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Mini-Review

Animal models for antiviral chemotherapy

H.J. Field and G.A. Brown

Department of Clinical Veterinary Medicine, University of Cambridge, Madingley Road, Cambridge, CB3 OES, U.K.

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Summary

Traditionally animal models have formed a vital part of the preclinical evaluation of new forms of antiviral therapy. A variety of models used in the past or potentially useful in the future are considered in this short review. Several valuable and complex questions concerning virus-drug interactions in vivo have been successfully addressed by means of animal models. Better understanding of drug modes of action and virus pathogenesis in the models enable even more accurate predictions to be made for the outcome of antiviral therapy in man. The complexity of virus infections in man is such that animals are likely to remain an important part in drug evaluation for many years. To this end, new developments such as improved techniques in the production of transgenic animals are opening up a variety of completely novel methods for studying inhibitors of a wider group of viruses in vivo including the human immunodeficiency virus. However, the correct interpretation of animal data requires the critical evaluation of animal models. This review will identify several important difficulties which confront those working on antiviral chemotherapy in animals and which must continue to be addressed if confidence in animal data is to be maintained.

Animal model; Inhibitor; Virus infection; HSV; HIV

The traditional role for animal models

Laboratory animal models have for many years played a crucial part in the development and assessment of antiviral agents. To date herpes simplex virus (HSV) has been the most successful target for chemotherapy and much of the progress

Correspondence to: H.J. Field, Department of Clinical Veterinary Medicine, University of Cambridge, Madingley Road, Cambridge, CB3 OES, U.K.

here can be attributed to animal models. Indeed, animal models for the various manifestations of herpes simplex in man have been available since early in this century (Goodpasture and Teague, 1923), long predating the first antiviral agents. The situation relating to many other important viruses, including the retroviruses, is more complicated as good models are not as readily available. However, when developing antiviral strategies for these viruses, we can learn much from past experience with HSV on both the potential advantages and likely limitations of animal model data.

Currently animal models might be considered to fit into the scheme of discovery and development of antiviral chemotherapy as shown in Fig. 1. Toxic side effects have been encountered commonly in antiviral chemotherapy and this means that a strong case for potential efficacy has to be made before new drugs can be tested in man. The demonstration of efficacy in one or more animal models has usually been an integral part of this preclinical evaluation of new antiviral drugs and is illustrated by the range of publications concerned with acyclovir (ACV) therapy of HSV infections in small laboratory animals (Cuatrecasas, 1982). The benefit of therapy can then be balanced against the risks of side effects either known or speculative.

The study of an animal model necessarily involves either the infection of an un-

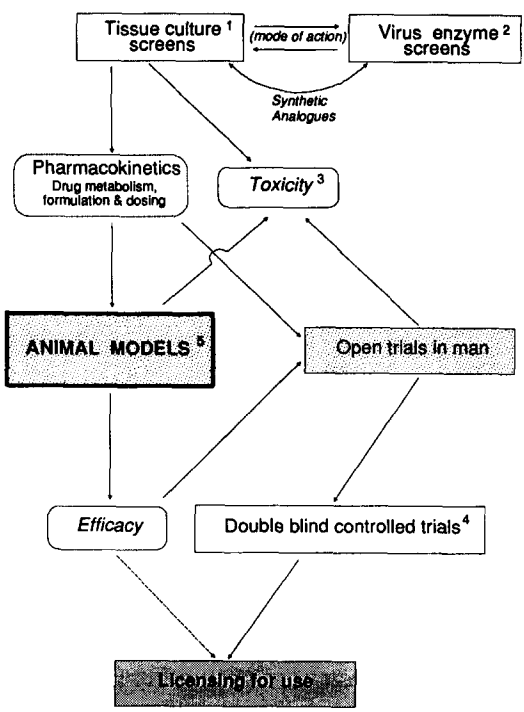

Fig. 1. Discovery and development of antiviral compounds. [1]Newton (1988); [2]Oberg and Johansson (1984); [3]Dayan and Anderson (1988); [4]Rees and Brigden (1988); [5]Field (1988).

natural host by a human virus; which we refer to as the 'classical model' or the study of a similar infection in its natural host, or 'surrogate model'. These facts place obvious constraints upon assessment of the data. In both cases the dose, route of inoculation and strain of virus used may differ markedly from the natural infection and this may be necessary in order to obtain a reproducible acute infection in a relatively short time span (Field, 1988). In addition to these 'conventional' models, modern genetic techniques are further extending the range of virus infection models. These developments include transgenic animals incorporating genes which make them susceptible to unusual viruses, and recombinant viruses of which the host range has been extended. Such methods are already being employed in relation to HIV (see below) and it is likely that many more models will be proposed in the near future.

In order that the results may provide accurate extrapolation to chemotherapy in man, both the conventional models and these new developments require critical appraisal. Many factors must be taken into account when evaluating the models and several important ones will be considered later in this review.

The range of available animal models

Classical models

These animal models today have a major role in evaluating the many hundreds of compounds which are potentially valuable as antiviral agents in man. Some of the models which have been particularly useful or are currently being considered for use in relation to virus chemotherapy are listed in Tables 1 and 2. Table 1 attempts to summarize the many models described in recent years spanning the majority of RNA virus families and several DNA viruses. At least one key reference to each model is cited in Table 1, in most cases referring to the use of the model for testing an antiviral compound. In some cases (e.g. rabies in the mouse), the models have been used for other purposes such as testing the effects of a vaccine preparation, there being few if any effective anti-rabiesvirus compounds available at present.

As mentioned before, HSV has been a particularly important target in the development of many new antiviral compounds and some of the many models which have been used in this work are shown in more detail in Table 2. The accumulation of many studies concerned with HSV in animals has enabled a number of complex questions relating to the outcome of chemotherapy in man to be addressed. The following are particular examples: (a) late therapy can ameliorate advanced disease; (b) reversal of established herpes encephalitis is possible; (c) an intact immune system is not essential for clinical benefit; antiviral agents are effective in the immunocompromised host; (d) the establishment of latency can be prevented with early therapy; (e) recurrences are suppressible with chronic therapy whilst latency is not eradicated; (f) circumstances arise where resistance develops in vivo; (g) many resistant mutants have demonstrably altered pathogen-

TABLE 1

Examples or animal models which have been used (or may potentially be useful) for the study of virus chemotherapy

Virus family	Example	Species employed	Source[b]
RNA viruses			
Arena-	Pichinde virus	Hamster, Guinea-pig	[1]
	Machupo virus	Guinea-pig	
		Rhesus monkey	[2]
	Lassa fever virus	Rhesus monkey	[3]
	Junin virus	Guinea-pig	[4]
Orthomyxo-	Influenza A	Mouse, Ferret	[5,6]
	Fowl plaque	Chicken	[7]
Paramyxo-	Respiratory syncytial virus	Ferret	[8]
Picorna-	Poliomyelitis	Cynomolgus monkey	
		Chimpanzee	[9]
	Encephalomyocarditis	Mouse	
		Hamster	[10]
Reo-	Colorado tick fever	Mouse	[11]
Retro-	HIV	Rabbit	[12]
		Simian	[13]
	Visna-maedi	Sheep	[14]
	Feline immunodeficiency virus	Cat	[15,16]
	Type-C viruses	Mouse	[17]
		Cat	[18]
Rhabdo-	Rabies	Mouse	[19]
	Vesicular stomatitis	Mouse	[20]
Toga-	Venezuelan encephalitis	Mouse	[21]
DNA viruses			
Herpes-	Herpes simplex[a]	Mouse	
		Guinea-pig	
		Rabbit	
	Varicella-zoster	Guinea-pig	[22]
	Simian varicella	African green monkey	[23]
	Guinea-pig cytomegalovirus	Guinea-pig	[24]
	Murine cytomegalovirus	Mouse	[25]
	Virus B (herpes simiae)	Rabbit	[26]

Virus family	Example	Species employed	Source[b]
Hepadna-	Hepatitis B	Chimpanzee	[27]
	Woodchuck hepatitis	Woodchuck	[28]
	Duck hepatitis	Duck	[29,30]
Pox-	Vaccinia	Mouse	
		Rabbit	[31]

[a]See Table 2

[b][1] Canonico et al. (1984)
[2] Stephen et al. (1980)
[3] Jahrling et al. (1980)
[4] Raut et al. (1975)
[5] Oxford et al. (1970)
[6] Barber and Small (1978)
[7] Beard et al. (1987)
[8] Pinto et al. (1969)
[9] Barrera-Oro and Melnick (1961)
[10] Altrock et al. (1986)
[11] Smee et al. (1981)
[12] Filice et al. (1988)
[13] Alter et al. (1984)
[14] Frank et al. (1987)
[15] Pederson et al. (1987)
[16] Harbour et al. (1987)
[17] Ruprecht et al. (1986)
[18] Onions (1987)
[19] Baer and Cleary (1972)
[20] De Clercq and Merigan (1969)
[21] Canonico et al. (1984)
[22] Chen et al. (1988)
[23] Soike and Gerone (1981)
[24] Myers et al. (1980)
[25] Sandford et al. (1985)
[26] Boulter et al. (1980)
[27] Barker et al. (1975)
[28] Abe et al. (1988)
[29] Zuckerman (1987)
[30] Thompson et al. (1953)
[31] Zeidner et al. (1988)

icity. These general findings are reviewed by Field (1988) and for each of these propositions it can be argued that the data obtained in the animal models has proven to be a more or less accurate prediction of the results obtained subsequently in man.

Clearly the animal models for HSV have a vital role to play in the development of strategies to counter aspects of the infection which are not readily modelled accurately in vitro, such as latency, disease in the immunocompromised, and the circumstances leading to resistance.

Surrogate models

One alternative to these 'classical' models is to study a similar but different virus in its natural host. Much important work in the past has been published based on this type of model [e.g. murine cytomegalovirus (Sandford et al., 1985) or simian varicella (Soike and Gerome, 1981; Felsenfeld and Schmidt, 1977)] in the investigation of nucleoside analogues such as ACV and bromovinyldeoxyuridine. Clearly care is required to assess these data due to differences between the viruses in their interactions with antiviral compounds as discussed below in the section on the assessment of animal model data.

More recently, the surrogate approach has been increasingly popular for experimental models for HIV infection. A special problem with all the models involving animal infections with dangerous human pathogens is the considerable risk to the experimenters from contaminated bite or scratch injuries and the obvious require-

TABLE 2

Examples of the many models which have been used to study inhibitors of herpes simplex virus

Disease manifestations	Species	Route of inoculation	Source[a]
Oral-facial herpes	Guinea pig	Flank	[1]
	Mouse	Ear pinna	[2]
		Flank	[3,4]
		Lip	[5]
		Foot pad	
	Hairless mouse	Flank	[6]
	Nude mouse/human skin		[7]
Genital herpes	Guinea pig (female)	Intravaginal	[8,9]
Herpes encephalitis	Mouse	Intranasal	[10,11,12]
		Intravaginal	[13]
		Intracerebral	[14]
		Intravenous	[15]
		Intraneural (sciatic nerve)	[16]
		Intraperitoneal	
	Rabbit	Intraneural (olfactory bulb)	
Ocular herpes	Rabbit	Corneal	[17,18,19]
	Mouse	Corneal	[20]
Disease in the immunocompromised host	Mouse (X-irradiated; Cyclosporin A-treated; Nude mice)	Various routes	[21]

[a][1] Alenius and Oberg (1978)
[2] Field et al. (1979)
[3] Simmons and Nash (1984)
[4] Kristofferson et al. (1988)
[5] Overall (1981)
[6] Klein et al. (1978)
[7] Genderen et al. (1987)
[8] Kern (1982)
[9] Alenius and Nordlinder (1979)
[10] Kern et al. (1982)
[11] De Clercq and Luczak (1975)
[12] Field et al. (1984)
[13] Kern et al. (1981)
[14] Schinazi et al. (1983)
[15] Anderson and Field (1983)
[16] Field et al. (1979)
[17] Kaufman et al. (1970)
[18] Narang and Codd (1978)
[19] Nesburn et al. (1983)
[20] Tullo et al. (1982)
[21] Ellis et al. (1986)

ment for high security containment facilities for the experimental animals. In the search for more convenient and safer models for HIV in man, a number of natural retrovirus infections of animals are attractive as experimental models.

Among the best studied retroviruses are the C-type feline (Onions, 1987) and murine retroviruses (Schiff and Oliff, 1986) and a number of models have previously been developed in relation to tumour virus research and vaccine development. Unfortunately these viruses have a significantly different genomic strategy at the biochemical level and lack the complexity of the accessory genes found in members of the *Lentivirus* group (Haseltine et al., 1988). However, models such

as Rauscher's leukaemia have major advantages of reproducible clinical signs (lymphoproliferation, including splenomegaly leading to death in a few weeks) which can be coupled with methods for accurate measurement of virus titres in blood and other tissues (Ruprecht et al., 1986). Similarly, murine Maloney sarcoma in new-born mice has the advantages of requiring very small amounts of the material under test with rapid results which can be obtained within 7–10 days (Balzarini et al., 1989).

Among the numerous different murine retrovirus systems are to be found candidates for investigating interactions with neural tissue (e.g. hind limb paralysis in the infection by a murine ecotropic retrovirus (DesGroseillers et al., 1984) and the viraemic spread of virus leading to infections in utero). Such models may be particularly useful for the study of compounds which interact with early events in retrovirus replication including those involving activity of the reverse transcriptase. However, the models all suffer from the major disadvantages of molecular difference in the genome and its expression compared with the human virus, HIV. The fact that mice contain many endogenous retroviruses which could interfere, and the knowledge that inocula may in some cases be heterogeneous containing both defective and helper viruses are further potential difficulties in the understanding of these models. For these and other reasons there is much interest in the animal lentiviruses as an alternative.

Maedi-visna, a natural infection of sheep and goats is an obvious candidate for AIDS research although these hosts, because of their size, are less convenient for laboratory study. Thus the more recently discovered immunodeficiency viruses such as feline immunodeficiency virus (FIV) (Pederson et al., 1987; Harbour et al., 1987), simian immunodeficiency virus (SIV) (Kestler et al., 1988) and bovine immunodeficiency virus (BIV) (Gonda et al., 1987) are now attracting much attention.

Novel approaches

The urgent need for suitable models to test potential inhibitors of HIV has led to novel approaches to the development of model systems for this virus; these new, high-technology systems which are currently under development may have application for other viruses for which no convenient model currently exists.

One such model involves the use of transgenic technology to introduce entire, infectious genomic clones of HIV or specific HIV genes into the germ line of mice. Vogel et al. (1988) have generated a murine transgenic model in which the HIV transactivating (tat) gene is expressed in the skin giving rise to histopathological changes similar to those observed in Kaposi's sarcoma. Leonard et al. (1988) have produced transgenic mice containing HIV proviral DNA. The transgenic animals described to date either have a very short life expectancy of less than a month or, in the case of the mice carrying the tat gene, develop characteristic lesions after a minimum of 10 months. Despite their limitations, these models and further developments of this type are certain to be used in the study of at least some aspects of AIDS chemotherapy.

Transgenic mice which express human or other genes that could make the animals susceptible to HIV infection have been suggested. Already murine cells expressing the human CD4 receptor have been developed, however the cultures remained refractory to HIV (Maddon et al., 1986), so it is uncertain whether mice expressing the human CD4 receptor can be exploited as a model for HIV infection.

Another interesting method for producing an experimental model for HIV employs a strain of mice with congenital severe combined immunodeficiency (SCID) (Bosma et al., 1983). Consequent upon devising a technique for implanting human foetal lymphoid tissue into SCID mice, Namikawa et al. (1988) have reported that inoculation of HIV directly into the human xenografts resulted in virus replication in those tissues. However, it is not clear whether any symptoms of HIV infection were observed in the inoculated animals and if there was any spread of infection to homogenic organs. Considering the large costs involved in keeping SCID mice and surgically transplanting human tissues, it may not be possible to take full advantage of this model system if it does not offer substantial benefits over traditional organ culture techniques.

Viruses without convenient models

HSV is an example of a virus which is unusual in that it will readily infect and produce typical clinical signs in a variety of laboratory animals. The challenge of HIV is resulting in the development of many potential models for chemotherapy. However there remain several important viruses for which no models exist. As mentioned above there are no convenient laboratory animals which can be infected directly with the human cytomegalovirus although this is an important target for antiviral therapy (Morris, 1988; Jeffries, 1989). Similarly, hepatitis B can only be studied directly in non-human primates. While surrogate models have been used for both infections, there is no adequate model for the human papillomavirus infection giving rise to genital warts. The lack of adequate models for these common and important human pathogens is a major bar to researching potential inhibitors.

General constraints

Even when animal models are thoroughly characterized there still remain a number of disadvantages. In particular 'standardized' animals (including small laboratory rodents) are expensive to produce and maintain compared with tissue cultures. The presence of intercurrent infections and other biological variables are difficult to control and as mentioned above new virus targets for chemotherapy have been identified for which there are no models yet available. An additional constraint is that in the U.K. and elsewhere there has been a trend over the last decade to reassess the ethics of the use of animals for research and this has been accompanied by tough legislation governing the use of animals for research and testing (Paton, 1984).

Organ cultures as an alternative?

A tempting approach which could obviate the need for live animals in antiviral research is the use of organ cultures of 'organotypic' cultures such as neuroblastoma cell lines. This approach generally suffers from a number of problems: (a) differentiated cells are more difficult to culture than established cell lines; (b) the cultures are fastidious, requiring special features such as collagen substrate, and complex media constituents such as growth factors; in some cases these can interfere with potential virus inhibitors; (c) organ cultures tend to dedifferentiate in culture as a result of the lack of the natural environment of the host in terms of biological factors and connections with other tissues. Notwithstanding these difficulties, neural tissues (especially explanted peripheral nerve ganglia) and skin cultures and cilliated epithelial cells (e.g. tracheal rings) have been exploited to address particular questions of drug/virus interactions with such cells (Nedrud et al., 1982; De Long and Reed, 1980). Notably, the effects of nucleoside analogues on latently-infected explanted neural tissue has been studied by these means (Wohlenburg et al., 1979). While forming an attractive alternative strategy, the problems of organ culture are so great as to severely limit further development in the near future. In addition to the direct damage caused by viruses to cells and their functions, virus interactions in vivo involve complex processes in which immune and inflammatory responses play a major role. This being so, animal experiments are essential and likely to remain so for the foreseeable future.

Important factors in the interpretation of animal model data

Pharmacokinetics

When designing an experimental model to investigate a drug, the correct interpretation of animal results depends on a thorough understanding of the drug pharmacokinetics in that species coupled with a complete knowledge of the normal translocation, replication and production of pathology and clinical signs by the virus. The same drug may be metabolized very differently in different species (Glazko et al., 1975; Larsson et al., 1986; De Miranda et al., 1982). Indeed it is well known that nucleoside analogues which require conversion to the nucleotides by means of kinase enzymes show widely differing activities against the same virus strain when tested in different cells (De Clercq, 1982; Suzutani et al., 1988). For example dihydroxypropoxymethylguanine (DHPG; gancyclovir) is very effective in the treatment of established murine encephalitis in an intranasal inoculation model (Field et al., 1984). However, evidence has been reported which suggests that the processing of DHPG in murine cells is unusual (Collins and Oliver, 1985; Field, 1985). In contrast to DHPG, the antiretrovirus nucleoside dideoxycytidine (DDC) is poorly activated in murine compared with human cells (Balzarini et al., 1988). These are examples of factors which when taken into account may greatly enhance the conclusions drawn when assessing the results of such animal experiments.

Other factors that can have a great influence on the outcome of antiviral therapy relate to the degradation in vivo of antiviral compounds or their conversion to less active (or in the case of prodrugs, more active) metabolites. For example, the conversion of the nucleoside analogue Ara-A to Ara-hypoxanthine by means of deamination (Glazko et al., 1975), or the conversion of bromovinyldeoxyuridine to the bromovinyluricil by means of phosphorylase attack (De Clercq et al., 1979; Desgranges et al., 1983). The latter probably accounts for the very short half life for the active compound in mice and the poor antiviral activity shown in some animal species, including mice, compared with other compounds of equal or lesser activity measured in cell culture (Field and De Clercq, 1981). Currently there is much interest in prodrugs which are converted to active antiviral nucleoside analogues in vivo, for example desciclovir (Krenitsky et al., 1984; Peterslund et al., 1987). It follows from the above that difficulties exist in studying such compounds in species other than man; notwithstanding such difficulties, by applying the appropriate interpretation, valuable conclusions can yet be obtained.

Drug metabolism in virus-infected cells

The interpretation of animal data is further complicated by the fact that particular animals may naturally have different levels of a molecule which interferes with antiviral activity. A good example is thymidine which readily reverses the antiviral effect of certain nucleoside analogues, for example acyclovir (Larsson et al., 1983), while pyrophosphate analogues such as phosphonoformate are unaffected. It has been shown that tissues of the guinea-pig, including skin, contain relatively high levels of thymidine (Harmenberg et al., 1985a,b). It may therefore be very misleading to compare a thymidine nucleoside analogue with a pyrophosphate analogue in this system. This does invalidate the guinea-pig models but these facts need to be taken into account when drawing conclusions from the experimental data.

Virus pathogenesis

Finally, if the pathogenesis of the infection is not completely understood the results of changes in clinical parameters following therapy may be misleading. For example, the intranasal inoculation of mice with HSV-1 leads to encephalitis and death (De Clercq and Luczak, 1975) and this model has been used for the study of herpes encephalitis. However, when mice are intranasally inoculated with HSV-2 while encephalitis is produced, virus also readily spreads to and involves the lung (Kern et al., 1982) possibly causing a fatal pneumonia. This could then become an important but unmonitored target for the chemotherapy. Similarly, the simple inoculation procedure required for intraperitoneal infection, has made this a popular model for herpes encephalitis. The pathogenesis likely involves early interactions with murine macrophages prior to eventual spread of the infection to the CNS (Johnson, 1964). There are therefore many possible tissue sites for interactions between the antiviral compound and virus-infected cells and some of these may not be taken into account by simple measurement of death or survival.

In order to draw conclusions from animal models, the following points concerned with pathogenesis should be emphasised: first, it is generally important to avoid simply using death rate, or cumulative frequency of death (or probability of survival) without reference to direct measurements of virus replication in particular tissues. This is especially important since clinical signs (and in some cases death) may primarily result from the inflammatory changes consequent on immune responses to the virus. It follows from the above that immunopotentiating, immunosuppressive or anti-inflammatory effects of a drug may ameliorate the disease without necessarily producing any direct reduction of virus growth. This may be suspected when chemotherapy produces a significant reduction in clinical signs without a concomitant reduction in virus titre in the target organs. In such a case it may be possible to employ drug-resistant strains of virus to rule out non-specific effects. If there is good correlation between the degree of reduction in clinical signs relative to the sensitivity of the virus strains in vitro (Field and Darby, 1980) this would be good evidence for a direct effect of the drug on virus growth in vivo. If no such correlation is apparent, another explanation may be sought such as an anti-inflammatory effect.

Animal models to compare drug efficacy

In general it has been easier to learn about the interactions between a particular drug and the target virus in a single host than to accurately compare the relative efficacy of two or more different drugs in a single model. Indeed, different models or species may yield conflicting data in this regard. The factors described above explain why this is so. However, this need not be a major disadvantage and studies have attempted to reconcile several of these difficulties. For example, the investigation of buciclovir and related acyclic guanosine analogues reported by Datema et al. (1987) takes into account the differences in drug pharmacokinetics, metabolism and antiviral activity of a series of closely related compounds in order to make an educated prediction of their relative potential. In the future we should be able to gain yet more useful information in the light of an increased understanding of existing models.

General conclusions

When the mode of action and pharmacokinetics of a drug in a particular animal species is thoroughly known and when the pathogenesis of the target virus in the same species is also well understood in terms of virus replication, translocation and production of tissue damage and clinical signs, then the system can be used with confidence to investigate chemotherapy. Even if features of the infection, or metabolism of the drug in a particular species are quite different from that occurring in the natural host, providing these factors can be taken into account and quantified, it is possible that the model can provide valuable data. This may include

data which are impossible to obtain in man such as the effects on establishment or maintenance of a latent infection deep in the nervous tissue. In contrast, failure to acknowledge the limitations of particular models or recognize different features from the natural infection can lead to misinterpretation of the experimental results such that useful therapies may be discarded, or patients subjected to forms of therapy which are useless or damaging.

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