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TITLE:
Efficacy of Antiviral Drugs against Feline Immunodeficiency Virus

ABSTRACT:
Feline immunodeficiency virus (FIV) is one of the most common infectious agents affecting cats worldwide .FIV and human immunodeficiency virus (HIV) share many properties: both are lifelong persistent lentiviruses that are similar genetically and morphologically and both viruses propagate in T-lymphocytes, macrophages, and neural cells. Experimentally infected cats have measurable immune suppression, which sometimes progresses to an acquired immunodeficiency syndrome. A transient initial state of infection is followed by a long latent stage with low virus replication and absence of clinical signs. In the terminal stage, both viruses can cause severe immunosuppression. Thus, FIV infection in cats has become an important natural model for studying HIV infection in humans, especially for evaluation of antiviral compounds. Of particular importance for chemotherapeutic studies is the close similarity between the reverse transcriptase (RT) of FIV and HIV, which results in high in vitro susceptibility of FIV to many RT-targeted antiviral compounds used in the treatment of HIV-infected patients. Thus, the aim of this article is to provide an up-to-date review of studies on antiviral treatment of FIV, focusing on commercially available compounds for human or animal use.

Reverse Transcriptase Inhibitors ::: 1. Introduction:
The most commonly used antiretroviral drugs in human and veterinary medicine are RTIs. There are three categories of RTIs: nucleoside analogue RTIs (NARTIs, Section 1), nucleotide analogue RTIs, Section 2, and non-nucleoside RTIs (NNRTIs, Section 3) [5,7]. A nucleoside consists of a nitrogenous base covalently attached to a sugar (ribose in RNA, 2-deoxyribose in DNA), and a nucleotide consists of a nitrogenous base, a sugar, and a phosphate group. Nucleic acid (RNA and DNA) contains a chain of nucleotides covalently linked to form a sugar-phosphate backbone with protruding nitrogenous bases. Prior to linkage of a new nucleotide (or monophosphate) to the nucleic acid, three phosphate groups must be bound to the nucleoside (triphosphate), two of which are removed, releasing energy during elongation of the nucleic acid chain [5].

2.1. Zidovudine ::: 2. Nucleoside Analogue Reverse Transcriptase Inhibitors:
Zidovudine (3′-azido-2′,3′-dideoxythymidine, AZT) was first synthesized in the 1960s [11] as a potential anticancer drug. In 1985 it was shown to be effective against HIV [12] and became the first drug approved for treatment of HIV infection [13].
The anti-FIV activity of zidovudine has been assessed in numerous in vitro studies in different cell systems [14,15,16,17,18,19,20,21,22,23,24,25,26]. The first in vitro study was carried out in 1989, when North and coworkers showed that zidovudine inhibited FIV replication in Crandell-Rees feline kidney (CRFK) cells. The susceptibility of FIV to zidovudine was similar to that of HIV [27]. There is evidence that FIV can become resistant to nucleoside analogues, as is the case in HIV. Zidovudine-resistant FIV mutants can arise after only six months of use, and a single-point mutation in the FIV gene is responsible for resistance [10].
In vivo, zidovudine can reduce plasma viral load, improve the immunologic and clinical status of FIV-infected cats, increase quality of life, and prolong life expectancy [16]. In placebo-controlled trials, zidovudine improved stomatitis and increased the CD4/CD8 ratio in naturally FIV-infected cats. In some cats with FIV-associated neurologic signs, marked improvement was reported within the first days of therapy [28,29].
Zidovudine not only inhibits RT, but also cellular polymerases, and this can lead to bone marrow suppression. Regular blood cell counts are necessary during zidovudine treatment because non-regenerative anemia is a common side effect [28]. Cats with bone marrow suppression should not be treated with zidovudine. Most FIV-infected cats treated with zidovudine for as long as two years tolerated the drug well. The hematocrit can decline within three weeks of initiating treatment to approximately 50% of baseline but increases afterwards in most cases, even without discontinuation of treatment. If the hematocrit drops below 20%, discontinuation of treatment is recommended, and anemia usually resolves within a few days. Other side effects in cats, including vomiting or anorexia, are rare [28].

2.2. Stavudine ::: 2. Nucleoside Analogue Reverse Transcriptase Inhibitors:

Stavudine (2′,3′-didehydro-2′,3′-dideoxythymidine, d4T) is another drug effective against HIV. It was approved for treatment of HIV infection in 1994, but in recent years has been replaced in most multi-drug treatment protocols by compounds with fewer side effects [30,31,32,33,34]. Stavudine is active against FIV in vitro [18,19,20,23,26,35,36]. Mutants of FIV that are resistant to stavudine and cross-resistant to several other antivirals, including zidovudine, have been detected. Resistance is caused by a single-point mutation in the RT-encoding region of the pol gene [26]. No in vivo data in FIV-infected cats have been published.

2.3. Didanosine ::: 2. Nucleoside Analogue Reverse Transcriptase Inhibitors:
Didanosine (2′,3′-dideoxyinosine, ddI) was shown to be active against HIV in 1986 [37]. In the United States, it was the second drug to be approved for treatment of HIV and has been on the market since 1991 [5].
Didanosine is active against FIV in vitro [14,18,20,21,22,23,24,26,38]. In one experimental in vivo study, FIV replication was significantly suppressed in animals treated with didanosine, but treatment contributed to the development of antiretroviral toxic neuropathy [39].

2.4. Lamivudine ::: 2. Nucleoside Analogue Reverse Transcriptase Inhibitors:
Lamivudine (2R,cis-4-amino-l-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one, 3TC) is also an anti-HIV drug, approved in 1995 [40].
Lamivudine is active against FIV in vitro [3,20,21,23,38,41]. A combination of zidovudine and lamivudine had synergistic anti-FIV activities in cell cultures [41]. FIV mutants resistant to lamivudine and containing a point mutation in the RT gene were selected in vitro and showed cross resistance to zidovudine [23].
In one in vivo study, experimentally FIV-infected cats were treated with a high-dose zidovudine/lamivudine combination, which protected some cats from infection when treatment was started before virus inoculation. However, zidovudine/lamivudine treatment showed no anti-FIV activity in chronically infected cats. Severe side effects, including fever, anorexia, and marked hematologic changes, were observed in some of the cats with this high-dose dual-drug treatment [41]. Thus, high-dose lamivudine treatment alone, or in combination with zidovudine, is not recommended in naturally FIV-infected cats.

2.5. Emtricitabine ::: 2. Nucleoside Analogue Reverse Transcriptase Inhibitors:
Emtricitabine (2',3'-deoxy-5-fluoro-3'-thiacytidine, FTC) is structurally similar to lamivudine and was licensed by the FDA in 2003 [40]. In vitro, antiviral efficacy has been demonstrated against FIV [17,20,21,22], but to date there have been no in vivo studies in FIV-infected cats.

2.6. Abacavir ::: 2. Nucleoside Analogue Reverse Transcriptase Inhibitors:
Abacavir ((1S,4R)-4-[2-amino-6-(cyclopropylamino)-9H-purin-9-yl]-2-cyclopentene-1-methanol, ABC) was shown to be active against HIV in 1986 and belongs to the FDA-approved anti-HIV compounds [40]. Abacavir is active against FIV in vitro, but had higher levels of cytotoxicity than other compounds, such as didanosine and amdoxovir [16,20]. There are no in vivo studies of this drug in FIV-infected cats.

3.1. Adefovir ::: 3. Nucleotide Analogue Reverse Transcriptase Inhibitors:
Adefovir (2-(6-amino-9H-purin-9-yl)-ethoxy-methyl-phosphonic acid, PMEA) is active against herpesviruses, hepadnaviruses (hepatitis B), and retroviruses [44]. Adefovir is not licensed as an HIV drug, but is currently available as an oral formulation (bis-POM PMEA) approved for the treatment of chronic hepatitis B. Adefovir belongs to the acyclic nucleoside phosphonates, in which the alkyl side chain of purines and pyrimidines is linked to a modified phosphate moiety and a C-P phosphonate linkage replaces the normal O5′-P phosphate linkage [43,45]. This phosphonate bond is not hydrolysable, which makes it more difficult to cleave off these compounds once they have been incorporated at the 3′-terminal end of the elongating proviral DNA strand [5]. Adefovir inhibits FIV replication in vitro [46].
Several studies have investigated the efficacy of adefovir in either experimentally and naturally FIV-infected cats [47,48,49,50,51,52,53]. A few of those studies showed some efficacy, but also reported severe side effects, mainly non-regenerative anemia. In a recent study, adefovir was administered to FIV-infected cats in a six-week placebo-controlled, double-blinded clinical trial; ten cats received adefovir (10 mg/kg SC twice weekly) and ten cats received placebo. There was no decrease in proviral or viral loads in treated cats, and treated cats developed a progressive, sometimes life-threatening anemia, which is a common adverse effect of NtARTIs [53]. This shows

that results obtained in experimental studies cannot always be applied to a field situation and emphasizes the importance of controlled clinical field trials. Based on the lack of efficacy in the recent placebo-controlled field trial and the side effects, adefovir cannot be recommended for treatment of FIV-infected cats.

3.2. Tenofovir ::: 3. Nucleotide Analogue Reverse Transcriptase Inhibitors:
Currently, the only approved NtARTi for the treatment of HIV infection is tenofovir disoproxil fumarate (TDF), the prodrug of tenofovir ((R)-9-(2-phosphonylmethoxypropyl)adenine, (R)-PMPA), which is also a member of the acyclic nucleoside phosphonates [43,45]. The antiviral spectrum of tenofovir (2R-1-(6-amino-9H-purin-9-yl)-propan-2-yl-oxy-methyl-phosphonic acid, PMPA) is narrower than that of adefovir; it does not encompass herpesviruses, but is confined to hepadna- and retroviruses [44]. Tenofovir disoproxil fumarate has become one of the most commonly used drugs in HIV therapy since its licensing in 2001 [5,9].
Tenofovir is effective against FIV in vitro [25,45], and there is some evidence that tenovovir might have greater anti-FIV efficacy with less cytotoxicity than other antiretroviral compounds, including adefovir [45,54]. However, in vivo studies are lacking and should be a focus of future research.

Suramin ::: 4. Non-Nucleoside Reverse Transcriptase Inhibitors:
Suramin (1-(3-benzamido-4-methylbenzamido)-naphthalene 4,6,8-trisulfonic acid sym-3′-urea sodium salt), a sulfated naphthylamine and trypan red derivative, is one of the oldest known antimicrobial agents. It has been used as an antitrypanosomal agent and for the treatment of some tumors, such as prostate cancer [58]. It also has an inhibitory effect on the RT activity of retroviruses and has also been used in humans with HIV infection [59]. Suramin inhibits RT by interacting with the template-primer binding site of the enzyme. Thus, it competitively binds to the primer binding site (without being a nucleoside analogue) and inhibits the template-primer binding that is necessary for DNA elongation. Suramin can therefore be classified as an NNRTI [60].
Suramin is effective against feline leukemia virus (FeLV) in vivo [61,62], and thus, could potentially be active against FIV, although this has not been investigated.
Suramin is associated with a significant number of severe side effects in humans, such as nausea and anaphylactic shock as immediate reactions during administration and peripheral neuritis leading to palmar-plantar hyperesthesia, photophobia, skin reactions, agranulocytosis, hemolytic anemia, and destruction of the adrenal cortex as later side effects [58,59,63,64,65]. In cats with FeLV infection, the major adverse effects of suramin were transient vomiting and anorexia [61].

5.1. Foscarnet ::: 5. Nucleotide Synthesis Inhibitors:
Foscarnet (phosphonoformic acid, PFA) has broad-spectrum antiviral activity against DNA and RNA viruses, including retroviruses. It is FDA-approved for the treatment of HIV-associated cytomegalo and herpes simplex virus infections in humans [67]. Foscarnet is usually administered intravenously by continuous intravenous infusion because of its short half-life, which has also been demonstrated in cats [68]. Oral administration of the drug is possible but can result in irritation of mucous membranes and oral bleeding. Foscarnet has many side effects, including nephrotoxicity and myelosuppression, in both humans and cats. It also is toxic to epithelial cells and mucous membranes, resulting in gastrointestinal side effects and genital epithelium ulceration. In addition, it chelates various cations, which can lead to hypocalcemia, hypomagnesemia, and hypokalemia [69,70].
In vitro, foscarnet has been shown to be active against FIV, but foscarnet-resistant FIV strains can develop [14]. No in vivo studies in FIV-infected cats have been carried out, likely because of the severe side effects and necessity for continuous intravenous administration of the drug.

5.2. Ribavirin ::: 5. Nucleotide Synthesis Inhibitors:
Ribavirin (1-β-d-ribofuranosyl-1 H-1,2,4-triazole-3-carboxamide, RTCA) has marked in vitro antiviral activity against a variety of DNA and RNA viruses [71]. Systemic administration of ribavirin is limited in cats because of side effects [72]. Sequestration of ribavirin within erythrocytes results in hemolysis, even when low doses of the drug are used [73,74]. In addition, there is a dose-related toxic effect on bone marrow, primarily on megakaryocytes, resulting in thrombocytopenia and hemorrhage. With prolonged ribavirin treatment or at higher doses, the production of erythrocytes and neutrophils also is suppressed. Ribavirin also can induce hepatic toxicity. An attempt to decrease the toxicity of ribavirin by incorporating it into lecithin-containing liposomes and administering it at lower doses was not successful [75].

Ribavirin is active against many viruses in vitro, including FIV [23,76]. Therapeutic concentrations are difficult to achieve in vivo because of toxicity [74]. To date, the efficacy of ribavirin has not been investigated in FIV-infected cats.

Plerixafor ::: 6. Receptor Homologues/Antagonists:
Plerixafor (1,1′-(1,4-phenylenbismethylene)-bis(1,4,8,11-tetraazacyclotetradecane)-octachlo-ride dehydrate, AMD3100) is the bicyclam prototype compound. It is not marketed as an anti-HIV drug, but is used in humans for stem cell mobilization [84].
Plerixafor is active against FIV in vitro [82]. In a placebo-controlled double-blinded clinical trial, treatment of naturally FIV-infected cats with plerixafor resulted in a significant decrease in proviral load in treated cats when compared to the placebo group. There was a concomitant decrease in serum magnesium levels, which did not produce any clinical consequences. Development of resistance of FIV isolates to plerixafor did not occur during treatment [53]. In cats, plerixafor is administered at a dosage of 0.5 mg/kg every 12 h. Monitoring of magnesium and calcium levels should be performed at regular intervals during treatment [53]. Further studies investigating the potential of this promising drug are needed.

7.1. Tipranavir ::: 7. Protease Inhibitors:
Tipranavir (N-[3-[(1R)-1-[(2R)-6-hydroxy-4-oxo-2-(2-phenylethyl)-2-propyl-3H-pyran-5-yl]propy l]phenyl]-5-(trifluoromethyl)pyridin-2-sulfonamid) was approved in 2005, and is used as an anti-HIV compound. The drug was shown to be active against FIV in vitro.Tipranavir completely prevented FIV replication [85,86]. No studies in FIV-infected cats exist so far, and further studies are needed to investigate the potential of tipranavir in naturally infected cats.

7.2. Lopinavir ::: 7. Protease Inhibitors:
Lopinavir (2S)-N-[(2S,4S,5S)-5-[2-(2,6-dimethylphenoxy)acetamido]-4-hydroxy-1,6-diphenylhex-an-2-yl]-3-methyl-2-(2-oxo-1,3-diazinan-1-yl)butanamide) is an anti-HIV compound, approved in 2001. The drug was shown to be active against FIV in vitro but did not prevent FIV replication completely [85]. There are no in vivo studies in FIV-infected cats.

7.3. Atazanavir ::: 7. Protease Inhibitors:
Atazanavir (methyl N-[(2R)-1-[2-[(2S,3S)-2-hydroxy-3-[[(2R)-2-(methoxycarbonylamino)-3,3-di-methylbutanoyl]amino]-4-(2,3,4,5,6-pentadeuteriophenyl)butyl]-2-[(4-pyridin-2-ylphenyl)methyl]hy-drazinyl]-3,3-dimethyl-1-oxobutan-2-yl]carbamate) was licensed by the FDA in 2003 and is also used as an anti-HIV compound. Similar to lopinavir, some efficacy of atazanavir was shown against FIV in vitro, but there are no in vivo studies published so far [85].

Raltegravir ::: 8. Integrase Inhibitors:
Raltegravir is used as an anti-HIV compound. The drug was shown to be active against FIV in vitro [92], but FIV was less susceptible to raltegravir than HIV [92].
No studies in FIV-infected cats exist so far. Although there are no in vivo studies on the efficacy of raltegravir in FIV-infected cats, the drug recently was shown to be effective against FeLV and was safe in cats [93].

9.1. Human Interferon-α ::: 9. Interferons:
Recombinant human interferon-α (rHuIFN-α) has antiviral and immune-modulatory activity. IFN-α is active against many DNA and RNA viruses [98]. There are two common treatment regimens for use of rHuIFN-α in cats: SC injection (104 to 106 U/kg every 24 h) or oral application (1 to 50 U/kg every 24 h).
Human IFN-α becomes ineffective after three to seven weeks of parenteral use in cats because of the production of neutralizing antibodies [100]. Anti-IFN-α antibody production does not occur with oral administration of IFN-α and therefore this route allows for a longer period of treatment. IFN-α is inactivated by gastric acid and destroyed by trypsin and other proteolytic enzymes in the duodenum [101], which means that direct antiviral effects are unlikely after oral application. However, oral IFN-α appears to have immuno-modulatory activity, because it can stimulate local lymphoid tissue. The release of cytokines by lymphatic cells in the oropharyngeal area triggers a cascade of immunologic responses with systemic effects [102,103,104].
RHuIFN-α has been shown to be active against FIV in vitro [105]. Although frequently used in the field for treating FIV-infected cats, controlled studies evaluating the effect of parenteral administration of rHuIFN-α in FIV-infected cats have not been conducted.

Use of oral rHuIFN-α in 24 ill, naturally FIV-infected cats (50 U/kg applied to the oral mucosa daily for seven days on alternating weeks for six months, followed by a two-month break, and then repetition of the six-month treatment) resulted in improvement of clinical signs (e.g., fever, lymphadenopathy, opportunistic infections) in a placebo-controlled, double-blinded study [106]. However, proviral and viral loads were not monitored during thiat study and therefore it is impossible to conclude whether treatment with rHuIFN- α had indeed an effect on FIV, or rather on secondary infections.

9.2. Feline Interferon-ω ::: 9. Interferons:
Recombinant feline interferon-ω (rFeIFN-ω), the corresponding feline interferon, is licensed for use in veterinary medicine in Japan, Australia, and some European countries. It can be used in cats for long periods without antibody development, and no major severe side effects have been reported [107].
IFN-ω inhibits FIV replication in vitro [105]. One placebo-controlled, multicenter study that investigated the effect of parenteral rFeIFN-ω against FIV infection in 62 naturally FIV-infected cats (treated with 106 U/kg SC q 24 h on five consecutive days) did not find a difference in the survival rate in treated cats. However, some improvement in clinical scores, including eight categories of clinical signs (rectal temperature, behavior, appetite, thirst, dehydration, mucous membrane appearance, stomatitis, and death) as well as improvement in laboratory abnormalities (leukopenia, leukocytosis, and anemia) occured [107]. In another study, which evaluated naturally FIV-infected cats housed in a shelter, some clinical improvement was observed after parenteral rFeIFN-ω (106 U/kg SC q 24 h on FIVe consecutive days for three cycles), but this study lacked a placebo control. In that same study, hematologic values remained within reference intervals, and there were no biochemical abnormalities associated with rFeIFN-ω treatment [96].
A recent study evaluated the use of oral administration of rFeIFN-ω for the treatment of eleven client-owned, naturally FIV-infected cats with clinical signs [108]. The treatment protocol was 105 U/cat PO q 24 h for 90 consecutive days, administered by the cats' owners. A historical retrospective group was used as a control for comparison (106 U/kg SC q 24 h on five consecutive days for three cycles), but a placebo group was not included. Treatment with oral rFeIFN-ω resulted in a significant improvement in clinical scores (e.g., oral lesions, coat appearance, body condition score, and ocular discharge) after treatment. In addition, there was no significant difference between the SC historical control group and the PO group, suggesting that oral administration of rFeIFN-ω might be a viable and less expensive alternative [109]. In a recently published study that assessed viremia, provirus load, and blood cytokine profile in naturally FIV-infected cats treated with oral rFeIFN-ω (105 U/cat PO q 24 h for 90 days) or with subcutaneous rFeIFN-ω (106 U/cat SC q 24 h for 5 consecutive days in three courses), no change in the level of viremia or in most cytokine levels was found; a placebo control group was not included [109]. The fact that virus load remained unchanged but some clinical improvement was observed in earlier studies suggests that rFeIFN-ω has an effect on secondary infections rather than on FIV itself [94]. As there are major differences in outcomes of the different studies on feline IFN-ω in FIV-infected cats. Thus, a definitive conclusion cannot be drawn without additional randomized, placebo-controlled, and double-blinded studies that include a sufficiently high number of naturally FIV-infected cats.

10. Conclusions:
Unfortunately, the efficacy of antiviral compounds for the treatment of FIV in cats has been generally poor. The duration of treatment in many clinical trials was relatively short and might have been inadequate for infections with a long clinical course. In addition, it is difficult to compare treatment results of cats infected experimentally and kept under laboratory conditions and pet cats infected with field strains of FIV. Therefore, further well-designed double-blinded, placebo-controlled trials using antiviral drugs in naturally FIV-infected cats are needed to determine the efficacy and side effects of different antiviral compounds.