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CHAPTER 7

# Update on Antiviral Therapies

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Antiviral chemotherapy use is still relatively uncommon in veterinary medicine. Controlled studies evaluating the efficacy of antiviral drugs in cats are lacking, or, if studies have been done, in many cases, the data are insufficient to determine effective dosing for these drugs. With the exception of the recombinant feline interferon (rFeIFN)-omega, so far no antiviral drugs are specifically licensed for veterinary medicine, which leaves the veterinary community with the option to use off-label antivirals made for humans to combat viral diseases in feline patients.

The goal of research in antiviral chemotherapy is the discovery of antiviral agents that are specific for the inhibition of viral multiplication without affecting normal cell division; however, because viruses are dependent on host cell machinery for replication, drug targets are often nonspecific. This makes antivirals inherently more toxic than antimicrobials are because the antiviral drugs are damaging to not only the virus but also the host cells as well. In addition, agents considered safe for human use are not always safe when administered to cats.[1] Antivirals made for systemic use often require host and/or viral metabolism to be active. Therefore, agents designed for use in humans are neither reliably nor predictably metabolized by cats or their viruses. Thus antiviral agents should always be tested first *in vitro* for efficacy and safety, and then followed by pharmacokinetic studies in cats.[1] Systemic antivirals often have a relatively narrow safety margin, and special considerations should always be given to patients with reduced hepatic or renal function. Well-designed blinded, placebo-controlled studies in client-owned animals should follow studies in laboratory-bred, experimentally infected cats to confirm results in genetically diverse cats.[1]

Most of the human antivirals are specifically intended for treatment of human immunodeficiency virus (HIV) or human herpesvirus infections. Therefore, feline immunodeficiency virus (FIV) and feline herpesvirus type 1 (FHV-1) infections have been the most important indications for antiviral chemotherapy in veterinary medicine. Topical antiviral therapy has been mainly used for herpetic ocular disease, but studies have evaluated a systemic antiviral compound (famciclovir) for treatment of multiple clinical syndromes associated with FHV-1 infections. Even though combination antiviral therapy has been successful in slowing disease progression in people with HIV, similar therapy has not been thoroughly evaluated in cats.[2] Recent studies have focused on combination therapy and evaluation of additional HIV drugs that have not been previously evaluated in feline cells. It is hoped that expanding the number of drugs that are shown to be effective for FIV will lead to effective combination therapy for feline patients.

Some additional feline infections that have been the focus of current antiviral studies are feline leukemia virus (FeLV) infection and feline infectious peritonitis (FIP). Some of the HIV antivirals, such as raltegravir, are nonspecific, and they display activity against additional retroviruses, including FeLV, in *in vitro* studies. Identification of the human coronavirus that causes severe acute respiratory syndrome (SARS) has led to evaluation of antivirals for treatment of various coronaviruses, including the feline coronaviruses (FCoVs) that cause FIP, although testing is mainly in *in vitro* stages. Several studies have also evaluated the use of rFeIFN-omega for treatment of multiple feline viruses. A review of the literature for antiviral treatment in cats, including current recommendations for drug dosages and use, is given in Table 7-1.

## FELINE IMMUNODEFICIENCY VIRUS

Feline immunodeficiency virus infects lymphocytes, cells of the monocyte-macrophage lineage, and cells of the central nervous system causing a variety of clinical signs (Figure 7-1). The viral replication cycle of FIV is highly similar to HIV. Feline immunodeficiency virus binds to host cells by an initial interaction of the FIV envelope (Env) glycoprotein with the CD134 molecule on the host cell, resulting in subsequent interaction with the co-receptor CXCR4 on the host cell, followed by viral envelope fusion with the host cell membrane. This allows entry of the viral nucleocapsid into the cytoplasm. The viral RNA is released into the cytoplasm and transcribed to complementary DNA (cDNA) by the reverse transcriptase (RT) enzyme, which is specific to retroviruses. The cDNA is subsequently synthesized to double-stranded DNA, transported to the nucleus, and integrated into the host genome by another virus-specific enzyme, the integrase. Viral messenger ribonucleic acid (mRNA) and genomic RNA are then transcribed and transported to the cytoplasm. Viral proteins are translated and processed by a third virus-specific enzyme, the protease. The immature virion moves to the cell membrane and acquires the viral envelope and glycoproteins and then is finally released from the cells.[2]

Antiretroviral drugs studied extensively in HIV infection have targeted the three virus-specific enzymes (protease, RT,

**Table 7-1 Some Current Recommendations for Antiviral Administration in Cats**

| Drug | Dose | Indication |
|---|---|---|
| Zidovudine (AZT) | 5-10 mg/kg every 12 h PO or SC (the higher dose may cause nonregenerative anemia) | FIV or FeLV |
| rHIFN-α* | $10^4$ to $10^6$ IU per kg SC every 24 h (associated with development of neutralizing antibodies within 3 wks with the higher dose)<br>Oral application of low-dose (1 to 50 IU per kg every 24 h)—no antibody development | FIV, FeLV, FCV (oral dose), or ±FHV-1 |
| rFelFN-omega† | Licensed protocol: 3 cycles of injections at day zero, day 14, and day 60; each treatment cycle consists of $10^6$ IU/kg/day SC for 5 consecutive days<br>Recently used oral protocol: $10^5$ IU/cat PO every 24 h for 90 consecutive days | FIV, FeLV, panleukopenia (parvovirus), ±FCV (oral protocol), ±FCoV, or ±FHV-1 |
| L-lysine | 500 mg every 12 h PO (twice daily important to maintain efficacy); must be given as a bolus and not in food; only an adjunctive therapy | FHV-1, long-term (likely lifelong) treatment in cats with recurring clinical signs to prevent reactivation of latent infection |
| Famciclovir | 40 mg/kg PO 3 times daily (most recent recommendation; definitive dose and rate have not been established)[19] | FHV-1-associated clinical disease |

*Recombinant human interferon alpha.
†Recombinant feline interferon omega.
*FCoV,* feline coronavirus; *FCV,* feline calicivirus; *FeLV,* feline leukemia virus; *FHV-1,* feline herpesvirus type 1; *FIV,* feline immunodeficiency virus; *IU,* international unit; *PO,* orally; *SC,* subcutaneous(ly) .

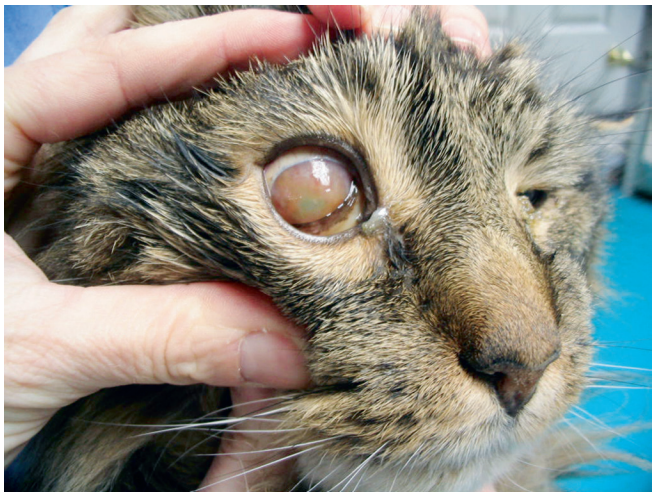

**Figure 7-1:** Severe anterior uveitis in a cat diagnosed with feline immunodeficiency virus infection. Photo courtesy of Dr. Susan Little.

and integrase), as well as some additional targets, interfering with different steps of the virus replication cycle.[3] As of 2014, approximately 30 compounds are approved by the U.S. Food and Drug Administration (FDA) for treatment of different stages of HIV infection.[2] Some of these drugs can also be used for FIV, and steps that can be inhibited include: (1) virus entry into susceptible cells by blocking attachment to the host cell co-receptor CXCR4; (2) reverse transcription of viral genomic RNA; (3) viral DNA integration into host genomes; and (4) proteolytic processing of precursor viral proteins into mature viral proteins (Figure 7-2).[2,3]

## Reverse Transcriptase Inhibitors

Close similarities exist between the RT of HIV and FIV, and it has been shown that several RT-targeted antiviral compounds active against HIV are also effective in inhibiting FIV replication *in vitro*.[4] The RT of HIV is actually the target for three classes of inhibitors: nucleoside RT inhibitors (NRTI), nucleotide RT inhibitors (NtRTI), and nonnucleoside RT inhibitors (NNRTI). Nucleoside RT inhibitors and NtRTI interact with the catalytic site (the substrate-binding site) of the RT enzyme, whereas NNRTI interact with an allosteric site located at a short distance from the catalytic site. For the NRTI and NtRTI to interact with the substrate-binding site, they need to be phosphorylated.[3]

All of the NRTI (zidovudine [AZT], didanosine [ddI], zalcitabine [ddC], stavudine [d4T], lamivudine [3TC], abacavir [ABC], and emtricitabine) can be considered as nucleoside analogues, and they act in a similar fashion. After they have been taken up by the cells, they are phosphorylated three times to the active triphosphate form, and they act as competitive inhibitors of the normal deoxynucleoside triphosphate (dNTP) substrates, which are used by the cell to make DNA. Unlike dNTP substrates, NRTI lack a 3′-hydroxyl group on the deoxyribose moiety. Once incorporated into the DNA chain, the absence of a 3′-hydroxyl group, which normally forms the 5′- to 3′-phosphoester bond with the next nucleic acid, blocks further extension of the DNA by RT, resulting in DNA chain termination. The analogues cannot be cleaved from the active center and thus block the RT enzyme.[3] Nucleoside analogues are not only accepted as false substrates by viral enzymes, but also by cellular enzymes, and

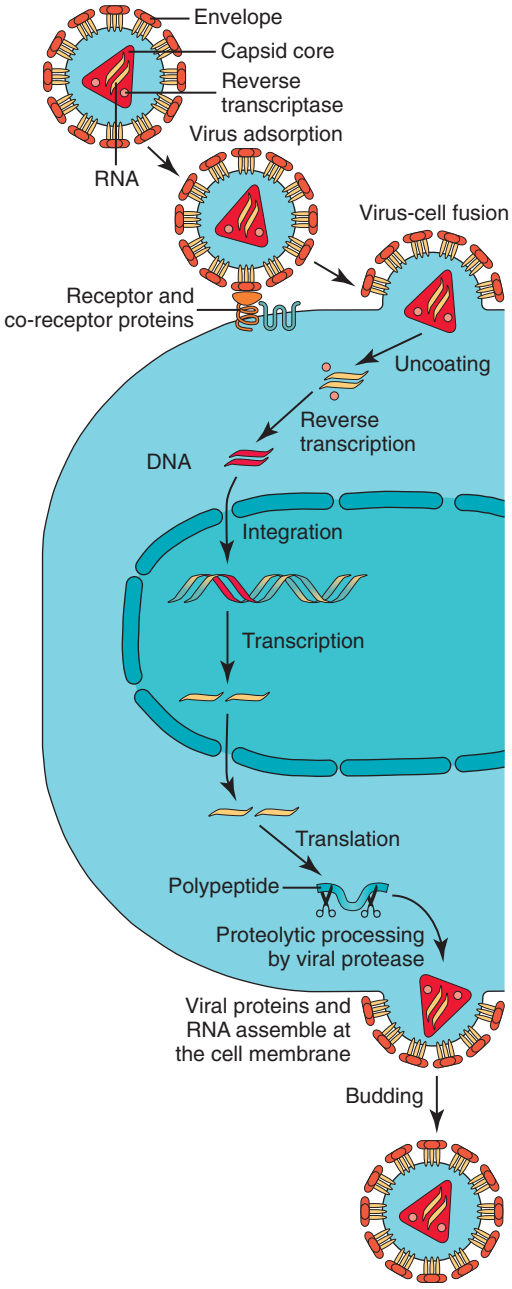


**Figure 7-2:** Replicative cycle of feline immunodeficiency virus and human immunodeficiency virus, demonstrating targets for therapeutic intervention, including co-receptor interaction, reverse transcription (by reverse transcriptase), integration, and proteolytic processing (by viral protease).

this is the major cause of their toxicity. Zidovudine is the NRTI most studied in cats, including *in vivo* studies evaluating the clinical response of experimentally and naturally FIV-infected cats treated with the drug. Zidovudine can increase the CD4+/CD8+ ratio and improve clinical condition scores in FIV-infected cats; however, it can result in adverse effects, such as dose-dependent nonregenerative anemia and neutropenia.[4,5] In addition, mutations producing resistance against the drug can develop.[4,5] Therefore, a study evaluated nine NRTI to inhibit FIV replication in feline peripheral blood mononuclear cells.[4] Six of these drugs (ABC, ddI, emtricitabine, 3TC, d4T, and AZT) had been previously evaluated in feline cells, and three (amdoxovir, racivir, and dexelvucitabine) had not. Significant differences among the drugs were not found, but based on the data obtained, amdoxovir, dexelvucitabine, and racivir appear to be options for future studies investigating their potential use in FIV-infected cats. Though pharmacological data for cats are not available for these drugs, cytotoxic properties of these compounds suggest they could likely be used *in vivo* at dosages comparable to that for AZT.[4]

Nucleotide RT inhibitors are distinguished from NRTI as they are nucleotide analogues (not nucleoside analogues), which means that they only need two (not three) phosphorylation steps to be converted to their active form. Most importantly, they contain a phosphonate group that cannot be cleaved by hydrolases (esterases), which would make it more difficult to cleave off these compounds, once incorporated at the 3′-terminal end, compared with their regular nucleotide counterparts. Use of these compounds also results in DNA chain termination. One of these drugs, cidofovir, is active against virtually all DNA viruses, including polyoma-, papilloma-, adeno-, herpes-, and poxviruses. Cidofovir has been used for treatment of FHV-1 (see Feline Herpesvirus Type 1). Adefovir (9-(2-phosphonylmethoxyethyl)adenine [PMEA]) has a spectrum of activity that partially overlaps with cidofovir, in that both are active against herpesviruses, but adefovir is also active against hepadnaviruses (hepatitis B) and retroviruses, including FIV and FeLV. The antiviral activity spectrum of tenofovir (PMPA) is narrower than that of PMEA, in that it no longer extends to herpesviruses but is confined to hepadna- and retroviruses.[6] This drug has been tested *in vitro* against FeLV (see Feline Leukemia Virus).

Adefovir has been tested in FIV-infected cats in a 6-week placebo-controlled, double-blinded, clinical trial; 10 cats received adefovir (10 mg/kg subcutaneously [SC] twice weekly) and 10 cats received a placebo.[7] There was no decrease in the proviral or viral loads in treated cats, and the cats developed a progressive, life-threatening anemia. This is a common adverse effect of some nucleotide analogues.[7] Adefovir was also tested in combination with the co-receptor inhibitor plerixafor (see Co-receptor Inhibitors) in the same study, producing the same outcome as seen with use of the adefovir alone.[7]

A related drug, (*R*)-9-(2-phosphonylmethoxypropyl)-2,6-diaminopurine (PMPDAP), has been shown previously to be a potent inhibitor of FIV replication in cell culture and has reduced the viral load in three of four cats experimentally infected with FIV when treated at 20 mg/kg SC three times per week for 6 weeks. There were no changes in the red blood cell counts or hemoglobin values with treatment.[8] A recent study evaluated the efficacy of this drug in a placebo-controlled, double-blind study with a population of 20 cats naturally infected with FIV.[8] No significant differences were found between PMPDAP-treated (25 mg/kg SC twice weekly for 6 weeks) and placebo-treated cats, although cats treated with PMPDAP showed a tendency for improvement

in their clinical signs and CD4+/CD8+ ratios. Mild hematological side effects (slight decline in packed cell volume and hemoglobin values) were seen in the treatment group. Compared with other NtRTI, PMPDAP seems to be slightly less toxic.[8]

Unlike the NRTI and NtRTI, NNRTI are an active form, with no dependence on intracellular metabolic pathways. NNRTI inhibit the RT by binding to the enzyme in a hydrophobic pocket that is located away from its catalytic site. The interaction of the compounds with the RT induces conformational changes that affect the catalytic activities of the enzyme.[9] Nonnucleoside RT inhibitors are considered highly specific inhibitors of HIV-1, and thus not active against other retroviruses, including FIV.[9] This is due to differences in the structure and/or flexibility of FIV RT that prevent NNRTI from interacting with the FIV RT.[10]

## Protease Inhibitors

Protease inhibitors are based on the "peptidomimetic" principle, that is, they contain a hydroxyethylene scaffold that mimics the normal peptide linkage (cleaved by the HIV protease) but which itself cannot be cleaved. They thus prevent the HIV protease from carrying out its normal function, which is the proteolytic processing of precursor viral proteins into mature viral proteins.[3] Despite similarities between the HIV and FIV proteases, all but one of the currently available HIV protease inhibitors have failed to inhibit the protease of FIV. The one compound of interest, tipranavir, has only been tested against FIV *in vitro* so far.[4] However, studies have demonstrated that these compounds can be used to inhibit FCoV replication (see Feline Coronavirus).

## Co-Receptor Inhibitors

Co-receptor inhibitors block viral attachment by binding to receptors on the host cell membrane to obscure the site of interaction of Env with the receptor.[2] Most of the receptor homologues or antagonists are highly selective for HIV and not useful for veterinary medicine. One exception can be used in cats with FIV infection, the class of bicyclams (e.g., plerixafor). Plerixafor (1,1′-[1,4-phenylenbismethylene]-bis(1,4,8,11-tetraazacyclotetradecane)-octachloride dehydrate, [AMD3100], [JM3100]), is the prototype compound among the bicyclams. Bicyclams are dimeric low-molecular weight nonpeptidic compounds that bind selectively to the chemokine receptor CXCR4. This is the cell surface co-receptor used by both HIV and FIV for attachment and infection of susceptible CD4+ lymphocytes, and the amino acid sequences of human and feline CXCR4 are highly similar. Drug binding inhibits attachment of the viral envelope to the host cell. The efficacy of plerixafor against FIV was recently investigated in naturally FIV-infected cats that were treated in a placebo-controlled, double-blind clinical trial.[7] Plerixafor was administered at 0.5 mg/kg SC every 12 hours. Treatment of FIV-infected cats with plerixafor caused a significant decrease in the provirus load but did not lead to improvement of clinical or immunological variables. A statistical decrease in serum magnesium levels was observed in the treatment group, without clinical consequences. No development of resistance of FIV isolates to plerixafor was found during the treatment period, making it a potential treatment for FIV-infected cats.[7] Limited oral bioavailability and short half-life preclude clinical use of plerixafor in HIV infection,[2,7] but additional CXCR4 antagonists are under development and should be tested for efficacy against FIV when available.

## Integrase Inhibitors

Integrase catalyzes strand transfer (3′-end joining), which inserts both viral DNA ends into a host cell chromosome.[3] Integrase inhibitors are used to treat HIV infection. One of the integrase inhibitors (raltegravir) has been shown to be effective for inhibition of FeLV (see Feline Leukemia Virus).

## Highly Active Antiretroviral Therapy

Administration of a combination of drugs from different classes, termed highly active antiretroviral therapy (HAART), to HIV-infected patients has turned an invariably fatal disease into a chronic but manageable condition.[2,3] The goals associated with the use of combinations of three (or more) anti-HIV compounds are: (1) to obtain synergism among different compounds acting at different molecular targets; (2) to lower the individual drug dosages to reduce their adverse side effects; and (3) to diminish the likelihood of development of drug resistance.[3] Combination therapy has not been thoroughly investigated for treatment of FIV infection in cats,[2] and use of multiple classes of drugs is more difficult in cats because some of the drug classes that are effective for HIV do not work for FIV.[2,4] However, the need for combination antiretroviral therapy for feline patients has been the focus of recent studies.

The goal of antiviral therapy should be improvement of the cat's clinical status. This is not always correlated with virus replication, as measured by a plasma viral load.[9] It has been suggested that antiretroviral therapy should be administered to FIV-infected cats in the later stages of the asymptomatic phase of infection, during which the cat does not show clinical signs and the immune system is relatively normal and more likely to respond to treatment.[5] After experimental infection, when the CD4+/CD8+ ratio decreases, viral load increases markedly, and clinical signs of immunosuppression begin to appear. However, the situation in naturally infected cats is different, and the quality of life is not associated with the viral load.[11] Therefore, it is debated at which time point antiviral therapy should be started and whether it should be administered to asymptomatic cats. In a recent study, antiretroviral therapy was initiated during the later stages of the asymptomatic phase of infection in naturally infected cats. The cats were defined as being in the later stages of the

asymptomatic phase of infection when the CD4+/CD8+ ratio reached 0.9, because at this stage of infection, the viral load increased markedly, and clinical signs of immunosuppression began to appear. The ratios were calculated every 4 months for 2 to 5 years prior to initiation of the antiviral therapy, and viral loads of all cats were quantified once a year. The cats were randomly assigned to treatment groups of eight cats each. Treatment included combination therapy, but no placebo group was used, and the study was not blinded.[5] The follow-up was performed over 1 year, through clinical evaluation and the determination of viral loads and CD4+/CD8+ ratios. Comparisons of pretreatment and post-treatment values from the cats were performed, as well as comparison of values between treatment groups. A combination of two NRTI (AZT + 3TC, 25 mg/kg every 12 hours orally [PO]) was compared to treatment with AZT alone (5 mg/kg every 12 hours PO). The combination of AZT and 3TC is often used in HIV-infected patients, given that both drugs show a synergistic effect. Treatment with AZT alone or in combination with 3TC induced a significant increase in the CD4+/CD8+ ratio and a significant decrease in viral load within and among groups, with an even greater reduction with combination therapy than with AZT alone. Only mild side effects, including vomiting in one of eight cats, anorexia in two of eight cats, and anemia in one of eight cats, were seen with this treatment combination, but therapeutic interventions resolved the problems, and treatment did not have to be stopped.[5] However, the lack of a control group and lack of blinding make the results of the study very difficult to interpret. Therefore, treatment of asymptomatic FIV-infected cats with antivirals cannot be generally recommended based on the currently available data. An earlier *in vivo* study was performed in experimentally FIV-infected cats that were treated with a high-dose AZT and 3TC combination (100 or 150 mg/kg/day PO for each drug). The combination had no anti-FIV activity in these chronically infected cats. Severe side effects, which included fever, anorexia, and marked hematologic changes, were observed in some of the cats with such high-dose dual-drug treatment, but the toxic effects were reversed when the dose was lowered to 20 mg/kg every 24 hours.[12]

Ideally, combination therapy for feline patients will contain at least two to three drugs from at least two different classes, as recommended for human patients.[9] As previously mentioned, PMEA (an NtRTI) was tested in combination with the co-receptor inhibitor plerixafor; however, because of the toxicity associated with the PMEA, this combination cannot be recommended.[7] Therefore, use of plerixafor in combination with other NtRTI that are less toxic than PMEA or compounds of other drug classes are should be further investigated in the future.

### Immunomodulator

Lymphocyte T-cell immunomodulator (LTCI), a protein produced by a bovine-derived thymic stromal epithelial cell line, is conditionally licensed by the United States Department of Agriculture (USDA) as a treatment aid for cats infected with FIV or FeLV. The primary therapeutic effect is activation of progenitor CD4 T-cells to mature cells, which then produce cytokines, including interleukin (IL)-2 and interferon (IFN). A few studies performed by the manufacturer are highlighted in a review article.[13] The studies suggest reduced virus load, improved clinical signs, and improved hematological parameters with treatment. However, the data for placebo-controlled studies were not shown, and a field study with naturally infected cats lacked a control group. Independent placebo-controlled, blinded studies are warranted. Additional information about immunomodulators and immunostimulants is provided in the feline herpesvirus type 1 and feline coronavirus sections.

## FELINE LEUKEMIA VIRUS

Feline leukemia virus, like FIV, is a member of the family *Retroviridae*, but unlike FIV, FeLV is a gammaretrovirus and not a lentivirus. Feline leukemia virus causes a wide variety of clinical signs in infected cats (Figure 7-3). Structural differences affect the susceptibility of gammaretroviruses to anti-HIV drugs, but the similarities in mechanism of replication suggest that some of these drugs can also inhibit FeLV. This is true of most NRTI.[14] Zidovudine effectively inhibits FeLV replication *in vitro*, and *in vivo* in experimental infections. However, in naturally FeLV-infected cats, it did not reduce plasma virus load, improve immunological and clinical status, increase quality of life, nor prolong life expectancy.[15] Its bone marrow toxicity can also cause adverse side effects (e.g., nonregenerative anemia) that are more pronounced in FeLV-infected cats than in FIV-infected cats. Therefore, it is not recommended as a first line of therapy for FeLV infection.[16]

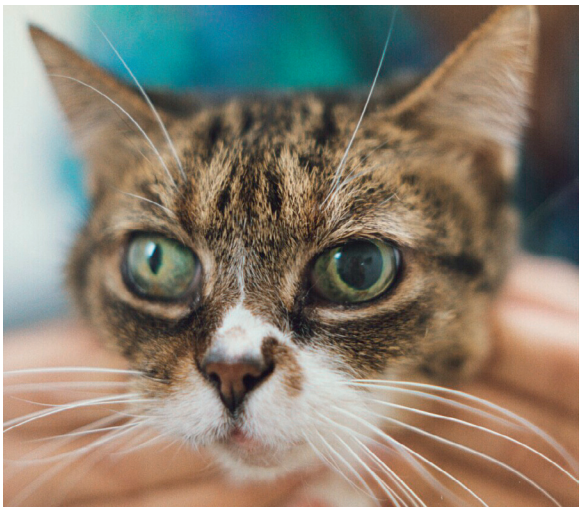

**Figure 7-3: Anisocoria in a Cat Infected with FeLV.** (Photo courtesy of Dr. Susan Little.)

Tenofovir, an NtRTI used for treatment of HIV, has been shown to be effective against FeLV *in vitro*.[14] The anti-FeLV mechanism of tenofovir is probably similar to what has been described for HIV-1. Tenofovir is given in the form of a prodrug, which is converted to an acyclic nucleoside phosphate. Once converted to the active diphosphate form, tenofovir is incorporated by RT into viral DNA, where it acts as a chain terminator to inhibit further elongation of the viral DNA.[14] However, *in vivo* studies in FeLV-infected cats are lacking.

### Raltegravir

Another compound currently used for human HIV therapy, raltegravir, could be considered for the treatment of FeLV-infected cats.[14] The high degree of conservation across lentiviruses, betaretroviruses, gammaretroviruses, and alpharetroviruses of integrase active sites suggests that FeLV might be highly sensitive to integrase inhibitors.[16] The mechanism of action against FeLV is the same as for FIV, inhibition of integration of the viral dsDNA that is produced by reverse transcription of the viral RNA genome.[14]

An *in vitro* study evaluated the effective 50% inhibitory concentration ($EC_{50}$) for FeLV inhibition of raltegravir in several feline cell lines and found these values are in the range of that observed for HIV and a related gammaretrovirus, xenotropic murine leukemia virus, and are well below the minimal plasma concentrations found in humans.[16] The effective concentration of raltegravir had no appreciable effect on cell viability nor induced apoptosis, suggesting that this could be an effective and safe drug also *in vivo*.[16] However, raltegravir is partly eliminated as glucuronide, a metabolic pathway that is not very efficient in cats, and it would increase the risk of toxicity resulting from drug accumulation.[16] As of 2014, no *in vivo* studies have been published.

## FELINE HERPESVIRUS TYPE 1

Feline herpesvirus type 1 is a member of the subfamily *Alphaherpesvirinae*, order *Herpesvirales*. Herpes simplex viruses 1 and 2 and varicella zoster virus are also members of this subfamily, and antivirals developed for the treatment of these human viruses have been used for treatment of FHV-1 in cats. Feline herpesvirus type 1 typically infects epithelial and mucosal surfaces and travels retrograde along sensory axons to establish latency in the trigeminal ganglia. Reactivated virus travels down those same axons to infect similar tissues to those that were originally infected, potentially resulting in recurrent or chronic sequelae, including keratitis, conjunctivitis, rhinosinusitis, dermatitis (Figure 7-4), and potentially blindness.[17] Whereas drug combinations have become standard procedure for the treatment of HIV infections, the treatment of other virus infections, including herpesviruses, is routinely based on the use of a single antiviral drug.[18]

A group of antiviral drugs known as acyclic nucleoside analogues are used for the systemic treatment of human

**Figure 7-4:** Herpetic dermatitis. (Photo courtesy of Dr. Elizabeth May, University of Tennessee College of Veterinary Medicine.)

alphaherpesviruses, such as herpes simplex virus 1 (HSV-1). They have been investigated for treatment of FHV-1. Members of this group of antiviral agents include acyclovir (and its prodrug, valacyclovir), ganciclovir, and penciclovir (and its prodrug, famciclovir). All require three phosphorylation steps for activation. The first of these steps must be catalyzed by the FHV-1 viral enzyme, thymidine kinase. This makes the drugs less toxic *in vivo* compared to many of the other antiviral drugs. However, the activity of the thymidine kinase in FHV-1 is not equivalent to the enzyme of human herpesviruses. The second and third phosphorylation steps must be performed by host enzymes, which are not as effective in cats as they are in humans. This knowledge helps explain why the acyclic nucleoside antiviral agents developed for humans infected with HSV-1 are not predictably effective when administered to cats infected with FHV-1 and why pharmacokinetic and efficacy studies are always needed to establish appropriate dosing in cats.[1]

Acyclovir has been adequately tested in cats for the treatment of FHV-1, but it has a relatively low antiviral potency and poor bioavailability. A very high dose is required for effective treatment, which is associated with unacceptable toxicity, with signs related to bone marrow suppression and nephrotoxicity.[1] A prodrug of acyclovir, valacyclovir, was developed for increased bioavailability in humans, but use for FHV-1 treatment in experimentally infected cats induced fatal renal and hepatic necrosis and bone marrow suppression, and did not reduce viral shedding or clinical disease severity.[1,19] Therefore, despite its superior pharmacokinetics, valacyclovir should not be used in cats.[1]

### Ganciclovir

Ganciclovir appears to be at least 10-fold more effective against FHV-1 than acyclovir *in vitro*. Ganciclovir is available for systemic as well as topical use in the form of a 0.15% ophthalmic gel formulation in humans. Ganciclovir holds promise for feline FHV-1 infection and currently available formulations warrant safety and efficacy studies in cats.[1]

## Famciclovir

The most promising systemic drug for the treatment of FHV-1 is famciclovir, a prodrug of the active compound penciclovir, which has been shown to be highly efficacious in inhibiting FHV-1 replication *in vitro*. Penciclovir is absorbed poorly when given orally, so the oral form famciclovir was developed with increased bioavailability and uptake from the intestinal tract.[1] Famciclovir requires di-deacetylation, mainly in the blood, and oxidation by a hepatic aldehyde oxidase for conversion to the active compound penciclovir. Unfortunately, hepatic aldehyde oxidase activity is basically absent in cats, which makes the pharmacokinetics of this drug complex and results in lower than expected plasma penciclovir concentrations despite administration of relatively high doses of famciclovir.[20,21] Despite this, studies evaluating famciclovir *in vivo* have shown it to be safe and efficacious for use in feline patients.[20,22] Cats experimentally infected with FHV-1 and receiving famciclovir 90 mg/kg PO three times daily for 21 days had significantly improved outcomes for systemic, ophthalmic, clinicopathologic, virologic, serologic, and histologic variables when compared with placebo-treated cats. Treatment was initiated on day zero, the same day the cats were infected.[20] Even though this study did not mimic how cats with natural infection would be treated, results from a clinical case study suggested this drug is likely effective for treatment of clinical cases, though it was not blinded and placebo controlled.[22] Clinical cases with primary ocular disease, rhinosinusitis, and dermatitis each attributed to FHV-1 (though not definitively diagnosed), were treated with famciclovir at doses of 62.5 mg PO once or twice daily for ocular herpetic disease or rhinosinusitis or up to 125 mg PO three times daily for dermatitis. Famciclovir was well tolerated with each dose and had a positive effect on each clinical condition.[22]

A definitive dose rate has not been established for famciclovir. However, penciclovir has no appreciable *in vitro* effect if present for 24 hours prior to infection, suggesting that famciclovir should be administered more frequently than once every 24 hours to ensure exposure to penciclovir as additional epithelial cells become exposed to viral infection.[21] Current pharmacokinetic data suggest that dosing three times daily is required,[21] and 40 mg/kg PO three times daily has been suggested for treatment of cats infected with FHV-1, based on effective concentrations obtained in *in vivo* studies[20,22] and determination of new *in vitro* 50%-inhibitory concentrations.[21] The most commonly reported adverse effects of famciclovir treatment in humans include urticaria, hallucinations, headaches, and confusion (especially in elderly humans), which would likely be more difficult to detect in animals. For these reasons, judicious use of this drug is recommended in client-owned cats, especially those with preexisting hepatic or renal insufficiency.[21]

Pharmacokinetic studies have also evaluated the concentration of penciclovir in tears, and treatment with an oral dose of 40 mg of famciclovir/kg three times daily achieves a penciclovir concentration at the ocular surface likely to be effective against FHV-1.[23] This is potentially an alternative therapy to the use of topical drugs, the majority of which require multiple daily applications.[23] However, an implantable silicone polymer device impregnated with penciclovir has been developed that holds promise for long-term, steady-state subconjunctival delivery of the drug for the treatment of ocular herpetic disease.[17]

## Cidofovir

Although herpetic ocular disease is commonly treated with topical antiviral ophthalmic solutions or ointments (including idoxuridine, vidarabine, or trifluridine),[1] these antivirals do not require a virus-specific phosphorylation step for activation. Moreover, they damage host cells, specifically resulting in bone marrow suppression. Therefore, they should not be used systemically.[1] For good reviews of these topical drugs, see the reports of Maggs[1] and Gould.[24] Cidofovir, a member of the NtRTI class of drugs, has been tested for topical treatment of FHV-1 ocular disease but not for systemic use. It appears to be efficacious topically and is a newer drug (therefore it is included in this section). Cidofovir requires the typical two host-mediated phosphorylation steps without virally mediated phosphorylation.[1] Its safety when given topically arises from its relatively high affinity for HSV DNA polymerase compared with human DNA polymerase. It is commercially available only in injectable form in the United States for treatment of a human betaherpesvirus. When applied topically as a 0.5% solution twice daily to cats experimentally infected with FHV-1, it led to reduced viral shedding and improvement of clinical disease compared to the placebo group.[25] Its efficacy with only twice daily administration (despite being virostatic) is believed to be due to the long tissue half-lives of the metabolites of this drug. There are reports of its experimental topical use in humans and rabbits being associated with stenosis of the nasolacrimal duct, but this has not been shown in cats. The fact that a twice-daily topical treatment is sufficient, whereas all other topical antivirals require application every 3 to 4 hours, makes cidofovir a useful alternative for ocular topical treatment.[1,24,25]

## Small Interfering RNA

Small interfering RNAs (siRNAs) designed to target the FHV-1 DNA polymerase[26] and glycoprotein D[27] have been used *in vitro* to induce RNA interference in an immortalized cell line and in primary feline corneal epithelial cells to inhibit FHV-1 replication. RNA interference is a post-transcriptional, RNA-guided gene-silencing mechanism present in eukaryotes.[28] Interference of the FHV-1 essential genes resulted in reduction of virus replication up to 98 ± 1%. This type of therapy is intended for topical treatment of chronic herpetic disease. However, a preliminary *in vivo* study evaluating topical delivery of siRNAs to feline corneas was unsuccessful.[29] The lack of delivery was likely the result of siRNA dilution and rapid removal by tear film and blinking. Studies are ongoing to identify a means of increasing

contact time between the corneal cells and siRNAs to allow delivery.

## Lysine

Twice-daily oral L-lysine bolus administration, initiated prior to experimental infection, reduced the severity of conjunctivitis in cats undergoing primary infection. L-lysine bolus administration also reduced viral shedding in latently infected cats experimentally infected with FHV-1, following changes in husbandry and housing but not following corticosteroid administration. *In vitro*, lysine supplementation led to reduction of FHV-1 replication.[1] Arginine exerts a substantial growth-promoting effect on FHV-1 and is an essential amino acid for viral protein synthesis, and lysine antagonizes this effect. Lysine and arginine competitively inhibit transport of each other by using a common transport system, and lysine induces arginase, an enzyme that causes the degradation of arginine. Arginine deficiency inhibits synthesis of infectious viral particles and downregulates synthesis of viral proteins. However, unlike the protocol for HSV-1-infected humans, owners of cats receiving lysine for FHV-1 should not be advised to restrict their cat's arginine intake[1] because feeding a diet lacking L-arginine is associated with a severe risk of hyperammonemia and encephalopathy.[30]

It has been suggested that the ratio of L-lysine to L-arginine, rather than the concentration of each amino acid, is critical in achieving an inhibitory effect on viral replication. Dietary supplementation increases mean plasma concentrations of L-lysine without reducing L-arginine concentrations and has been shown to be safe for use in cats, up to 86 g/kg of diet. Supplementation with higher doses has been shown to result in reduced food intake.[30,31]

Despite promising initial *in vitro* data and *in vivo* results from experimental studies, current studies question whether viral inhibition with increased lysine concentrations, in the absence of decreased arginine concentrations, can be biologically important. A new study evaluating the effect of various ratios of L-lysine and L-arginine on FHV-1 DNA replication *in vitro* demonstrated only a modest reduction in viral DNA (less than 1 log) at ratios considered difficult to obtain *in vivo* in healthy cats.[31]

A lack of efficacy of L-lysine supplementation has also been demonstrated *in vivo* in shelter settings.[1,32] Dietary supplementation was unsuccessful, likely because the cats were anorexic during peak disease and were not ingesting the lysine when they needed it the most. Bolus administration was also unsuccessful, likely because of stress associated with the lysine administration.[1,32] The stress of bolus administration in shelter situations could negate its effects and even cause transfer of pathogens among cats by shelter workers administering the lysine. However, data do not support dietary supplementation.[1,32]

Unfortunately, no studies to date have been conducted on client-owned cats; however, anecdotal evidence suggests that there is a benefit from administration of lysine in individuals. Dosing is 500 mg PO twice daily, which should be given as a bolus and not added to food. Any benefit from lysine therapy is likely only possible with daily, lifelong treatment of cats with chronic herpetic disease, rather than use of lysine as a treatment during acute or recrudescent episodes. Potentially, daily therapy would reduce episodes of viral recrudescence. However, clinical studies in pet cats are lacking. The cost of this therapy should be weighed against the potential benefits. Owners should be made aware that this is only an adjunctive therapy and that administration of antiviral drugs might be necessary to gain better control of signs.

## Polyprenyl Immunostimulant

Polyprenyl immunostimulant (PI) is an immunomodulator that has a conditional license in the United States for treatment of FHV-1 infection. In blinded, placebo-controlled, experimental challenge studies, PI started on the day of virus exposure significantly reduced the severity and duration of rhinitis and conjunctivitis associated with acute FHV-1 disease (Legendre and Kuritz, manuscript in preparation). According to the manufacturer, PI upregulates the innate immune system and modulates the immune response toward a cellular response. This activity was attributed to positive effects associated with treatment of FHV-1, which requires a cell-mediated immune response for control. Viral titers were not compared between treatment and control groups in the studies, but based on the reduced signs associated with treatment, clinical studies are warranted.

# FELINE CORONAVIRUS AND FELINE INFECTIOUS PERITONITIS

Feline infectious peritonitis is associated with clinical signs that can affect almost any body system (Figure 7-5). Currently, there is no effective treatment for FIP despite its importance as the leading infectious cause of death in young cats.[33] Following the discovery that SARS is caused by a coronavirus (SARS-CoV), efforts to find an antiviral drug for coronaviruses increased. A few antiviral agents that target different steps in the replication cycle have been tested against feline FCoV. Coronavirus spike proteins on the viral envelope initially bind to receptors on the host cell membrane.[33] The spike protein mediates fusion of the viral envelope with host cell membranes. During this process, heptad repeats 1 and 2 (HR1 and HR2) of the spike protein assemble to form a complex, resulting in a conformational change that is necessary for fusion.[34] Peptides have been used as antivirals by inhibiting the HR1-HR2 interaction, thus preventing membrane fusion.[34] The spike protein must be cleaved for entry of the virus into the cytoplasm. Feline coronavirus infection is dependent on cathepsin B, a host cysteine protease found within the cell, making this the likely protease responsible for spike protein cleavage. Therefore, cathepsin B can serve as a potential target for the development of therapeutic drugs against FCoV. Following entry into the cell, FCoV produce viral polyproteins that are processed into

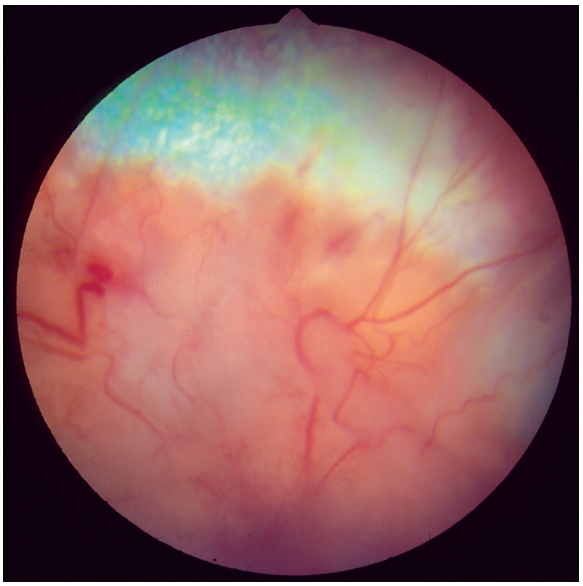

**Figure 7-5:** Chorioretinitis in a cat diagnosed with feline infectious peritonitis. (Photo courtesy of Drs. Dan Ward and Diane Hendrix, University of Tennessee College of Veterinary Medicine.)

mature proteins by viral-specific proteases, the main protease (3C-like [3CL] protease) and the papain-like protease. Because the cleavages of viral polyproteins are an essential step for virus replication, blockage of viral protease is also an attractive target for therapeutic intervention.[33]

## Protease and Cathepsin Inhibitors

In an *in vitro* experiment in Crandell-Rees feline kidney cells, 3CL protease inhibitors and cathepsin inhibitors were tested for their ability to inhibit FCoV replication.[33] Both types of drugs produced effective inhibition with $EC_{50}$ values in the nanomolar range and each drug tested was nontoxic to the cells at effective concentrations. The 3CL protease inhibitors were more effective than the cathepsin inhibitors and when used in combination, these drugs had strong synergic effects. There have not been any *in vivo* studies with these drugs in cats to date. In one *in vitro* study, 16 antiviral compounds, including nucleoside analogues used to treat herpesviruses, NRTI used for HIV, and protease inhibitors also used for HIV, were tested for their ability to inhibit FCoV in cell culture.[35] Among the 16 drugs tested, two showed significant inhibition of FCoV when compared to the untreated cells. These were nelfinavir and *Galanthus nivalis* agglutinin (GNA). Nelfinavir is a HIV protease inhibitor that has been shown to target the 3CL protease of SARS-CoV by interacting with 18 residues of the protease. The drug was slightly less effective against FCoV than against SARS-CoV, likely because only seven of the corresponding residues of the 3CL protease of FCoV are identical to the SARS-CoV protease.[35] GNA, a carbohydrate-binding agent, exhibits its antiviral effect by binding to coronavirus glycosylated envelope glycoproteins, thereby inhibiting viral attachment to the host cell. The antiviral effects were concentration dependent, and nelfinavir displayed cellular toxicity at higher doses. GNA was a better inhibitor of FCoV, and when the two agents were added together, a synergistic antiviral effect was produced. The results suggest that the combined use of GNA and nelfinavir could have therapeutic potential in the treatment of cats with FIP.[35]

## Fusion Inhibitors

Viral fusion has also been targeted effectively with a synthetic peptide based on the putative HR2 sequence of FCoV. Virus replication was significantly inhibited *in vitro* compared to controls, and the peptide was nontoxic.[34] This peptide was also used in combination with human IFN-alpha. The two displayed a synergistic effect, but the cells were pretreated with IFN prior to infection by the virus.[34] See the section on interferon for further information about interferon treatment for FIP.

## Polyprenyl Immunostimulant

Immunomodulators have been considered because FIP is an immune-mediated disease.[35]

A drug that has shown promise for immunomodulation is PI.[36] This drug has a conditional license in the United States for treatment of FHV-1 infection. In a case series of three cats, PI was associated with prolonged survival in cats with noneffusive FIP.[36] No placebo group was included for comparison, so definitive conclusions about the effectiveness of this drug for treatment of FIP cannot be drawn.

Additional immunostimulants such as ImmunoRegulin (*Propionibacterium acnes*), an inactivated bacterin, and a T-cell receptor peptide (manufactured by Imulan Biotherapeutics), have been suggested for treatment of FIP. These are not antiviral drugs; instead, each of these products is reported to stimulate the immune response toward a cell-mediated response or to reduce an overactive type 2 helper T-cell (Th2) response. An imbalance in T-cell versus B-cell immune response has been suggested to contribute to the development of FIP. It has been proposed that a strong cell-mediated immune response protects a cat from the development of FIP, whereas the production of antibodies is counterproductive, enhancing the uptake and replication of feline infectious peritonitis virus (FIPV) in macrophages and contributing to the pathology by producing a type III hypersensitivity vasculitis. However, this hypothesis has been questioned.[37] Therefore, even though the use of these types of drugs for stimulation of a cell-mediated response might seem a logical approach for the treatment of FIP, there is currently no data to support their use.

An additional non-antiviral drug that has been evaluated for treatment of FIP is propentofylline. This drug appears to downregulate proinflammatory cytokines, which in turn can reduce vasculitis. Vasculitis, as stated earlier, is responsible for pathology associated with FIP. However, in a placebo-controlled, double-blind study in cats with late stage FIP,

there was no statistically significant difference in the survival time, the quality of life, or any clinical or laboratory parameter in cats treated with this drug versus cats receiving a placebo.[38] Of the cats in the study, 21 of 23 cats displayed effusion at the start of the study. The drug might be more useful in cats without effusion because it may have a chance to prevent vasculitis and therefore effusions, but studies are lacking.

## INTERFERON

Interferons are molecules produced by vertebrate cells in response to viral infections or certain inert substances, such as double-stranded RNA, and other microbial agents. There are three types of IFNs. Type I IFNs comprise the largest subfamily and include IFN-α, IFN-β, and IFN-omega. Type I IFNs are produced by various cell types, such as leukocytes and fibroblasts, in direct response to virus infection.[39] There is only one member of the type II IFN subfamily, IFN-γ, that is an immunomodulatory cytokine, produced in response to recognition of infected cells by T lymphocytes and natural killer cells of the host's immune system.[39] Type III IFNs, which contain three ILs, (IL-28A, IL-28B, and IL-29), are identified. This subfamily also has the ability to interfere with virus replication and has been suggested to be the ancestral antiviral system of vertebrates.[39] Interferons are not virucidal; rather, they trigger expression of various antiviral proteins and thus induce an antiviral state within the host cell to limit replication and spread of viruses. Further, type I IFNs have been shown to potently enhance innate and adaptive immune responses *in vivo*, through various immunomodulatory effects, such as activation of dendritic cells (DCs), amplification of antibody response, and enhancement of T-cell and natural killer cell cytotoxicity.[40] Viruses causing lysis of their target cell are most effectively inhibited by IFN through their antiviral activity in noninfected cells. Therefore, IFNs have their highest utility in the prophylaxis or early postexposure management for virus infections. Given that IFNs are not specific for a particular virus, they have been tested for the treatment of multiple feline viruses, including FHV-1, FIV, FeLV, feline calicivirus (FCV), and FCoV.[40]

Two molecules of type I IFNs are currently being used for therapy in cats: human recombinant interferon alpha (rHuIFN-α), and rFeIFN-omega, which is licensed for use in cats and dogs in Europe, Australia, and some Asian countries. IFNs are not strictly species-specific in their effects; however, their biologic activity and toleration are greater in cells of genetically related species. *In vitro* results suggest that rFeIFN-omega would likely be more effective than rHuIFN-α *in vivo*, although both IFNs have been shown to have therapeutic value in cats.[40]

There are two common treatment regimens for use of rHuIFN-α in cats: injection of a high dose ($10^4$ to $10^6$ International Unit per kg SC every 24 hours) or oral application of a low dose (1 to 50 International Unit [IU] per kg every 24 h). When given parenterally to cats, rHuIFN-α becomes ineffective within a few weeks because of the development of neutralizing antibodies that limit its activity.[40,41] rHuIFN-α can be given orally for a longer period because no antibodies will develop during oral treatment. Unlike rHuIFN, rFeIFN-omega, being a feline recombinant product does not induce neutralizing antibodies when administered SC. This means that the high-dose parenteral protocol can be used safely and efficiently even if repeat administration is required. This is an important factor to consider in a condition where management needs to be lifelong.[42]

Given PO, IFNs are inactivated by gastric acid and destroyed by trypsin and other proteolytic enzymes in the duodenum and therefore are not absorbed and cannot be detected in the blood after oral administration. Direct antiviral effects are unlikely after oral application; however, IFN still seem to have immunomodulatory activity. Type I IFNs likely bind to mucosal receptors in the oral cavity, stimulating the local lymphoid tissue, leading to cytokine release from lymphatic cells in the oropharyngeal lymphoid tissues, triggering a cascade of immunologic responses that act systemically.[43]

### Feline Retroviruses

Interferons have been used for the treatment of feline retrovirus infections. Treatment with IFN improved the clinical condition scores of cats infected with FeLV and FIV, but not because of a reduction in viral load. This suggests that the improved clinical condition seen with treatment is not specific to an antiviral effect, at least not for FIV and FeLV, but instead is a result of immunomodulation, potentially associated with the innate immune response.[40,42,44]

Some clinical signs in FIV-infected cats are caused by immunopathological reactions, such as gingivostomatitis and uveitis. Immunomodulation might be the cause of improvement of some clinical signs associated with IFN treatment, probably the result of an effective control over inflammatory cytokines in diseased organs.[44] It has been suggested that a nonspecific stimulation of the immune system with IFN therapy might be contraindicated in FIV-infected cats because it could lead to a rise in viral replication produced by the activation of lymphocytes and macrophages harboring latent infections and therefore accelerate disease progression in these cats, and the use of IFN in HIV-infected humans is controversial.[5] However, use of low-dose oral HuIFN (natural, not recombinant in this study) in ill FIV-infected cats (50 IU per kg on the oral mucosa daily for 7 days on alternating weeks for 6 months, followed by a 2-month break, and then repetition of the 6-month treatment) resulted in improvement of clinical signs in a placebo-controlled, double-blind study.[44] Parenteral rFeIFN-omega used according to the licensed protocol (Table 7-1) resulted in decreased mortality rates in FeLV-infected cats, compared with the control group in another placebo-controlled study.[40,45] In another study evaluating FIV- and/or FeLV-infected cats housed in a shelter, hematologic values remained within reference ranges, and there were no biochemical abnormalities

associated with rFeIFN-omega treatment used according to the licensed protocol.[41] These findings suggest that IFN treatment is safe for treatment of FIV- and FeLV-infected cats,[40,41,44,45] but further studies are required to clearly demonstrate its efficacy against FIV and FeLV *in vivo*.

A recent study evaluated the use of oral administration of rFeIFN-omega for the treatment of symptomatic naturally infected, client-owned FIV-infected cats.[42] The treatment protocol was $10^5$ IU/cat PO every 24 hours for 90 consecutive days, administered by the cats' owners. A historical group that was treated SC with the licensed protocol[41] was used as a control for comparison, but no placebo group was included. Treatment resulted in significant improvement of clinical scores between pretreatment and post-treatment values, and there was no significant difference between the SC historical control group and the PO group, suggesting that PO administration of rFeIFN-omega could be used effectively as an alternative to the licensed protocol, at a significantly reduced cost.[42]

An additional benefit of using IFN therapy for FIV and FeLV treatment could be the effect of IFN on opportunistic infections by other viruses, including FHV-1 and FCV.[41] In fact, the effect of IFN on these additional viral infections might be the cause of the improved clinical scores associated with IFN treatment.[40,41] Both FIV and FeLV replicate in lymphoid and monocytoid cell subsets and cause immunosuppression. In FIV-infected cats, most of the clinical signs are not directly caused by the FIV itself but are the result of secondary infections, as well as neoplasia.[40,46] Although FeLV causes more severe clinical syndromes than FIV does, diseases secondary to immunosuppression account for a large portion of the syndromes seen in FeLV-infected cats as well.[11] Considering that IFN therapy seems to have no effect on FIV and FeLV virus load but it is immunomodulatory, it would seem advisable to treat retrovirus-infected cats with IFN when they have clinical signs, as they would benefit from its effects in improving their clinical condition.[40]

## Feline Herpesvirus Type I and Feline Calicivirus

A recent study attempted to evaluate the hypothesis that improvement in clinical scores with IFN treatment in FIV- and FeLV-infected cats might be a reflection of reduction in viral shedding of secondary viruses in these cats.[41] Sixteen naturally infected FIV- and/or FeLV-infected cats (seven FIV, six FeLV, and three coinfected) were followed during rFeIFN-omega therapy (used according to the licensed protocol) to monitor clinical signs and to correlate them with excretion of concomitant viruses (FCV, FHV-1, FCoV, and feline parvovirus [FPV]). Shedding of these viruses was evaluated by real-time quantitative polymerase chain reaction (PCR) (FHV-1 and FCoV) or conventional PCR (FCV and FPV). Pretreatment and post-treatment samples were compared. Feline calicivirus shedding was detected in 13 of 16 cats on day 0 and not detected on day 65. The amount of FHV-1 shedding was significantly reduced in the cats at the end of the study (day 65), compared with the beginning. Feline coronavirus shedding was reduced but not significantly, and there was not enough FPV detected in the population to draw any significant conclusions.[41] However, there was no placebo group used for this study, and without a placebo group, it is difficult to determine definitively if the results are due to antiviral effects of IFN or are just consistent with the natural resolution of viral shedding.

In a separate study, 36 cats with naturally acquired upper respiratory tract disease housed in a humane society facility were treated with one drop of rFeIFN-omega solution ($10^6$ unit/mL), rHuIFN-α solution ($10^6$ IU/mL), or saline (0.9% NaCl) solution (12 cats/group) in each eye twice daily for 14 days for the treatment of keratoconjunctivitis.[46] There was no statistical difference between the treatment groups and the placebo group with regard to clinical scores or viral shedding (FHV-1 and FCV), determined by real-time quantitative PCR from oropharyngeal and conjunctival swabs. Feline herpesvirus type 1 shedding was lower, though not statistically significant, on day 14 compared with day 0 for all groups (including the placebo group), and clinical scores were significantly decreased on day 14 compared to day 0, again for all groups including the placebo group.[46] Therefore, comparing results between days 0 and 14 in the treated cats without the inclusion of the placebo group would have resulted in a different conclusion for this study. These cats were not infected with FeLV, and even though the FIV status was unknown for all the cats, the ones that were tested were negative. However, this study highlights the need for a placebo group for accurate evaluation of the effect of IFN therapy on FHV-1 and FCV shedding and associated disease.

Oral and SC IFN therapy has been associated with an improvement in oral ulcers and gingivitis and gingivostomatitis in cats infected with FIV,[40,41] a condition that is common in cats with FCV infections.[41] Feline calicivirus is also associated with chronic gingivostomatitis in cats not infected with FIV or FeLV,[43] and a study evaluated the efficacy of rFeIFN-omega ($10^5$ IU/day for 90 days by topical oromucosal administration) for the treatment of FCV-associated feline chronic gingivostomatitis (FCGS) and caudal stomatitis in FIV-/FeLV-negative cats.[43] Cats were included in the study if they continued to show persistence of clinical signs of FCGS at least 2 months after periodontal treatment (scaling, subgingival débridement, and polishing), tooth extraction, and 3 weeks of antibiotics with analgesic and anti-inflammatory drugs as needed. Twenty-four cats were treated with the IFN, and the effect was compared with a positive control group that received a standard corticosteroid therapy. The results suggested that the IFN therapy was as effective as the corticosteroid treatment for this condition for improvement in clinical signs.[43] Feline calicivirus viral loads were not evaluated in this study, and there was no placebo group used for comparison. However, assuming the IFN therapy was the cause of the improvement seen in these cats, the results add to the hypothesis that improvement in oral lesions in FIV- and/or FeLV-infected cats likely is associated with the effect of IFN on opportunistic viral infections. Differences in the outcome of the different studies could be due to different

application methods (e.g., ocular versus oral); however, definitive conclusions cannot be drawn without additional studies that also evaluate viral load in naturally infected cats that are randomized, placebo-controlled, and double-blinded.

## Feline Coronavirus and Feline Infectious Peritonitis

IFN has also been evaluated for treatment of FIP. In a randomized placebo-controlled, double-blinded treatment trial, 37 cats with FIP were treated with rFeIFN-omega or placebo.[47] In all cats, FIP was confirmed by histology and/or immunohistochemical or immunofluorescence staining of FCoV antigen in effusion or tissue macrophages. All cats received glucocorticoids, either as dexamethasone in case of effusion (1 mg/kg intrathoracic or intraperitoneal injection every 24 hours) or prednisolone (2 mg/kg PO every 24 hours). Cats also received either a placebo or rFeIFN-omega at $10^6$ IU/kg SC every 24 hours for 8 days and subsequently once a week. There was no statistically significant difference in the mean survival time of cats treated with rFeIFN-omega versus the placebo. Cats survived for a period of 3 to 200 days before euthanasia with a mean survival time of 18 days. There was only one long-term survivor (>3 months) in the rFeIFN-omega group. Interferon treatment might be more effective if started earlier, but this is not of relevance in the treatment of cats with FIP in the field.[47] However, IFN therapy might be useful for treatment of cats with chronic FCoV shedding, but further studies are required. As previously mentioned, treatment with rFeIFN-omega (licensed SC protocol) was associated with a decrease in FCoV shedding in FIV- and/or FeLV-infected cats; however, the results were not compared with a placebo group.[41]

## SUMMARY

In conclusion, antivirals are still in their infancy for the treatment of feline diseases. However, as new drugs are produced for human viral diseases that can be used for feline patients, and testing of currently available drugs continues, it is hoped that determination of effective protocols for treatment of feline viral diseases will be possible in the future.

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