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Evaluation of new antiviral agents: II. The use of animal models

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Summary

Antiviral chemotherapy has become a reality in the 1980s. Since the use of animal models in the testing of new antiviral agents is an inevitable step prior to clinical trial in human patients, it is important to understand the basic principles of using model systems. Briefly reviewed in this paper are the heterologous and homologous animal models which have been used for studies of various herpesvirus infections in humans. Discussions of the use of the guinea pig models mainly, for members of the Herpesviridae are presented in more detail. Precautions needed for the development of new animal models, and suggestions proposed for the use of animal models for testing new antiviral agents are outlined. It is hoped that new animal models will be developed in the foreseeable future for evaluating the much needed effective but less toxic antiviral agents for a variety of human viral diseases.

Animal model; Guinea pig; Mice; Herpesvirus infection; Antiviral agent testing in vivo

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Introduction

Testing a new antiviral compound for its inhibitory activity against a specific viral infection or a group of viruses is generally done first in vitro using cell culture systems (Sidwell, 1986; Newton, 1988; Hu and Hsiung, 1989a). In each and every instance, in vivo testing of antiviral agents in animal models follows the in vitro tests prior to clinical trial in humans. Bypassing in vivo testing in animal model systems is more of an exception, i.e., only when there is no suitable animal model to be used and the disease is debilitating and life-threatening; then and only then will clinical trial in humans be justifiable without the benefit of data from animal model systems. Prototype of this particular instance is typified by the clinical trials of anti-human immunodeficiency virus (HIV) compounds in AIDS patients.

Some antivirals that are active in cultured cells may not be active in experimental animals, and the converse is also true. Furthermore, relative potencies of various antivirals determined in vitro may not be the same in vivo especially if, unavoidably, the cell culture system that was utilized is of a different species from that of the animal model system to be used for in vivo testing. Although testing done in animal models will never replace clinical trials in humans, much reliance is put on the former not only due to ethical considerations, but because of its important roles in antiviral chemotherapy, foremost of which is its predictive value for human risk. By testing in animal model systems, new antivirals are screened for their efficacy and toxicity and compared to priorly approved antivirals. The best route and regimen of administration can be determined and the elucidation of complex questions involving pathogenesis of viral infections can be investigated (Field, 1988).

The present paper addresses the cautions that are needed to be taken in the: (1) choice of animal model, (2) development of animal models for testing antiviral agents, and (3) steps needed in the in vivo testing of new antivirals. By way of examples, the use of guinea pigs for testing antiherpes agents is described.

Choice of animal models

Because each experimental animal model may show a different pattern of effectiveness of an antiviral agent, it is preferable to use more than one animal model whenever feasible. Moreover, familiarity with the pathogenesis of a virus infection in a given animal model system is essential prior to its application for antiviral evaluation. Several important factors must be taken into account before an animal model system is chosen. It is crucial that: (1) the disease produced in the animal species closely mimics human disease; (2) the animal species is easy to handle in the laboratory; (3) the animal species is commercially available at all times; and (4) the animal species is not costly. The response to the virus infection including clinical signs, or the survival time of the infected animals should be correlated with the dose of virus inoculum. As a result, suppression of the response will indicate the effective reduction in the virus infectivity, thus, an antiviral effect. The sex and

age of the animals used and route of inoculation mainly depend upon the virus strain used and clinical disease being studied.

Animal model systems

There are two animal model systems that are commonly employed, i.e. heterologous and homologous systems (Table 1 and Fig. 1). Examples of heterologous systems are herpes encephalitis induced by a human virus, herpes simplex virus type 1 (HSV-1), in rodents including mice, rats and hamsters, and HSV-2 in mice; genital herpes induced by HSV-2 in mice, guinea pigs, and monkeys; and varicella (chicken pox) induced in guinea pigs by human varicella-zoster virus (VZV). Thus, a disease induced by a virus of human origin in an experimental animal that mimics the human disease is considered to be a heterologous system. On the other hand, there are some viruses that are host specific, therefore, a homologous system has to be used. For example, cytomegalovirus (CMV) infection is host specific, i.e. the human CMV (HCMV) only infects humans, mouse CMV (MCMV) only infects mice, and guinea pig CMV (GPCMV) only infects guinea pigs. Therefore, in order to study a HCMV-induced disease in an animal model, the homologous virus of that animal species must be used (Fig. 1).

Over the years, many other animal models have been developed for the study of human viral diseases. An excellent comprehensive report of animal models for the evaluation of antiviral activities against both DNA and RNA viruses using both homologous and heterologous systems has been reviewed (Sidwell, 1986). A wide variety of animal species, such as rodent, avian, swine, simian, up to and including chimpanzee, has been presented in the review as models for human viral infections that are life-threatening, and of high-priority targets in public health. Since the outbreak of HIV infections, animal models have been sought for testing anti-HIV agents. Recently, it has been reported that rabbits can be infected with HIV-1 (Filice et al., 1988). The first workshop on animal models in the evaluation of chemotherapeutic agents against HIV was held in Germany in 1988. Several animal species, such as mouse, cat, sheep, goat, monkey and chimpanzee, were reported to be useful as animal models for the evaluation of chemotherapeutic agents against HIV infections (Schlumberger and Schrinner, 1989).

Animal models for human diseases caused by members of the herpesvirus group

Animal models listed in Table 1 include only those that have been characterized, and utilized in the evaluation of antivirals against members of the Herpesviridae. The corresponding references are those of the description of the animal model and/or representative report(s) of its utilization in antiviral testing. Readers are referred to additional publications for more detailed coverage of animal models for other human viral diseases. Published recently, is a brief review centered on animal models used for the evaluation of antivirals against herpes simplex virus infections (Field, 1988). Mouse, guinea pig (female) and rabbit models have been cited for one or more diseases due to herpes simplex virus infections.

TABLE 1

Animal models for human viral infections caused by members of the Herpesviridae

Model system	Clinical disease in human	Virus strain	Animal species	References (description and/or first utilization of model)
Heterologous	Herpes encephalitis	HSV-1	Mouse	Renis and Hollowell, 1968, DeClercq and Luczak, 1976, Field et al., 1979, Seal and Jamison, 1984
			Rat	Renis, 1973
			Hamster	Sidwell et al., 1968
			Newborn guinea pig	Tenser and Hsiung, 1977
			Rabbit	Parnitsch and Baringer, 1973
			Marmoset	Cho et al., 1973
		HSV-2	Mouse	Kern et al., 1973, Seal and Jamison, 1984
	Herpes genitalis	HSV-1	Mouse	Nahmias et al., 1967, Overall, Jr et al., 1975
			Guinea pig	Landry et al., 1982
		HSV-2	Guinea pig (female)	Lukas et al., 1974, Scriba, 1975, Pronovost et al., 1982, Richards et al., 1982, Shannon et al., 1982, Zheng et al., 1983
		HSV-2	Guinea pig (male)	Stephanopoulous et al., 1989
		HSV-2	Monkey	Nahmias et al., 1971
	Ocular herpes	HSV-1	Rabbit	Kaufman et al., 1962, 1970
	Herpesvirus neural spread	HSV-2	Guinea pig	Bernstein and Stanberry, 1986
	Herpesvirus cervicovaginal shedding	HSV-2	Guinea pig	Myers et al., 1988
	Chickenpox	VZV	Guinea pig	Myers et al., 1980, 1985
Homologous	CMV congenital infection	MCMV	Mouse	Kelsey et al., 1976
		GPCMV	Guinea pig	Griffith et al., 1982

Model system	Clinical disease in human	Virus strain	Animal species	References (description and/or first utilization of model)
Homologous	CMV mononucleosis syndrome	GPCMV	Guinea pig	Griffith et al., 1981
	CMV interstitial pneumonia	MCMV	Mouse	Shanley and Pesanti, 1982, 1985; Shanley et al., 1985
		GPCMV	Guinea pig	Bia et al., 1982
	Chickenpox	Simian VZV	Cynomolgus monkey	Wenner et al., 1985
			Erythrocebus patas	Allen et al., 1974
			Cercopithecus aethiops	Felsenfeld et al., 1978, Soike et al., 1980, 1981
	Epstein-Barr virus-associated infectious mononucleosis	GPHLV	Guinea pig	Dowler et al., 1984

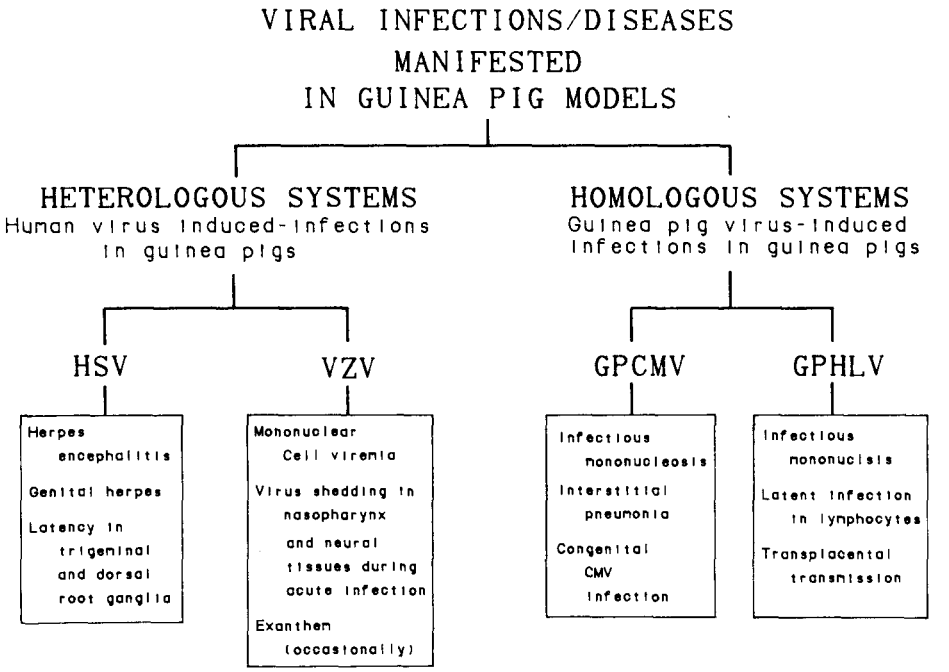

Fig. 1.

New animal models for herpes simplex virus infections have been developed, surprisingly all in guinea pigs. These are the guinea pig model for HSV-2 neural spread (Bernstein and Stanberry, 1986), cervicovaginal virus shedding (Myers et al., 1988), and a male guinea pig model for genital herpes (Stephanopoulous et al., 1989).

In our review of literature, we noted that there is no single animal species that universally serves as an animal model for infections by most or all the members of the Herpesviridae. In the prevailing situation, the commonality of the use of an animal species depends very much on the host specificity and degree of animal susceptibility to herpesvirus infection.

Among the herpesviruses that require homologous animal models are the HCMV, VZV and Epstein-Barr virus (EBV). GPCMV infections in guinea pigs are commonly used because they closely mimic those of HCMV infections in humans with more features in common. Simian models are available for VZV infections while a guinea pig model has been characterized for EBV infection. Using these animal model systems, the pathogenesis of a particular virus can be studied under well-defined and controlled conditions. Consequently, much information that is not available from studies in cultured cells can be obtained and new antiviral agents can be tested. It is beyond the scope of this paper to discuss all the animal models, therefore only the guinea pig models that have been developed over the years and have been used extensively for evaluations of antiherpes agents in both heterologous and homologous systems are discussed in the following sections.

The use of guinea pig models for evaluation of antiherpes agents

Fig. 1 shows schematic presentation of homologous and heterologous model systems in which various infections and/or disease entities that mimic closely herpesvirus pathogenesis in humans are inducible in guinea pigs. Examples of heterologous animal model systems are the human herpes simplex virus (HSV) infections which are inducible in guinea pigs including herpes encephalitis, genital herpes, and herpes latency in ganglion; VZV infection in guinea pig characterized by mononuclear cell viremia, virus shedding in nasopharynx and neural tissues during acute infection, and occasionally, exanthem (Myers et al., 1985). Examples of homologous animal model systems are those of GPCMV, and the guinea pig lymphotropic herpesvirus (GPHLV) infections that mimic clinical entities in humans which are inducible in guinea pigs.

In the following sections, each of the guinea pig models will be discussed in greater detail in order to illustrate the establishment of an animal model system and its application for antiviral assay.

Animal species

Several animal species including mice, monkeys, and guinea pigs have been used as models for genital herpes induced by the human HSV. Although the mouse provides a valuable model for fatal herpesvirus encephalitis, genital lesions are rarely noted in mice (Nahmias et al., 1967; Overall et al., 1975). Therefore, the mouse model would be unsuitable to be used for antiviral evaluation if genital lesions were considered as one of the criteria for treatment (Hsiung et al., 1984). Cebus monkeys genitally infected with HSV-2 produce clinical lesions and latent infection similar to that seen in humans (Nahmias et al., 1971; Felsberg et al., 1972; London et al., 1974; Reeves et al., 1976). However, cost limits the number of these animals that can be used in a single experiment. On the other hand, guinea pigs, following genital infection, produce herpetic lesions on the external genitalia and establish latency in both the peripheral nervous system (dorsal root ganglia) and central nervous system (spinal cord, brain) with recurring genital lesions (Stanberry et al., 1982). The clinical, histological, cytological, and virological features of the guinea pig genital infection are very similar to those of the human genital infection and have been reviewed elsewhere (Hsiung et al., 1984). In addition to the familiarity of the pathogenesis of HSV infection in guinea pigs, several other factors need to be determined prior to drug testing.

Virus strains

Within each virus type, significant strain variability is often noted (Table 2). When female guinea pigs are inoculated intravaginally by swabbing with HSV-1 strains, they generally exhibit milder clinical disease compared to animals inoculated with HSV-2 strains. Mortality is not observed in animals inoculated with HSV-1 strains (Landry et al., 1982). Even among HSV-2 strains, some may produce se-

TABLE 2

Genital infection with different strains of herpes simplex virus types 1 and 2 (HSV-1 and HSV-2) in guinea pigs: clinical disease during acute infection

Strain	Original source of isolation	Inoculum (log PFU)	Genital lesions severity[a]	Paralysis and/or death (%)
HSV-1				
NYU-78	Brain tissue	5.1	1 to 3+	0
D1745	Penile lesion	5.0	1 to 3+	9
D1758	Nasolabial lesion	5.3	1 to 3+	0
HSV-2				
WT-186	Penile lesion	5.5	3 to 4+	50
D160	Penile lesion	4.5	2 to 3+	7
D1868	Penile lesion	5.0	2 to 3+	20

[a]Lesion scoring system: 1+, friability, erythema, 1–2 lesions; 2+, 3–10 lesions; 3+, 11–20 lesions; 4+, > 20 lesions, loss of bladder control, or paralysis. Modified from Landry et al., 1982.

vere infection while others may produce mild infection only. Moreover, variability of antiviral susceptibilities among different strains has been noted both in vitro and in vivo (Landry et al., 1982; Myerson and Hsiung, 1983). Thus, the choice of virus strains must be made before testing, and preferably it should include both antiviral-sensitive as well as antiviral-resistant strains.

Dose-response

The severity of genital lesions in guinea pigs following genital inoculation with HSV correlates with the quantity of virus inoculated (Anderson et al., 1980). High doses of virulent strain of virus generally induce severe infections which may obliterate the activity of the antiviral agent. With low doses of the virus, clinical disease is milder, fewer deaths occur, and during acute infection, the frequency of virus isolations from the various nerve tissues of inoculated animals is also decreased, thus experimental results are doubtful, vague, and unreliable. It is therefore advisable to use a virus challenge dose of moderate strength.

Parameters for antiviral evaluation

In our laboratory, several parameters have been employed for evaluating the activity of antiviral agents using the guinea pig genital herpes model (Landry et al., 1982; Pronovost et al., 1982; Mayo et al., 1983; Myerson and Hsiung, 1983; Zheng et al., 1983; Mayo and Hsiung, 1984). The following virus-induced responses have been shown to be altered and/or suppressed by one treatment or another.

Clinical manifestations. Infected animals are observed for: severity of genital lesions (number, size and nature of lesions), recovery time, frequency of recurrence, neurologic sequelae (such as hindlimb paralysis and loss of sphincter control), and death (especially in HSV-2 infection).

Virus recovery. Vaginal swabs, lesion swabs, neural tissues (brain, spinal cord, lumbosacral dorsal root ganglia, etc.), and non-neural tissues (cervix and vagina) are used for virus isolations. Virus infectivity titer, frequency, and duration of virus recovery are then determined and recorded.

Cytological and histological studies. Vaginal pap smears are examined for the presence of virus-induced changes (multinucleated giant cells, virus-induced inclusions, giant cell koliocytic changes) and atypical cells. Moreover, tissues from vagina, spinal cord, and dorsal root ganglia are fixed and stained for routine histological examination for evidence of changes (Lucia and Hsiung, 1981).

Latency. During the latent stage following genital herpes infection in guinea pigs, the virus can be recovered from neural tissues, such as the spinal cord and dorsal root ganglia (Anderson et al., 1980; Stanberry et al., 1982). Drug treatment has been shown to reduce virus recovery during the latent infection period (Pronovost et al., 1982).

Drug administrations

Route. Many antiviral agents such as acyclovir (ACV), phosphonoformate (PFA), 2′-fluoro-5-methyl-arabinosyl-uracil (FMAU), etc., have been used systemically and topically for the treatment of genital herpes in guinea pigs with various degrees of success (Alenius and Nordliner, 1979; Kern, 1982; Landry et al., 1982; Pronovost et al., 1982; Richards et al., 1982; Mayo et al., 1983; Myerson and Hsiung, 1983; Zheng et al., 1983; Mayo and Hsiung, 1984). In our early study, parenteral therapy with ACV appeared to be more effective than oral or topical treatment (mainly in reducing virus recovery from the genital lesions or vaginal secretions) (Landry et al., 1982; Pronovost et al., 1982). Systemic administrations of the antiviral agent can be carried out intraperitoneally (IP), intramuscularly (IM), subcutaneously (SC), by continuous infusion or per orem (PO). Topical treatment is carried out by application of drug ointment or gel (prepared in glycerine, propylene glycol, agar, ethyl alcohol, etc.) both in the vagina and on the external genitalia.

Timing and dosage. Responses to drug treatment have been shown to vary depending on the time when the drug is administered relative to the time of infection, and the drug dosage (Alenius and Nordliner, 1979; Richards et al., 1982; Hsiung et al., 1984). Both prophylactic (before virus infection) and therapeutic (during infection) treatments have been studied (Myerson and Hsiung, 1983). For therapeutic treatments, the antiviral agent is administered either immediately after virus inoculation, within 24 h postinfection, or delayed until the first herpetic genital lesion is evident. Treatment usually lasts for several days. Although prophylactic treatment or treatment soon after infection has been shown to be more effective compared to the treatment that is delayed until the appearance of genital lesions (usually three days postinfection), the latter is more applicable for the treatment of primary disease in humans. Although prophylactic treatment is not commonly feasible in HSV infections in humans except in cases where the viral reactivation may be predicted, it has been proved to be effective in preventing herpetic lesions in guinea pigs (Myerson and Hsiung, 1983).

Varicella-zoster virus infection in the guinea pig

Both homologous and heterologous animal model systems are available for varicella-zoster virus (VZV) infection. Simian varicella has been developed in three different species of monkeys and used for anti-VZV testing (Allen et al., 1974; Wenner et al., 1975; Felsenfeld et al., 1978; Soike et al., 1980, 1981), while human VZV infection has been also developed in guinea pigs (Myers et al., 1980, 1985). The cost of monkeys and their maintenance precludes the use of large numbers of these animals as dictated by statistics, besides the occupational hazard from and cumbersome handling of these animals.

VZV infection in small animals was first induced successfully in guinea pigs with human isolates of VZV (Myers et al., 1980). This guinea pig model for VZV infection was further characterized in weanling strain 2 guinea pigs (Myers et al., 1985). Following an intramuscular inoculation of weanling strain 2 guinea pigs, with

a VZV adapted in fetal guinea pig cells in vitro, mononuclear cell viremia developed that may persist for as long as three weeks, during which time VZV may be recovered from the nasopharynx and a variety of tissues. In the absence of viremia, virus may even be recovered from neural tissues, but infectious virus was not recoverable from neural tissues 23 days postinfection. Exanthem was occasionally observed. Thus, a strain 2 guinea pig model is available for pathophysiological studies as well as testing of new antivirals against VZV infection.

Cytomegalovirus infection and evaluation of antiviral agents

Animal species

As mentioned before, CMV is species specific and a homologous virus host system in a readily available laboratory animal species is needed. Currently, there are two animal species commonly used for HCMV infection study: MCMV infection in mice (Shanley and Pisanti, 1982, 1985) and GPCMV infection in guinea pigs (Griffith et al., 1981; Bia et al., 1983; Fong et al., 1983).

Guinea pig strain commonly used is the adult randomly-bred Hartley guinea pig; GPCMV in this strain usually produces a mild transient CMV mononucleosis syndrome with transient viremia, splenomegaly, lymphadenopathy and peripheral lymphocytosis, interstitial pneumonia, and intrauterine infection. The latter does not occur in MCMV infections. Chronic infection following acute infections caused by GPCMV is restricted to the salivary glands (Griffith and Hsiung, 1980; Bia et al., 1983; Fong et al., 1987). However, when the animals are pregnant or immunosuppressed, more severe fatal disease can occur (Griffith and Hsiung, 1980; Bia et al., 1982). Recently, it has been found that GPCMV can also induce severe generalized fatal infection in the newborn guinea pigs (unpublished data). As opposed to Hartley guinea pigs, inbred strain 2 guinea pigs infected with GPCMV develop severe disseminated disease with high mortality, including interstitial pneumonia and involvement of other visceral organs such as the spleen, liver, and kidneys (Bia et al., 1982; Fong et al., 1983).

Virus strains

While GPCMV serially passaged in the salivary gland (so called GPCMV-SG) retains its virulence and induces various diseases in guinea pigs, it has been shown to lose its virulence following serial passages in tissue cultures (Griffith et al., 1981; Bia et al., 1983; Fong et al., 1983). In fact, tissue culture-passaged GPCMV (GPCMV-TC) was once used as a live vaccine for inducing protection against the more virulent GPCMV-SG infections (Bia et al., 1982). Thus, only GPCMV-SG is suitable for inducing infections in guinea pigs for pathological and antiviral studies.

Parameters for antiviral evaluation

Clinical evaluations. All inoculated animals are examined daily for signs of clinical disease such as weight loss in adult animals, retardation of weight gain in young animals, ruffled fur, and delayed response to stress. Death and survival time are

also recorded. Blood samples are obtained at certain intervals after infections for hematocrit, total leukocyte and differential counts including atypical lymphocyte determinations.

Virus distribution in animal tissues. For viremia studies, blood is obtained by cardiac puncture and mixed with anticoagulants other than heparin since the latter prevents CMV replication in cultured cells (Choi et al., 1978). The whole blood sample or mononuclear cells obtained after Ficoll-Hypaque separation are used for virus isolation and titration. At the time the animal is sacrificed, portions of tissues from spleen, liver, lung, salivary gland, etc., are removed aseptically. Minced tissues or cell suspensions (10% W/V) are prepared from each tissue and used for virus isolation and assay. All virus isolates are identified by a plaque reduction neutralization test using GPCMV-specific antiserum.

Histological examinations. Portions of tissue samples from the spleen, liver, lung, cervical lymph nodes, salivary glands, etc. are fixed in a buffered formalin or Bouin's fixative and processed for routine hematoxylin-eosin (H&E) staining procedures. Histologically, CMV induced lesions can be scored based upon the lesion severity as reflected by the amount of tissue necrosis, inflammation, the number of viral inclusions, etc. (Fong et al., 1983, 1987).

Drug administration. As yet, only a few drugs ACV, PFA, 9-(1,3-dihydroxy-2-propoxymethyl) guanine (DHPG) etc. have been used in the experimental treatment of non-lethal CMV infection (transient mononucleosis) in the Hartley guinea pig model (Fong et al., 1983, 1987; Lucia et al., 1984; Chen et al., 1988; Li et al., 1989; Yang et al., 1989). Drugs are usually administered intraperitoneally or subcutaneously one day after virus infection and continued for four to seven days. Treatment of guinea pigs with congenital CMV infection or of strain 2 guinea pigs with severe disseminated infections has not been attempted because most of the drugs tested had no significant therapeutic efficacy even when tested in immunocompetent Hartley guinea pigs.

Adverse effects of antiviral agents in guinea pigs infected with GPCMV

Among all the drugs tested in the treatment of GPCMV infection, only DHPG was shown to be somewhat effective (Fong et al., 1987); ACV, PFA and FMAU were not effective (Fong et al., 1984; Lucia et al., 1984). In fact, adverse effects were noted in GPCMV-infected guinea pigs receiving ACV or PFA (Lucia et al., 1984). Severe disseminated infections, including interstitial pneumonia were observed in infected guinea pigs treated with ACV and PFA. More recently, when 9-[2-hydroxy-1,3,2-dioxaphosphorinan-5-yl]-guanine P-oxide (2′-nor-cGMP) and (*s*)-1-(3-hydroxy-2-phosphonylmethoxypropyl) cytosine (HPMPC) were tested in guinea pigs, severe toxicity was noted in guinea pigs receiving either the drugs alone or GPCMV-infected animals (Yang et al., 1989; Li et al., 1989). Body weight losses were severe and atypical-tubular necrosis was found in the kidneys at autopsy. However, mice infected with MCMV treated with ACV (Overall et al., 1976;

TABLE 3

Comparison of mice with guinea pigs for evaluation of antiviral agents against HSV and/or CMV infection

Antiviral agent	Animal model	Virus strain	Maximum tolerated dose (mg/kg/day)	Route administered	No. of dose/day (No. days)	References
ACV	Mice	MCMV	60.0	SC	2 (3)	Wingard et al., 1981
	Mice	MCMV	40.0	IP	2 (5)	Glasgow et al., 1982
	Mice	MCMV	400.0	PO	1 (7)	Glasgow et al., 1982
	Guinea pigs	GPCMV	50.0	IP	2 (5)	Lucia et al., 1984
2′-nor-cGMP	Mice	HSV-2	25.0	PO	2 (10)	Field et al., 1986
	Guinea pigs	GPCMV	5.0	SC	1 (5)	Yang et al., 1989
HPMPC	Mice	HSV-1	200.0	IP	2 (5)	Bronson et al., 1989b,c
	Mice	MCMV	33.3	IP	2 (5)	Bronson et al., 1989b
	Guinea pigs	GPCMV	2.5	SC	1 (5)	Li et al., 1989
PMEG	Mice	HSV-1	5.0	IP	2 (5)	Bronson et al., 1989a
	Mice	HSV-2	1.0	IP	2 (5)	Bronson et al., 1989a
	Guinea pigs	GPCMV	0.5	SC	1 (5)	Feng, Li and Hsiung, unpublished data

Glasgow et al., 1982), 2′-nor-cGMP (Tolman et al., 1985), and HPMPC (Bronson et al., 1989b,c) showed good response (Table 3). In addition, the combination of GPCMV infection and drug treatment results in more adverse effects in guinea pigs. The exact mechanism of these distinct differences between the mouse and the guinea pig in response to these antiviral agents is not clear.

Pathogenesis of lymphotropic herpesvirus infection in the guinea pig: a model for human Epstein-Barr virus (EBV) infection

Earlier studies have shown that leukemic as well as normal guinea pigs harbor GPHLV with a high percentage rate of natural infection among inbred strain 2 guinea pigs (Hsiung et al., 1971b). However, randomly bred Hartley strain guinea pigs only showed 4% infection rate when they reached 6 months of age (Hsiung et al., 1980). Infectious virus can be recovered from a wide variety of tissues but only by cocultivation with susceptible cells (Hsiung et al., 1971b). The highest vi-

rus titer has always been associated with the spleen (Tenser and Hsiung, 1976). Neither clinical disease nor pathological changes in tissues were observed in infected guinea pigs, but once infected, they remain infected for life; thus establishing a latent infection. In pregnant guinea pigs with latent GPHLV infection, intrauterine infection of the fetuses via the placenta can occur (Lam and Hsiung, 1971).

Further studies on GPHLV (Hsiung, 1977) dealt with the comparison of GPHLV and EBV; showing similarities between the two viruses. Leukocytes from GPHLV-infected guinea pigs were found negative for GPHLV antigens by immunofluorescence; neither intranuclear inclusions nor virus particles were found by either light or electron microscopy (Hsiung et al., 1971a). However, when such latently infected cells were cultured in vitro, viral inclusions and viral particles were evident. These sequences of events were considered as closely resembling those of human leukocytes bearing EBV.

In view of the similarities between GPHLV and EBV, it is reasonable to consider GPHLV infection in guinea pigs as a model for human EBV infection (Hsiung, 1977). Recently, it was found that 2′-nor-cGMP inhibits GPHLV replication in GPE cells (Hu and Hsiung, 1989b); it is possible that such a compound can be tested in guinea pig for its effectiveness in the inhibition of GPHLV latency in guinea pigs.

Precautions needed in the development of new animal models and steps required for testing new antiviral agents

Experimental virology including animal models is responding very well to the pressures of patient care (see Fig. 2). Once a viral agent is isolated and identified, control of the infection is amended. Much time and effort have been expended in the testing of new antivirals; however, development and validation of new animal models have not been addressed hand-in-hand. There are still a large number of human viral infections that are of high priority for antiviral chemotherapy, but absence of suitable animal models offers a roadblock in the in vivo testing of new antivirals. The following precautions and steps are suggested in the development of animal models.

Choice of animals

Conceivably, inbred animals will ease and hasten up the virus adaptation process, but they are more expensive than outbred animals. The question of specific pathogen-free animals should be considered particularly when adapting a human virus in the animal species by serial passage. One must recognize, if possible rule out the presence of indigenous virus infections in the animal species to be used (Hsiung et al., 1980); it may be the cause of experimental results that are difficult-to-interpret. For example, indigenous viruses including cytomegalovirus, herpesvirus, retrovirus, adenovirus and paramyxovirus have been found in guinea pigs

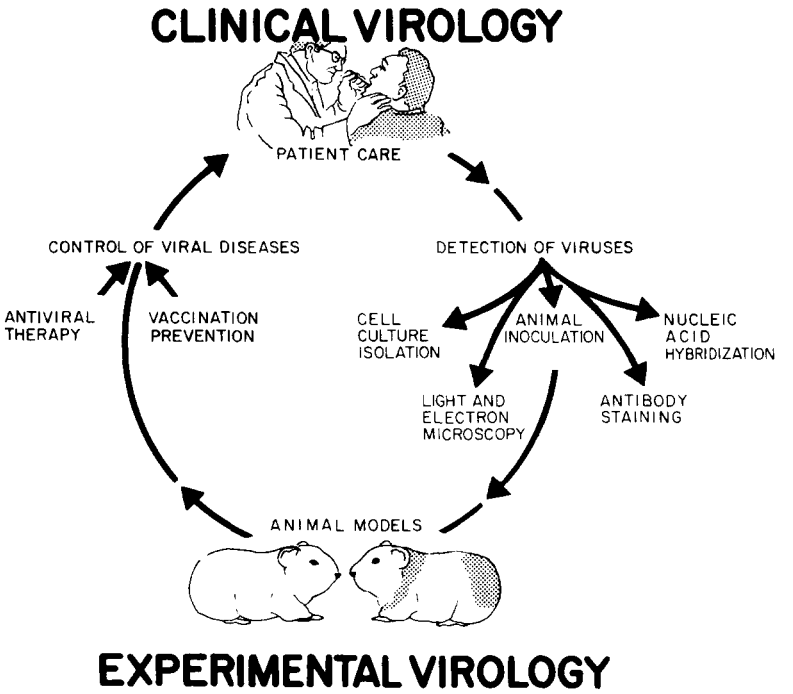

Fig. 2.

(Hsiung et al., 1986). Therefore, recognition of their presence is necessary. Third generation immunocytochemistry methods and/or ultrastructural examinations for detecting viral antigens and/or viral particle have the sensitivity and specificity to recognize the presence of indigenous viral infection.

Selection of virus stocks

If the viral infection to be studied is a homologous type, the question of adaptation is not a problem; however, if it is a heterologous type, adaptation of the human virus in the animal species of choice must be done in order to establish regularly reproducible disease or lethality. Usually, it is advisable to start with highly virulent low-passage level of the pathogenic wild strain. A purified virus stock by in vitro cloning in cultured cells helps in the adaptation process whereby disease or lethality are observable within a narrow peak-days rather than in a broad-plateau-days; the former being highly desirable for an animal model.

Familiarity with the pathogenesis of the viral infection

A thorough knowledge of the pathogenesis of a specific virus infection in humans is mandatory before one can investigate if the pathogenesis of the same given

viral infection mimics closely the human disease or clinical syndrome. The familiarity with the pathogenesis of certain virus infection in the animal species selected is one of the major points of concern. Presumably, the more congruent they are, the more predictive the animal model will be. As much as possible, the route of inoculation must be the same as the natural route of infection in humans; nevertheless, all routes of inoculation should be studied. With the viral infection fully established in the animal species of choice, the ultimate goal is to trace the entire sequence of virus infection from inoculation site through the course of the disease. It may be that only a certain specific syndrome is mimicked and not the whole spectrum of pathogenesis. The more features in common, the better it is for the animal model. This aspect of the development process requires much time and effort, and is fraught with difficulties.

Validation of the usefulness of the animal model

Usually, the usefulness of an animal model is validated by the use of an established effective antiviral drug if currently available. Otherwise, novel drug(s) are to be tried empirically. The following steps are required for testing new antiviral agents.

Selecting a suitable animal model

If both heterologous and homologous animal models of a given viral infection are available, the choice would be the heterologous animal model system for the simple reason that the new antiviral activity will be tested against the same human virus causing the disease which antiviral treatment is being sought for. When the human virus strain does not infect any animal species, the correlate or equivalent animal virus infection in its natural animal host (a homologous animal model system) is the choice. When selecting the animal model system to work with, bear in mind the following: (1) choose the animal model known to have the most features (clinical, histological, cellular and virological) in common, if not congruent, with the human disease, (2) cost of animals, (3) availability of supply at all times, (4) cost of maintenance and ease of handling, and (5) if possible, the animal species should be the same as the species of the cell culture used in the in vitro drug testing. In addition, each animal species may react to a given drug differently as illustrated in Table 3. Thus, it is advisable to test a new antiviral agent in more than one animal species.

Determining parameters for antiviral evaluation

The parameters employed in evaluating the activity of new antiviral agents can be grouped into three broad categories, namely: clinical, virological and histopathological examinations. Details of each of the parameters have been discussed in the preceding sections of this paper.

Establishing maximum and minimum dosages of antiviral activity and drug toxicity

In setting up dose-response experiments on antiviral activity, moderately high

virus challenge dose is used. The route of inoculation depends on the adaptation of the virus strain for a particular animal model. Infected animals are then treated usually with 24 h postinfection with low, and high concentrations in mg/kg body weight of the antiviral drug administered by the route which allows the drug to reach the virus target organ in high levels. Treatment is usually once or twice daily for four to eight days. Set up controls such as: (a) virus alone; (b) diluent used to dissolve the antiviral drug alone, and (c) sham-treatment. Treated and control animals are observed for 14 to 21 days after cessation of antiviral treatment. Record daily observations as to the parameter being used in the evaluation process. Determine statistical significance between virus-infected, drug-treated animal responses and virus-infected, untreated animal responses. Similar protocols can be used for uninfected animals as a parameter for drug toxicity tests.

Comparing relative potency of the new antiviral agent with established drugs

In this experiment, the effectivity and toxicity of the new antiviral drug is statistically compared with those of established antiviral drugs. New drugs found more potent but less toxic merit further studies such as best regimen of administration and mode of action.

In perspective, the development of new animal models for human viral infections needs much time and effort. During the process of development, if the precautions are taken and the various steps are followed, a new animal model can be established for testing available agents. The call for new antiviral agents is urgent for many human viral diseases.

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