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Combined effect of zidovudine (ZDV), lamivudine (3TC) and abacavir (ABC) antiretroviral therapy in suppressing in vitro FIV replication

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Abstract

In view of close similarities at the molecular and clinical levels, feline immunodeficiency virus (FIV) infection of the domestic cat is subject of increasing attention as an animal model for human immunodeficiency virus (HIV) infection. A range of reverse transcriptase inhibitors effective against HIV are also active against FIV, allowing successful use of the cat model to investigate drug interactions and resistance development. Nevertheless, while combined nucleoside analog and protease inhibitor usage has proven remarkably effective in treating HIV infection, combination antiretroviral therapy of FIV infection has been hampered by lack of protease inhibitors specific for FIV. In an attempt to circumvent this problem, we have examined the feasibility of applying in the FIV system combination protocols lacking a protease inhibitor. We now report that, as observed during HIV infection, the nucleoside analog abacavir (ABC or 1592U89) is able to effectively block in vitro FIV-replication. Furthermore, we demonstrate that combined usage of ABC with the nucleoside analogs zidovudine (ZDV or AZT) and lamivudine (3TC) also blocks in vitro FIV replication in a synergistic manner. However, in contrast to its effect on HIV replication, the ribonucleotide reductase inhibitor hydroxyurea (HU) is unable to effectively control in vitro FIV replication.

Keywords: FIV; In vitro; Antiretroviral; Zidovudine; Lamivudine; Abacavir; Hydroxyurea

1. Introduction

Of the different animal models for human immunodeficiency virus (HIV) infection, feline immunodeficiency virus (FIV) infection of the domestic cat has seen increasing application in view of close similarities at both the molecular

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and clinical levels (Willett et al., 1997). A range of antiretroviral compounds capable of controlling HIV replication are also effective against FIV, leading to suggestion that the cat model is of value in exploring new therapeutic strategies (North and LaCasse, 1995). Nevertheless, while combination therapy consisting of two nucleoside reverse transcriptase inhibitors (NRTIs) and a protease inhibitor has proven remarkably effective in controlling HIV replication (Ledergerber et al., 1999; Opravil et al., 2000), lack of protease inhibitors specific for FIV has prevented extension of this approach to include treatment of FIV infection (Kervinen et al., 1998).

Combined antiretroviral therapies substituting the protease inhibitor with a non-nucleoside reverse transcriptase inhibitor (NNRTI) or a third nucleoside reverse transcriptase inhibitor (NRTI) have been effectively used to control HIV replication (Moyle, 2000). In particular, the NRTI abacavir (ABC or 1592U89) has proven to be a useful adjunct to zidovudine (ZDV or AZT) and lamivudine (3TC), in view of its unique activation pathway (Faletto et al., 1997) and synergistic action with ZDV (Daluge et al., 1997). Combined ZDV, 3TC and ABC therapy of HIV infection is known to be a viable alternative to protease inhibitor-containing combinations (Staszewski et al., 1998; Wang et al., 1999). In addition, nucleoside analog-only approaches have the added advantage of requiring lower tablet numbers, are less likely to induce drug interactions and allow greater central nervous system penetration, while preserving the option for protease inhibitor use as a second line of therapy (Moyle, 2000). Alternatively, the ribonucleotide reductase inhibitor hydroxyurea (HU) has also been shown to block HIV replication by decreasing intracellular deoxynucleotide availability (Lori et al., 1994). Application of combined didanosine (ddI), stavudine (d4T) and HU therapy is also undergoing evaluation as an alternative treatment for HIV infection (Rutschmann et al., 1998, 2000).

In attempt to apply current HIV-based combination antiretroviral approaches in the cat model, we have investigated the efficacy of protease inhibitor-lacking regimens in controlling FIV infection. In view of the reported failure of NNRTIs to inhibit the FIV reverse transcriptase (North and LaCasse, 1995), we have focused on the ability of combined antiretroviral therapy comprised of ZDV and 3TC, together with either ABC or HU, to suppress in vitro FIV replication. It is anticipated that eventual use of combined antiretroviral therapy to block in vivo FIV replication should allow extension of the cat model to safely investigate proposed HIV-based therapy adjuncts, such as structured therapy interruption (Friedrich, 2000), targeting of therapy initiation (Yerly et al., 2000) and immune-based modulation (Pantaleo, 1997), ultimately directed at eradicating long-lived reservoirs of virus (Finzi et al., 1999; Ramratnam et al., 2000).

2. Materials and methods

2.1. Cells and virus

Crandell feline kidney (CrFK) cells (ATCC CCL-94) were maintained at 37 °C/5% CO_2 in culture medium (RPMI-1640 (Life Technologies), 10% heat-inactivated fetal calf serum (FCS, Life Technologies), 20 mM HEPES (Life Technologies), 4 mM L-glutamine (Life Technologies), 100 U/ml penicillin (Life Technologies) and 100 μg/ml streptomycin (Life Technologies)).

CrFK cells chronically infected with FIV-Petaluma (FIV_{Pet})(ATCC CRL-9761) were also maintained at 37 °C/5% CO_2 in culture medium. Prior to splitting, FIV-containing supernatant was harvested, filtered through a 0.2 μ filter, aliquoted and stored at −70 °C. All experiments were undertaken using the same batch of FIV-containing supernatant.

2.2. Antiretroviral compounds

Stocks of ZDV, 3TC, ABC (Glaxo Wellcome) and HU (Sigma) were prepared as 0.1 mM solutions in dimethylsulfoxide (Pierce) and stored at −70 °C. Stock solutions were further diluted in culture medium immediately prior to use.

2.3. Viral replication assay

Uninfected CrFK cells were maintained at 37 °C/5% CO_2 until confluent, detached by trypsinization and resuspended in culture medium at 2×10^4 cells per ml into 25 cm^2 tissue culture flasks (Falcon). The CrFK cells were allowed to adhere overnight at 37 °C/5% CO_2 and the culture supernatant was replaced with an equivalent volume of FIV-containing culture medium derived from CrFK cells chronically infected with FIV_{Pet}. After overnight incubation at 37 °C/5% CO_2, the FIV-containing culture supernatant was removed and the adherent CrFK cells washed four times in RPMI-1640 (Life Technologies). Antiretroviral compounds alone or in three-drug combinations were added to the appropriate flasks at individual concentrations of 50, 5, 0.5, 0.05 or 0 μM and the infected CrFK cells incubated in the presence of antiretroviral drug at 37 °C/5% CO_2 for 8 days. Immediately after addition of the antiretroviral compound and after 2, 4, 6 and 8 days, 50 μl of culture supernatant was removed and stored at −70 °C for evaluation in an RT-PCR assay specific for the *pol* region of FIV_{Pet}. Each assay was undertaken in duplicate.

2.4. FIV pol RT-PCR assay

Quantification of FIV_{Pet} levels in culture supernatants was undertaken using a one-tube real-time RT-PCR assay specific for the *pol* region of FIV_{Pet} and incorporating direct RNA isolation (Bisset et al., 2001). Forward and reverse primer sequences consisted of CCATTCCTCTT-GATCCAGATTAT and AAATCCAGCCTT-GTGGTAGACTACA, respectively. A probe labeled at the 5′-end with the reporter-dye FAM and at the 3′-end with the quencher-dye TAMRA consisted of the sequence TACTTTACCTAG-GAAGAATAATGCGGGACCAGGAA. Culture supernatant was dispensed in 1 μl amounts into a 96-well plate (Perkin–Elmer) prior to the addition of 49 μl of reaction mix containing 1 × AMV/*Tfl* Reaction Buffer (Promega), 0.2 mM each of dATP, dCTP, dGTP and dTTP (Promega), 1 μM forward-primer (Microsynth), 1 μM reverse-primer (Microsynth), 1 mM $MgSO_4$ (Promega), 0.1 μM flourogenic probe (Eurogentec), 2 mM DTT (Promega), 0.4% Triton-X 100 (Pierce), 0.5 U/μl RNase inhibitor (Promega), 0.1 U/μl AMV-RT (Promega), 0.1 U/μl Tfl DNA polymerase (Promega) and nuclease-free H_2O (Promega). All manipulations of potential template-containing supernatants were undertaken at 4 °C. Each reaction was done in duplicate.

Reverse transcription and amplification were carried out using an ABI Prism 7700 (Perkin–Elmer) sequence detection system. Samples were initially incubated at 48 °C for 45 min to facilitate first strand cDNA synthesis by reverse transcription. A subsequent incubation at 95 °C for 2 min was undertaken to inactivate the heat-labile AMV-reverse transcriptase and initiate RNA, cDNA and primer denaturation. Second strand cDNA synthesis and PCR amplification was undertaken during 40 cycles of denaturation at 94 °C for 30 s and annealing and extension at 60 °C for 1 min. Increase in reporter dye emission was examined in real-time by collecting data during each annealing and extension cycle. Sample threshold cycle measurements (Ct) were expressed in relation to a standard-curve derived from a dilution series of FIV_{Pet} plasmid standard (Phillips et al., 1992) which was included in each assay. Standardized Ct values were then expressed as the mean of duplicate samples.

2.5. Cytotoxicity assay

The possible cytotoxic effect of the antiretroviral compounds tested was examined using a 3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay (Mossman, 1983). Mock-infected CrFK cells were grown at 37 °C/5% CO_2 for 6 days in the presence of antiretroviral compounds alone or in three-drug combinations at individual concentrations of 50, 5, 0.5, 0.05 or 0 μM, before removal of the supernatant and replacement with 0.25 mg/ml MTT (Sigma) in phenol red-free RPMI-1640 (Life Technologies). After incubation at 37 °C/5% CO_2 for 1 h, the MTT-containing supernatant was removed and the cells lysed with 5 ml of isopropanol:1M HCl (96:4 v/v). Triplicate 100 μl volumes of dye-containing supernatant were transferred to a 96-well

ELISA-plate (Nunc) and the absorption measured at 570 nm, using background subtraction at 630 nm.

2.6. Data analysis

Using the CalcuSyn 1.1 (Biosoft) software package (Chou and Hayball, 1996), median effect plots were derived from dose-effect curves obtained for each antiretroviral compound alone and for three-drug combinations using the median-effect equation:

$$\frac{fa}{fu} = \left(\frac{D}{Dm}\right)^m$$

where D is the dose, Dm is the dose required for a 50% effect, fa is the fraction affected by the dose, fu is the unaffected fraction and m is a coefficient of the sigmoidicity of the dose-effect curve. The median-effect dose (Dm) was used to determine both the 50% effective dose (ED_{50}) and 50% toxic dose (TD_{50}). Multiple drug effect analysis was also undertaken using the CalcuSyn 1.1 (Biosoft) software package (Chou and Hayball, 1996) using the combination index (CI)-isobologram equation:

$$CI = \frac{(D)_1}{(D_x)_1} + \frac{(D)_2}{(D_x)_2} + \frac{(D)_3}{(D_x)_3}$$

where $(D)_1$, $(D)_2$ and $(D)_3$ represent the inhibitory doses of drug 1, 2 and 3 in combination, and $(D_x)_1$, $(D_x)_2$ and $(D_x)_3$ represent the inhibitory doses of drug 1, 2 and 3 alone. A CI of <1, 1 or >1 is indicative of synergism, additive effect or antagonism, respectively.

3. Results

3.1. Effect of single antiretroviral drugs on 'in vitro' FIV replication

The antiretroviral drugs ZDV, 3TC, ABC and HU were tested for their ability to inhibit in vitro replication of FIV_{Pet} in feline CrFK cells. In order to simulate treatment of established infection, antiretroviral drugs were added after infection of CrFK cells with FIV_{Pet} and the resulting inhibition of viral replication calculated during exponential viral growth after 6 days of incubation (Fig. 12). Similarly, the potential toxic effects of ZDV, 3TC, ABC and HU on CrFK growth were evaluated in mock-infected cells following 6 days incubation (Fig. 2).

The nucleoside analogs ZDV, 3TC and ABC all effectively inhibited viral replication, demonstrating ED_{50} values of 2.13, 2.44 and 3.07 μM, respectively, (Table 1). ZDV and 3TC have previously been shown to effectively inhibit in vitro FIV

Table 1
In vitro antiretroviral effect of zidovudine (ZDV or AZT), lamivudine (3TC), abacavir (ABC, 1592U89) and hydroxyurea (HU) on FIV_{Pet} replication in feline CrFK cells

Compound	ED_{50} (μM)[a]	m[b]	r[c]	Combination index (CI) at[d]		
				ED_{50}	ED_{75}	ED_{90}
ZDV	2.13 ± 0.83[e]	-0.13 ± 0.02	0.97 ± 0.02	–	–	–
3TC	2.44 ± 0.64	-0.16 ± 0.01	0.97 ± 0.01	–	–	–
ABC	3.07 ± 1.39	-0.19 ± 0.02	0.97 ± 0.01	–	–	–
HU	14.52 ± 4.47	-0.17 ± 0.01	0.80 ± 0.09	–	–	–
ZDV+3TC+ABC (1:1:1)	0.57 ± 0.39	-0.13 ± 0.02	0.96 ± 0.02	0.61 ± 0.24	0.24 ± 0.09	0.16 ± 0.02
ZDV+3TC+HU (1:1:1)	1.80 ± 0.88	-0.16 ± 0.04	0.96 ± 0.02	>1	>1	>1

[a] 50% effective dose (based on median-effect dose).
[b] Slope of median-effect plot.
[c] Correlation coefficient.
[d] CI <1, $=1$, >1 indicate synergism, additive effect and antagonism, respectively.
[e] Mean ± standard error (S.E.) of two consecutive experiments.

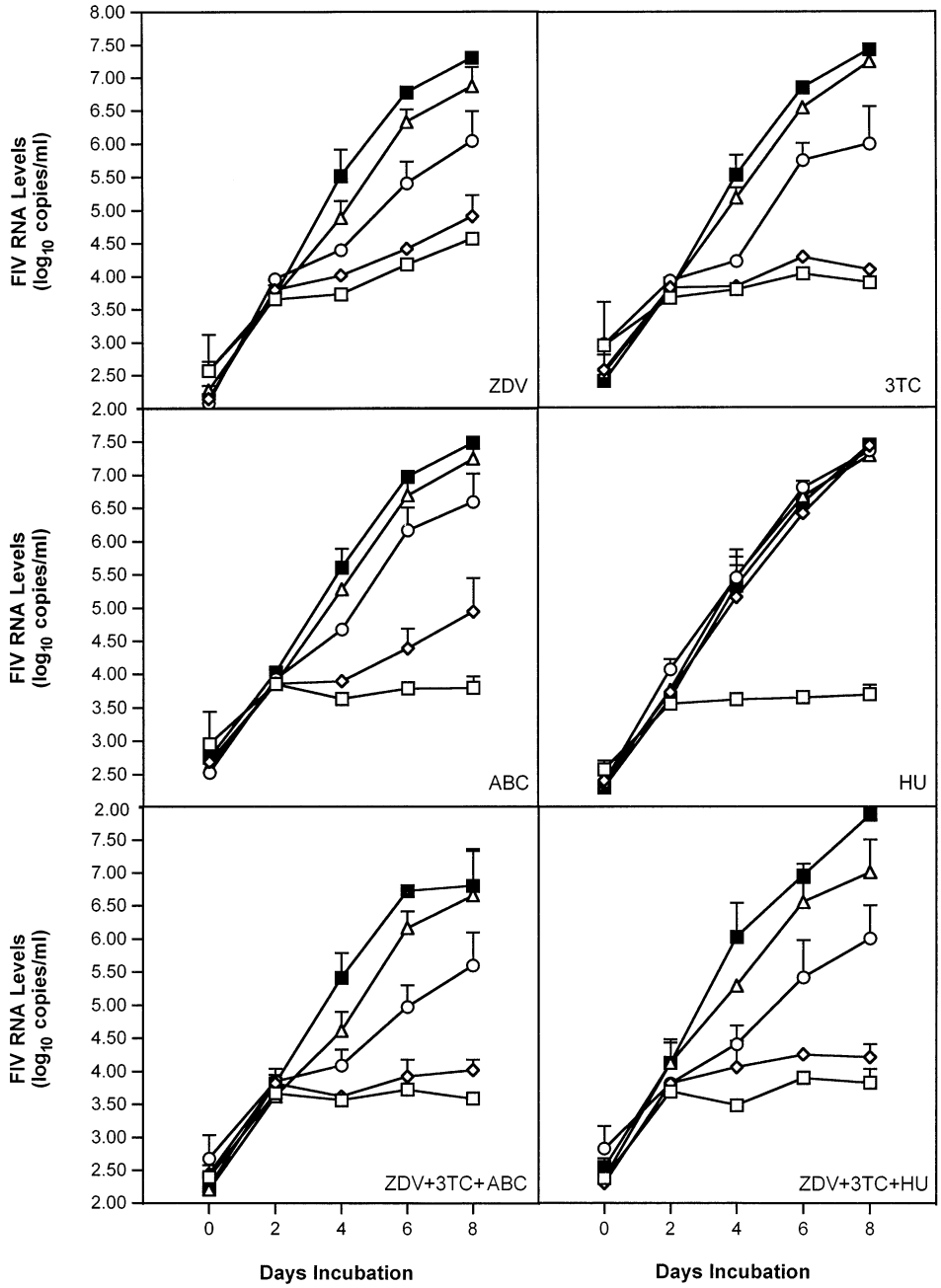

Fig. 1. Kinetics of in vitro FIV_{Pet} replication in feline CrFK cells in the presence zidovudine (ZDV or AZT), lamivudine (3TC), abacavir (ABC, 1592U89) and hydroxyurea (HU). Antiretroviral drugs were applied following infection of CrFK cells with FIV_{Pet} at final concentrations of 50 (□), 5 (◇), 0.5 (○), 0.05 (△) and 0 (■) μM. FIV-RNA levels in the culture supernatant were assessed using a one-tube real-time RT-PCR assay incorporating direct RNA preparation (Bisset et al., 2001). Results are expressed as the mean ± S.E. of two consecutive experiments.

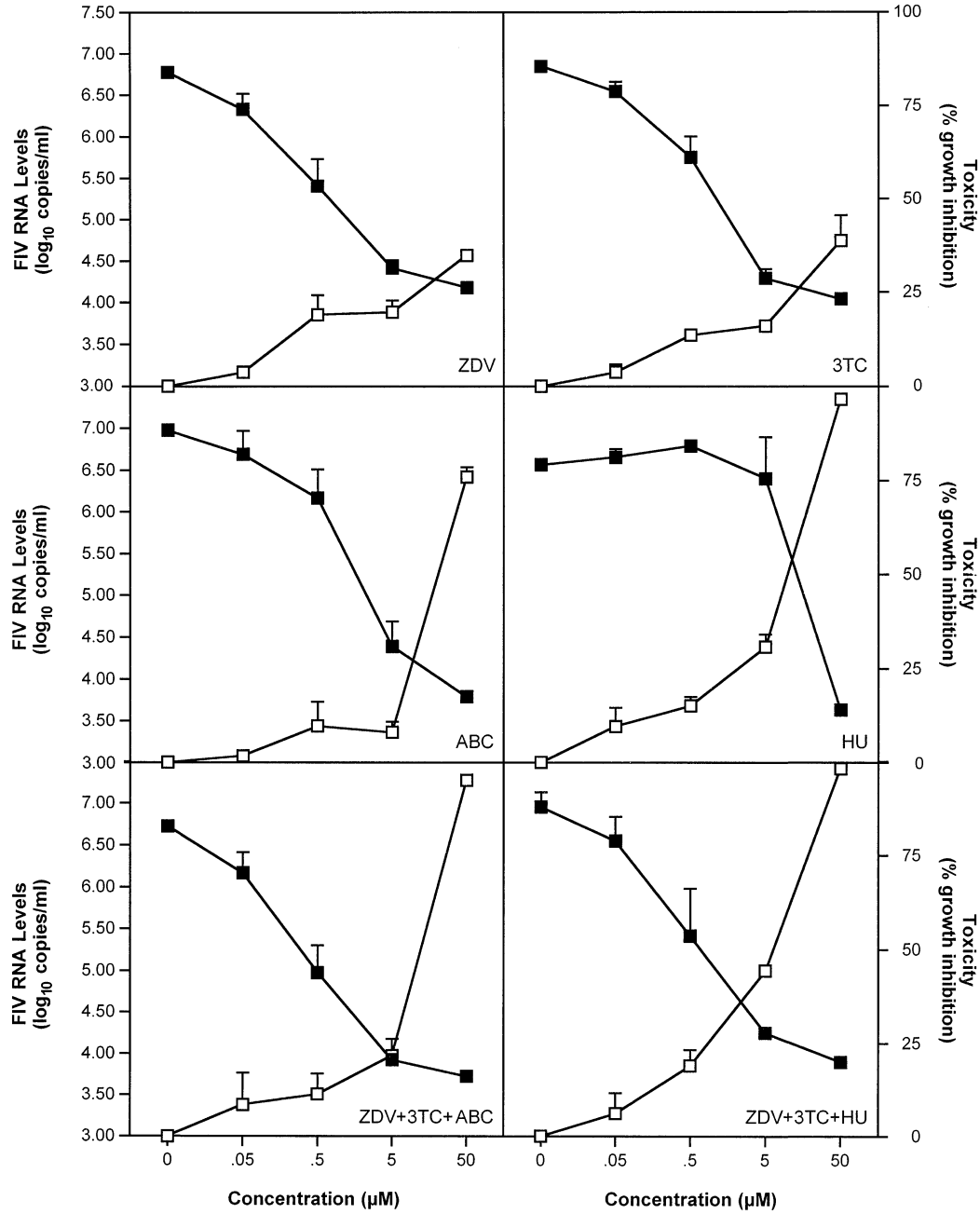

Fig. 2. In vitro antiretroviral (■) and cytotoxic (□) effects of varying concentrations of zidovudine (ZDV or AZT), lamivudine (3TC), abacavir (ABC or 1592U89) and hydroxyurea (HU) on FIV_{Pet} replication in CrFK cells and CrFK cell growth, respectively. FIV-RNA levels in the culture supernatant were assessed following 6 days incubation using a one-tube real-time RT-PCR assay incorporating direct RNA preparation (Bisset et al., 2001). CrFK cell proliferation and viability were assessed following 6 days incubation using an MTT assay (Mossman, 1983). Results are expressed as the mean ± S.E. of two consecutive experiments.

Table 2
In vitro cytotoxic effect of zidovudine (ZDV or AZT), lamivudine (3TC), abacavir (ABC, 1592U89) and hydroxyurea (HU) on mock-infected feline CrFK cells

Compound	TD_{50} (μM)[a]	m[b]	r[c]	Selectivity index (SI)[d]
ZDV	216.79 ± 54.83[e]	0.35 ± 0.02	0.92 ± 0.04	101.78
3TC	170.53 ± 10.39	0.40 ± 0.06	0.94 ± 0.01	69.89
ABC	22.89 ± 2.63	0.97 ± 0.42	0.92 ± 0.04	7.46
HU	2.51 ± 0.69	0.77 ± 0.07	0.91 ± 0.03	0.17
ZDV + 3TC + ABC (1:1:1)	5.31 ± 2.59	1.13 ± 0.51	0.90 ± 0.08	9.32
ZDV + 3TC + HU (1:1:1)	2.12 ± 0.78	1.03 ± 0.21	0.95 ± 0.03	1.18

[a] 50% toxic dose (based on median-effect dose).
[b] Slope of median-effect plot.
[c] Correlation coefficient.
[d] $SI = TD_{50}/ED_{50}$.
[e] Mean ± S.E. of two consecutive experiments.

replication in CrFK cells at comparable ED_{50} values of between 1.3 μM (Smith et al., 1998) and 4.0 μM (Vahlenkamp et al., 1995) for ZDV and 1.5 μM for 3TC (Smith et al., 1998). Exponential viral replication observed between 0 and 2 days incubation in nucleoside analog-treated cultures, independent of drug concentration (Fig. 1), is likely to reflect a time delay during which these initially inactive compounds undergo sequential intracellular phosphorylation (Morse et al., 1993).

In addition, ZDV, 3TC and ABC did not demonstrate appreciable inhibition of mock-infected CrFK cell growth, as reflected in TD_{50} values of 216.79, 170.53 and 22.89 μM, respectively, (Table 2). In conjunction with the observed ED_{50} values, these TD_{50} values yielded selectivity indices for ZDV, 3TC and ABC of 101.78, 69.89 and 7.46, respectively, (Table 2). ZDV and 3TC have previously been shown to be toxic for CrFK cell growth at comparable TD_{50} values of 160 μM (Smyth et al., 1994) and > 200 μM (Vahlenkamp et al., 1995) for ZDV and 480 μM for 3TC (Smyth et al., 1994).

In contrast, the deoxynucleotide synthesis inhibitor HU was unable to effectively inhibit viral replication. Although demonstrating a ED_{50} value of 14.52 μM (Table 1), any inhibition of viral replication is likely to reflect the negative effect of HU on CrFK cell growth, as reflected in a TD_{50} value of 2.51 and a selectivity index of 0.17 (Table 2).

3.2. Effect of triple antiretroviral drug combinations on 'in vitro' FIV replication

The antiretroviral drug combinations ZDV + 3TC + ABC and ZDV + 3TC + HU were tested at a constant ratio for their ability to inhibit in vitro replication of FIV_{Pet} in feline CrFK cells following 6 days incubation (Figs. 1 and 2).

The combination of ZDV, 3TC and ABC was able to effectively inhibit viral replication, demonstrating an ED_{50} value of 0.57 μM (Table 1). Although demonstrating higher toxicity than each compound alone, this combination yielded a TD_{50} value of 5.31 μM and a selectivity index of 9.32 (Table 2). Multiple drug effect analysis-derived combination index (CI) values were indicative of drug synergy (Table 1), with increasing degrees of synergism being apparent at higher drug dosages (Table 1).

The combination of ZDV, 3TC and HU was also able to inhibit viral replication, demonstrating an ED_{50} value of 1.80 μM (Table 1). Nevertheless, given the low TD_{50} and selectivity index values observed for this combination (Table 2), any effect on viral replication is likely to reflect primarily nucleoside analog-derived antiretroviral activity. Combination index (CI) values indicative of drug antagonism at a range of drug dosages (Table 1) further suggest a negative influence originating from the presence of HU, reflecting the low TD_{50} value observed for this compound alone.

4. Discussion

Infection of the domestic cat with FIV has proven to be a compelling animal model for HIV infection in view of parallels at both the molecular and clinical levels. However, while the success of combined reverse transcriptase and protease inhibitor therapy in treating HIV infection has lead to widespread adoption of this approach (Carpenter et al., 1998; Gazzard and Moyle, 1998), combination therapy of FIV infection has been hampered by lack of protease inhibitors effective for FIV (Kervinen et al., 1998). In an attempt to circumvent this problem, we have examined the feasibility of applying combination therapy lacking a protease inhibitor to control in vitro FIV replication. We now report that, as observed during in vitro HIV replication (Daluge et al., 1997; Faletto et al., 1997), antiretroviral therapy comprised of the nucleoside analogs zidovudine (ZDV or AZT), lamivudine (3TC) and abacavir (ABC or 1592U89) is able to effectively inhibit in vitro FIV replication. We also demonstrate that, in contrast to its reported effect on in vitro HIV replication (Lori et al., 1994), use of the deoxynucleotide synthesis inhibitor hydroxyurea (HU) fails to control in vitro FIV replication, precluding its use as a potential substitute for a FIV-specific protease inhibitor or NNRTI.

Nucleoside analogs designed for HIV been used successfully in treating FIV infection (North et al., 1989). In particular, the nucleoside analog ZDV decreases in vitro FIV replication at a concentration similar to that required to inhibit in vitro HIV replication (North et al., 1990; Tanabe-Tochikura et al., 1992; Smyth et al., 1994). This susceptibility of FIV to ZDV has allowed the cat model to be successfully used in identifying the first lentiviral drug-resistance mutation (Remington et al., 1991). Additional nucleoside analogs exhibiting in vitro inhibitory activity against FIV include lamivudine (3TC) (Smyth et al., 1994), 9-(2-phosphonomethoxyethyl)adenine (PMEA) (Egberink et al., 1990), zalcitabine (ddC), didanosine (ddI) and stavudine (d4T) (Smyth et al., 1994). Monotherapy of in vivo FIV replication using either ZDV (Hayes et al., 2000) or PMEA (Egberink et al., 1990) has been successful in reducing plasma FIV-RNA levels, although the positive influence of treatment on $CD4^+$ T-cell loss was not as pronounced. These results are reminiscent of the lack of significant benefit, in terms of survival or disease progression, deriving from ZDV monotherapy of HIV infection (Aboulker and Swart, 1993). In contrast, stabilization of $CD4^+$ T-cell values during FIV infection appears to be more effective following treatment protocols employing both ZDV and PMEA (Hartmann et al., 1992).

Despite a high degree of resemblance in terms of both sequence and structure (Wlodawer et al., 1995), protease inhibitors designed for HIV do not function effectively in blocking the FIV protease (Schnolzer et al., 1996), most likely reflecting variation among residues involved directly in enzyme–ligand interactions (Li et al., 2000). While progress has been made in designing universal inhibitors capable of blocking both the FIV and HIV protease (Wlodawer et al., 1995; Kervinen et al., 1998; Li et al., 2000), routine availability and application of these compounds has yet to be established. This lack of applicable FIV protease inhibitor has limited the use of combination antiretroviral therapy in treating FIV infection per se and prevented consideration of the cat model as a means to examine modifications aimed at enhancing current drug-based therapy of HIV-infection.

We now report that the nucleoside analog ABC is able to effectively inhibit in vitro FIV replication and that inhibition takes place at an ED_{50} equivalent to that observed for both ZDV and 3TC. The relative effectiveness of ABC must be contrasted with that observed during treatment of in vitro HIV replication, where ABC has been reported to be 2-fold less potent than 3TC and 100-fold less potent than ZDV (Daluge et al., 1997). The observed TD_{50} for ABC on feline CrFK cells allows for a selectivity index indicative of genuine antiretroviral activity, despite the fact that ABC toxicity was relatively higher than that observed for either ZDV or 3TC. This level of toxicity must again be contrasted to that observed in humans, where ABC has been reported to exhibit lower in vitro hematopoietic cytotoxicity than ZDV (Daluge et al., 1997) and suggests that

eventual application of ABC to block in vivo FIV replication will need to be accompanied by appropriate assessment of adverse effects. Additionally, we now also report that the combined action of ZDV, 3TC and ABC in blocking in vitro FIV replication yields a combination index indicative of synergism. Furthermore, it is evident that this synergism increases in conjunction with increasing dosage. This observation parallels the synergistic action observed for this nucleoside analog combination in suppressing in vitro HIV replication (Daluge et al., 1997). Eventual use of ABC, in conjunction with ZDV and 3TC, to block in vivo FIV replication may also be advantageous in view of the in vitro evidence for a novel feline RT point mutation conferring resistance to the combination of ZDV and 3TC (Smith et al., 1998).

Substituting the protease inhibitor component in combination protocols with the ribonucleotide reductase inhibitor HU is also able to effectively block in vivo HIV replication (Lori et al., 1999; Rutschmann et al., 2000). Although originally used as a cytostatic drug for treating myeloproliferative disorders, the observation that HU effectively decreases intracellular deoxynucleotide availability has also encouraged application as an antiretroviral compound (Lori et al., 1994; Lori, 1999). In contrast to compounds inhibiting viral proteins, targeting of a cellular protein by HU is therapeutically advantageous in that the probability of developing resistance mutations is extremely small (Donehower, 1992). Nevertheless, reservations remain regarding the in vivo use of HU in view of reports describing bone marrow toxicity in 5–7% of treated HIV-patients and less than optimal post-treatment recovery in $CD4^+$T-cell values (Lori et al., 1999; Rutschmann et al., 2000).

We now also demonstrate that, in contrast to its reported influence on in vitro HIV replication (Lori et al., 1994), HU alone is ineffective in controlling in vitro FIV replication. In particular, inhibition of in vitro FIV replication by HU was restricted to dosages inhibiting cell proliferation, suggesting the absence of genuine antiretroviral activity. These results must be contrasted with the effective blockage of in vitro HIV replication observed in macrophage and activated T-cells at HU concentrations as low as 50 μM (Lori et al., 1994). Moreover, a lack of toxic effect in macrophage and activated T-cells was observed at HU concentrations as high as 1000 μM (Lori et al., 1994). In this regard, the observed lack of antiretroviral effect by HU on in vitro FIV replication may be attributed to hypersensitivity of CrFK cells to the cytostatic effects of this compound, preventing application of HU at a dosage likely to exert a true antiretroviral effect. Similarly, the combined action of ZDV, 3TC and HU in blocking in vitro FIV replication yielded a combination index indicative of drug antagonism. The diminished effect of combined ZDV, 3TC and HU usage is likely to again reflect the inhibitory effect of HU on CrFK cell proliferation and must be contrasted to the observed synergistic effect of ZDV, 3TC and HU usage in blocking in vitro HIV replication (Palmer and Cox, 1997).

In conclusion, it is apparent that development of combination antiretroviral therapies effective in controlling HIV infection has not been matched by parallel advancement in the treatment of FIV infection. This imbalance has especially limited use of the cat model to test alternative treatment strategies useful in combating HIV infection. Our observation that nucleoside-only combination therapy is able to effectively inhibit in vitro FIV replication circumvents the current lack of FIV-specific protease inhibitors and provides the basis for a treatment approach equivalent to current therapies targeted against HIV. Eventual use of ZDV, 3TC and ABC to control in vivo FIV replication should ultimately allow realistic emulation of HIV-specific combination antiretroviral therapy and introduce the cat model as a practical and ethical means to investigate treatment schedules directed at a durable suppression of retroviral infections.

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