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Treatment of FeLV-induced immunodeficiency syndrome (FeLV-FAIDS) with controlled release capsular implantation of 2′,3′-dideoxycytidine

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Summary

2′,3′-dideoxycytidine (ddC) inhibits replication of the immunodeficiency inducing strain of feline leukemia virus (FeLV-FAIDS) in vitro at concentrations ranging from 1–10 μg/ml. Additive antiviral effect is achieved when ddC is combined with either human recombinant alpha interferon (IFNα) or tumor necrosis factor (TNF) plus IFNα. Initial in vivo pharmacokinetic studies in cats, utilizing bolus intravenous administration of ddC (20 mg/kg), resulted in peak plasma concentrations of 15 μg/ml 1 min after administration and a half-life of approximately 1 h. These values could not be augmented with high levels of the deaminase blocker tetrahydrouridine administered prior to or concurrently with ddC. In vivo trials utilizing multiple, daily intravenous injections of ddC could not prevent the development of persistent viremia in cats infected with FeLV-FAIDS.

To enhance ddC pharmacokinetics and antiviral activity, controlled release capsular implants were developed by blending ddC with a copolymer consisting of DL-lactide glycolide and hydroxypropyl cellulose, which was melt-spun into fibers and encapsulated in a sheath of polyethylene glycol for subcutaneous implantation. Pharmacokinetic studies, conducted in cats receiving an average dose of 600 mg of ddC, indicated an average peak plasma concentration of 17 μg/ml achieved at 6 h post implantation with 3.5 μg/ml noted at 48 h; and an extension of plasma half-life from 1.5 (bolus subcutaneous injection) to 20 h. Sustained plasma concentrations of 1.5 to 10 μg/ml, equivalent to ddC levels previously shown to have anti-FeLV activity in vitro, were maintained throughout a 72 h period. Implantation

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devices could be replenished every 48 h and elevated plasma levels were sustained for four weeks without signs of clinical toxicity, sepsis or significant alterations in the hemogram. Initial clinical trials employing controlled release capsular ddC implants in vivo indicate significant retardation of FeLV-FAIDS replication throughout a four week treatment period.

Feline leukemia virus; Immunodeficiency; Antiviral therapy

Introduction

Feline leukemia virus is a horizontally transmitted, C-type retrovirus which causes lymphosarcoma, myeloproliferative disease and aplastic anemia in the cat (Gilden and Oroszlan, 1971; Hardy and McClelland, 1977). The virus is highly immunosuppressive and is responsible for numerous opportunistic infections (Hardy, 1980; Hardy, 1982). In fact FeLV induced immunodeficiency syndrome is the most frequent cause of death in infected pet cats. Eighty-three percent of persistently infected cats die within 3.5 years of initial diagnosis, usually from secondary viral and bacterial diseases which are refractory to conventional therapy (Hardy, 1980).

We have recently characterized a naturally occurring isolate of FeLV (FeLV-FAIDS) which induces persistent viremia and fatal immunodeficiency syndrome (FAIDS) without concomitant leukemogenesis when inoculated into specific pathogen-free cats (Hoover et al. 1987). Cats greater than 15 weeks of age develop a chronic syndrome characterized by a long asymptomatic period (6 months to 1 year), progressive lymphopenia with decreased lymphocyte colony formation in vitro, emaciation and opportunistic infections during the terminal stages of disease (Hoover et al., 1987; Quackenbush et al., 1988). With a mean survival time of approximately 400 days after inoculation, the clinical signs and immunopathology associated with the chronic form of this disease resembles that seen in humans with the acquired immunodeficiency syndrome (Curran et al., 1985).

Studies of the sequential pathogenesis of FeLV-FAIDS has revealed intense viral replication in the bone marrow and in hyperplastic lymphoid follicles at three weeks post inoculation followed by diminution of virus replication corresponding to lymphoid depletion (Hoover et al., 1987). Concurrent with the onset of clinical immunodeficiency syndrome, high levels of the FeLV-FAIDS pathogenic variant are detected as unintegrated viral DNA in bone marrow, intestine and systemic lymphoid tissues (Mullins et al., 1986). Studies utilizing chimeras formed between the highly replicative apathogenic and replication defective variant components comprising FeLV-FAIDS have implicated the envelope-3′ LTR of the pathogenic variant as the region encoding the lymphocytopathic determinants of FeLV-FAIDS (Overbaugh et al., 1988). Like FeLV-FAIDS, disease progression in humans may correlate with expression of more cytopathic variants of HIV (Rubsammen-Waigman et al., 1986, 1988; Cheng-Mayer et al., 1988).

Many chemotherapeutic agents are currently under investigation as possible

treatment modalities for retrovirus-induced immune deficiency syndrome in humans (Sarin, 1988; Hirsch and Kaplan, 1987). One such agent, 2′,3′-dideoxycytidine (ddC), is a potent in vitro inhibitor of the infectivity and cytopathic effect of HIV-1 and HIV-2 in a variety of human cell types (Mitsuya and Broder, 1988; Mitsuya and Broder, 1986). To inhibit retrovirus infectivity ddC must be converted intracellularly to its 5′-triphosphate derivative which successfully competes with binding of normal cellular nucleotides to retroviral reverse transcriptase and thus is preferentially incorporated into the proviral DNA chain (Waqar et al., 1984; Coony et al., 1986). Once incorporated the substitution of a hydroxyl group at the 3′ portion of the sugar moiety allows ddC to act as a chain terminator of nascent proviral DNA. Previous studies have documented the ability of ddC to inhibit infectivity of a diverse group of mammalian retroviruses dependent on the relevant levels of host intracellular deoxycytidine kinases (Dahlberg et al., 1987). Phase I studies in humans as well as pharmacokinetic studies in macaques and mice indicate a plasma half-life of 1–2 h after intravenous infusion (Kelly et al., 1987; Yarchoan et al., 1988). Because of the distribution, rapid urinary clearance and relative lack of systemic accumulation of ddC, these studies appear to indicate that repeated administration or continuous infusion may provide some therapeutic advantage in the host (Kelly et al., 1987).

The broad spectrum and potent antiviral activity of ddC against a variety of mammalian retroviruses prompted us to study the effect of this nucleoside analogue in the feline model of retroviral induced immunodeficiency disease. In the present studies we first investigated the capacity of ddC, alone and in combination with biological response modifiers, to inhibit the de novo FeLV-FAIDS infectivity in vitro. We then investigated the pharmacokinetics of ddC in cats utilizing intravenous or subcutaneous bolus injection with and without the use of a potent deaminase inhibitor, tetrahydrouridine, to modify the half-life of ddC in vivo. Finally, we report on the use of a novel controlled release subcutaneous implantation system for delivery of ddC at sustained therapeutic levels over an extended period to enhance antiviral activity in vivo. The potential usefulness of this system for delivery of ddC, other nucleoside analogues, and biologic response modifiers as a model for therapeutic intervention of HIV infection in humans is discussed.

Materials and Methods

Animals

All animals used in this study were from a breeding colony of cesarian-derived SPF cats maintained at Colorado State University. These animals were age-matched, eight months of age at the time of inoculation and free of infection and immunity to horizontally transmitted feline viruses, including FeLV. Cats derived from this colony have been used previously to characterize the pathogenicity of FeLV-FAIDS.

Virus inoculation

The inoculum used in these studies was the molecularly cloned pathogenic but replication-defective 61C rescued by its replication-competent companion virus clone 61E (Overbaugh et al., 1988) thus reconstituting within molecular clones the highly infectious and pathogenic inoculum represented by the original tissue-origin field isolate of FeLV-FAIDS (Hoover et al., 1987). FeLV-FAIDS 61 E/C is produced from single cell clones of AH 927 feline fibroblasts stably cotransfected with clones 61C and 61E which are expressed in approximately equal representation (Overbaugh et al., 1988). The infectious inoculum used in vivo contained 6×10^5 focus forming units (FFU) injected intraperitoneally. The 81C cell focus induction assay in vitro employed 20 FFU; the inoculum used in the Crandell feline kidney cell infectivity assay consisted of 3×10^4 FFU.

Antiviral compounds

2′,3′ dideoxycytidine (ddC) was obtained through the Developmental Therapeutics Branch, AIDS Program, National Institutes of Allergy and Infectious Disease, Bethesda, MD, under the auspices of Drs. John J. McGowan and Charles L. Litterst. Human recombinant alpha interferon (IFN α) was provided by Schering Plough Research Inc., Montclair, NJ by Dr. Jerome Schwartz via Dr. Margaret I. Johnston, DTB, AIDS Program, NIAID. Human recombinant tumor necrosis factor alpha (24×10^6 units/mg) was generously provided by Dr. Jack Nunberg, Cetus Corporation, Emeryville, Ca.

In vitro antiviral assays

The capacity of ddC and recombinant cytokines to inhibit replication of FeLV-FAIDS 61C/E in vitro was determined by an enumerative focus induction assay described by Fischinger et al. employing S+/L clone 81 (81C) feline fibroblasts (Fischinger et al., 1974) and by antigen ELISA for FeLV p27 produced by virus-inoculated Crandell feline kidney cells (CrFK) four days after virus inoculation. In either case antiviral compounds were placed in direct contact with cellular monolayers 24 h prior to infection, removed during the period of virus absorption and replaced for contact with cell monolayers for the duration of these assays. Supernatants from at least three test wells were pooled and these samples for ELISA were then run in triplicate.

Formulation and use of controlled release capsular implants

Polymeric ddC-releasing capsular implantation devices were designed in collaboration with Drs. Janna D. Strobel and Donald L. Hill, Southern Research Institute, Birmingham, AL. Dry blends of ddC, copolymer (50:50 DL-lactide/glycolide) and hydroxypropyl cellulose (HPC) were melt-spun into fibers, which were bundled together within a polyethylene glycol matrix to maintain internal in-

tegrity and stability of the implant. Bundles of 17 fibers loaded with 150 mg of ddC were then cut into rod-shaped implantation devices measuring 0.63 by 2.5 cm. These implants were inserted and replaced under local anesthesia through a small cutaneous incision along the dorsal midline of each animal. Each animal received four implantation devices (600 mg ddC) per implantation period.

Treatment protocol

In the case of multiple bolus infusion therapy, cats were treated with 20 mg/kg ddC intravenously three times daily for 24 h before being challenged with FeLV-FAIDS. Tetrahydrouridine in these studies was given at a dose of 100 mg/kg three times daily concurrently with ddC injections. Serial blood sampling was done in the first 24 h for ddC pharmacokinetic studies, before inoculation with FeLV-FAIDS.

Using controlled release capsular implantation of ddC, cats were treated 48 h prior to inoculation with FeLV-FAIDS and four implantation devices (600 mg ddC) were replaced with four new devices every 48 h thereafter for 24 days. Tumor necrosis factor alpha (TNF) and human recombinant alpha interferon (IFNα) were started with ddC treatment and TNF was delivered subcutaneously at 50 $\mu g/m^2$ every 72 h for the first week and then every 96 h until day 24. Likewise IFNα was given subcutaneously at a dosage of 1.6×10^6 units/kg daily for the first week and then every 72 h until day 24. FeLV status, hematologic parameters and clinical chemistry data were monitored weekly in treated and non-treated groups (three cats per group in all studies) for the duration of the study.

Determination of ddC in plasma

The pharmacokinetics of ddC delivered by intravenous or subcutaneous injection was determined by serial blood sampling over a 24 h period post injection. In the case of subcapsular implantation of ddC blood sampling was done over the course of a single, 72 h implantation period. Concentrations of ddC in post treatment plasma samples were determined by high pressure liquid chromatography using methods described by Kalin and Hill (Kalin and Hill, 1988). Pharmacokinetic analysis of plasma ddC was determined using methods developed by Yeh and Kwan (Yeh and Kwan, 1978).

Detection of infectious FeLV and circulating FeLV p27 antigen in serum

Sera were examined for infective FeLV using the clone 81 focus induction assay described by Fischinger et al. (Fischinger et al., 1974).

FeLV p27 antigen was identified by ELISA using a murine monoclonal antibody developed by Lutz et al. (1983) and supplied by Pitman Moore Corporation, Washington Crossing, NJ. Antigen-binding antibody at 2 μg/well was placed in 96-well microtiter plates and serial dilutions of serum and 50 μl of horseradish peroxidase-conjugated FeLV p27 monoclonal antibody was added per well and in-

cubated for 90 min. The plates were rinsed, blot-dried and 50 μl of *o*-phenylenediamine and citric acid substrate was added per well in the presence of 30% hydrogen peroxide for 10 min. One hundred microliter per well of 2N sulfuric acid was added and the absorbance was determined at 490 nm. Test well reactions were considered positive if an absorbance value of at least 1.67 times that of a standard control of normal SPF cat serum was attained.

Statistical analysis

Significant differences in the mean levels of plasma p27 were determined by the Student's *t*-test. *P* values less than 0.05 were considered significant.

Results

In vitro inhibition of FeLV-FAIDS infectivity with 2',3' dideoxycytidine (ddC) alone or in combination with immune modulators

A viral infectivity assay and an antigen (p27) ELISA were modified to determine anti-FeLV-FAIDS activity of specific nucleoside analogues in vitro. Results

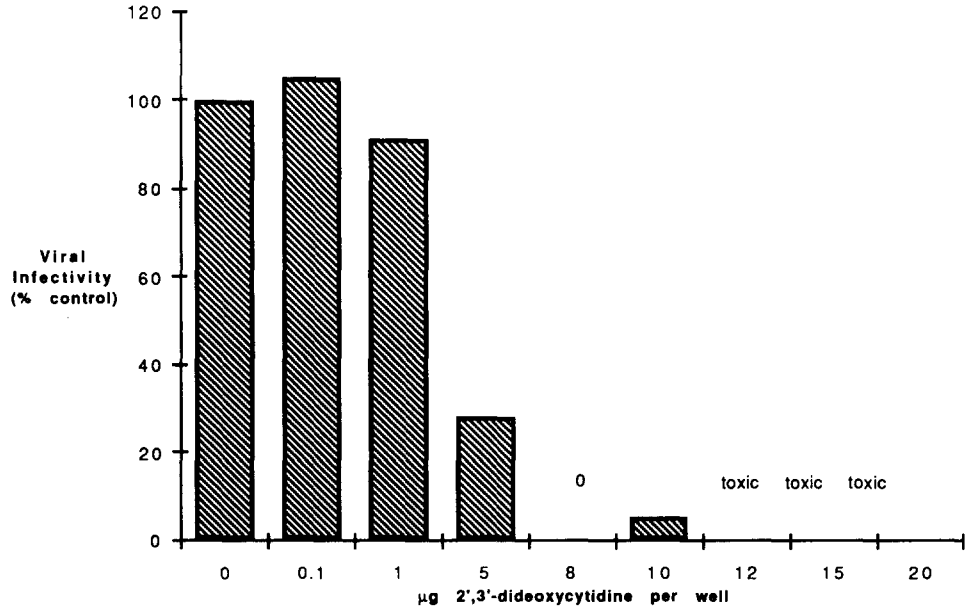

Fig. 1. Inhibition of FeLV-FAIDS infectivity by ddC. Eighty-one clone indicator cells were treated with ddC 24 h prior to inoculation with 20 FFU of FeLV-FAIDS. Focus forming colonies were counted 10 days post inoculation with FeLV-FAIDS and bars represent the percent inhibition of focus forming units compared to virus inoculated control wells.

indicate anti FeLV-FAIDS activity for ddC between 1 μg and 10 μg per ml (Fig. 1). Concentrations greater than 20 μg/ml produced toxicity to feline target cells. Fig. 2 illustrates that dosages of 1 μg/l and 5 μg/ml reduced infectivity to levels 20% and 80% of virus controls. Antiviral activity was enhanced by the addition of human recombinant alpha interferon (IFNα) at concentrations of 500 to 2000 units/ml. With concentrations between 1500 and 2000 units/ml of IFNα FeLV-FAIDS infectivity was reduced by 50–60% compared to ddC alone. Similar inhibition of viral replication, although to a lesser extent, was attained when ddC, at 5 μg/ml, was used alone or in combination with IFNα, immediately post infection (data not shown). IFNα alone only marginally inhibits viral replication; i.e., a 25% reduction at 100 units/ml to a 60% reduction at 2000 units/ml, levels approaching toxicity to indicator cells. Thus ddC and IFNα appear to be additive in their effect on FeLV-FAIDS infectivity in vitro.

The inhibition of FeLV-FAIDS replication was augmented to produce 95% suppression when ddC (5 μg/ml) was used in combination with both tumor necrosis factor (TNF) and IFNα (Fig. 3). TNF used alone, or in combination with only IFNα or ddC, did not alter the activity of each of these antivirals when compared to their use alone. No toxicity to indicator cells was noted at levels of 0.001 μg/ml of TNF. Moderate toxicity to feline indicator cells was noted at levels greater than 0.01 μg/ml TNF.

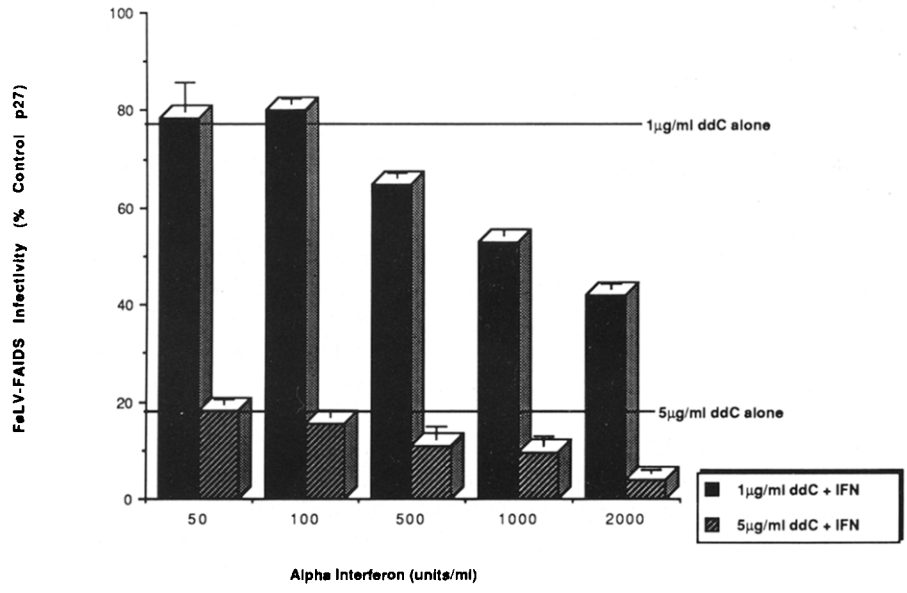

Fig. 2. Inhibition of FeLV-FAIDS infectivity by ddC plus IFNα. CrFK indicator cells were treated with ddC and alpha interferon 24 h prior to inoculation with 3×10^4 FFU of FeLV-FAIDS. Supernatants from treated and non-treated wells were collected four days post inoculation, pooled and assayed by ELISA for p27. Pooled samples were run in triplicate and inhibition as percent p27 of virus infected control wells.

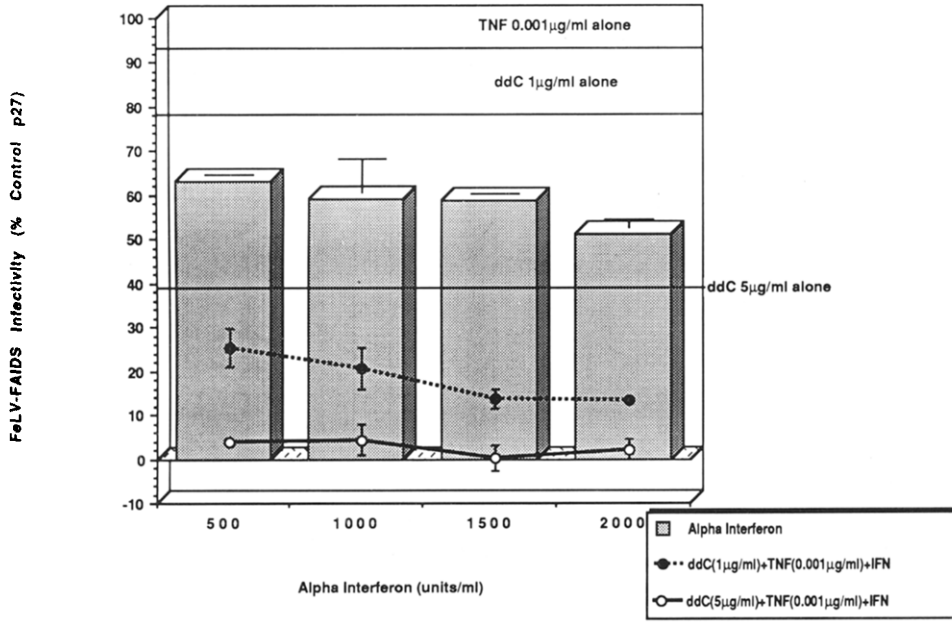

Fig. 3. Inhibition of FeLV-FAIDS infectivity by ddC in combination with IFNα and TNF. CrFK monolayers were treated 24 h prior to inoculation with 3×10^4 FFU of virus and supernatants were collected four days post inoculation with FeLV-FAIDS and assayed in triplicate by ELISA for p27. Horizontal lines represent nucleoside analogue or cytokine treatment given alone. Samples are scored as percent inhibition of p27 detected in virus infected control wells.

Pharmacokinetics and anti-FeLV-FAIDS activity of ddC administered by bolus infusion

Bolus intravenous administration of 20 mg/kg of ddC resulted in peak plasma levels of 15 μg/ml at 1 min post inoculation and a plasma half-life of approximately 45 min. No drug was detectable in the plasma at 6 h post administration. Attempts to increase the plasma half-life with high levels (100 mg/kg i.v. t.i.d.) of a deaminase blocker, tetrahydrouridine, were unsuccessful (Fig. 4). Approximately 25% of the total plasma concentration of ddC entered the blood leukocyte compartment post injection (data not shown).

Subcutaneous administration of 20 mg/kg of ddC produced a prolonged curve, with peak plasma levels of 3.5 μg/ml attained at 1 h post inoculation and a plasma half-life of approximately 2.5 h. As with intravenous administration, no drug was detectable in plasma by 6 hours post inoculation (Fig. 4).

Attempts to prophylactically block the onset of FeLV-FAIDS infection in vivo utilizing multiple short term bolus infusion (20 mg/kg given intravenously three times daily), were unsuccessful. No difference in circulating antigen (p27) levels or time of onset of viremia could be detected in treated versus non treated groups. Clinical toxicity was not evident at this dosage level of ddC.

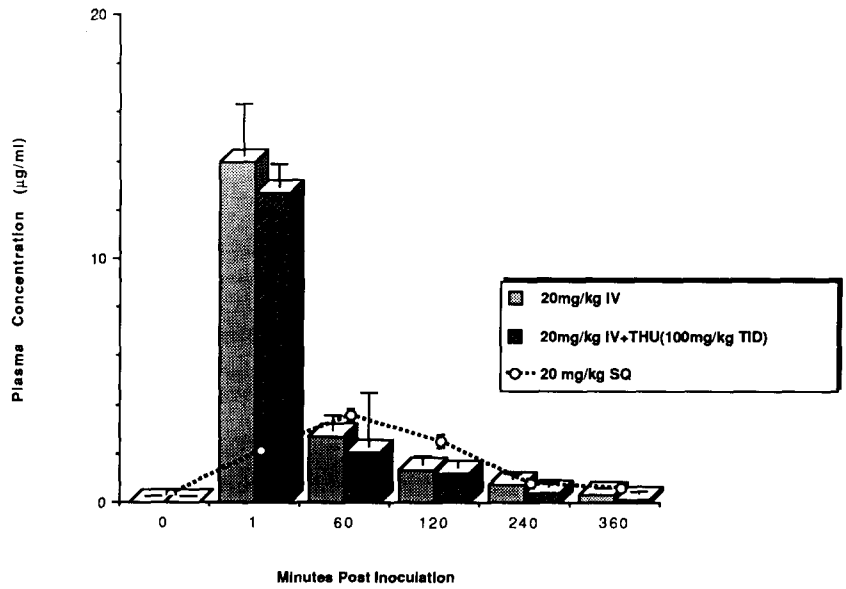

Fig. 4. Pharmacokinetics of ddC injected into cats. HPLC analysis of intravenous injection of ddC is represented by the bars comparing plasma kinetics of ddC injected alone versus ddC injected concurrently with tetrahydrouridine. The dotted line is representative of a subcutaneous bolus injection of ddC alone.

Pharmacokinetic studies employing controlled release capsular implantation of ddC

Copolymer controlled release implants were formulated based on pharmacokinetic data indicating a half-life of 1 h in vivo and in vitro assay results suggesting a sustained dosage of 1–10 µg/ml is needed for effective block of FeLV-FAIDS infectivity. Initial studies utilized subcapsular implantation of ddC with individual fiber diameter of 0.25 cm and incorporation of 130 mg of ddC within 3 individual fibers. A total dose of 520 mg was delivered using 4 implants per animal which produced peak plasma levels of 1.5 µg/ml at 6 h post implantation with a decline to 0.25 µg/ml at 72 h. Adequate sustained release was achieved by combining an individual fiber diameter of 0.12 cm with an initial ddC loading of 150 mg per implant contained within 17 individual fibers. Fig. 5 illustrates the comparative pharmacokinetics of this implantation device in comparison with subcutaneous bolus injection of 20 mg/kg ddC. A total dose of 600 mg, released over a 72 h period, was given per animal and a peak plasma level of 17 µg/ml was achieved at 6 h post implantation. Plasma levels were maintained between 1–10 µg/ml, levels shown to have an inhibitory effect on FeLV-FAIDS activity in vitro, for 72 h. Plasma concentrations ranging from 3.5–4 µg/ml were consistently detected at 48 h post implantation and the effective half-life of ddC was extended to 18 h, a 17-fold increase over intravenous infusion pharmacokinetics. Total area under the curve (A.U.C.) calculated for this implantation device through 24 h was 449.5 µg/ml/h

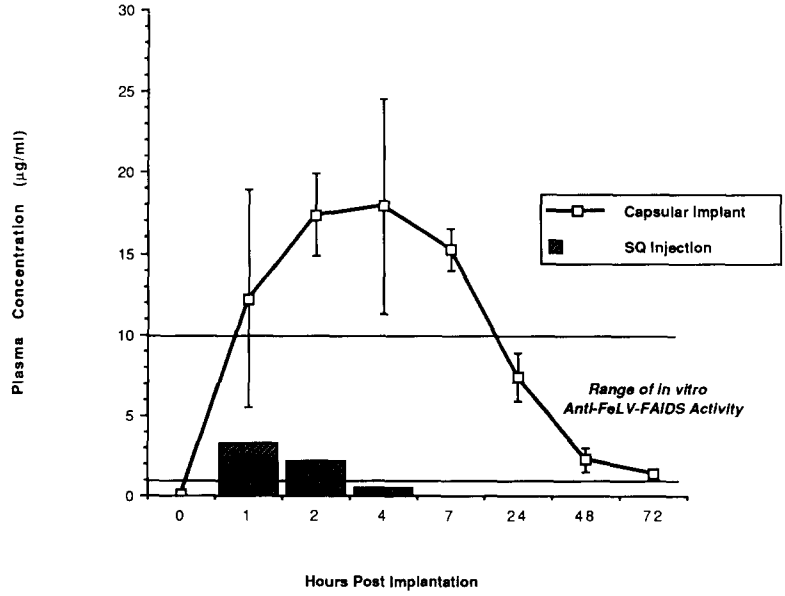

Fig. 5. Comparative pharmacokinetics of bolus subcutaneous injection of ddC versus controlled release subcutaneous implantation. Horizontal lines represent the dosage range of inhibitory activity of ddC in vitro. Striped bars represent subcutaneous bolus injection and the line is representative of capsular implantation of ddC. Standard error bars represent the standard deviation of the mean.

compared to 11.9 and 22.7 for intravenous and subcutaneous bolus injection. Sequential pharmacokinetic studies done over a four week period (implants were replenished every 72 h) demonstrated that sustained plasma levels can be maintained in cats with no evidence of clinical or hematological toxicity. ddC extraction data derived from implants recovered from treated cats indicated a release of 75–80% of available ddC within the first 48 h (data not shown).

In vivo anti-FeLV-FAIDS activity of controlled release capsular ddC implants alone and in combination with human recombinant alpha interferon and tumor necrosis factor

Based on preliminary pharmacokinetic data copolymer capsular implants delivering controlled release of 600 mg ddC were inserted and replenished every 48 h in order to sustain plasma ddC levels between 3.5–17 µg/ml. Because available quantities of ddC were limited, therapy was stopped at day 24. Virus infected control animals received mock implants consisting of copolymer and hydroxypropyl cellulose. A significant delay in the onset of viremia ($P<0.02$) was achieved in all ddC treatment groups compared with untreated virus-inoculated controls throughout the duration of the treatment period (Fig. 6). Only that group receiving ddC alone had a significant reduction in circulating viral antigen ($P<0.02$) and a delay in the onset of viremia through four weeks after inoculation with FeLV-FAIDS.

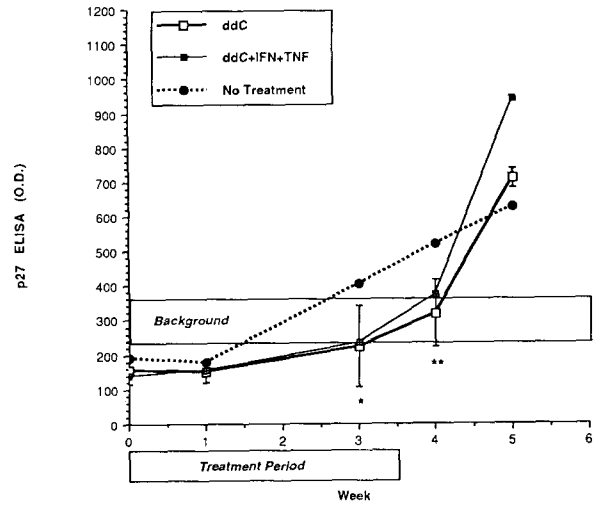

Fig. 6. Circulating p27 levels in cats treated with ddC subcutaneous capsular implantation in combination with IFNα and TNF. Lines are representative of weekly ELISA results of each treatment group. Treatment was stopped at day 24. Standard error bars represent the standard deviation of the mean. * ddC and ddC + IFNα + TNF groups are statistically different from mock treated controls ($P < 0.02$; Student's t-test). ** ddC treated group is statistically different from mock treated controls ($P < 0.02$).

Moreover, no significant difference in circulating antigen levels was noted between those animals receiving ddC alone versus ddC in combination with IFNα plus TNF at four weeks. At five weeks post inoculation, 10 days after therapy was stopped, amplification of virus replication was evident and all groups displayed similar levels of circulating p27 antigen in serum.

Those animals receiving combined cytokine therapy did have a considerable reduction in circulating antigen levels (not statistically significant) compared to virus controls at week four. All cats gained weight during the course of this study and no clinical toxicity or significant alterations in hematologic or clinical chemistry data were noted in either treatment group.

Discussion

The studies reported here indicate that anti-FeLV-FAIDS activity can be attained in vitro with 2′,3′ dideoxycytidine (ddC). Used alone in an antigen (p27) ELISA or enumerative focus induction assay anti-FeLV activity was seen at a dose range of 1–10 μg/ml. This antiviral activity can be potentiated 50–60% with the use of alpha interferon (IFNα) in combination with ddC (5 μg/ml), at dosages ranging from 500 to 2000 units/ml, levels which can be maintained in vivo when injected subcutaneously into cats (unpublished results). Similar results can be obtained in vitro using this combination therapy immediately post infection. Potentiation of the action of nucleoside analogues using IFNα in vitro has been reported with AZT (Hartshorn et al., 1987). To our knowledge this is the first report of the additive anti-retroviral effect of IFNα plus ddC. Using the combined biological re-

sponse modifier tumor necrosis factor (TNF) and IFNα, a virtual complete block of FeLV-FAIDS infectivity in vitro was attained at dosages of 0.001 μg/ml TNF, 5 μg/ml ddC, and 500–2000 units/ml IFNα. No toxicity was noted with these drug combinations in vitro. Multiple cytokine inhibition of HIV infectivity in vitro has been achieved using tumor necrosis factor and gamma interferon (Wong et al., 1988). Although tumor necrosis factor alone has been shown to inhibit HIV infectivity in vitro (Wong et al., 1988) the replication of FeLV-FAIDS in feline cells was only minimally inhibited at concentrations of TNF bordering on toxic levels.

The half life of ddC in cats, approximately 45 min after intravenous injection, is comparable to that noted in humans. Subcutaneous bolus injection can extend this half-life to 2.5 h, although peak plasma levels are approximately 20% that of intravenous administration. Attempts to prophylactically block or retard FeLV-FAIDS infectivity in vivo with bolus injection ddC therapy were unsuccessful. The use of sustained release capsular implants to administer ddC subcutaneously effectively extended the half-life of this drug 17-fold, producing plasma levels previously shown to have anti-FeLV-FAIDS activity in vitro throughout the duration of implantation (72 h). By replenishing implants every 48 h plasma levels between 3.5–17 μg/ml can be maintained for a four-week period without signs of clinical toxicity or sepsis. Results from the initial in vivo antiviral experiment utilizing copolymer implantation of ddC alone or in combination with IFNα and TNF indicate a significant retardation of the onset of persistent viremia in treated groups compared to mock implant controls. ddC treatment given alone also achieved a significant reduction in circulating viral antigen and a delay in the onset of viremia throughout a four-week treatment period. Amplification of virus replication in vivo was noted in all groups when treatment was withdrawn. This is not surprising as similar results have been shown in cats treated with zidovudine against FeLV (Tavares et al., 1987). Moreover, it has been shown that DNA chain terminators like AZT delivered in increasing concentrations in vitro inhibit but do not completely block de novo virus infection (Smith et al., 1987; Tochikura et al., 1987). Resumption of antigenemia in the face of continuous chemotherapy has also been demonstrated in AZT treated AIDS and ARC patients probably reflecting the in vitro observation that complete copies of proviral DNA can be established in the continual presence of elevated levels of AZT (Reiss et al., 1988).

The significance of the controlled release capsular implantation system lies in the simplicity of its design and delivery of nucleoside analogues. As was apparent in this study minor alterations in the composition and geometry of these implants can lead to greatly enhanced pharmacokinetics of ddC, allowing us to mimic drug levels shown to be virostatic in vitro. It appears that within this model an increase in the absolute bioavailability of ddC within a predetermined therapeutic range enhances virostatic effect in vivo without amplification of toxicity. Evidence gleaned from several studies dealing with nucleoside analogue treatment of AIDS patients suggests that sustained levels of antivirals may be responsible for reduction in circulating viral antigen and that reduced delivery of antiviral therapy appears to be followed by increased antigenemia, decreased $CD4^+$ counts and recurrent symptomatology (Yarchoan et al., 1986; Jackson et al., 1988). If this is the case, the

ability to deliver sustained levels of nucleoside analogues, in combination with individual or multiple cytokine treatment, may be a means of bypassing retroviral escape mechanisms as well as drug induced toxicity by taking advantage of additive antiviral effects and permitting lower total drug dosages. Moreover, this technology can be extended to include other more potent nucleoside analogues and cytokines, which traditionally have been compromised in their effect in vivo because of inordinately short half-lives. We are currently exploring this methodology in the feline retrovirus immunodeficiency model.

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