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Inhibition of bovine immunodeficiency virus by anti-HIV-1 compounds in a cell culture-based assay

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Abstract

The bovine immunodeficiency virus (BIV) and human immunodeficiency virus types 1 and 2 (HIV-1 and -2) are members of the lentivirus genus of retroviruses. Although DNA sequences of these viruses have diverged considerably, the BIV genome organization, function of structural and regulatory genes, and replication cycle are very similar to that of HIV-1, making BIV a potentially useful model to study compounds with anti-HIV-1 activity. A cell culture-based antiviral assay was developed to test compounds for inhibition of BIV replication. The assay uses an embryonic rabbit epithelial (EREp) cell line that is highly sensitive to BIV infection and cytopathology. The 50% effective concentrations (EC_{50}) at which the virus was inhibited in EREp cells were determined for 13 nucleoside analog, non-nucleoside, tumor-suppressive, or membrane-surface inhibitory compounds. The nucleoside analogs (3′-azido-2′,3′-dideoxythymidine, 2′,3′-dideoxyinosine and 2′,3′-dideoxycytosine), surface-membrane inhibitors (dextran sulfate, hypericin, Chicago Sky Blue and quinobene), the nucleoside reductase inhibitor (hydroxyurea), and a tumor-suppressive phorbol ester (prostratin) inhibited BIV with EC_{50} values similar to those derived in HIV-1 lymphocyte ($CD4^+$)-based assays. BIV was markedly more resistant to inhibition with HIV-1-specific non-nucleoside reverse transcriptase inhibitors (NNRTIs) (thiazolobenzimidazole, oxathiin carboxanilide and thiocarbamate) than was HIV-1, which parallels results with NNRTIs in HIV-2 assays.

Keywords: Bovine immunodeficiency virus; Human immunodeficiency virus type 1; Cell culture-based inhibition assay; Antiviral compounds

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1. Introduction

The replication cycle of the human immunodeficiency virus type 1 (HIV-1) presents several targets for antiviral intervention therapies (reviewed in Gonda and Weislow, 1992; De Clercq, 1995). Several antiviral compounds have been found to inhibit virus attachment and penetration, maturation of the Gag and Gag–Pol polyproteins by the viral protease, or synthesis of virion DNA by the viral reverse transcriptase (RT). Two basic approaches have been used in the identification of antiviral compounds: high-throughput screening of natural and synthetic compounds using HIV-1-infectible cell culture assays (Weislow et al., 1989) and gene-specific targeting through the rational design of inhibitors based upon the three-dimensional structure of the target's active site (Erickson et al., 1990). Cell culture-based screens have identified hundreds of inhibitors, a handful of which fulfills criteria for entrance into clinical trials (Clanton et al., 1991; Gruszecka-Kowalik et al., 1992; Gustafson et al., 1992; Buckheit et al., 1993, 1994; De Clercq, 1995). The rational design approach favors the development of inhibitors that bind tightly to the active sites of small, well-defined molecules such as the HIV-1 protease. Several protease inhibitors, which show promise as therapeutic agents for HIV-1-infected patients, have been independently developed by several groups (Dorsey et al., 1994; Vella, 1994; Winslow et al., 1994; Kempf et al., 1995).

The bovine immunodeficiency virus (BIV) is a lentivirus that is associated with lymphadenopathy, lymphocytosis, central nervous system lesions, progressive weakness, opportunistic infections, and emaciation in cattle (Van der Maaten et al., 1972; Gonda et al., 1987, 1994; Snider et al., in press). BIV is related to HIV-1 structurally, genetically and immunologically (Gonda et al., 1987, 1990; Garvey et al., 1990; Horzinek et al., 1991; Battles et al., 1992; Jacobs et al., 1992; Tobin et al., 1994). In addition to the obligate retroviral structural genes *gag*, *pol* and *env*, BIV encodes six putative accessory genes. Three of these, *tat*, *rev* and *vif*, are closely related in function to those found in HIV-1. Additional BIV accessory genes (*tmx*, *vpy* and *vpw*), whose functions are under investigation, may also have analogs in the HIV-1 genome (Gonda, 1992; Gonda et al., 1994).

BIV replicates in cultured cells of bovine, lapine and canine origin (Gonda et al., 1990) but does not appear to infect humans, as no virus-specific antibodies have been observed in human samples and efforts to infect a wide variety of human cell lines have failed (Kashanchi et al., 1991; Pifat et al., 1992; Whetstone et al., 1992). Like HIV-1 infection in humans, BIV infection in cattle appears to replicate in both neural and lymphoid tissues. The in vivo host range of BIV is broader than that of most lentiviruses. Goats, sheep and rabbits appear to be infectible by experimental inoculation as a persistent antibody response to BIV can be demonstrated (Whetstone et al., 1990; Jacobs et al., 1992; Pifat et al., 1992; Van der Maaten and Whetstone, 1992). However, with the exception of cattle, only in rabbits is there a strong and persistent immune response to BIV proteins, recoverable virus and demonstrable levels of viral DNA in brain and immune tissues indicative of chronic virus replication (Pifat et al., 1992). BIV infection in rabbits is under further investigation in our laboratory for its utility as a small animal model that may have a use in testing antiviral therapies relevant to HIV-1. Thus, the complexity, function of structural and regulatory proteins, and replication cycle of the BIV genome is similar to that of HIV-1 and should present similar targets to test antiviral strategies. In the present study, we have developed a BIV-infectible rabbit cell culture-based assay and tested a number of effective inhibitors of HIV-1 for their antiviral activity with BIV.

2. Materials and methods

2.1. Virus and cell culture

Uninfected embryonic rabbit epithelial (EREp; CRL 6498) and canine thymus (Cf2Th; CRL 1430) cultures were obtained from the American Type Culture Collection and propagated as monolayers in Dulbecco's modified Eagle's media

(DMEM) supplemented with 7% fetal bovine serum (FBS), 1% penicillin/streptomycin and 1% glutamine in a humidified atmosphere of 5% CO_2 at 37°C. Cf2Th cells were transfected with an infectious proviral molecular clone (BIV R29-127) plasmid DNA and passaged several times to produce chronically infected cultures (Braun et al., 1988; Garvey et al., 1990). DMEM was replaced with chemically defined, serum-free growth medium (Opti-MEM, Life Technologies, USA) 20 h prior to harvesting virus. Supernatant virus was centrifuged for 15 min at $15\,000 \times g$ at 4°C to remove cell debris prior to inoculation of test cultures. EREp cells were seeded onto 24-well culture dishes at 10% confluence and cultured in DMEM with 10% FBS for 16–20 h prior to inoculation with virus.

2.2. Antiviral compounds

Pharmaceutical-grade compounds were obtained for this study from the Drug Synthesis and Chemistry Branch (National Cancer Institute). The compounds tested were representatives of several classes of agents with known inhibitory activity against HIV-1. RT inhibitors included the nucleoside analogs 3′-azido-2′,3′-dideoxythymidine (AZT), 2′,3′-dideoxycytosine (ddC) and 2′,3′-dideoxyinosine (ddI) and the non-nucleoside RT inhibitors (NNRTIs) oxathiin carboxanilide (UC84) (Bader et al., 1991), thiocarbamate (UC38) (Strickley and Anderson, 1993), and thiazolobenzimidazole (1,3-TBdf) (Buckheit et al., 1993). The DNA synthesis inhibitor hydroxyurea (Gao et al., 1994; Lori et al., 1994; Torres, 1995) was tested. Virus attachment and membrane-acting inhibitors included 5-kDa dextran sulfate (Chang et al., 1988), Chicago Sky Blue (Clanton et al., 1991), hypericin (Lenard et al., 1993; Lavie et al., 1995), and quinobene (Gruszecka-Kowalik et al., 1992). In addition, a tumor-suppressing phorbol ester, prostratin, was tested (Gustafson et al., 1992). Antiviral compounds were serially diluted in Opti-MEM to concentrations 10-fold greater than used in each well of an assay. During assays of the photoreactive compound, hypericin, fluorescent light was directed down upon the culture plates.

2.3. EREp drug cytotoxicity assays

The EREp minimum cytotoxic concentration of each compound was initially determined microscopically 3–4 days after drug inoculation. As toxic concentrations of the test compound were reached, cell growth was inhibited and the cells became more refractile as they began to detach from the culture surface. The minimum cytotoxic concentration was determined to be that concentration of drug at which detectible changes in EREp growth or morphology were first observed. The morphological changes correlating with drug toxicity also were evaluated for most compounds by a quantitative ^{3}H-thymidine incorporation assay. For this assay, cells were incubated with varying concentrations of inhibitors in the absence of BIV. After 3 days, the cells were pulsed for 18 h with 1 μCi of ^{3}H-thymidine per well, the wells were washed with phosphate buffered saline (PBS) and the cells were removed with 100 μl of a 0.05% trypsin solution at 37°C for 30 min. Radioactive incorporation was quantitated by scintillation counting. The counts from wells containing drugs were compared to drug-free control wells to determine the drug concentration that inhibited the cell growth (DNA replication) by 50% (IC_{50}).

2.4. Virus inhibition assays

At the start of the virus inhibition assay, DMEM was aspirated from target cultures of EREp cells and replaced with 350 μl of Opti-MEM, 50 μl of $10\times$ concentrated test compound, and 100 μl of clarified virus stock, in the order cited. After a 3–4 day incubation at 37°C, the wells were microscopically examined for signs of infection, and the cells were lysed in 200 μl of a solution containing 50 mM Tris (pH 7.8), 5 mM EDTA, 100 mM NaCl, 0.5% CHAPS and 0.5% deoxycholate. Cell lysates were clarified by centrifugation for 5 min at $18\,000 \times g$ and transferred to 96-well plates for quantitation by a BIV capsid (p26) antigen-capture enzyme-linked immunosorbent assay (ELISA) (Battles et al., manuscript in preparation). Negative and positive control wells for virus infection were incorporated into each assay and contained either no virus or virus with-

out inhibitor, respectively. Duplicate wells were prepared for each compound dilution; at least three independent assays were performed to determine inhibitory concentrations of each compound.

2.5. End-point assays

This study initially measured virus inhibition by syncytia, BIV p26 antigen, RT activity, and the XTT-formazan cell cytotoxicity reduction assay (Weislow et al., 1989). BIV infection of EREp cells is acute with the formation of giant multinucleated cells or syncytia resulting from the fusion of plasma membranes of infected with uninfected cells (Pifat et al., 1992). The number and size of syncytia in each culture well were scored visually under low magnification ($\times$ 100) for comparison to drug-free control wells. Syncytia scores were assessed on a scale of 0–5, with 5 representing the syncytia score of the drug-free wells. Use of the BIV p26 antigen-capture ELISA appeared to give more reproducible results and was favored over the RT and XTT-formazan assays, as the latter end-point tests were either less consistent from experiment to experiment or more labor intensive. The BIV p26 antigen-capture ELISA was performed as described (Battles et al., manuscript in preparation). Briefly, 96-well plates were coated overnight at 4°C with BIV p26-specific murine monoclonal antibodies and blocked for 1 h with 0.25% human serum albumin in a solution of PBS (pH 7.2), 0.75% polyvinyl propylene-40 and 0.5% Tween-20 (blocking solution). Cell lysate (50 μl) from the antiviral assays was diluted to 200 μl in blocking solution with 1% FBS, placed into the antibody-coated wells and incubated overnight at 4°C. The wells were then washed with PBS and 0.02% Tween-20, and incubated for 1 h at 37°C with a polyclonal rabbit antiserum raised against bacterially expressed BIV p26 protein. The wells were rewashed five times with PBS and incubated for 1 h at 37°C with peroxidase-conjugated goat anti-rabbit IgG. After the final wash in PBS, the wells were developed with ABTS peroxidase substrate (Kirkegaard and Perry Labs Inc.), and the optical density determined at 405 nm on a Molecular Devices Vmax reader. The assays were processed for analysis in the window of time between the appearance of significant numbers of syncytia and the destruction of the cell monolayer by virus-induced cytopathy. Virus inhibition was quantitated for each drug-treated well by comparison of p26 antigen-capture ELISA or syncytia values with those derived from drug-free BIV-infected control wells. The concentration of a compound that resulted in a 50% reduction of p26 antigen or syncytia was termed the 50% effective concentration (EC_{50}). The results were compared with values obtained from the literature or from data derived by the AIDS Drug Screening and Development Laboratory. Because of the differences in methodologies used in the BIV inhibition assay and the variations of protocols used by different groups to derive HIV-1 EC_{50} values, EC_{50} results can differ by more than a factor of 10. Thus, a BIV EC_{50} value that was within one $\log_{10}$ of that obtained with HIV-1 was considered analogous in our study.

3. Results

3.1. EREp cytotoxicity

To ensure that a potential reduction in virus end-point parameters was not due to the cytotoxic effects of the test compounds, the cell monolayers were microscopically examined for morphological changes in the absence of virus. Most compounds did not produce any visible changes in the cells at the concentrations used in the antiviral assays. However, at the highest concentrations tested, ddC produced a slight morphological change and UC84, UC38, 1,3-TBdf, hypericin and prostratin caused noticeable toxicity (Table 1). The cytotoxicity of nine compounds was measured by a more quantitative ^{3}H-thymidine-incorporation cell growth assay to confirm the cell morphology assay. The highest concentrations of hydroxyurea (20 mM), dextran sulfate (200 μg), quinobene (30 μM) and prostratin (5 μg/ml) tested did not slow cell growth, as no decline in ^{3}H incorporation was seen. Relatively high concentrations of AZT, ddC, UC84, 1,3-TBdf and Chicago Sky Blue did slow cell growth and IC_{50}

Table 1
Summary of activities of anti-HIV-1 compounds in BIV cell culture-based assays

Inhibitor class/compounds	EREp MCC[a]	EREp IC_{50} [b]	BIV syncytia EC_{50} [c]	BIV p26 EC_{50}	HIV-1 EC_{50} [d]
Nucleoside analogs					
AZT	>10 μM	30 μM[e]	0.07 μM	0.1 μM	0.1 μM
ddC	10 μM	3 μM	2 μM	2 μM	0.2 μM
ddI	>10 μM	ND[f]	0.2 μM	0.1 μM	0.2 μM
NNRTIs/DNA synthesis inhibitors					
UC84	500 μM	75 μM	300 μM	200 μM	0.5 μM
UC38	200 μM	ND	30 μM	20 μM	<0.5 μM
1,3-TBdf	200 μM	90 μM	15 μM	25 μM	1 μM
Hydroxyurea	>5 mM	>20 mM	0.06 mM	0.2 mM	0.2 mM
Membrane inhibitors					
Dextran sulfate	>50 μg/ml	>200 μg/ml	0.1 μg/ml	0.25 μg/m	l0.8 μg/ml
Quinobene	>12.5 μM	>30 μM	0.5 μM	0.6 μM	1.6 μM
Chicago Sky Blue	>10 μM	50 μM	1 μM	1 μM	5.1 μM
Hypericin	40 ng/ml	ND	3 ng/ml	2 ng/ml	<500 ng/ml
Tumor suppressor					
Prostratin	1.25 μg/ml	>5 μg/ml	0.05 μg/ml	0.2 μg/ml	0.4 μg/ml

UC84, oxathiin carboxanilide; UC38, thiocarbamate; 1,3-TBdf, thiazolobenzimidazole.
[a] MCC, minimum cytotoxic concentration determined by microscopic examination of cell morphology.
[b] IC_{50}, inhibitory concentration determined by ^{3}H-tridium incorporation assay and defined as the concentration of a compound that results in 50% reduction in DNA synthesis.
[c] EC_{50}, effective concentration for 50% virus inhibition.
[d] HIV-1 EC_{50} values were derived from published literature cited in Section 2 using CEM-SS cell cultures (Nara and Fischinger, 1988).
[e] The presence of AZT may compete with ^{3}H-thymidine and result in higher reported IC_{50} values using this methodology.
[f] ND, not done due to limited supplies of specific compounds.

values of 30, 3, 75, 90 and 50 μM, respectively, were derived for these compounds (Table 1).

3.2. Nucleoside analogs

The first compounds tested in the EREp cell culture-based assay for anti-BIV activity were the nucleoside analog RT inhibitors AZT, ddC and ddI. The incorporation of nucleoside analogs into DNA replicative intermediates using viral RNA templates results in chain termination. AZT, ddC and ddI inhibited BIV syncytia and p26 production with EC_{50} values in the range 0.1–2 μM (Fig. 1). These values are consistent with the HIV-1 EC_{50} values previously published (Mitsuya et al., 1990) and values derived with the same compound lots used by the Anti-AIDS Virus Drug Screening Laboratory of the National Cancer Institute (data not shown).

3.3. NNRTIs

Most NNRTIs are effective against HIV-1 RT, but not HIV-2 RT, a virus more closely related to simian immunodeficiency virus than HIV-1 (Hizi et al., 1993; Buckheit et al., 1994). To assess the activity of this class of compounds against BIV, four NNRTIs were tested (Fig. 2). Two related NNRTI compounds, UC84 and its more soluble derivative, UC38, were found to inhibit BIV at relatively high BIV p26 EC_{50} values of 200 and 20 μM, respectively. 1,3-TBdf had a BIV EC_{50} of 25 μM when inhibition was measured by the BIV p26 antigen-capture assay and 15 μM when the syncytia-reduction assay was employed. The EC_{50} values of these three NNRTIs approached the concentrations that caused cell toxicity. The specificity of the NNRTIs for HIV-1 was again demonstrated by this study as the BIV EC_{50} val-

ues derived for each of the above three NNRTIs were 25–400-fold greater than reported values for HIV-1 (Table 1). Two additional NNRTIs, TIBO and a TIBO derivative (Pauwels et al., 1990), were also tested for inhibition of BIV. Neither compound inhibited BIV replication at nontoxic concentrations (data not shown). Hydroxyurea, on the other hand, demonstrated relatively low BIV EC_{50} values of 0.2 mM in the BIV p26 antigen-capture ELISA assay and 0.06 mM in the syncytia-reduction assay, which is in close agreement with the reported HIV-1 EC_{50} of 0.2 mM (Gao et al., 1994). The EC_{50} values for hydroxyurea were well below the concentrations that cause toxicity (Table 1).

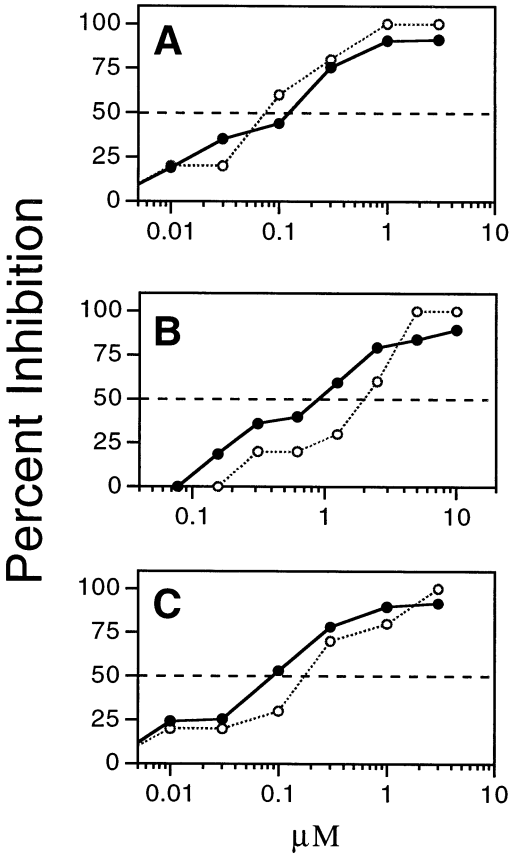

Fig. 1. Inhibition of BIV by nucleoside analogs. EREp cultures were infected with BIV in the presence of increasing concentrations of (A) AZT, (B) ddC or (C) ddI. After 3 or 4 days, the cultures were microscopically examined for the presence of syncytia (○) and measured for BIV p26 antigen (●) in an antigen-capture ELISA. Percent inhibition was calculated as described in Section 2. Intersection of horizontal broken line denotes EC_{50} for each test compound.

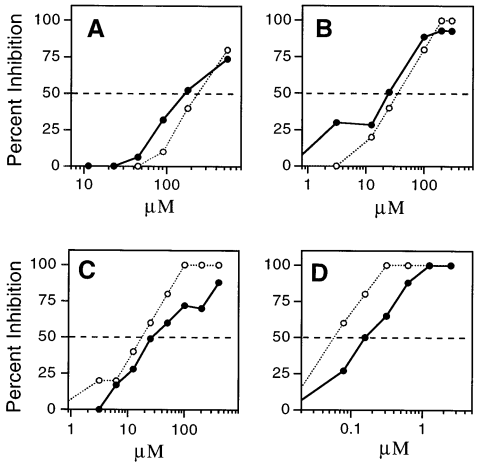

Fig. 2. Inhibition of BIV by NNRTIs or DNA synthesis inhibitors of HIV-1. EREp cultures were infected with BIV in the presence of increasing concentrations of (A) UC84, (B) UC34, (C) 1,3-TBdf or (D) hydroxyurea. Cultures were microscopically examined for the presence of syncytia (○) and measured for BIV p26 antigen (●) in an antigen-capture ELISA 4 days postinfection. Percent inhibition was calculated as described in Section 2. Intersection of horizontal broken line denotes EC_{50} for each test compound.

3.4. Surface-membrane inhibitors

Inhibitors of virus–cell interactions have been investigated for use against HIV-1 and were tested for inhibition of BIV (Fig. 3). Dextran sulfate is a sulfonated polysaccharide that inhibits HIV-1 absorption to cells with a reported EC_{50} of 0.8 μg/ml (Chang et al., 1988). We found that dextran sulfate inhibited BIV with an EC_{50} of 0.1–0.25 μg/ml. Quinobene is a synthetic stilbene-linked sulfonated compound that includes aromatic azo and hydroxyquinoline groups. Quinobene was equally effective in inhibiting both BIV and HIV-1, and EC_{50} values of 0.5 and 0.6 μM in the syncytia and antigen-capture assays, respectively, were obtained (Table 1). Chicago Sky Blue is a sulfonyl compound that binds strongly to gp120 to inhibit surface-membrane interactions (Clanton et al., 1991). Chicago Sky Blue also inhibits RT activity, most likely by

chelating necessary divalent cations. Titration of this compound resulted in a BIV EC_{50} of 1 μM, which corresponded well to the HIV-1 EC_{50} of 5.1 μM. A fourth inhibitor, hypericin, is a photoreactive virucidal agent that binds cell and viral membranes and cross-links the capsid proteins (Lenard et al., 1993; Lavie et al., 1995). Hypericin inhibited BIV with an EC_{50} of 2 ng/ml, which is well below the reported HIV-1 EC_{50} of 0.5 μg/ml and its minimum cytotoxic concentration in EREp cells.

3.5. A tumor-suppressing natural product

Prostratin is a nonpromoting phorbol ester that has been identified as the active compound in the medicinal plant *Homalanthus nutans* (Gustafson et al., 1992). Prostratin binds to and activates protein kinase C, yet interferes with phorbol-ester-induced hyperplasia and inflammation. The specificity of viral inhibition was assessed in the BIV cell culture-based assay. The BIV EC_{50} values of 0.2 and 0.05 μg/ml obtained from BIV p26 and syncytia assays, respectively (Fig. 4), were in agreement with the reported HIV-1 EC_{50} value of 1 μM (0.4 μg/ml).

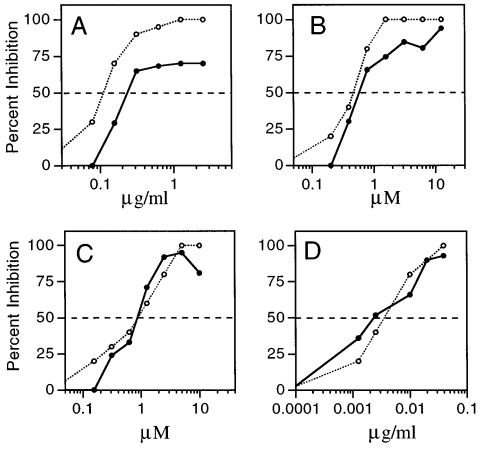

Fig. 3. Inhibition of BIV by membrane-surface inhibitors of HIV-1. EREp cultures were infected with BIV in the presence of increasing concentrations of (A) dextran sulfate, (B) quinobene, (C) Chicago Sky Blue or (D) hypericin. Cultures were microscopically examined for the presence of syncytia (○) and measured for BIV p26 antigen (●) in an antigen-capture ELISA 4 days postinfection. Percent inhibition was calculated as described in Section 2. Intersection of horizontal broken line denotes EC_{50} for each test compound.

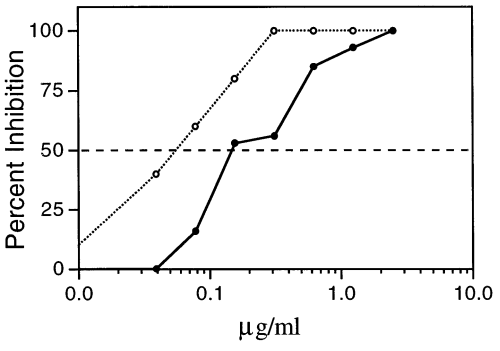

Fig. 4. Inhibition of BIV by a tumor-suppressing phorbol ester. EREp cultures were infected with BIV in the presence of increasing concentrations of prostratin. Cultures were microscopically examined for the presence of syncytia (○) and measured for BIV p26 antigen (●) in an antigen-capture ELISA 4 days postinfection. Percent inhibition was calculated as described in Section 2. Intersection of horizontal broken line denotes the EC_{50}.

4. Discussion

To validate the use of BIV in testing drug- and gene-based therapeutic strategies, we developed a cell culture-based BIV inhibition assay and endpoint assays. We determined the EC_{50} values of compounds that have been identified as HIV-1 inhibitors. The BIV EC_{50} values derived for three nucleoside analog RT inhibitors (AZT, ddC and ddI) tested in this study were within a log of the range previously reported for HIV-1 (Table 1). In other studies, using the ovine lentivirus, visna virus (Thormar et al., 1993, 1995), simian immunodeficiency virus (Mitsuya and Broder, 1988) and feline immunodeficiency virus (North et al., 1989; Remington et al., 1994), AZT was shown to inhibit these genetically diverse lentiviruses with EC_{50} values in the range 0.1–19.0 μM with visna virus having the highest reported EC_{50} of 1–19.0 μM (Thormar et al., 1993, 1995) and feline immunodeficiency virus having an intermediate EC_{50} of 1.4 μM (Remington et al., 1994). Some of the differences in inhibitory values between various lentiviruses may be explained by the cell-culture

systems or the end-point assay used. The differences in primary sequences for the RT domains of these viruses no doubt also play an important role in the sensitivity of each virus to nucleoside RT inhibitors. Despite sequence divergences in the RT domains of each lentivirus, essential functions are maintained and each lentivirus system was able to measure an inhibitory effect of antiviral compounds far below its cell cytotoxicity.

The inhibitors that act upon virus–cell interactions at the surface membrane (Chicago Sky Blue, hypericin, dextran sulfate and quinobene) also inhibited BIV at similar concentrations as for HIV-1 (Chang et al., 1988; Clanton et al., 1991; Hudson et al., 1991; Gruszecka-Kowalik et al., 1992; Lenard et al., 1993). The present report represents the first study of surface-membrane inhibitors on a nonprimate lentivirus. Consistent with the findings of Lenard et al. (1993), these data suggest that these inhibitors target structures and virus–cell interactions that are conserved among lentiviruses or throughout a broad range of enveloped viruses. Because of this broad inhibition, mutational escape from these compounds may be rare. In contrast, the BIV EC_{50} values derived for the NNRTIs (UC84, UC38 and 1,3-TBdf) were at least 50-fold greater than EC_{50} values reported for HIV-1. The BIV EC_{50} values for these three NNRTIs were close enough to their toxicity values that the slight inhibition observed in these assays could be attributed to test-compound toxicity rather than virus inhibition. This result is consistent with previous reports that demonstrate the specificity of several NNRTIs for HIV-1 (Hizi et al., 1993; Thormar et al., 1995).

Hydroxyurea is an inhibitor of cellular DNA synthesis and thus can be loosely classified with NNRTIs. Hydroxyurea has been reported to inhibit HIV-1 with an EC_{50} of 0.2 mM in peripheral blood mononuclear cells (Gao et al., 1994; Torres, 1995). Although there are no other reports of hydroxyurea inhibiting nonhuman lentiviruses, it appeared to demonstrate no specificity for HIV-1, as did the other NNRTIs tested, since it was effective with BIV and had an EC_{50} similar to that reported for HIV-1. Hydroxyurea depletes the cellular pool of deoxynucleotides by binding to and interfering with the cellular enzyme ribonucleotide reductase (Lori et al., 1994). Our present data reflect the more generalized mechanism of hydroxyurea inhibition. Hydroxyurea is orally available and thought to be relatively nontoxic. It is rapidly cleared by the body, is considerably less expensive than other synthetic compounds, and has been approved for use in treating such diseases as chronic myelogenous leukemia since 1960. The BIV and HIV-1 EC_{50} values for the tumor-suppressive compound, prostratin, were similar (Table 1). Although prostratin binds to protein kinase C, the mechanism whereby this inhibits HIV-1 has not been elucidated (Gustafson et al., 1992). We would predict that this inhibitor's interference with BIV may operate in a manner similar to its anti-HIV-1 activity.

The greatest impediment to effective drug treatment appears to be the high mutation rate which facilitates the generation of resistant strains and subsequent virus escape. Nonhuman lentivirus structural and enzymatic genes already contain an array of natural variations in sequence that do not affect gene function, and only a limited number of these variations map to the active domains of similar gene products found in HIV-1. BIV is the closest nonprimate lentivirus to HIV in terms of genome organization and functions of nonstructural regulatory gene products (Gonda, 1992). Thus, the sensitivity of HIV-1 and BIV to each compound may help elucidate the molecular mechanisms involved in antiviral activity and provide a guide to improving the activity of inhibitors. Several investigators have reported the usefulness of visna virus (Frank et al., 1987; Thormar et al., 1995) and feline immunodeficiency virus (Egberink et al., 1990; Goobert et al., 1994) in the study of compounds with anti-HIV-1 activity both in vitro and in vivo. The availability of both a cell culture-based assay, as described in the present report, and an infectible small rabbit model (Pifat et al., 1992) to measure the antiviral activity of compounds on BIV, offers an attractive alternative to the use of primates in pre-clinical drug testing.

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