

USE OF FAMCICLOVIR FOR THE TREATMENT OF ENDOTHELIOTROPIC HERPESVIRUS INFECTIONS IN ASIAN ELEPHANTS (*ELEPHAS MAXIMUS*)

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Abstract: Two juvenile Asian elephants (*Elephas maximus*) presented with an acute onset of facial edema and lethargy. Examination of the oral cavity of each animal revealed cyanosis of the tip and distal margins of the tongue suggestive of endothelial inclusion body disease (EIBD) of elephants. Whole-blood samples were obtained, and polymerase chain reaction tests confirmed the presence of elephant herpesvirus. The animals were administered famciclovir (Famvir, SmithKline Beecham Pharmaceuticals, Philadelphia, Pennsylvania 19101, USA), a potent human anti-herpesvirus drug, in the course of their disease, and recovery followed a treatment regime of 3–4 wk. These are the first known cases of elephants surviving EIBD.

Key words: Elephant, *Elephas maximus*, endothelial inclusion body disease (EIBD), famciclovir, herpesvirus, penciclovir.

INTRODUCTION

Since 1985, there have been eight confirmed deaths of captive Asian elephants in North America that were attributed to a novel herpesvirus.⁶ Asian elephants that have succumbed to the virus ranged from 1 to 26 yr, with the majority of cases under 7 yr of age. The syndrome has been called endothelial inclusion body disease (EIBD)⁶ because of a predilection of the herpesvirus for capillary endothelial cells of the heart, liver, and tongue. Signs of the disease include acute onset of lethargy, reluctance to move, intermittent anorexia, decreased stool formation, and cyanosis of the distal tongue. Postmortem findings include extensive myocardial hemorrhages, hydropericardium, and mesenteric and serosal petechiae throughout the peritoneal cavity.^{6,7} The animals described in this report are the first two elephants in which EIBD was identified antemortem and successfully treated.

CASE REPORT

Case 1

On 12 November 1997, Dickerson Park Zoo elephant keepers noted an acute onset of lethargy and

anorexia in a 16-mo-old female Asian elephant calf (elephant E1). Samples for complete blood count (CBC) and serum chemistries were obtained the morning signs first appeared. The CBC revealed an elevated white blood count (WBC) of $19.5 \times 10^3/\mu\text{l}$ (normal female Asian elephant = $13.46 \pm 3.3 \times 10^3/\mu\text{l}$),^{3,4} lymphopenia of $3.5 \times 10^3/\mu\text{l}$ (normal = $6.6 \pm 6.6 \times 10^3/\mu\text{l}$),³ and decreased platelets of $166 \times 10^3/\mu\text{l}$ (normal = $601 \pm 97.9 \times 10^3/\mu\text{l}$).^{3,4} Serum chemistries indicated elevated lactate dehydrogenase (LDH) levels of 2571 IU/L (normal = $409.1 \text{ IU/L} \pm 221.3$).⁴ Selected normal blood parameters for female Asian elephant and for E1 during the course of the disease are summarized in Table 1. Upon examination late that afternoon, subcutaneous bilateral edema of the face, neck, and flanks was noted. The distal 5 cm of the tongue was cyanotic and slightly swollen. EIBD was considered to be the primary diagnosis. Blood for baseline serum samples was taken for serologic and chemistry analyses. In addition, a lithium heparin tube of whole blood for polymerase chain reaction (PCR) was obtained to detect herpesvirus viremia and for possible viral isolation attempts prior to initiation of any treatment.⁸ The viral samples were submitted to Dr. Laura Richman at Johns Hopkins School of Medicine. Although herpesvirus infection was not yet confirmed, there was consensus among veterinary staff and keepers to instigate treatment with antiviral medication.

Data regarding treatment of elephants with acyclovir-type drugs were unavailable; however, treatment with famciclovir (Famvir, SmithKline Beecham Pharmaceuticals, Philadelphia, Pennsylvania 19101, USA) was initiated. Treatment was based on

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Table 1. Blood parameters of elephant E1 during acute onset and treatment of endotheliotropic herpesvirus disease.

Parameter	Normal value		Elephant E1					
	Mean female ^a [standard deviation] [sample size]	Mean female ^b [standard deviation] [sample size]	Day 1	Day 2	Day 3	Day 5	Day 9	Day 14
WBC	$13.46 \times 10^3/\mu\text{l}$ [3.3] {557}	$14.32 \times 10^3/\mu\text{l}$ [4.4] {1,602}	19.5	19.8	18.5	16.1	17.1	24.4
RBC	$3.10 \times 10^6/\mu\text{l}$ [0.5] {503}	$3.06 \times 10^6/\mu\text{l}$ [0.5] {1,364}	2.55	2.40	2.20	2.04	1.78	2.44
HCT	37.8% [6.3] {569}	37.0% [5.9] {1,712}	31.6	29.8	27.3	25.6	21.9	30.6
Platelets	$601 \times 10^3/\mu\text{l}$ [97.9] {141}	$470 \times 10^3/\mu\text{l}$ [218] {380}	166	143	226	276	594	602
Neutrophils	$4.71 \times 10^3/\mu\text{l}$ [2.1] {401}	$4.76 \times 10^3/\mu\text{l}$ [2.9] {1,352}	9.55	13.5	12.8	9.0	7.7	17.1
Lymphocytes	$6.60 \times 10^3/\mu\text{l}$ [6.6] {404}	$5.21 \times 10^3/\mu\text{l}$ [3.2] {1,363}	3.5	4.3	3.5	3.1	5.5	5.1
Monocytes	$2.44 \times 10^3/\mu\text{l}$ [2.8] {323}	$3.73 \times 10^3/\mu\text{l}$ [2.9] {1,156}	1.6	2.0	2.0	3.7	3.9	2.0
CPK	299.8 IU/L [251.0] {219}	230 IU/L [173] {419}	345	1,057	2,064	3,316	466	210
LDH	409.1 IU/L [221.3] {286}	671 IU/L [743] {425}	2,571	2,906	3,534	1,372	3,537	3,398

^a From Mikota, Sargent, and Ranglack.⁴^b From International Species Information System.³

information extrapolated from human oral dosages of 500 mg/70 kg body weight (BW) or 7 mg/kg, t.i.d., with a loading dose of 1,000 mg/70 kg (14 mg/kg) BW.¹ Favorable responses with famciclovir were noted in human immunodeficiency virus cases with secondary herpesvirus infections (Dr. Alistar Haddow, St. Johns Medical Center, Springfield, Missouri 65804, USA, pers. comm.), and side effects were reported to be minimal compared with acyclovir.

On day 1 of the clinical onset of disease, a loading dose of 8 g (12.8 mg/kg) of famciclovir was administered orally. Other treatments administered for possible secondary bacterial infection, pain, and supportive purposes included 500,000 IU (800 IU/kg) benzathine penicillin G and 500,000 IU (800 IU/kg) procaine penicillin G in a combined formulation (Twin-Pen, Agri-Labs Ltd., St. Joseph, Missouri 64505, USA; i.m., q48hr), flunixin meglumine (Banamine, Schering-Plough Animal Health Corp., Kenilworth, New Jersey 07033, USA; 0.88 mg/kg i.m. b.i.d., furosemide (Lasix injectable, Hoechst-Roussel Agri-Vet Company, Somerville, New Jersey 08876-1258, USA; 0.8 mg/kg i.m. b.i.d.), and 5 ml B-complex (B Comject 150, Vetus, AM-VET Pharmaceuticals, Yaphank, New York 11980, USA; i.m. s.i.d.).

On day 2, the elephant was still very lethargic but nursing. Facial and cervical edema was still evident, and the distal 10 cm of the tongue was cyanotic. Urination and defecation were normal. Multiple serum samples showed an elevation of creatinine phosphokinase (CPK) of 1,057 IU/L (normal female Asian elephant = 299.8 ± 251 IU/L),^{3,4} indicative of muscle injury. The WBC was $19.8 \times 10^3/\mu\text{l}$, with lymphopenia and monocytosis (Table 1). Platelets were decreased to $143 \times 10^3/\mu\text{l}$. Famciclovir (4,000 mg [6.4 mg/kg] p.o. t.i.d.) and furosemide (Lasix tablets, Hoechst-Roussel Agri-Vet Company; 0.8 mg/kg p.o. b.i.d.) and flunixin meglumine (1.2 mg/kg p.o. b.i.d.) were administered to reduce fluid accumulation and pain.

On day 3, the PCR test (from blood sample obtained day 1) result for herpesvirus was reported to be positive, confirming the diagnosis of EIBD. The animal was more active and was nursing, urinating, and defecating normally. Its heart rate was increased at 92 bpm, and heart sounds were loud upon auscultation as compared with normal juvenile elephants. Body temperature was 36.9°C. At this time, its CBC revealed a WBC count of $18.5 \times 10^3/\mu\text{l}$ and a declining RBC count of $2.20 \times 10^6/\mu\text{l}$ (normal female Asian elephant = $3.1 \times 10^6/\mu\text{l}$). The hematocrit (hct) was 27.3% and serum LDH

and CPK remained very high at 3,534 mg/dl and 2,064 mg/dl, respectively.

On day 4, the elephant's appetite remained fair, but its activity level decreased. The heart rate was elevated at 88 bpm, and heart sounds were still easily auscultated. Famciclovir and furosemide were continued twice daily at the same doses.

By day 5, the lingual cyanosis was decreasing and the elephant's activity was improving. However, the weight loss was 12 kg (from 625 kg to 613 kg). The WBC was $16.1 \times 10^3/\mu\text{l}$ with segmented neutrophils (41%), bands (17%), and mild monocytosis. The RBC count had decreased to $2.04 \times 10^6/\mu\text{l}$ and the hct was 25.6%. Mucus obtained in a loose stool was found to contain degenerative neutrophils with 5–6 neutrophils/100 \times field. Additional therapy included 1,000,000 IU of long-acting penicillin (q48hr i.m.) and sulfamethoxazole/trimethoprim (Sulfa/Trim D/S, Mutual Pharmaceutical Company Inc., Philadelphia, Pennsylvania 19124, USA; 20 mg/kg p.o. s.i.d.).

On day 9, the elephant's attitude improved enough that it was stealing pellets from two adult elephants in the same stall. The heart rate was still at 88 bpm with increased heart sounds, and the tongue appeared normal. The RBC had decreased to $1.78 \times 10^6/\mu\text{l}$ and the hct was 21.9%. Neutrophils were not detected in subsequent stool samples.

Allometric scaling was used to adjust the famciclovir dose. Allometric scaling⁹ for conversion of the human famciclovir dosage to a 625-kg elephant resulted in a calculated dose of 2.5 g (4.06 mg/kg) t.i.d. Over the next few days, an increasing WBC with a continuing left shift was noted. Cefotiofur (Naxcel, Upjohn Company, Kalamazoo, Michigan 49001, USA; 2.24 mg/kg i.m. s.i.d.) was added to counter possible secondary bacterial infection.

On day 14, PCR-amplified DNA for EIBD from whole blood was barely detectable. The elephant's attitude and activity were considered normal. The dosage of famciclovir was changed to 4 g (6.4 mg/kg) b.i.d. p.o. for the next 5 days as a withdrawal dose and then discontinued. Twice-weekly blood sampling was continued for the next 3 wk to monitor CBC and serum chemistry values. By day 54, the heart rate had stabilized at 56 bpm, and the heart sounds were normal. Whole-blood samples were submitted to Dr. Richman to perform PCR for the presence of herpesvirus. The samples became negative by day 56. Serum penciclovir levels on treatment dates were measured by F. Hamzeh and A. Shahkolahi (Johns Hopkins School of Medicine, Baltimore, Maryland 21205, USA). Serum levels

varied from 97 ng/ml to 4,365 ng/ml and were comparable with known therapeutic treatment levels described for a 500-mg dose of famciclovir.^{1,10}

At the time of this report (1 July 2000), the elephant continues to be normal.

Case 2

A 21-mo-old, captive-born male Asian elephant (E2) with no prior history of medical problems was presented for acute onset of lethargy. The calf was estimated to weigh 750 kg. On 8 October 1998, staff at the Center for Elephant Conservation (Polk City, Florida 33868, USA) noted the elephant was mildly lethargic and listless. Further observations revealed increased sensitivity to touch in the area of the tusks and decreased food and water consumption. It was first thought that the clinical signs were due to eruption of the tusks. On the third day, staff noted cyanosis of the tip of the elephant's tongue and reluctance to eat and drink. On the fifth day, the elephant was examined and determined to be alert, responsive, and eating small amounts of the food offered. At rest, however, it was lethargic and tended to rest its trunk tip on the ground. Further examination of the oral cavity revealed that the cyanosis was particularly marked at the margins. Subjectively, the tongue appeared swollen and painful. Clinical signs suggested a tentative diagnosis of EIBD. Treatment with oral famciclovir (8 g [10.6 mg/kg] p.o. b.i.d.) was initiated; however, the elephant refused all medications. The following morning, a decision was made to administer the famciclovir rectally. Although pharmacokinetic data were not available to document rectal absorption of famciclovir in elephants or humans, other drugs have had good rectal absorption in elephants² (R. Isaza, unpubl. data on isoniazid). Famciclovir (8 g [10.6 mg/kg]) was administered rectally b.i.d. as a liquid paste. Additional medication included flunixin meglumine (150 mg [0.2 mg/kg] i.m. b.i.d.). Samples for CBC and serum chemistries were obtained on day 6. The CBC revealed anemia (hct = 23.3%, hemoglobin = 9.7 g/dl) and an elevated WBC of $15.8 \times 10^3/\mu\text{l}$ with a normal differential.^{3,4} Serum chemistries indicated a mild azotemia (BUN = 20 mg/dl, creatinine = 1.9 mg/dl), assessed to be due to dehydration. Hypoproteinemia (total protein = 6.0 g/dl) was noted and considered significant in the face of dehydration. Abnormal liver indices included mild elevations in the total bilirubin (4.2 mg/dl) and aspartate aminotransferase (52 IU/L). Urinalysis was performed (sp. gr. = 1.023) and found to be normal. Whole-blood samples were drawn in lithium heparin tubes and

submitted to Dr. Richman for PCR analysis for elephant EIBD.

On the day 6 (the second day of rectal administration with famciclovir), the clinical signs had progressed to complete anorexia with no nursing activity, and the elephant's face was swollen in the cheek areas. The PCR test for elephant EIBD was reported as positive. Treatment continued with famciclovir (8 g [10.6 mg/kg] rectally b.i.d.) and flunixin meglumine (0.67 mg/kg i.m. b.i.d.). Ceftiofur (0.8 mg/kg i.m. s.i.d.) and furosemide (500 mg [0.67 mg/kg] i.m. s.i.d.) were added to the drug regimen. On day 7 (the third day of famciclovir administration), the head and face were more swollen, the conjunctiva of both eyes were edematous and purple, and the elephant was increasingly lethargic. The anorexia continued, and an increase in water consumption was noted. Treatment consisted of famciclovir (5 g [6.7 mg/kg] rectally b.i.d.), flunixin meglumine (0.67 mg/kg i.m. b.i.d.), furosemide (0.6 mg/kg i.m. s.i.d.), and ceftiofur (0.8 mg/kg i.m. s.i.d.).

On day 8 of illness (the fourth day of famciclovir treatment), the clinical signs became less severe. Nursing and food consumption increased, and the elephant was noticeably more active and was seen moving around the pen and interacting with its mother and the staff. The lingual cyanosis and swelling began to resolve, and the tongue seemed less painful when touched. The swelling of the head and the discoloration of the conjunctiva around the eyes appeared unchanged. Treatment consisted of famciclovir (6.7 mg/kg rectally b.i.d.), flunixin meglumine (0.67 mg/kg i.m. b.i.d.), furosemide (0.6 mg/kg i.m. s.i.d.), and ceftiofur (0.8 mg/kg i.m. s.i.d.).

Between days 9 and 15 of clinical illness (fifth to 11th days of treatment), the clinical signs gradually diminished. On the 20th day of illness, the elephant was active and clinical signs had resolved except for the conjunctival discoloration. Samples for CBC and serum chemistries were obtained on the 20th day of the illness. The CBC revealed that the anemia was improving (hct = 33.7%, hemoglobin = 12.0 g/dl); however, WBC was elevated ($24.9 \times 10^3/\mu\text{l}$), with a monocytosis. Serum chemistries indicated a resolution of the previously noted azotemia (BUN = 9 mg/dl, creatinine = 1.5 mg/dl); hypoproteinemia (7.4 g/dl) was still present but improved. All liver-related indices were considered to be normal. Whole-blood samples were drawn in lithium heparin tubes and submitted for a second PCR analysis. Results were reported as a weak positive. Treatment consisted of famciclovir (6.7 mg/kg rectally b.i.d.). The flunixin meglumine and fu-

roseamide were discontinued on day 9 of treatment. The ceftiofur was discontinued on day 11 of treatment.

The next 14 days (days 12–26 of treatment) were unremarkable except for the resumption of normal behavior and appetite and the slow resolution of the conjunctival discoloration. Famciclovir (6.7 mg/kg rectally b.i.d.) was continued until day 30 of treatment. To date (1 July 2000), E2 continues to be clinically normal with no recurrence of clinical signs or apparent sequela to the infection. A third PCR test was collected on Day 99, and results were negative for the presence of the elephant EIBD.

DISCUSSION

These are the first two reported cases of EIBD in elephants to be confirmed antemortem. This confirmation was possible because of an awareness and detection of the symptoms associated with the disease by keeper and/or veterinary staff and the recent development of an accurate PCR to detect the viremia that occurs in clinically affected elephants. Because of treatment with famciclovir, recovery was possible.

Famciclovir is an orally administered pro-drug of the anti-herpes agent penciclovir. Famciclovir undergoes rapid biotransformation to the active anti-herpes compound penciclovir, which has known inhibitory activity against herpes simplex and varicella zoster through downregulation of herpesvirus synthesis and replication.¹ Support for the beneficial effect of famciclovir on EIBD is that both animals were found to be viremic by PCR early in the disease and both had all the classic clinical signs; yet, at a stage of the disease when elephants normally die, they began to recover. One of the elephants (E1), in a semiquantitative PCR, showed decreasing detectable blood viral DNA load that coincided with serum famciclovir at therapeutic levels. The recovery of the second elephant (E2) after rectal administration of famciclovir provides anecdotal evidence that there may be an alternative to oral administration of famciclovir. In elephants unable to consume the medication because of edema of the face and tongue or the handler's inability to administer the drug orally, this route of administration may be an important factor in survival of the patient. These are the first known cases of elephants surviving EIBD.

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