Effect of oral administration of L-lysine on conjunctivitis caused by feline herpesvirus in cats

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Objective—To determine whether oral administration of L-lysine to cats would lessen the severity of conjunctivitis caused by feline herpesvirus (FHV-1).

Animals—Eight healthy young adult cats.

Procedure—Cats received oral administration of lysine monohydrochloride (500 mg, q 12 h) or placebo (lactose) beginning 6 hours prior to inoculation of virus. The left conjunctival sac received a 50-µl suspension of FHV-1 grown in cell culture (1.8 × 10^6 tissue culture infective dose [TCID50]) on day 1. Cats were evaluated and scores given for clinical signs each day for 21 days. Samples for virus isolation were collected from the eye and throat every third day. Plasma lysine and arginine concentrations were measured prior to the study and on days 3, 14, and 22.

Results—Cats that received lysine had less severe conjunctivitis than cats that received placebo. Virus isolation results did not differ between the groups. Plasma lysine concentration was significantly higher in cats that received lysine, compared with control cats, whereas plasma arginine concentrations did not differ between groups.

Conclusions and Clinical Relevance—Oral administration of 500 mg of lysine to cats was well tolerated and resulted in less severe manifestations of conjunctivitis caused by FHV-1, compared with cats that received placebo. Oral administration of lysine may be helpful in early treatment for FHV-1 infection by lessening the severity of disease. (Am J Vet Res 2002;63:99–103)

Feline herpesvirus (FHV-1) infection is common and is one of the most frequently encountered causes of ocular disease in cats. Because FHV-1 develops neuronal latency, an infected cat may have recurring bouts of disease. Numerous treatments have been used for cats with ocular herpesvirus-associated disease, including topically or parenterally administered antiviral agents, topically administered anti-inflammatory agents, topically or parenterally administered interferon, and parenterally administered L-lysine.

Oral administration of L-lysine in cats has been adopted from human medicine; reduced frequency or severity of outbreaks of herpes simplex virus (HSV)-associated disease in humans has been reported with the use of orally administered l-lysine. The mechanism of action is thought to be reduced viral replication attributable to antagonism of arginine by excess lysine. Protein fraction I of the histone layer around the DNA of the eukaryotic or host cell is 28% lysine and 3 to 4% arginine. The protein in the capsid coat around the DNA core of the herpesvirus is in reverse proportions, with approximately 3 times more arginine than lysine. Elimination of arginine from the media of HSV grown in cell culture results in lack of viral replication. Excess lysine in the media also has an inhibitory effect on viral replication. Similar in vitro results have been documented for FHV-1. Replication of FHV-1 was almost completely inhibited when the growth media was free of arginine. Excess lysine in the media inhibited viral replication but only when arginine concentration was reduced to 2.5 µg/ml.

Data specifically related to the effect of lysine on the clinical course of FHV-1-induced disease in cats have not been reported. The purpose of the study reported here was to assess the effect of oral administration of L-lysine monohydrochloride on the course of experimentally induced conjunctivitis caused by FHV-1.

Materials and Methods

Cats—Eight young adult random-source cats (5 neutered males, 2 spayed females, 1 sexually intact female) were included in the study. All cats were in good systemic health and had normal ocular examination findings by use of slit-lamp biomicroscopy and indirect ophthalmoscopy prior to the study. Cats had negative results of tests for serum FeLV antigen and antibodies against FIV. Results of CBC and serum biochemical analyses were within reference ranges in all cats. Results of attempts at isolation of FHV-1 from the left conjunctival sac and the pharynx were negative for all cats prior to the study. Cats were housed primarily in individual cages in 1 room but were allowed to interact as a group for a portion of each day. Cats had been vaccinated against diseases caused by FHV-1 (viral rhinotracheitis), calicivirus, panleukopenia virus, and rabies 6 months prior to the study. Other historical information about the cats was unavailable.

Experimental design—A field strain of FHV-1 was grown in cell culture, using Crandell feline kidney cells. Viral infectivity assessed by use of quantal titration was 1.84 × 10^6 tissue culture infective dose (TCID50)/ml. Cats were randomly assigned to 1 of 2 groups. On day 1 of the study, 50 µl of virus suspension was placed in the left conjunctival sac of each cat. Beginning 6 hours prior to inoculation of virus, 4 cats received 500 mg of l-lysine monohydrochloride orally...
twice daily (group 1), and 4 cats received a placebo (lactose, 500 mg) twice daily (group 2). Investigators were not aware of group assignments. The powdered medications were given in a small aliquot of moist food, which all cats ate readily.

Cats were evaluated each day for the 3-week study period. Clinical scores (0 = typical or negative findings, 1 = mild, 2 = moderate, and 3 = severe) were given individually for signs of conjunctival hyperemia, chemosis, ocular discharge, blepharospasm, and fluorescein retention by the cornea. Mean scores were calculated to obtain a single value for each cat for each day. All examinations and scores were performed by 1 investigator (JS). Sterile moistened rayon swabs were used to take samples from the left conjunctival sac and the pharynx for virus isolation every third day. Samples were immediately transported on ice to the laboratory. Plasma samples for lysine and arginine concentrations were obtained from blood samples drawn the week before the study, on days 3 and 14 of the study, and immediately after the study. Complete blood counts and serum biochemical analyses were performed before and at the end of the study. All protocols were approved by the Purdue University Animal Care and Use Committee.

**Analysis of plasma lysine and arginine concentrations**—Lysine and arginine analyses were performed by use of an automated amino acid analyzer. Plasma stored at −20 C or colder was thawed, mixed with an equal volume of 6% sulfosalicylic acid, and centrifuged at 10,000 X g for 10 minutes. The supernatant was prepared with an internal standard, and the equivalent of 40 µl of plasma was injected onto the ion exchange column. The quantity of each amino acid was determined colorimetrically, using ninhydrin for color development.

**Statistical analyses**—Clinical scores of cats in group 1 and 2 were compared by use of a Friedman nonparametric ANOVA. This test was also used to compare differences within groups among days. Comparison of the virus isolation results of both groups was by use of the χ² test. Comparisons of plasma lysine and arginine concentrations between groups were performed by use of the Student t-test. Plasma lysine and arginine concentrations from blood collected on days 3 and 14 from cats were combined and the mean values were compared with prestudy mean values. This was done because values may vary depending on the time of blood sampling after administration of lysine, and the combined values were thought to be more representative. A value of P < 0.05 was considered significant for all comparisons.

**Results**

**Lysine administration**—All cats ate lysine or placebo mixed with moist food readily. No gastric upset or other adverse signs attributable to lysine intake were noted during the study period.

**Clinical disease**—All cats developed left-sided conjunctivitis, sneezing, and left-sided submandibular lymphadenopathy. None of the cats developed corneal ulceration. Cats in group 2 developed more severe and more rapid onset of conjunctivitis than cats in group 1 (Fig 1). Two cats in group 2 developed erosions of the conjunctiva and symblepharon formation. There was a highly significant difference between groups for mean clinical score (Friedman test statistic = 9; 1 df; P < 0.001) on days 5 to 15. There were no significant differences among days within groups for mean clinical score (Friedman test statistic = 12; 8 df; P > 0.10).

Three of 4 cats that received lysine developed only mild conjunctivitis, and 1 cat developed severe conjunctivitis, although there was no erosion of conjunctiva or symblepharon formation (Fig 2). Of the 4 cats that received placebo, 3 developed rapid-onset severe conjunctivitis, whereas 1 cat developed moderate conjunctivitis (Fig 3). Submandibular lymphadenopathy, although not quantitated, did not appear to differ between group-1 and group-2 cats. Sneezing developed earlier in cats in group 2, although by day 7, all cats
were sneezing and, when observed by the investigators, frequency of sneezing did not appear to differ between groups, although no attempt to quantitate this clinical feature was made. The length of time cats were affected with conjunctivitis did not differ between groups. By day 21 of the study, all cats had resolution or only mild conjunctivitis.

Virus isolation—All cats had positive results of virus isolation from the left eye and the throat on days 3 and 6 of infection. Results of subsequent virus isolations from cats of both groups were variably positive and negative from the eye and throat. Comparison of virus isolation results from group 1 and 2 did not reveal significant difference between the groups ($\chi^2 = 2.46; 1 \text{ df}; P > 0.10$).

Plasma lysine and arginine concentrations—

Prestudy mean plasma lysine concentration in group-1 cats was $115 \pm 21 \text{ nmol/ml}$, and that of group-2 cats was $85 \pm 16 \text{ nmol/ml}$; difference between the groups was not significant ($t = 1.13; 6 \text{ df}; P > 0.05$). Prestudy mean plasma arginine concentration of group-1 cats was $144 \pm 18 \text{ nmol/ml}$, and that of group-2 cats was $117 \pm 22 \text{ nmol/ml}$; the difference was not significant ($t = 0.94; 6 \text{ df}; P > 0.05$).

Mean plasma lysine concentration of group-1 cats for samples drawn on days 3 and 14 of the study was $669 \pm 101 \text{ nmol/ml}$. Compared with the prestudy mean value, the difference was significant ($t = 3.78; 10 \text{ df}; P < 0.001$). Mean plasma arginine concentration for group-1 cats on days 3 and 14 was $205 \pm 15 \text{ nmol/ml}$. Compared with the prestudy mean value, the difference was significant ($t = 2.65; 10 \text{ df}; P < 0.05$).

Mean plasma lysine concentration of group-2 cats for samples drawn on days 3 and 14 of the study was $193 \pm 11 \text{ nmol/ml}$. Compared with the prestudy mean value, the difference was significant ($t = 5.51; 10 \text{ df}; P < 0.001$) Mean plasma arginine concentration of group-2 cats for samples drawn on days 3 and 14 was $175 \pm 12 \text{ nmol/ml}$. Compared with the prestudy mean value, the difference was significant ($t = 2.65; 10 \text{ df}; P < 0.05$).

Mean plasma lysine concentration of group-1 cats for samples drawn on days 3 and 14 of the study (669 nmol/ml) was significantly ($t = 4.49; 14 \text{ df}; P < 0.001$) different from that of group-2 cats (193 nmol/ml). Mean plasma arginine concentration of group-1 cats for samples drawn on days 3 and 14 of the study (205 nmol/ml) was not significantly different ($t = 1.57; 14 \text{ df}; P > 0.05$) from that of group-2 cats (175 nmol/ml).

Samples were drawn on day 22, 1 day after cessation of oral administration of lysine. Mean plasma lysine concentration for group-1 cats was $207 \pm 41 \text{ nmol/ml}$ and for group-2 cats was $134 \pm 33 \text{ nmol/ml}$. Each group had significantly greater mean lysine concentration at the end of the study than during the prestudy period (group 1: $t = 1.99, 6 \text{ df}, P < 0.05$; group 2: $t = 2.44, 6 \text{ df}, P < 0.05$). Mean plasma arginine concentration on day 22 for group-1 cats was $146 \pm 15 \text{ nmol/ml}$ and for group-2 cats was $116 \pm 15 \text{ nmol/ml}$. There were no significant differences between these values and the prestudy concentration for either group (group 1: $t = 0.09, 6 \text{ df}, P > 0.05$; group 2: $t = 0.03, 6 \text{ df}, P > 0.05$).

CBC and serum biochemical values—Values for CBC and serum biochemical analyses did not differ significantly between prestudy evaluations and those taken at the end of the study.

Discussion

Findings of the study reported here are similar to those of human clinical trials in which daily oral administration of l-lysine reduced outbreaks of HSV-related disease and lessened the severity when outbreaks did occur. Cats that received l-lysine orally in our study developed less severe FHV-1-induced conjunctivitis than cats that received placebo, although the time course to resolution of conjunctivitis did not differ between groups.

Cells infected with HSV or FHV-1 in vitro require arginine in the media for successful viral replication.\textsuperscript{13,16,17} Cells deprived of arginine fail to develop cytopathic effects associated with viral replication. Analysis of herpesvirus proteins synthesized in cells deprived of arginine reveals that the major structural proteins are present but that these proteins do not migrate from their cytoplasmic site of synthesis to their assembly site in the nucleus.\textsuperscript{18} Lysine is not required for viral growth and, when present, has a partially inhibitory effect on HSV or FHV-1 replication, presumably by competitive inhibition of arginine.\textsuperscript{13,17} In the study reported here, cats began receiving lysine several hours prior to infection, which may have reduced viral replication sufficiently in the early phase to result in less severe conjunctivitis.

In experimental studies\textsuperscript{19-22} in chickens, lysine competes with arginine for the transport system across the wall of the intestine and also with the reabsorption of arginine by the transport system of the renal tubules. The net result of oral administration of excess lysine in chickens is reduced arginine absorption across the intestine and increased excretion of arginine in the urine. In growing puppies, administration of excess lysine does not antagonize intestinal absorption of arginine, although lysine-arginine antagonism is detected, and clinical signs of arginine deficiency such as decreased urea formation, reduction in weight gain, hyperammonemia, and emesis are observed.\textsuperscript{23} Non-specific amino aciduria is detected, although induction of liver arginase does not occur. Lysine is reported to be a potent arginase inducer, resulting in catabolic degradation of arginine.\textsuperscript{24,25} In young pigs, oral administration of lysine at twice the normal required amount does not affect weight gain.\textsuperscript{26} At 3 or 4 times basal concentration, lowered weight gain and feed intake are detected, although tissue arginase is unaffected.

Arginine restriction in cats is not recommended. Arginine is an essential amino acid in cats and plays an active role in the urea cycle. Arginine is transformed to ornithine through the action of the enzyme arginase, giving off urea and, thus, disposing of ammonia. Reduction below the minimal requirement of 0.83% arginine in the diet of cats allows ammonia to increase to toxic concentrations. Cats fed slightly less than half the dietary requirement of arginine develop vomiting and severe lethargy within 4 hours.\textsuperscript{27} Cats fed diets
devoid of arginine rapidly develop hypersalivation, hyperesthesia, emesis, muscle tremors, ataxia, tetanic spasms, and, in some instances, coma and death.\textsuperscript{28,29}

Plasma arginine concentration in cats that received orally administered lysine in our study were significantly different from prestudy values, although this was not clinically relevant, because the arginine concentrations on days 3 and 14 were greater than those at baseline rather than lesser. Arginine concentrations were not significantly different on days 3 and 14 between groups 1 and 2; this suggests that absorption of arginine across the intestines was not affected by excess lysine intake, nor was increased urine excretion or induction of arginase an important factor. Signs of arginine deficiency such as depression, anorexia, or neurologic signs were not observed at any time. Although competition for arginine uptake into viral proteins may be occurring at the cellular level, suppression of arginine function in the urea cycle does not appear to be a concern in cats that received 1 g of L-lysine daily, at least in the short term.

The focus of human clinical trials of orally administered lysine in double blind crossover studies\textsuperscript{4,30,32} has been to evaluate its ability to reduce the frequency of recurrences of HSV-associated disease. Although the results of the trials have been somewhat mixed, most reported a positive influence of lysine in dosages of 1 to 3 g/d to reduce the frequency of HSV outbreaks and to lessen the severity of signs when an outbreak did occur. Lysine is an essential amino acid for cats and humans. Dietary intakes of lysine in adult cats eating a commercial diet as determined at the Amino Acid Analysis Laboratory, School of Veterinary Medicine, University of California-Davis, Calif.\textsuperscript{4} have been reported to be approximately 1 to 3 g/d, with kittens eating approximately half this amount.\textsuperscript{31} A dosage of 500 mg given twice daily was chosen as a starting point in our study. Many clinicians prescribe 250 to 500 mg of lysine twice daily for cats with active FHV-1 lesions or for cats prone to recurrences. One gram of lysine was chosen, because it represents a similar percentage increase in lysine, compared with that shown to be effective in humans. Assuming a cat eats 1.5 g of lysine daily, an additional 1 g represents an increase of 66%.

In a pilot study undertaken prior to the study reported here, in which 300 to 1,000 mg of lysine was given to cats after food was withheld, approximately 50% of the cats vomited within 1 hour. Typically, lysine could be seen within the vomitus. Thus, for the study reported here, lysine was given with a small amount of moist food, and vomiting did not occur. Cats were allowed access to dry food after the moist food containing the test substances was consumed. The hydrochloride form of lysine may contribute to vomiting, and trials using the free-base form of lysine may be warranted if vomiting is a problem in individual cats. Typical feline plasma lysine concentration is 100 ± 16 nmol/ml, and values up to 200 nmol/ml are not unusual after feeding a high protein diet (eg, some canned foods). Typical plasma arginine concentration\textsuperscript{4} is 138 ± 24 nmol/ml, although values up to 200 nmol/ml are not unusual in cats after eating canned food. In the study reported here, cats that received lysine had plasma concentrations that ranged from 318 to 1,137 nmol/ml. The variability probably relates to time of blood sampling relative to ingestion of lysine, time cats took to consume a meal, and a variability of absorption among cats. Plasma concentration of arginine in this study ranged from 76 to 274 nmol/ml. The mean lysine concentration of group-1 cats in samples taken immediately after the study (day 22) was 207 nmol/ml, which is within the postprandial reference range. This suggests lysine concentration will quickly return to reference range when excess lysine is withdrawn.

One of the limitations of this study was the small sample size. Individual cats may have variable susceptibilities and responses to FHV-1 infection. The importance of innate variability in responses to the virus could be lessened by having a much larger sample size, although that was not possible in the study reported here. The effect of vaccination of the cats in this study 6 months prior is unknown. Protection afforded against FHV-1 is usually incomplete and temporary\textsuperscript{3} and probably had little bearing on the results of the study. It has been noted in human clinical trials that some individuals may benefit more than others from oral administration of lysine in regards to suppressing viral replication. This likely will hold true for cats as well. In this study, 1 cat in the lysine group developed severe conjunctivitis, and 1 cat in the placebo group developed only moderate conjunctivitis. Despite this finding, when compared as 2 groups, it was clear that cats that received lysine had markedly less severe disease. The findings of this study suggest that orally administered lysine in a dosage of 500 mg administered twice daily is well tolerated and lessens the severity of FHV-1–induced conjunctivitis. Although a shortened course of disease has been noted in humans, cats that received lysine in our study had conjunctivitis for the same time period as cats that received placebo. Prevention or early treatment with lysine may be more efficacious in cats than treating an infection that is already established.

\textsuperscript{a}Sigma Chemical Co, St Louis, Mo.
\textsuperscript{b}Viral culturette, Becton-Dickinson Co, Sparks, Md.
\textsuperscript{c}Beckman Instruments Inc, Fullerton, Calif.
\textsuperscript{d}Typical concentration for adult cats fed a commercial dry expanded diet as determined at the Amino Acid Analysis Laboratory, School of Veterinary Medicine, University of California-Davis, Davis, Calif.

References


