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Evaluation of Antiviral Activity and Toxicity of Dextran Sulfate in Feline Leukemia Virus-Infected Cats

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The feline leukemia virus (FeLV) disease model was used to conduct a toxicity and antiretrovirus efficacy trial of dextran sulfate (DS; molecular mass, 7,000 to 8,000 Da). In vitro, FeLV infection of feline lymphoid cells was inhibited by 10 μg of DS per ml. DS was administered to cats by continuous intravenous infusion at doses of 600, 120, 24, or 4.8 mg/kg of body weight per day, beginning 24 h before FeLV challenge. Doses of 24 mg/kg/day and more were excessively toxic, causing intestinal lesions and death. Similar changes were observed in unchallenged animals receiving 24 mg/kg/day, indicating that toxicity was DS mediated. The dosage of 4.8 mg/kg/day was subtoxic but did not prevent the induction and persistence of FeLV viremia. The results demonstrate that DS by continuous intravenous infusion is excessively toxic at high doses and ineffective at preventing FeLV infection at a subtoxic dose in the FeLV cat model.

Dextran sulfate (DS) is a glucose homopolymer with a molecular mass of 7,000 to 8,000 Da and contains 17 to 20% sulfur in the form of sulfate. It inhibits replication of human immunodeficiency virus type 1 (2, 3, 8, 12, 13, 21, 23) and other retroviruses in vitro (5, 9, 17, 22) by reportedly blocking virus attachment (12, 14), infection (12, 13), syncytium formation (2, 12, 20), and reverse transcriptase (3, 12, 13). DS has been used for more than 20 years as an anticoagulant and antilipemic agent in humans.

The objective of the present investigation was to conduct a preliminary evaluation of DS for toxicity and antiretroviral prophylactic efficacy in the feline leukemia virus (FeLV) cat model. DS was administered by continuous intravenous infusion to assure adequate plasma concentrations. Previous studies with AIDS and AIDS-related complex patients given oral DS (1) were difficult to interpret because of low oral bioavailability of the compound (10). Using the animal model also permitted examination of postmortem tissues to determine possible organ toxicity.

The in vitro antiviral activity of DS was evaluated against FeLV-infected feline lymphoid cells (3201 cell line). 3201 cells were incubated for 48 h in medium (41% RPMI 1640, 41% Lebovitz-15, 15% heat-inactivated fetal bovine serum, 2% L-glutamine, 1% penicillin-streptomycin) containing DS at concentrations of 0, 0.1, 0.5, 5, 10, 50, 100, 500, and 1,000 μg/ml. DS was provided by the AIDS Research and Reference Reagent Program and Developmental Therapeutics Branch, AIDS Program, National Institute of Allergy and Infectious Diseases. Cells were then subcultured in duplicate into 24-well culture dishes at a density of 7.5×10^5 cells per well in 0.2 ml of medium. Cultures were inoculated with the Kawakami-Theilen strain of FeLV collected from cell-free culture fluids of FL-74 cells (300 focus-forming units per well) (20). Virus was allowed to attach for 2 h at 37°C, after which 3 ml of medium containing the appropriate DS concentration (DS medium) was added. Cultures were split at days 3 and 7 with DS medium. After 10 days, cell-free supernatant fluids were assayed for viral p27 antigen by a commercial enzyme-linked immunosorbent assay (ELISA) (Virachek/FeLV; Symbiotics, San Diego, Calif.). In three replicate experiments, DS suppressed FeLV infection of 3201 cells by >70% at concentrations of 10 μg/ml as indicated by FeLV antigen in culture supernatants (Fig. 1). By comparison, 3′-azido-3′-deoxythymidine (AZT) inhibited FeLV antigen expression >80% at a concentration of 1.5 μg/ml in the same assay (Fig. 1, inset).

The in vivo prophylactic studies were designed to evaluate toxicity and prophylactic antiviral activity of DS in a small number of animals and to increase the number of animals at the dosage found to be subtoxic. DS was administered by continuous intravenous (i.v.) infusion by using the technique of Swenson et al. (18). DS was dissolved in pyrogen-free saline, adjusted to pH 7.4 with 1 M NaOH, and passed through a 0.44-μm (pore size) filter. DS solution was infused at a constant rate and dosage (600, 240, 120, 24, or 4.8 mg/kg of body weight per day) based on animal weight by external infusion pumps (Micro Flo-Gard 8500; Travenol Laboratories, Inc., Deerfield, Ill.). DS treatment began 48 h prior to FeLV challenge and continued for various lengths of time depending on toxicity and for 4 weeks in cats receiving 4.8 mg/kg/day. Challenge virus consisted of 0.1 ml of pooled infectious plasma from cats viremic with the Rickard strain of FeLV and contained 6×10^6 focus-forming units per ml assayed on 81c cells (4). Animals were monitored for an additional 4 weeks following the drug treatment period. Efficacy evaluation was based on prevention of viremia. FeLV viremia status was assessed weekly by using a commercial p27 ELISA kit (Virachek/FeLV) and indirect immunofluorescence technique on blood smears (6, 7). Weekly complete blood counts, plasma biochemical profiles, and postmortem histopathologic examinations were used to evaluate toxicity. Animals were monitored for virologic and toxic effects during the DS treatment period and for up to 4 weeks following discontinuance of treatment of the surviving animals.

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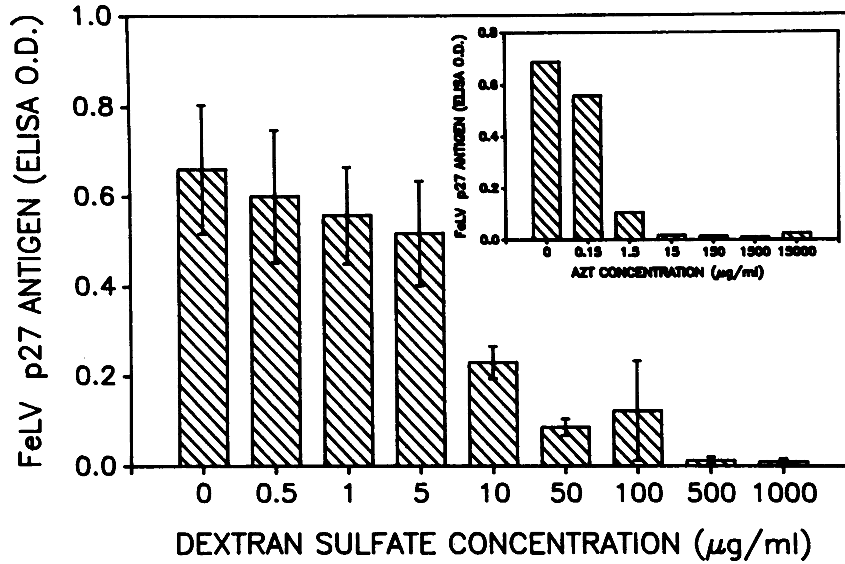

FIG. 1. Effects of DS and AZT (inset) on FeLV infection of the feline lymphoid cell 3201. 3201 cells were preincubated with the indicated concentrations of DS beginning 48 h before FeLV inoculation. Cell-free culture fluids were collected 10 days after inoculation and assayed for FeLV p27 antigen by ELISA. The DS results are a compilation of three separate assays. Values were expressed as mean optical density (O.D.) ± standard deviation. An optical density reading of 0.5 U was equal to 0.275 μg of protein from disrupted FeLV, or approximately 2.2×10^8 viral particles.

A total of 11 cats were treated prophylactically with DS. One cat received 600 mg/kg/day; one cat received 240 mg/kg/day, which was subsequently reduced to 120 mg/kg/day; five cats received 24 mg/kg/day; and four cats received 4.8 mg/kg/day. The effects of DS administration are shown in Table 1. Dosages of 600 and 240 (120) mg/kg/day were excessively toxic, causing deaths of both cats. The 24-mg/kg/day dosage was also toxic, causing deterioration of all five cats, which lead to euthanasia at 18 to 26 days after DS treatment began. The major organs targeted in these animals were the small and large intestines, which contained severe mucosal epithelial degeneration and necrosis with collapse of the lamina propria. Clinical signs of toxicity included lethargy, anorexia, anemia, and a slight left shift. One cat given 24 mg/kg/day also developed diarrhea after 15 days of therapy. The mean DS plasma concentration in the cats given 24 mg/kg/day was 47.7 μg/ml (range, 10.3 to 82.6), based on coagulation times (10).

Four additional cats serving as uninfected controls received 24 mg/kg/day by continuous i.v. infusion as described above. All four cats developed clinical signs similar to those of the virus challenge animals and were euthanized after 19 days of treatment. Gross and histologic examinations revealed intestinal lesions identical to those observed in the FeLV-challenged cats receiving DS at 24 mg/kg/day. Coagulation profiles were conducted on the unchallenged and challenged DS-treated (24 mg/kg/day) control animals on days 0 and 19 or 20 of DS infusion. Coagulation screening tests at day 19 or 20, for both infected and uninfected animals, were significantly longer than those for age-matched untreated controls (Fig. 2). As a group, the mean one-stage prothrombin time (OSPT) was significantly increased from a pretreatment (day 0) mean ± standard deviation of 12.8 ± 0.62 s to 29.45 ± 7.8 s at day 19 or 20 of DS infusion ($P = 0.0012$ by paired Student t test [PST]).

TABLE 1. In vivo prophylactic effects of dextran sulfate on development of FeLV viremia

DS dosage (mg/kg/day)	No. of cats	No. of cats with FeLV antigenemia[a] at wk postinfection								
		0	1	2	3	4	5	6	7	8
600	1	0	D[b]							
240 (120)	1	0	0	D						
24	5	0	0	3	5	E[c]				
4.8	4	0	0	1	4	4	4	4	4	4
0	6	0	0	3	6	6	6	6	6	6

[a] FeLV antigenemia was determined by ELISA for FeLV p27 antigen.
[b] D, died.
[c] E, euthanatized between 18 and 25 days after DS treatment began.

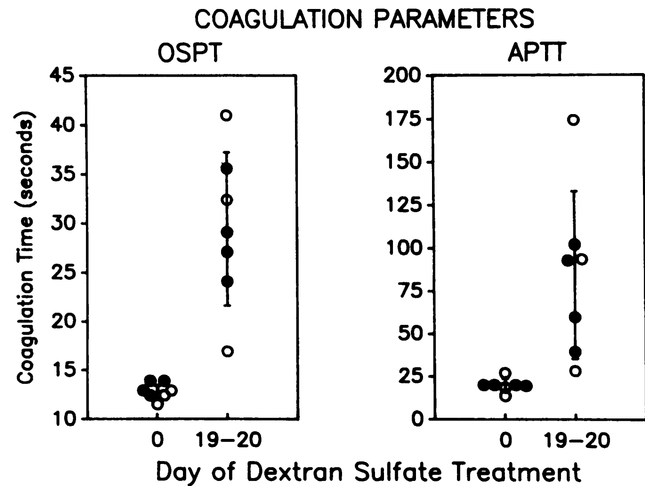

FIG. 2. Scattergram of OPST and APTT for DS-treated FeLV-infected (○) and noninfected (●) cats. Plasma samples were collected at days 0 (preinfusion) and 19 or 20 of DS infusion.

Likewise, the mean activated partial thromboplastin time (APTT) was significantly increased from 20.2 ± 3.16 s pretreatment to 84.3 ± 48.9 s on day 19 or 20 of infusion ($P = 0.01$ by PST) (Fig. 2). The prolongation of the OSPT and APTT reflected the anticoagulant effect of DS. No significant differences in the OSPT and APTT between FeLV-infected and uninfected animals were noted. No differences in platelet concentrations were detected ($P = 0.22$ by PST) (data not shown). The fibrinogen concentrations (not shown) were increased in four of six animals at day 19 or 20; however, the mean values for all six animals, 357 ± 161 mg/dl on day 19 or 20 compared with 233 ± 150 mg/dl prior to treatment, were not significantly different ($P = 0.174$ by PST). An increase in fibrinogen concentrations in some animals (not shown) was observed and attributed to increased production of acute-phase reactant proteins secondary to tissue injury.

The cats given DS at 4.8 mg/kg/day completed 4 weeks of treatment with no apparent drug-related toxicity. All four cats, however, became chronically viremic by week 2 or 3 postchallenge and remained viremic throughout the remainder of the 8-week postchallenge observation period. At the onset of viremia, these animals were still receiving DS therapy and remained on therapy for at least another week. The two cats on the higher doses did not survive long enough for viremia status to be determined. Cats given DS at 24 mg/kg/day became viremic by 3 weeks postchallenge. Challenge control cats also developed viremia by week 3 postchallenge (Table 1). The concentrations of viral antigen in plasma of the DS-treated cats (24- and 4.8-mg/kg/day groups) were not significantly different from those of challenge control cats (unpaired two-tailed *t* test) at any time following virus inoculation. However, cats given DS at 4.8 mg/kg/day had a uniformly lower mean plasma antigen concentration during DS treatment.

These studies showed that the maximum tolerable dose for cats receiving DS by continuous i.v. infusion was <24 mg/kg/day. The mechanism of DS toxicity was not immediately apparent. Because DS was administered i.v., it was assumed that drug delivery to the intestine was by the vascular route rather than transluminal. DS would presumably be available to other rapidly dividing cells, including bone marrow and lymphoid cells; however, on the basis of histologic examination, these target cells were not severely affected by DS. The possibility that DS acted synergistically with FeLV, which commonly infects rapidly dividing intestinal crypt epithelial cells (16), was discounted because DS-treated uninfected controls displayed similar lesions.

We and others have used the FeLV cat model to evaluate the prophylactic activities of a variety of antiviral agents, including AZT (11, 19), 2′,3′-dideoxycytidine (15), phosphonoformate (18a), and 9-(2-phosphonylmethoxyethyl)adenine (11). Each of these compounds demonstrated some degree of antiretroviral activity, ranging from delaying the onset of viremia to preventing chronic viremia induction. In the case of DS, no measure of efficacy could be demonstrated as a prophylactic treatment for retrovirus infection in the FeLV cat model. DS, when administered by continuous i.v. infusion, appeared to be extremely toxic at higher dosages and ineffective in preventing viremia at the lower dosage. Intestinal lesions in cats induced by DS treatment may account for the toxicity seen in human patients for whom gastrointestinal side effects were reported (1).

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