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In Vitro and In Vivo Evidence that the Antiviral Activity of 2′,3′-Dideoxycytidine Is Target Cell Dependent in a Feline Retrovirus Animal Model

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2′,3′-Dideoxycytidine (DDC) was evaluated for prophylactic antiviral activity in vitro and in vivo, using the feline leukemia virus (FeLV)-cat animal model. In vitro antiviral activity of DDC against FeLV was dependent upon the target cell used for infection. DDC (5 to 10 μM) inhibited FeLV infection of feline lymphoid cells by >80%, while 6.07 to 12.13 μM DDC was required to similarly inhibit infection of feline fibroblasts. However, 43 to 384 μM DDC was needed to inhibit FeLV infection of primary bone marrow cells by >80%. These in vitro results suggest that, although relatively low doses of DDC may be adequate to prevent infection of feline lymphoid cells, 8- to 80-times-higher doses may be necessary to block infection of bone marrow cells, a primary target cell type for FeLV infection. In vivo studies with DDC consisted of pharmacokinetic and toxicity determinations and evaluation of the prophylactic antiviral activity against FeLV in cats. Clearance and half-life values for DDC in cats were 6.5 ml/min per kg and 54.7 min, respectively. In the prophylactic studies, DDC was administered by continuous intravenous infusion at doses of 22, 15, 10, and 5 mg/kg per h for 28 days in most animals. Cats were challenged intravenously with FeLV 1 to 3 days after drug treatment began. Doses of 22 and 15 mg/kg per h were extremely toxic, causing death in 8 of 10 cats. The 10 mg/kg per h dose was slightly toxic, causing chronic progressive thrombocytopenia over the 28-day treatment period. Of 10 cats given 10 or 5 mg of DDC per kg per h, only one was completely protected from FeLV antigenemia. However, conversion to positive FeLV antigenemia status was delayed by 2 to 7 weeks in seven of nine remaining animals. Interestingly, FeLV infection of bone marrow cells, as indicated by FeLV antigen in peripheral blood neutrophils, was only slightly delayed by 0 to 2 weeks, except in the case of the one protected cat, and usually preceded conversion to antigenemia. This pattern of neutrophils becoming antigen positive before detection of antigenemia was not seen in FeLV challenge control animals and indicates that the antiviral activity of DDC may be incomplete during DDC treatment. Results of our in vitro and in vivo studies suggest that feline bone marrow cells may remain partially susceptible to FeLV infection at tolerated doses, while other somatic target tissues (i.e., lymphoid or epithelial tissues) may be protected from infection. Incomplete inhibition of FeLV infection permitted focal bone marrow infection to develop in cats given DDC. These loci of infection served as virus reservoirs which, subsequent to discontinuation of DDC treatment, permitted spread of infection to tissues previously protected during treatment.

The nucleoside analog 2′,3′-dideoxycytidine (DDC) is a potent in vitro inhibitor of the replication of human immunodeficiency virus, the etiologic agent of acquired immune deficiency syndrome (AIDS) (25). DDC was originally developed for use as an anticancer drug by Horwitz et al. (16). Like other nucleosides, DDC must be phosphorylated to the triphosphate form before utilization (1, 6, 32). Phosphorylated DDC is an analog of 2′-deoxycitidine-5′-triphosphate which normally serves as a substrate for cellular and viral DNA polymerase (34). The antiviral action of DDC is thought to occur by incorporation of the dideoxynucleotide 2′,3′-dideoxycytidine-5′-triphosphate at the 3′ end of growing chains of unintegrated viral DNA, resulting in chain termination (3, 10, 26, 32, 36). DDC suppresses the cytopathic effect and infectivity of human immunodeficiency virus in vitro at concentrations of 0.01 to 0.5 μM, while having no effect on the growth or immune function of cells (2, 25, 28). DDC has been studied extensively in vitro and is currently undergoing phase I and II trials in patients with AIDS and AIDS-related complex (7, 24, 35). Prophylactic evaluation of DDC, for ethical and practical reasons, will be limited to studies in animal models.

One such animal model is the feline leukemia virus (FeLV)-cat system. FeLV, a naturally occurring retrovirus of domestic cats, is horizontally transmitted and causes neoplastic disease and impairment of the immune system, with the majority of the animals dying from opportunistic infections (11, 27, 30). The extensive characterization of this disease and the similarity of its pathogenesis to that of human retroviral infection make it a suitable model for evaluating antiretroviral drugs for the treatment of AIDS and other retroviral diseases (4, 5, 9, 11, 30).

In this study, DDC was first tested for anti-FeLV activity in vitro and then evaluated as a possible treatment for preventing retrovirus infection in vivo by using the FeLV-cat model system. The study consisted of four phases: (i) assessment of antiviral activity of DDC in vitro with four different feline cell types, (ii) determination of DDC pharmacokinetics in cats, (iii) determination of in vivo toxicity of DDC to cats, and (iv) evaluation of DDC efficacy in vivo for

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preventing or delaying virus infection. We discovered that DDC has differential antiviral activity in vitro against FeLV, depending on the target cell for infection. This differential activity was also apparent in the in vivo studies in which cats were treated prophylactically with DDC.

MATERIALS AND METHODS

Cells. Four different cell types were used in this study. A feline lymphoid cell line, 3201 (provided by William Hardy, Jr., Memorial Sloan Kettering Cancer Center, New York, N.Y.) (31), was maintained in RPMI 1640–Lebovitz-15 medium with 15% heat-inactivated fetal bovine serum, 2% L-glutamine, and 1% penicillin–streptomycin at 37°C with 5% CO_2 and was subcultured twice weekly.

A sarcoma-positive, leukemia-negative feline lung fibroblast cell line, clone 81 (81C) (8), was cultured in McCoy 5A medium with 15% heat-inactivated fetal bovine serum, 2% L-glutamine, 2% $NaHCO_3$, and 0.1% gentamicin at 37°C and was subcultured every 5 days.

Bone marrow cells were obtained from femoral aspirates of three adult specific-pathogen-free cats by using bone marrow biopsy needles. Cells were layered over Percoll, washed twice in Dulbecco phosphate-buffered saline, and suspended in Dexter special medium in preparation for the infectivity assay.

The chronically FeLV-infected feline lymphoma cell line FL-74 (33) was maintained in RPMI 1640 medium with 15% heat-inactivated fetal bovine serum, 1% penicillin–streptomycin, and 2% L-glutamine. Cells were subcultured every 5 days.

Cats. The specific-pathogen-free cats used in the studies reported here were obtained from a hysterectomy-derived breeding colony maintained by the Department of Veterinary Pathobiology at The Ohio State University.

Reagent. DDC was provided by The National Institute of Allergy and Infectious Diseases AIDS Research and Reference Reagent Program, Bethesda, Md. The DDC used in the in vitro assays was diluted in each respective cell culture medium to the following concentrations: for 3201 cells, 0, 5, 10, 19, 38, 76, 152, 256, 303, and 384 μM; for FL-74 cells, 0, 0.1, 0.5, 1, 2, 4, 8, 16, 32, and 64 μM; for 81C cells, 0, 0.05, 0.10, 0.19, 0.38, 0.76, 1.52, 3.03, 6.07, 12.13, 24.27, and 48.53 μM; for bone marrow cells, 0, 5, 14, 43, 128, and 384 μM. DDC was dissolved in sterile water at a concentration of 1 mg/ml and passed through a filter (pore size, 0.45 μm) for the pharmacokinetics studies. The continuous-infusion prophylactic treatment studies used an 8-mg/ml solution of DDC in pyrogen-free normal saline adjusted to pH 7.4 with 1 M NaOH.

Virus and virus infection. An Fea-A strain of FeLV with an infectious titer of 4.6×10^3 focus-forming units (FFU) per ml was used for all assays (8). The FeLV used for the animal challenge was a plasma pool derived from FeLV-viremic cats inoculated with the Rickard strain of FeLV (FeLV-R) and contained 2×10^3 FFU of FeLV per ml (approximately 10 50% infective dose units). Cats, 8 to 10 weeks old, were inoculated intravenously with 0.1 ml of the infectious plasma pool. Seven age-matched untreated challenge control cats were included in this study.

81C direct focus reduction assay. A direct focus reduction assay was used to determine the antiviral activity of DDC on 81C cells. 81C cells were subcultured 1 day before the assay. Adherent cells were washed twice in Dulbecco phosphate-buffered saline and removed with trypsin. Cells were suspended to 10^5/ml. Equal volumes (0.5 ml) of medium containing the various DDC concentrations and cell suspension were added to each well of 24-well plates, giving a final concentration of 5×10^4 cells per ml. After incubation for 18 h at 37°C with 5% CO_2, cells were treated with DEAE-dextran for 30 min. The DEAE-dextran was removed, and 0.2 ml of FeLV (4.6×10^3 FFU/ml) at a 10^{-1} dilution was added to each well. After incubation for 2 h at 37°C, 0.8 ml of DDC medium was added to the wells. The plates were kept at 37°C with 5% CO_2 for 10 days. A 0.5-ml medium change was performed on days 4, 6, and 8. On day 10, supernatant solutions were discarded. Cells were fixed with 10% Formalin and stained with Giemsa stain. Viral inhibition by DDC was determined by counting the number of virus-induced FFU per milliliter in DDC-treated and control wells.

3201 cell indirect focus reduction assay. Pelleted 3201 cells were treated with 0.3 mg of DEAE-dextran (Sigma Chemical Co., St. Louis, Mo.) for 30 min and then suspended to 3×10^6/ml in complete medium plus DDC at various concentrations. A 0.25-ml sample of each cell suspension plus DDC was plated with 1 ml of virus suspension diluted 10^{-1} and incubated for 2 h at 37°C in 5% CO_2 for virus adsorption. Control wells containing virus-infected cells and uninfected cells in DDC-free medium were also plated. An additional 3 ml of DDC-medium was added to each well, and plates were incubated at 37°C with 5% CO_2. Cells and supernatant fluids were harvested on day 5 and tested for viral infectivity on 81C cells, with 3201 supernatant fluids as the inoculum.

Primary bone marrow indirect focus reduction assay. An indirect focus reduction assay was used to determine the antiviral activity of DDC on primary bone marrow cells. One-milliliter samples of bone marrow cells at 2×10^7/ml were centrifuged, the medium was aspirated, and the cells were treated with 1 ml of Fea-A FeLV containing 4 μg of Polybrene (Sigma) per ml and 1 ml of medium plus various concentrations of DDC. The bone marrow cells, DDC, and virus were incubated for 3 h at 37°C with 5% CO_2. Supernatant solutions were removed, and the cells were suspended in Dexter special medium with 2.5% bone marrow pokeweed mitogen condition medium and various concentrations of DDC, plated, and incubated at 37°C with 5% CO_2. On day 7, supernatant fluids were harvested and tested for the presence of infectious virus by the 81C infectivity assay described previously, with bone marrow supernatant fluids as the inoculum.

FL74 cell treatment with DDC. Chronically FeLV-infected FL-74 cells were pelleted and suspended in complete medium to 3×10^6/ml. A 0.25-ml sample of the cell suspension and 4 ml of DDC medium were added to each well of six-well plates. Plates were incubated at 37°C with 5% CO_2 for 10 days, with one-half of the DDC medium changed on days 3, 6, and 9. Cells and supernatant fluids were harvested on day 10 and tested for viral p27 antigen by an enzyme-linked immunosorbent assay (ELISA).

ELISA for FeLV p27 antigen. Cell pellets from the FL-74 assay were suspended in 200 μl of extraction buffer (0.05 M NaCl, 0.02M Tris hydrochloride, pH 7.4, 10% aprotinin, 0.5% Nonidet P-40, and 0.5% sodium deoxycholate) and tested for cell-associated antigen by VIRACHEK FeLV ELISA kits (Synbiotics Corp., San Diego, Calif.). Color changes were measured by using a Bio-Tek EIA reader, and the optical density was recorded.

Determination of DDC concentration in plasma. A high-performance liquid chromatography procedure for DDC was used to determine the concentration of DDC in the plasma from treated cats (23). Briefly, time-collected plasma samples from cats given a bolus dose of DDC and weekly

samples from cats treated by constant infusion were filtered by using Spin-X centrifuge filter units (Costar, Cambridge, Mass.). External calibration standards were prepared by dissolving DDC in prefiltered plasma from normal specific-pathogen-free cats and making serial twofold dilutions at concentrations beginning with 10 μM DDC per ml. Ten microliters of each standard or unknown plasma sample was injected directly into a liquid chromatograph (HP 1090; Hewlett Packard, Walbrann, Federal Republic of Germany). DDC was separated from plasma proteins on a 15-cm column (Pinkerton ISRP; Regis Chemical Company, Morton Grove, Ill.). After 15 min, the effluent was diverted by a switching valve onto a Cyclobond I column (ASTEC, Whippany, N.J.) for analysis by reversed-phase chromatography. The mobile phase consisted of a 0.1 M solution of phosphate buffer (1.0 M K_2HPO_4 and 1.0 M KH_2PO_4), pH 7.1, in high-performance liquid chromatography-grade water. The flow rate was 1.0 ml/min, with a total run time of 25 min and detection at 275 nm. Concentrations of DDC in the unknown plasma samples were calculated from the calibration curve obtained by linear regression analysis of DDC peak areas and DDC concentrations in external calibration standards.

Determination of pharmacokinetic parameters of DDC in cats. Three 8-week-old cats were injected intravenously with a single bolus dose of DDC in water (1 mg/ml) at a dosage of 1 mg/kg. Blood samples were collected at 1 min before injection and at 2, 15, 20, 45, 60, 120, and 200 min postinjection. Plasma was harvested and stored at −70°C until assayed. The DDC concentrations in plasma were determined by high-performance liquid chromatography (23). The data were analyzed using the PCNONLIN nonlinear estimation program (Statistical Consultants, Inc., Louisville, Ky.) to determine pharmacokinetic values for DDC in cats.

Prophylactic treatment with DDC. DDC was administered by continuous intravenous infusion to 8- to 9-week-old cats beginning 1 to 3 days before virus challenge. The procedure of Swenson et al. (32a) was used for catheter placement. In short, an indwelling catheter was placed in the jugular vein and tunneled subcutaneously to the dorsolateral thoracic region, where it exited through an externally located stainless steel button sutured to the skin. The tubing next passed through a stainless steel spring attached to the button on the cat and to a swivel at the top of the cage. Drug delivery through the catheter tubing was monitored by using a Flow-guard pediatric continuous infusion pump (Micro-90-Gard 8500; Travenol Laboratories, Inc., Deerfield, Ill.) and was adjusted for each individual cat depending on the desired dose. Doses were as follows: eight cats received 22 mg/kg per h, two cats received 15 mg/kg per h, six cats received 10 mg/kg per h, and four cats received 5 mg/kg per h. Dose rates during drug infusion were calculated on the basis of body weight at time zero. No adjustments were made for weight gain during the treatment period. On day 1 or 3 after drug treatment began, the cats were intravenously inoculated with 0.1 ml of the FeLV plasma pool. Drug treatment was intended for a period of 4 weeks postchallenge. Cats were monitored daily. Blood samples were calculated once a week and analyzed for concentrations of DDC in plasma, FeLV antigenemia, and presumptive bone marrow infection. Antigenemia was determined by using VIRACHEK FeLV ELISA kits (Synbiotics Corp.). Color change was measured in a Bio-Tek EIA reader, and the optical density value was recorded. The presumptive test for bone marrow infection was an indirect immunofluorescence assay on terminally differentiated neutrophils for group-specific antigen, modified (13) from that developed by Hardy et al. (12). The primary reagent was goat antiserum against ether-disrupted FeLV. Drug toxicity was assessed through hematologic analysis and plasma biochemical profiles performed by the Clinical Laboratory of The Ohio State University School of Veterinary Medicine and the Veterinary Diagnostic Laboratory, Columbus, Ohio.

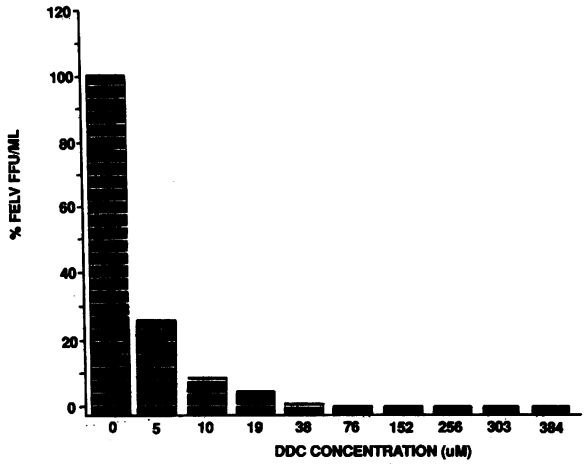

FIG. 1. Inhibitory effects of DDC on FeLV infection of 3201 (feline lymphoid) cells in vitro as determined by indirect focus reduction assay. 3201 cells were incubated with the indicated concentration of DDC beginning at the time of viral inoculation. Cell-free culture fluids were collected at day 5 postinfection and assayed for focus-forming virus on 81C cells. Results are expressed as a percentage of the infectivity of the control (no drug); 100% = 1.9×10^5 FFU/ml.

RESULTS

Effect of DDC on FeLV infection in vitro. The antiviral activity of DDC was measured in infectivity assays by using feline lymphoid cells (3201), feline lung fibroblasts (81C cells), fresh bone marrow cells, and chronically FeLV-infected cells (FL-74) as target cells. The inhibitory activity of DDC against FeLV-challenged 3201 cells is shown in Fig. 1. Viral infectivity was measured by an indirect focus reduction assay. DDC inhibited >80% of FeLV infection at doses between 5 and 10 μM. Inhibition of FeLV infection of 81C cells (feline lung fibroblasts) was measured by a direct focus reduction assay. DDC inhibited 80% of FeLV infectivity of 81C cells (Fig. 2) at concentrations between 6.07 and 12.13 μM. Inhibition of infection of primary feline bone marrow cultures was measured by an indirect focus reduction assay. Eighty percent focus reduction was seen at doses between 43 and 128 μM for two of three cultures (Fig. 3). FeLV-infected bone marrow from the third animal showed 80% inhibition of infectivity, between 128 and 384 μM DDC. Treatment of chronically infected FL-74 cells with up to 64 μM DDC had no inhibitory effect on viral antigen production (Fig. 4). DDC caused no significant toxicity in any cell lines at the drug concentrations used, as determined by viable cell counts (data not shown).

Pharmacokinetic parameters for DDC in cats. Three cats were administered a single intravenous dose of DDC (1 mg/kg), and plasma samples were collected at 1 min before injection and 2, 15, 30, 45, 60, 120, and 200 min postinjection. From the DDC concentration in plasma-versus-time curves generated for these animals (Fig. 5), pharmacokinet-

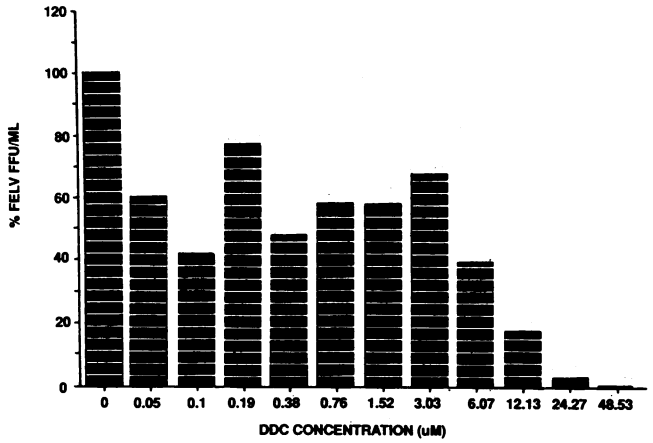

FIG. 2. Inhibitory effect of DDC on FeLV focus formation of 81C (sarcoma-positive, leukemia-negative feline lung fibroblast) cells in vitro. 81C cells were incubated with the indicated concentration of DDC beginning at the time of viral inoculation. Cultures were fixed, stained, and enumerated for focus formation on day 10 of culture. Results are expressed as a percentage of the infectivity of the control (no drug); 100% = 92 FFU/ml.

ics parameters were calculated. Clearance and half-life values for the cats are listed in Table 1. The mean pharmacokinetic values for clearance and half-life in cats given 1 mg of DDC per kg were 6.5 ml/min per kg and 54.7 min, respectively.

Plasma DDC concentrations during constant intravenous infusion were less than predicted (30.4 and 45.3 μM at doses of 5 and 10 mg/kg per h, respectively). Clearance was calculated for the eight cats receiving 5 or 10 mg of DDC per kg per h by continuous infusion for a period of ≥4 weeks from the average steady-state plasma DDC concentration

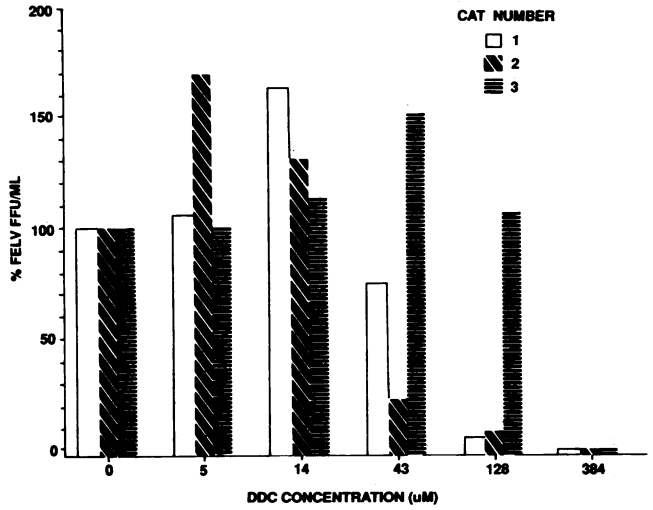

FIG. 3. Inhibitory effect of DDC on FeLV infection of primary feline bone marrow cells. Bone marrow samples were obtained from three cats. Primary feline bone marrow cells were incubated with the indicated concentration of DDC beginning at the time of viral inoculation. Tissue culture fluid from 7-day-old cultures was assayed for viral infectivity on 81C (sarcoma-positive, leukemia-negative) cells. Results are expressed as a percentage of the infectivity of the control (no drug) for each culture; 100% controls were 80 FFU/ml (cat 1), 65 FFU/ml (cat 2), and 80 FFU/ml (cat 3).

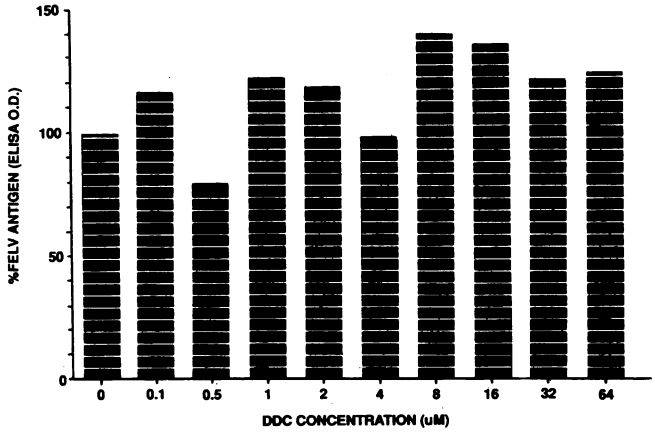

FIG. 4. Effects of DDC on FeLV expression in chronically infected FL-74 cells. FL-74 cells were incubated with the indicated concentration of DDC. Cell-free supernatants were collected at day 10 of culture and measured for FeLV p27 by ELISA. Results are expressed as a percentage of that of the control (no drug); 100% = 0.518 optical density (OD) units.

and the average dose rate, adjusted for body weight gain. The mean clearance during continuous infusion was 12.5 ml/min per kg.

Prophylactic activity of DDC in FeLV-inoculated cats. Infusion rates of 22, 15, 10, and 5 mg of DDC per kg per h were evaluated for prophylactic antiretroviral activity in cats challenged with FeLV. DDC was administered by continuous intravenous infusion beginning 1 to 3 days before FeLV inoculation to allow attainment of steady-state drug concentrations. Efficacy was evaluated on the basis of FeLV antigenemia conversion and presumptive bone marrow infection assays. The results are summarized in Table 2.

DDC doses of 22 and 15 mg/kg per h caused severe drug-related toxicity and death in 8 of 10 cats. For the surviving animals, drug therapy was discontinued at days 6 and 8 postchallenge. Both of these animals converted to FeLV antigenemia by week 2. Cats given 10 mg/kg per h were treated for 28 days postchallenge, except for one animal which received treatment for 42 days. FeLV antigenemia was delayed by 2 to 7 weeks in five of six cats in this group compared with untreated FeLV-challenged control animals. Prolonging drug therapy to 6 weeks in cat no. 3769

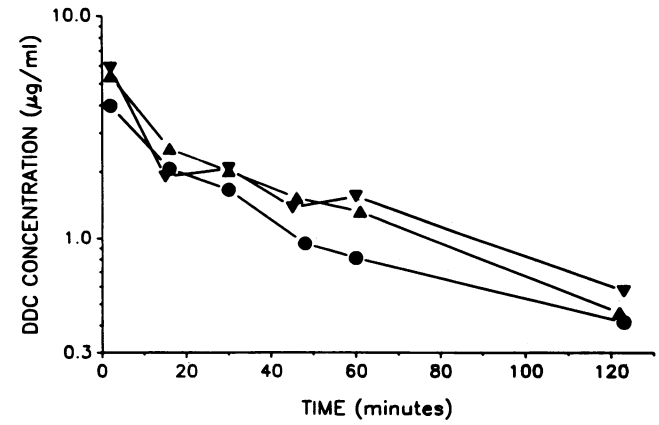

FIG. 5. DDC concentration in plasma-versus-time curve for three cats given a 1-mg/kg intravenous bolus dose of DDC.

TABLE 1. Pharmacokinetic values for DDC in cats

Cat no.	DDC clearance (ml/min per kg)	DDC half-life (min)
3542	5.9	57.8
3543	6.3	43.3
3544	7.2	63.0
Mean	6.5	54.7

extended the delay in FeLV antigenemia. In all six cats in this group, neutrophils and presumably bone marrow cells became FeLV positive before the development of antigenemia by 1 to 4 weeks postchallenge.

Two of the four cats given 5 mg/kg per h received therapy for 4 days postchallenge. The other two cats received the full 28-day treatment regimen. FeLV antigenemia was delayed in two cats and prevented entirely in a third. Interestingly, of the two cats that received only 8 days of treatment, disease induction was prevented in one and delayed by 2 weeks in the second.

All age-matched untreated control cats inoculated according to the protocol became antigenemic by week 2 or 3 postchallenge, with neutrophils showing infection either concurrently or 1 week later (Table 2). These animals remained viremic throughout the observation period.

DDC-related toxicity in cats. The higher DDC doses of 22 and 15 mg/kg per h caused severe toxicity resulting in death for all but two animals treated at these rates. The two surviving animals were removed from treatment after only 6 or 8 days because of obvious toxicity. Hematologic evaluations revealed severe thrombocytopenia. The cats receiving 15 mg/kg per h had less than 20,000 platelets per μl during the first week of treatment (normal cats had 300,000 to >1,000,000/μl). Megakaryocyte hypoplasia in several of the cats that died correlated with the extremely low platelet counts. Monitoring platelet counts proved valuable as a means of early detection of toxicity so that measures could be taken to increase survival by either decreasing the dose or discontinuing treatment. In the cats which died, other nonspecific lesions possibly related to drug toxicity were emaciation, mild periportal hepatocellular vacuolation, and acute hemorrhage, possibly due to drug-induced thrombocytopenia. Relatively mild drug-related toxicity occurred in animals given 10 mg of DDC per kg per h, as evidenced by reductions in platelet counts from baseline values. Only one cat had a platelet count below 100,000/μl during week 3 on the drug and recovered before the treatment period ended (Fig. 6). At necropsy, three of these cats also showed evidence of mild hepatic toxicity.

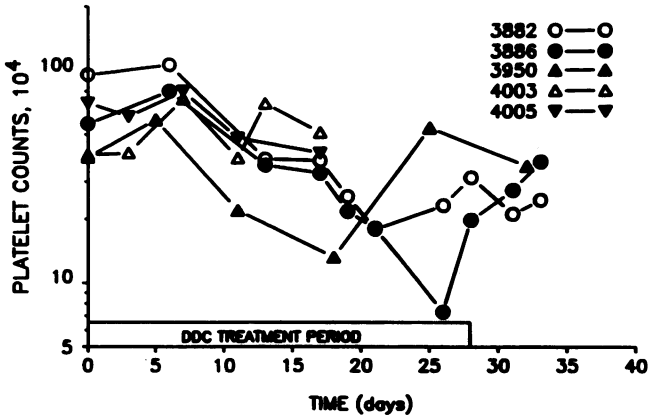

FIG. 6. Platelet count-versus-time graph for five cats given 10 mg of DDC per kg per h by continuous intravenous infusion. DDC administration began 1 or 3 days before FeLV challenge. Time zero is the day of virus challenge. DDC was administered for a period of 28 days postchallenge as indicated. Cat numbers are indicated at upper right.

DISCUSSION

A comprehensive evaluation of the antiviral activity of DDC against FeLV infection in cats was undertaken. In vitro

TABLE 2. Onset of FeLV antigenemia and drug levels in plasma of cats prophylactically administered DDC

Cat no.	Dose rate (mg/kg per h)	Level in plasma (μM)		Antigenemia status/presence of FeLV-positive neutrophils for wk:								
		Challenge	Avg	0	1	2	3	4	5	6	7	8
Expt 1												
3769	10	90.95	74.88	−/−	−/−	−/−	−/−	−/+	−/+	−/+	−/+[a]	−/+
3882	10	35.5	51.86	−/−	−/−	−/+	−/−	−/−[a]	−/+	+/+	+/+	+/+
3886	10	14.41	40.71	−/−	−/−	−/−	−/−	−/−[a]	−/+	+/+	+/+	+/+
3950	10	61.85	42.72	−/−	−/−	−/−	−/−	−/+[a]	ND[b]/−	+/+	+/+	+/+
4003	10	3.66	30.30	−/−	−/−	−/+	+/+	+/+[a]	+/+	+/+	+/+	+/+
4005	10	34.41	31.13	−/−	−/−	−/−	−/−	−/+[a]	+/+	+/+	+/+	+/+
3705	5	35.5	33.75	−/−	−/−	−/−	−/−	−/+[a]	−/−	+/+	+/+	+/+
3715	5	42.65	27.01	−/−	−/−	−/−	+/+	+/+[a]	+/+	+/+	+/+	+/+
3703	5	110.9	110.9	−/−	−/−	−/−	+/−	−/−[a]	+/+	+/+	+/+	+/+
3704	5	91.9	91.9	−/−	−/−	−/−	−/−	−/−[a]	−/−	−/−	−/−	−/−
Control												
3624				−/−	−/−	−/−	+/+	+/+	+/+	+/+	+/+	+/+
3632				−/−	−/−	+/−	+/+	+/+	+/+	+/+	+/+	+/+
3702				−/−	−/−	+/−	+/+	+/+	+/+	+/+	+/+	+/+
3712				−/−	−/−	+/−	+/+	+/+	+/+	+/+	+/+	+/+
3713				−/−	−/−	+/−	+/+	+/+	+/+	+/+	+/+	+/+
3726				−/−	−/−	−/−	+/+	+/+	+/+	+/+	+/+	+/+
3727				−/−	−/−	−/−	+/+	+/+	+/+	+/+	+/+	+/+

[a] Termination of drug treatment.
[b] ND, Not done.

testing demonstrated that DDC inhibited FeLV infection of a feline lymphoid cell line by >80% at concentrations of 5 to 10 μM. Eighty-percent inhibition of FeLV infection of primary bone marrow cells, however, required DDC concentrations of between 43 and 384 μM, suggesting that different target cells may phosphorylate DDC at different efficiencies. Human macrophage cultures are reported to be less sensitive to the antiviral activities of DDC and 3'-azido-2',3'-dideoxythymidine than are lymphocytes, accounting for the higher concentrations of these compounds required to inhibit HIV infection (29). However, in a subsequent report, fresh macrophage cultures were found to be almost as sensitive as lymphocytes to the antiretroviral activities of DDC and 3'-azido-2',3'-dideoxythymidine, despite lower cytosolic 2',3'-dideoxynucleoside-5'-triphosphate concentrations in macrophages than in T lymphocytes, at least in the case of 3'-azido-2',3'-dideoxythymidine. A parallel reduction in 2'-deoxynucleoside-5'-triphosphate concentration seemed to account for this antiviral activity (28). Our in vitro results are in agreement with the conclusion that different cell lineages may have variable sensitivities to inhibition of retrovirus infection by nucleotide analogs, in this case DDC. The in vivo results discussed below also tend to support this conclusion.

Our objective was to evaluate the prophylactic antiretroviral activity of DDC in cats by establishing steady-state DDC concentrations capable of inhibiting FeLV infection before virus challenge. In vitro results indicated that concentrations of 128 to 384 μM may be required to inhibit FeLV infection of bone marrow cells. This concentration should be more than adequate to prevent infection of lymphoid cells.

With this objective in mind, single-dose pharmacokinetic studies of DDC were performed in cats so that the appropriate dosage for continuous intravenous infusion could be calculated. The total plasma clearance of DDC in cats based on a single 1-mg/kg intravenous bolus injection was 6.5 ml/min per kg. Later calculations determined from cats receiving 5 or 10 mg of DDC per kg per h as a continuous intravenous infusion gave an average clearance of 12.5 ml/min per kg. The clearance of DDC in cats was comparable to the clearance shown by Kelley et al. (20) for rhesus monkeys (9.6 to 11.8 ml/min per kg) but was much less rapid than that reported for BDF mice (30.5 ml/min per kg). Half-life values of 102 to 113 min in monkeys and 69 min in mice were reported. Hoover et al. reported the DDC half-life in cats to be 50 min (15). We calculated the average DDC half-life in cats to be 54.7 min.

On the basis of a clearance of 6.5 ml/min per kg, we estimated that a continuous intravenous infusion rate of 22 mg/kg per h was required to establish the target steady-state concentration of 270 μM DDC, a dose between the 128 to 384 μM levels necessary to inhibit FeLV infection of bone marrow cells. Subsequent in vivo testing of DDC at dosage rates of 22 and 15 mg/kg per h resulted in excessive toxicity, causing severe thrombocytopenia, hemorrhage, and death in 8 of 10 animals. DDC was only mildly toxic at dose rates of 10 mg/kg per h and below, causing a gradual decrease in platelet counts over a period of 4 weeks. DDC reportedly inhibits hematopoiesis in vitro, causing suppression of the human megakaryoblastic leukemia cell line MEG-01 and human CFU-E and CFU-GM cell colony formation (17, 19). In phase I studies of human patients with AIDS or AIDS-related complex, toxicity symptoms included cutaneous eruptions, fever, mouth sores, thrombocytopenia, and neutropenia at DDC doses of 0.015 to 0.0225 mg/kg per h (0.06 to 0.09 mg/kg every 4 h) (35). Other investigators also reported toxicities of diffuse erythematous rash, fever, and aphthous stomatitis with rare hematopoietic suppression at DDC doses of 0.0075 to 0.015 mg/kg per h (0.3 to 0.6 mg/kg every 4 h) in phase I and II studies in patients with AIDS and AIDS-related complex (24). At a lower dose (0.00125 mg/kg per h), the skin rash, fever, and stomatitis were mild to absent. Both groups reported reversible painful peripheral neuropathy at the doses administered (7, 24, 35). In cats, thrombocytopenia was the only adverse effect observed. Although the treatment period was less than that in the human study, there was no evidence of any neurological disorder in cats treated with DDC at 1,000 times the dose administered to human subjects.

The efficacy of DDC treatment in cats was evaluated by measuring viral antigen in plasma (antigenemia) by an antigen capture assay and by measuring viral antigen expression in neutrophils by immunofluorescence. The first assay correlates very well with infectious virus in plasma and is a presumptive assay for viremia (22). The second assay also correlates with viremia (14) but is better viewed as an indicator of bone marrow infection in which the terminally differentiated neutrophils are infected as bone marrow progenitor cells. Challenged control animals became FeLV antigenemic during week 2 or 3 postinoculation; neutrophils became FeLV antigen positive during week 3 (Table 2). In no case did neutrophils become FeLV positive before the onset of antigenemia in the 7 untreated challenged control animals shown or in 17 additional challenged control animals not presented in this report.

Only 1 of 10 cats given DDC at a dose rate of either 5 or 10 mg/kg per h prophylactically by continuous infusion was protected from FeLV antigenemia. However, FeLV antigenemia conversion as measured by ELISA was delayed by 2 to 7 weeks in seven of nine remaining animals. Mean drug concentrations in plasma ranged between 27 and 74 μM DDC in cats given the drug for the full 4-week period. DDC concentrations in plasma of cats showing a delay in antigenemia were generally >14 μM at the time of FeLV challenge. One of two cats that did not show a delay in antigenemia (no. 4003) had an unusually low drug concentration in plasma of 3.66 μM DDC at the time of FeLV challenge, thus increasing the likelihood of successful virus infection.

In six cats given 10 mg of DDC per kg per h, neutrophils became FeLV positive 1 to 4 weeks before the development of antigenemia. As indicated above, this pattern of antigen expression was not seen in untreated challenged control animals and is not typical of what has been reported in the literature (18, 21).

Although DDC did not prevent the eventual expression of FeLV in cats given 10 mg/kg per h, viremia induction was delayed. However, the delay in virus expression was less evident in neutrophils, which are bone marrow-derived cells. The data indicate that DDC may block the infectious cycle of FeLV in unidentified tissues which are the predominant producers of virus and virus proteins detected as antigenemia. Other studies suggest that lymphoid or intestinal epithelial cells may be the unidentified cell population (unpublished data). Virus infection of bone marrow myelomonocytic progenitor cells that mature into neutrophils was inhibited to a lesser extent, with many cells becoming infected, but perhaps at a slower rate. Upon discontinuation of DDC treatment, bone marrow cells and possibly other cells that harbored virus during DDC treatment presumably release infectious virus which subsequently infects cells formerly protected by DDC. These results are compatible

with the differential activity of DDC observed with lymphoid and bone marrow target cells described in the in vitro studies.

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