% inhibition of Vero cell growth is 300 μM and for WI-38 human fibroblast it is greater than 3000 μM. This highly selective toxicity is based on a large degree of selective activation of the drug by the viral TK (Scholar and Pratt, 2000).

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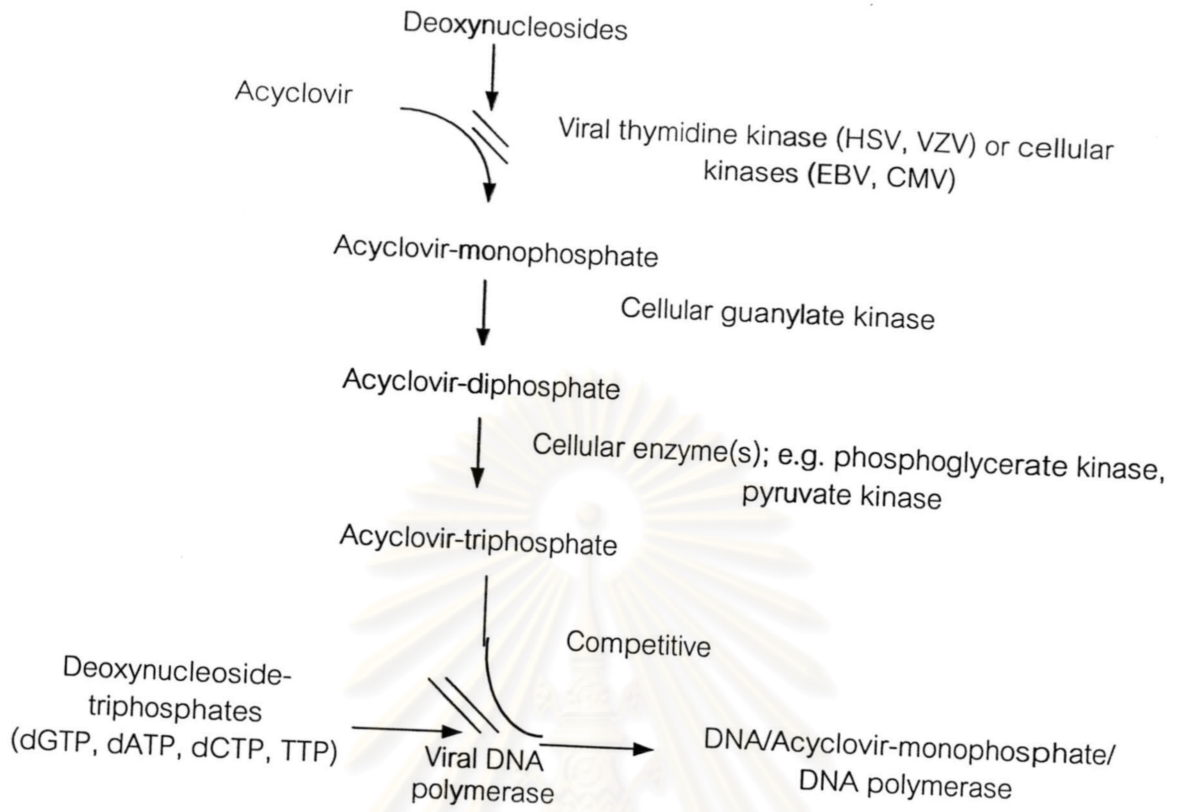

Figure 3 Acyclovir inhibition of viral DNA synthesis

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In general, HSV-2 strains are *in vitro* less susceptible to acyclovir than HSV-1 strains, although there nas been considerable overlap in the range of concentrations reported to inhibit the CPE of these 2 types of herpes simplex viruses by 50% (ID_{50} as measured by the plaque reduction assay or reduction in viral-induced cytopathogenicity) (O'Brien et al., 1989). As reviewed by Richards et al. (1983), in most studies ID_{50}s have been in the range 0.01 to 0.7 mg/l for herpes simplex type 1, and 0.01 to 3.2 mg/l for herpes simplex type 2. The 10- to 100-fold reduction in susceptibility of herpes simplex viruses growing in African green monkey kidney (Vero) cells compared with that in human fibroblast cells (Harmenberg et al., 1980) appears to be due, at least in part, to the substantially higher concentrations of thymidine in Vero cells (Harmenberg et al., 1985)

8. Medicinal Plants

Andrographis paniculata (Burm. f.) Nees (ฟ้าทะลายโจร) is in family Acanthaceae. Pharmacological activities of this plant were included antibacterial activity, antifertility activity, alkylating activity, antithelmintic activity, hypotensive activity, cardiac depressant activity, antiinflammatory antivity, smooth muscle stimulation, antispasmodic activity, antivenin activity, abortifacient effect, antitumor activity, antipyretic activity, sleeping time effect, larvicidal activity, antigastric ulcer activity, choleretic activity, and antitumor activity (Farnsworth and Bunyapraphatsara, 1992).

Barleria lupulina Lindl. (เสลดพังพอน) is in the family Acanthaceae. Pharmacological activities of this plant included antiherpes activity, antiviral activity and antiinflammatory (Farnsworth and Bunyapraphatsara, 1992).

Bridelia ovata Decne. (มะกา) is in the family Euphorbiaceae. Pharmacological activities of this plant included antibacterial activity and purgative activity (นันทวัน บุณยะประภัศรและอรนุช โชคชัยเจริญพร, 2539).

Cissus quadrangularis Linn. (เพชรสังฆาต) is in the family Vitaceae. Pharmacological activities of this plant included antifungal activity, antineoplastic

activity, antiinflammatory activity and mutagenicity (นันทวัน บุณยะประภัศรและอรนุช โชคชัยเจริญพร, 2539).

Citrus reticulata Blanco (ส้มเขียวหวาน) is in the family Rutaceae. Pharmacological activities of this plant included antibacterial activity, antifungal activity, antitumor activity, antiviral activity, carcinogenesis inhibition, cardiotonic activity, cyclic adenosine monophosphate (AMP) inhibition, cyclic AMP phosphodiesterase inhibition, cyclooxygenase inhibition and insect repellant activity (นันทวัน บุณยะประภัศรและอรนุช โชคชัยเจริญพร, 2539).

Clinacanthus nutans (Burm.b.) Lindau (พญายอ) is in the family Acanthaceae. Pharmacological activities of this plant were included antiherpes activity antiinflammatory activity, antivenin effect and antiviral activity (นันทวัน บุณยะประภัศรและอรนุช โชคชัยเจริญพร, 2539).

Clinacanthus siamensis Berm. (ลิ้นงูเห่า) is in the family Acanthaceae. Pharmacological activities of this plant included antiinflammatory activity and antivenin effect (วุฒิ วุฒิธรรมเวช, 2540).

Cocos nucifera Linn. (มะพร้าว) is in the family Palmae. Pharmacological activities of this plant included absorption enhancement effect, allergenic activity, amino acid level alteration, antibacterial activity, antifungal activity, antitumor activity, bile acid synthesis stimulation and carcinogenesis inhibition (นันทวัน บุณยะประภัศรและอรนุช โชคชัยเจริญพร, 2539).

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Costus speciosus (Koen.) J. E. Smith (เอื้องดิน) is in the family Costaceae. Pharmacological activities of this plant included antibacterial activity, antifungal activity, antiviral activity, cardiotonic activity and fish poison (นันทวัน บุณยะประภัศรและอรนุช โชคชัยเจริญพร, 2539).

Momordica charantia Linn. var *maxima* (มะระ) and *Momordica charantia* Linn. var *minima* (มะระขี้นก) are in the family Cucurbitaceae. Pharmacological activities of these plants included autoimmune deficiency syndrome (AIDS) therapeutic effects,

antibacterial activity, antifungal activity, antihyperglycemic activity, antimalarial activity, antioxidant activity, antiparasitic activity, antitumor activity, antiulcer activity, antiviral activity, apoptosis inhibition, immunomodulator activity, insect attractant activity, insect repellant activity, lipid metabolism inhibition and lipid peroxide formation inhibition (นันทวัน บุณยะประภัศรและอรนุช โชคชัยเจริญพร, 2539).

Nephelium lappaceum Linn. (เงาะ) is in the family Sapindaceae. Pharmacological activities of this plant included antibacterial activity, fish poison and sperm agglutination (นันทวัน บุณยะประภัศรและอรนุช โชคชัยเจริญพร, 2539).

Orthosiphon aristatus (Bl.) Miq. (หญ้าหนวดแมว) is in the family Labiatae. Pharmacological activities of this plant included antifungal activity, antihistamine activity, antihyperglycemic activity, antilithic activity and diuretic activity (นันทวัน บุณยะประภัศรและอรนุช โชคชัยเจริญพร, 2539).

Phyllanthus amarus Schum. & Thonn. (ลูกใต้ใบ) is in the family Euphorbiaceae. Pharmacological activities of this plant included antifungal activity, antibacterial activity, antiviral activity, anthelmintic activity, hepatitis B surface antigen inactivation, antihepatotoxic activity, reverse transcriptase inhibition, angiotensin-converting enzyme inhibition; protease (human immunodeficiency virus; HIV) inhibition, analgesic activity, antihyperglycemic activity, antiinflammatory activity, antimutagenic activity and antipyretic activity (นันทวัน บุณยะประภัศรและอรนุช โชคชัยเจริญพร, 2539).

Schefflera leucantha Vig. (หนุมานประสานกาย) is in the family Araliaceae. Pharmacological activities of this plant included antibacterial activity, bronchodilator activity and insect repellant (นันทวัน บุณยะประภัศรและอรนุช โชคชัยเจริญพร, 2539).

Thunbergia laurifolia Lindl. (รางจืด) is in the family Acanthaceae. Pharmacological activities of this plant included antibacterial activity, anti-HSV activity, antitoxin activity, insecticidal activity and hypotensive activity (นันทวัน บุณยะประภัศรและอรนุช โชคชัยเจริญพร, 2539).

Vitis vinifera Linn. (องุ่น) is in the family Vitaceae. Pharmacological activities of this plant included alkaline phosphatase stimulation, alpha-amylase inhibition, antibacterial activity, antifungal activity, antifungal activity (plant pathogens), antihypercholesterolemic activity, antihyperglycemic activity, antiinflammatory activity, antimutagenic activity, antinephrotoxic activity, antioxidant activity, antithiamine activity, antitumor activity, antiviral activity, capillary permeability increase, low density lipoprotein (LDL) oxidation inhibition, lipase inhibition, lipid peroxidase inhibition, nitric oxide release stimulation, platelet aggregation inhibition, radical scavenging effect and vasodilator (นันทวัน บุณยะประภัศรและอรนุช โชคชัยเจริญพร, 2539).

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