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Pharmacokinetics and analytical determination of acyclovir in Asian elephant calves (*Elephas maximus*)

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ABSTRACT

A therapeutic regimen that includes antiviral drugs is critical for the survival of Asian elephant (*Elephas maximus*) calves infected with elephant endotheliotropic herpesvirus hemorrhagic disease (EEHV-HD), with acyclovir showing considerable promise. The purpose of this study was to determine the pharmacokinetics and bioavailability of acyclovir following intravenous (IV) and oral (PO) administration in Asian elephants. A single dose of acyclovir (15 mg/kg, IV or 45 mg/kg, PO) was administered to four healthy elephant calves, with a minimum 2-week washout period between treatments. Serial plasma samples were collected after each injection for acyclovir analysis using a validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) technique. Maximum plasma acyclovir concentrations were 27.02 ± 6.79 μg/mL at 0.94 ± 0.31 h after IV administration, and 1.45 ± 0.20 μg/mL at 3.00 ± 0.70 h after PO administration. The half-life of the elimination phase ($T_{1/2}$) was 5.84 ± 0.74 and 8.74 ± 2.47 h after IV and PO administration, respectively. After IV administration, acyclovir concentrations were higher than the half-maximal inhibitory concentration (IC_{50}) of those found for herpes simplex virus (HSV) 1 and 2 in humans, and equid alpha herpesvirus-1 (EHV-1) for at least 12 h. By contrast, the bioavailability of oral administration was low, only 6.03 ± 0.87%, so higher doses by that route likely are needed to be effective. Due to the high concentration of plasma acyclovir after IV administration, the dose may need to be adjusted to prevent any negative side effects.

1. Introduction

Elephant endotheliotropic herpesviruses (EEHV) cause a highly fatal hemorrhagic disease (EEHV-HD) in young Asian (*Elephas maximus*) elephant calves, with a mortality rate of 70–80% in those less than 8 years of age (Boonprasert et al., 2019; Hayward, 2012; Latimer, Zong, Heaggans, Richman & Hayward, 2011). Asian elephants are categorized as endangered in the International Union for Conservation of Nature (ICUN) Red List with population declines due to habitat destruction, human-elephant conflict, and poaching for ivory. In captivity, deaths related to non-infectious and infectious diseases, including EEHV-HD (Kendall, Howard, Masters & Grant, 2016), are a hindrance to

; %CV, Mean precision; $AUC_{0\text{-inf}}$, Total area under the plasma concentration-time curve from time zero to infinity; $AUC_{0\text{-t}}$, Total area under the plasma concentration-time curve from time 0–48h; Cl, Total clearance; C_{max}, Peak plasma concentration; EEHV, Elephantendotheliotropic herpesviruses; EEHV-HD, Elephant endotheliotropic herpesvirus hemorrhagic disease; EHV, Equid alphaherpesvirus; F, Bioavailability; HSV, Herpes simplex virus; IV, Intravenous administration; K_{el}, Elimination rate constant; LC-MS/MS, Liquid chromatography-tandem mass spectrometry; LLOQ, Lower limit of quantitation; *m/z*, Mass-to-charge ratio; MAT, Mean absorption time; MRM, Multiple reaction monitoring; MRT, Mean residence time; PO, Oral administration; QC, Quality control; r^2, Coefficients of determination; S/N, Signal to noise ratio; $T_{1/2}$, Elimination half-life; T_{max}, Time to reach peak plasma; $V_{d(ss)}$, Steady-state volume of distribution.

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population sustainability. Early diagnosis of EEHV by molecular diagnostic and hematology techniques, and treatments that include fluid therapy, antiviral and anti-inflammatory drugs, antioxidants, diuretics, and plasma infusion are key to survival (Luz & Howard, 2016), but are costly, especially in range countries.

Successful treatment of EEHV with antiviral drugs has been reported; e.g. acyclovir (Khammesri et al., 2021; Sripiboon et al., 2017), famciclovir (Dastjerdi, Seilern-Moy, Darpel, Steinbach & Molenaar, 2016; Schmitt et al., 2000), and ganciclovir (Wissink-Argilaga, Dastjerdi & Molenaar, 2019). Acyclovir is widely used in the treatment of herpesvirus infections in other species, including humans (Brigden & Whiteman, 1983; Elion, 1982; Spector et al., 1981; Whitley, Blum, Barton & de Miranda, 1982), cats (Owens, Nasisse, Tadepalli & Dorman, 1996), dogs (Evermann, Ledbetter & Maes, 2011), and horses (Wong, Maxwell & Wilkins, 2010). In mammals, it is selectively converted to a monophosphate form by viral thymidine kinase, which is more effective (3000 times) in the phosphorylation of acyclovir than human thymidine kinase (Elion, 1982). Subsequently, the monophosphate form is further phosphorylated into the active triphosphate form, aciclo-GTP, by cellular kinases. Aciclo-GTP is a potent inhibitor of viral DNA polymerase, which has approximately 100 times higher affinity to a virus than cellular polymerase (Elion, 1982). This triphosphate form incorporates into the viral DNA, resulting in chain termination of virus DNA replication. It has also been shown that the viral enzymes cannot remove aciclo-GMP from the chain, which consequently inhibits further activity of DNA polymerase. Aciclo-GTP is rapidly metabolized within the cell, possibly by cellular phosphatases (Brigden & Whiteman, 1983). Based on EEHV genome sequences, thymidine kinase (Ehlers et al., 2006; Ling et al., 2013; Richman et al., 2014) may play an important role in the mechanism of action of acyclovir and penciclovir. In remote areas, where many captive Asian elephants reside, acyclovir is a better choice for treating EEHV as it is available in drugstores and much less expensive than other antiviral drugs. Moreover, it is available in both injectable and tablet forms, which offers more flexibility in treating calves that may not be trained to accept veterinary interventions (Boonprasert et al., 2019; Dastjerdi et al., 2016; Lopez et al., 2017; saza & Hunter., 2004; Sripiboon et al., 2017; Wissink-Argilaga et al., 2019).

Pharmacokinetic and bioavailability studies of acyclovir have been conducted in humans (Blum, Liaq & De, 1982; Jankowski, Jankowska & Lamparczyk, 1998; Laskin, 1983), dogs (Krasny, de Miranda, R Blum & B Elion, 1981), cats (Owens et al., 1996), and horses (Bentz et al., 2006; Garre et al., 2007; Wilkins, Papich & Sweeney, 2005), but are lacking in elephants. Thus, this study aimed to characterize the pharmacokinetics of both oral and intravenous (IV) acyclovir in Asian elephant calves. The second aim was to develop and validate a LC−MS/MS method to characterize and measure acyclovir in elephant plasma. To the best of our knowledge, this is the first study to report plasma acyclovir concentrations using this analytical method in elephants. This information will be important to optimize safe and effective dose regimens and routes of administration to treat EEHV-HD for successful survival outcomes.

2. Materials and methods

2.1. Analytical method development and validation

The LC-MS/MS method was developed and validated based on guidelines set forth by the EMEA (European Medicines Agency, 2011) and US-FDA (Food & Drug Administration, 2018).

To measure a wide range of concentrations, acyclovir standards were prepared by serial dilution in elephant plasma over a range of 0.01 – 5 μg/mL. A reliable method for acyclovir determination in elephants was then developed and validated as described below.

To test for selectivity and matrix effects, blank plasma ($n = 5$) samples were used to determine interference at the retention times for acyclovir and ganciclovir, with none detected. The multiple reaction monitoring (MRM) mode of the LC-MS/MS technique provided high analyte selectivity. To determine the lower limit of quantitation (LLOQ), five replicates of 0.1 μg/mL acyclovir in plasma were prepared and evaluated for accuracy, precision, and signal to noise (S/N) ratio. Linearity of calibration standards over a range of 0.01, 0.02, 0.1, 0.2, 1, 2, 4, and 5 μg/mL were evaluated in triplicate. Linear regressions with a weighting factor of $1/x^2$ were calculated, including slopes, intercepts, and coefficients of determination (r^2), as well as a back-calculation of calibration standards from the linear regression equation.

Four quality control (QC) samples [Low (LQC) (0.03 μg/mL), Medium 1 (MQC1) (0.15 μg/mL), Medium 2 (MQC2) (0.75 μg/mL) and High (HQC) (1.875 μg/mL)] were evaluated for accuracy and precision, both within-run (five replicate for each level) and between-run (three runs). The accuracy was calculated from the following equation: Accuracy = (measured concentration x 100)/theoretical concentration. The precision was calculated as%CV, which should not exceed 15%. For extraction recovery, four-levels of QC samples were evaluated by comparison of the area under the curve of extracted and unextracted samples.

Blank plasma spiked with acyclovir to a concentration of 9 μg/mL was used to test the dilution integrity at 1:2, 1:4, and 1.8. Five replicated samples for each dilution were evaluated against the calibration curve.

Stability of acyclovir (0.25 μg/mL) and ganciclovir (0.25 μg/mL) stock solutions was evaluated at 25 ± 5°C for 4 h and at 5 ± 3 °C for 7 days. Working solutions of acyclovir at 0.1 and 50 μg/mL and ganciclovir at 55 μg/mL were evaluated at 25 ± 5 °C for 6 h and at 5 ± 3 °C for 7 days. Additional short-term stability at 25 ± 5 °C for 5 h and long-term frozen stability at -30 ± 5 °C for 85 days were evaluated. To confirm the stability of acyclovir in blood before plasma was separated, samples were stored at 0 ± 5 °C in an ice-box cooler for 5 h. Freeze-thaw stability of acyclovir in plasma was determined after freezing (-30 ± 5 °C) and thawing (25 ± 5 °C) for three cycles. All acceptance criteria of the validated parameters are presented in Table 1.

2.2. Acyclovir analysis

Acyclovir (purity: 94.9%,) and ganciclovir (purity: 98.8%) were purchased from Sigma-Aldrich (Laramie, USA) and used as reference and international standard, respectively. Stock solutions of 250 μg/mL were prepared in 5% methanol and then serially diluted in 15% methanol to concentrations of 0.01, 0.02, 0.1, 0.2, 1, 2, 4 and 5 μg/mL with a mobile phase. All stock and working solutions were stored in a controlled temperature cabinet at 5 ± 3 °C and used within 7 days of preparation. Acyclovir concentrations were measured by high-pressure liquid chromatography with mass spectrometry (LC-MS/MS) based on analytical protocols modified from previous pharmacokinetic studies in humans and horses (Bentz et al., 2006; Garre et al., 2007; Maes et al.,

Table 1
Plasma concentration-time profile of the calves (E1 – E4) after a single dosage of acyclovir (15 mg/kg) IV administration determined in four Asian elephant calves.

sampling time (h)	Calculated Concentration (μg/mL)				Average	SD
	E1	E2	E3	E4		
0.25	7.41	3.59	9.14	18.56	9.68	6.36
0.5	18.73	23.50	9.50	25.24	19.24	7.05
0.75	21.19	25.65	13.77	24.14	21.19	5.28
1	21.82	36.99	13.29	18.50	22.65	10.18
1.5	18.84	17.70	24.04	14.35	18.73	4.02
2	14.42	11.42	11.88	11.76	12.37	1.38
3	11.16	9.96	9.39	8.07	9.65	1.28
4	8.20	7.47	7.06	6.38	7.28	0.76
5	6.30	6.46	6.11	4.72	5.90	0.80
6	4.76	6.02	4.32	3.49	4.65	1.06
12	1.86	1.57	2.04	1.77	1.81	0.20
24	0.41	0.41	0.36	0.23	0.35	0.09
36	0.09	0.07	0.07	0.07	0.07	0.01
48	0.04	0.05	0.03	0.02	0.04	0.01

2009; Patel et al., 2015; Wilkins et al., 2005; Xing, Gu, Zhang, Xu & Lu, 2016) at the Pharmacy Service Center, Faculty of Pharmacy, Chiang Mai University. The liquid chromatography system (1260 Infinity LC System, Agilant, Santa Clara, USA) consisted of a solvent delivery unit, on-line degasser, auto-injector, and thermostat-controlled column compartment. Optimal chromatographic separation utilized a column 250 × 4.6 mm (particle size 5 μm) (Silia chrom AQ C8 column, SiliCycle, Quebec, Canada) in combination with a pre-column 10 × 4.0 mm (particle size 5 μm) (Thermo BDS-hypersil-C8 filter, Thermo Scientific, Waltham, USA). The mobile phase was a mixture of acetonitrile and 0.1% formic acid (7:93 v/v). The temperature of the column was set at 30 °C and the flow rate was 0.7 mL/min. The mass spectrometer was a triple-quadrupole instrument (API 3200 AB, Watertown, USA), operated in a positive ion mode by electrospray ionization. The MS/MS parameters were set with: curtain gas, 35 psi; collision gas, 5 psi; ion spray voltage of 5500 V; ion source gas, 160 psi; and ion source gas, 260 psi. Detection of the ion abundance was monitored in the MRM mode by using the transition pairs of *m/z* 226.089 to 152.114 for acyclovir and *m/z* 256.08 to 151.95 for ganciclovir, respectively.

2.3. Pharmacokinetic analysis

Pharmacokinetic modeling was achieved using WinNonlin 8.0 (Certara, USA) and included calculation of drug concentration-time curves and noncompartmental pharmacokinetic modeling. Pharmacokinetic parameters, peak plasma concentration (C_{max}), time to reach peak plasma (T_{max}), total area under the plasma concentration-time curve from time 0–48 h ($AUC_{0\text{-}t}$) and infinity ($AUC_{0\text{-}inf}$), elimination rate constant (K_{el}), and elimination half-life ($T_{1/2}$), were calculated for each sample and then averaged. The bioavailability (F) of oral acyclovir was calculated as follows: $\%F = (AUC_{PO}/AUC_{IV}) \times (Dose_{IV}/Dose_{PO}) \times 100$, $AUC_{0\text{-}inf}$ of PO and IV will be used for F calculation.

2.4. Animals

Four healthy Asian elephant calves (2 males and 2 females, E1-E4) with an average age of 7.3 ± 1.5 years (range 5.3 – 8.8 years) and average weight of 1665 ± 209 kg (range 1440 – 1880 kg) in tourist camps of Northern Thailand were used in the study. The animals participated in a show 2–3 times per day, which lasted not more than 40 min each. When not working, the calves were tethered on 3 m chains under a shade structure, with trunk length physical access to other elephant calves. From 1700 h to 0600 h, they were tethered in an enclosure (6 × 6 m). Calves were fed primarily corn stalk, Napier grass (*Pennisetum purpureum*) and Bana grass (*Pennisetum purpureum X, P. americanum hybrid*), and supplements such as bananas and sugar cane, with free access to fresh water. Each calf was weighed the day before initial drug administration. All animal procedures were approved by the Institutional Animal Care and Use Committee, Faculty of Veterinary Medicine, Chiang Mai University, Chiang Mai, Thailand (license number S31/2561).

2.5. Drug administration and blood sample collection

Study 1: The acyclovir (USP 500 mg, Axovir-500, Samarth Life Sciences, Baddi, India) was dissolved in 10 ml of sterile water and then diluted in 0.9% physiological saline. Acyclovir was administered IV at a dose of 15 mg/kg of body weight through a single-lumen IV catheter placed in the left auricular vein at a rate of 76.25 ± 31.46 min, as described by Sripiboon et al. (2017). A single-lumen intravenous polyurethane catheter coated with heparin was placed in the right auricular ear vein for blood sampling. Blood samples were collected at 0, 15, 30, 45, and 60 min and 1.5, 2, 3, 4, 5, 6, 12, 24, 36, and 48 h after the start of the infusion into EDTA tubes, stored in an ice-box at 4 °C for transport to the laboratory, and the plasma separated within 4 h by centrifugation at 2000 g for 10 min. The plasma was frozen at −30 °C until analysis.

Study 2: Acyclovir (800 mg tablets, HERPENON 800, Polipharm, Samut Prakan, Thailand) was administered once PO in a banana at a dosage of 45 mg/kg of body weight as described by Khammesri et al. (2021). Blood samples were collected at 0, 20, 40, and 60 min and 1.33, 1.67, 2, 2.5, 3, 4, 6, 12, 24, 36, and 48 h post-administration.

There was a 2-week washout period between studies for each calf, *with treatments administered in* a Latin Square design.

3. Results

3.1. Analytical method development and validation

For selectivity and matrix effects after injection, peak retention times were observed at 7.7 min and 6.3 min for acyclovir and ganciclovir (internal standard), respectively. Blank plasma samples from five calves showed no peaks at those retention times, and there were no matrix effects. The LLOQ of this quantification method was 0.01 μg/mL. The calibration curves of acyclovir were linear over three orders of magnitude (0.01 – 5 μg/mL). The r^2 of all calibration curves was over 0.99 and all (100%) of the back-calculation of the calibration standards from the linear regression equation were within ± 15% of the nominal values, including the LLOQ samples. The mean values of accuracy for within-runs were 89.13 – 101.63%, whereas those for between-runs were 92.85 – 100.91%. The mean precision (%CV) for within- and between-runs ranged from 3.44 – 7.18% for all QC samples. The extraction recoveries of acyclovir from four levels of QC samples ranged between 62.87 – 79.81%, and for ganciclovir was 48.67%. The precision of all samples showed low variation with CVs in the range of 3.62 – 8.80% for acyclovir and 6.46% for ganciclovir. The results of dilution integrity tests showed an accuracy of 99.47, 100.07, and 101.94% at 1:2, 1:4, and 1:8 dilutions, and precision values of 2.01, 2.74, and 3.98 for dilutions of 1:2, 1:4 and 1:8, respectively. In the stability test, acyclovir and ganciclovir stock solutions (250 μg/mL) kept at 25 ± 5 °C for 4 h and at 5 ± 3 °C for 7 days had a deviation range of -6.87 – 3.70%. For working solutions, the maximum deviation was found for ganciclovir (55 μg/mL), with a deviation of -9.38%. Thus, working solutions of acyclovir at 0.1 and 50 μg/mL and ganciclovir at 55 μg/mL were stable after storage at 25 ± 5 °C for 6 h and at 5 ± 3 °C for 7 days. The acyclovir in plasma was stable after storage at 25 ± 5 °C for 5 h (short-term stability) and at -30 ± 5 °C for 85 days (long-term stability); the accuracy of quantification ranged from 95.33 – 104.84%. After three freeze-thaw cycles, acyclovir showed good stability with an accuracy of 94.15 – 97.99%. Moreover, acyclovir was stable after storage at 0 ± 5°C for 5 h in an ice-box, with an accuracy of 92.52 – 95.55%. Validation data are summarized and presented in Table 1, Supplemental data. Table 2

Table 2
Plasma concentration-time profile of the calves (E1 – E4) after a single dosage of acyclovir (45 mg/kg) PO administration determined in four Asian elephant calves.

sampling time (h)	Calculated Concentration (μg/mL)				Average	SD
	E1	E2	E3	E4		
0.33	0.05	0.12	0.09	0.01	0.07	0.05
0.67	0.28	0.24	0.32	0.21	0.26	0.05
1	0.62	0.42	0.72	0.56	0.58	0.12
1.33	0.86	0.59	1.01	0.73	0.80	0.18
1.67	1.01	0.73	1.30	1.06	1.02	0.23
2	1.16	0.96	1.43	1.11	1.17	0.20
2.5	1.16	1.04	1.58	1.52	1.33	0.26
3	1.37	1.15	1.52	1.45	1.38	0.16
4	1.54	0.85	1.27	1.23	1.22	0.29
6	1.06	0.63	0.81	0.84	0.84	0.18
12	0.52	0.42	0.50	0.48	0.48	0.04
24	0.36	0.28	0.27	0.28	0.30	0.04
36	0.13	0.09	0.08	0.09	0.10	0.02
48	0.07	0.05	0.02	0.03	0.04	0.02

3.2. Pharmacokinetics

Mean plasma acyclovir concentrations by the IV route increased rapidly and reach a peak within an hour, and thereafter decreased gradually over the next 12 h, but remained above LLOQ concentrations (0.01 μg/mL) during the sampling period. Mean plasma acyclovir concentrations by the PO route increased more slowly and peaked after 3 h (Fig. 1). A summary of data for all four calves is shown in Table 3, with no differences observed between males and females. The mean C_{max} and AUC values of the IV route were higher than those of the PO route. In addition, PO administration of acyclovir was associated with a bioavailability of 6%. However, the mean K_{el} and $T_{1/2}$ for both routes were similar.

3.3. Clinical effects

No adverse effects were observed in any of the calves after IV or PO administration of acyclovir. In addition, the varying IV infusion rates in Study 1 did not significantly affect the pharmacokinetic analysis results. Plotting the half-maximal inhibitory concentrations of acyclovir compared to other virusus showed that peak concentrations after a single PO dose of acyclovir stayed above the IC_{50} level of herpes simplex virus-1 (HSV-1), HSV-2 and equid alpha herpesvirus-1 (EHV-1) for 6 h (Fig. 2).

4. Discussion

This is the first study to characterize the pharmacokinetics of acyclovir in Asian elephant calves after IV and PO administration. Because of limited data using acyclovir in Asian elephants via either route, dosages were based on successful treatment of EEHV in other reports (Khammesri et al., 2021; Sripiboon et al., 2017). The lower limit of quantification using LC/MS-MS was 0.01 μg/mL with a calibration range of 0.01 – 5.0 μg/mL, which was suitable for quantification of acyclovir in plasma during absorption and after five elimination half-lives. The mean drug plasma concentrations after PO and IV administration were within range of detection, and the sample integrity validation demonstrated the feasibility of measuring acyclovir in samples diluted at least 1:8.

Table 3
Pharmacokinetic parameters (mean ± SD) for acyclovir after administration at 15 mg/kg IV or 45 mg/kg PO to four Asian elephant calves.

Pharmacokinetic parameters	IV administration (15 mg/kg)	Oral administration (45 mg/kg)
C_{max} (μg/mL)	27.02* ± 6.79	1.45 ± 0.20
T_{max} (h)	0.94 ± 0.31	3.00 ± 0.70
$AUC_{0\text{-}t}$ (μg.h/mL)	94.41 ± 7.39	17.00 ± 2.27
$AUC_{0\text{-}inf}$ (μg.h/mL)	94.61 ± 7.40	17.55 ± 2.43
K_{el}	0.12 ± 0.01	0.09 ± 0.03
Cl (mL/min/kg)	2.65 ± 0.21	–
$V_{d(ss)}$ (mL/min/kg)	0.88 ± 0.07	–
MRT (h)	5.39 ± 0.91	13.26 ± 0.97
MAT (h)	–	7.86 ± 1.06
$T_{1/2}$ (h)	5.87 ± 0.74	8.74 ± 2.47
% Bioavailability	6.03* ± 0.87	

C_{max}, peak plasma concentration; T_{max}, time to reach peak plasma; $AUC_{0\text{-}t}$, total area under the plasma concentration-time curve from time 0–48 h; $AUC_{0\text{-}inf}$, total area under the plasma concentration-time curve from time zero to infinity; K_{el}, elimination rate constant; Cl, total clearance; $V_{d(ss)}$, steady-state volume of distribution; MRT, mean residence time; MAT, mean absorption time.

Acyclovir plasma concentrations varied among the calves in the IV trial. The loading time from start to end points affected C_{max} and T_{max} pharmacokinetic parameters, but none of the other parameters were influenced by this factor. In this study, the rate of absorption and peak concentration of acyclovir after IV administration were markedly greater than those after PO administration, a finding that has also been reported in the horse (Bentz et al., 2006; Garre et al., 2007; Wilkins et al., 2005). Elephants are monogastric herbivors with hindgut fermentation and have a short gastrointestinal tract compared to other herbivorous species (Sukumar, 2006), which can contribute to challenges in giving medications via a PO route. Moreover, elephants exhibit

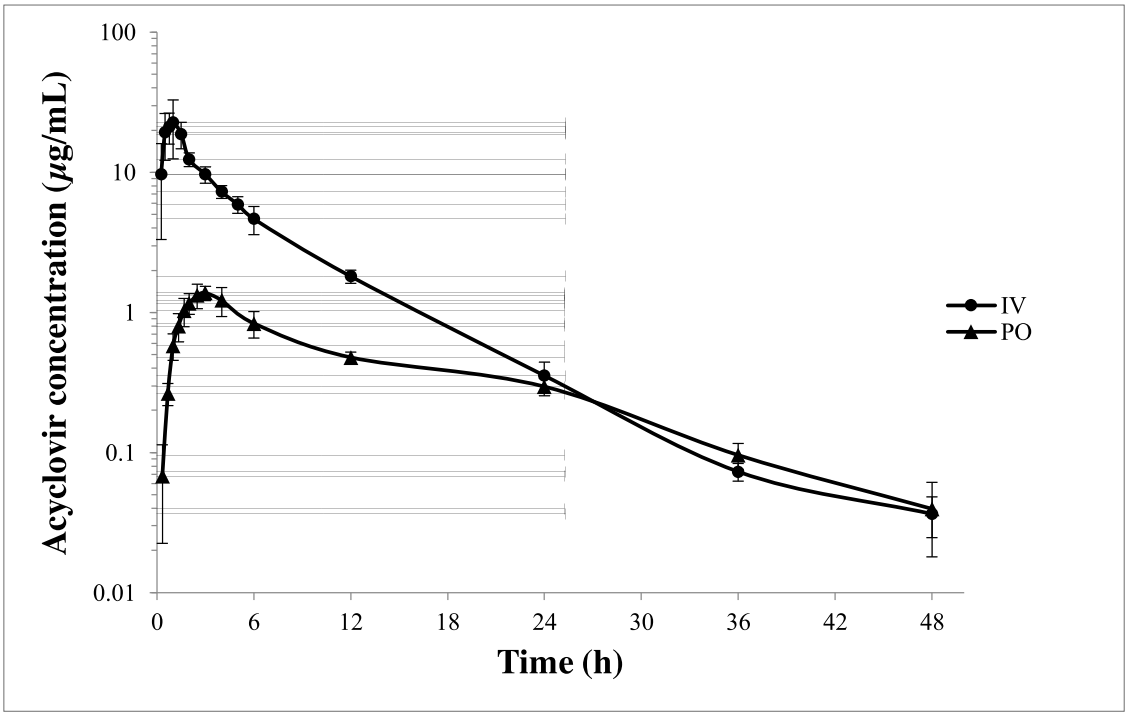

Fig. 1. Mean ± SD plasma acyclovir concentration of four captive Asian elephant calves at 0, 15, 30, 45, and 60 min and 1.5, 2, 3, 4, 5, 6, 12, 24, 36, and 48 h after intravenous (IV) administration of acyclovir at a dose of 15 mg/kg of body weight (●), and at 0, 20, 40, and 60 min and 1.33, 1.67, 2, 2.5, 3, 4, 6, 12, 24, 36, and 48 h post per oral (PO) at a dosage of 45 mg/kg body weight administration (▲).

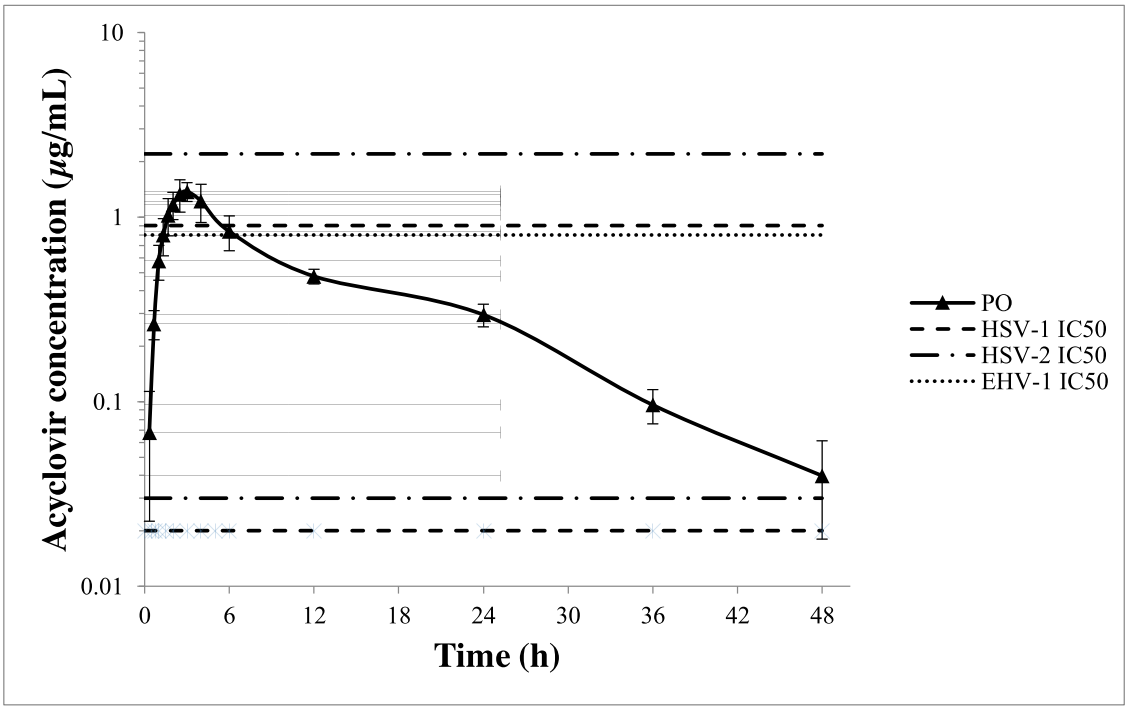

Fig. 2. Mean ± SD plasma acyclovir concentration of four captive Asian elephant calves at 0, 20, 40, and 60 min and 1.33, 1.67, 2, 2.5, 3, 4, 6, 12, 24, 36, and 48 h post per oral (PO) at a dosage of 45 mg/kg body weight administration (▲). Valued report showed the peak level was above the IC50 levels of HSV-1, HSV-2 and EHV-1 from previous studied in human and horse at least 6 h.

a low gut digestibility, only about 40 – 50% compared to other mammals (Loehlein et al., 2003; Sukumar, 2006). Therefore, elephants have to consume 1.5 – 2% of their body weight in food daily, and spend up to 80% of the day feeding (Sukumar, 2003). The presence of large quantities of food in the gut can raise gastric pH and decrease the solubility of acyclovir (Bruni et al., 2013). Thus, the bioavailability of PO acyclovir in this study was low (6.03 ± 0.87%), in agreement with other species where it declines with increasing doses (Laskin, 1983). Because its solubility requires an empty stomach with a low pH, it was not surprising that oral administration was associated with poor bioavailability. Furthermore, the lower blood concentration of acyclovir PO administration can also be related to low permeability and limited water solubility (Bergström et al., 2003; Fletcher & Bean, 1985; Wagstaff, Faulds & Goa, 1994). However, oral administration is still a viable alternative compared to other antiviral drugs that need to be given IV, for safety, availability, and economic reasons. Oral administration of acyclovir is limited in other species as well, due to poor and variable bioavailability, but has been effective and a drug of choice for treating *Herpesviridae* in humans and horses (De Clercq & Field, 2006; Kłysik, Pietraszek, Karewicz & Nowakowska, 2020; Maxwell, 2017).

In an equine pharmacokinetic study, the $T_{1/2el}$ of acyclovir was 7.21 ± 7.56 h (Bentz et al., 2006), which was comparable to that in our study (8.74 ± 2.47 h). Glomerular filtration and renal tubular excretion are the main elimination pathways for acyclovir (45 – 90%) (Laskin et al., 1982; Loregian, Gatti, Palu & De Palo, 2001; Spector et al., 1981). In other species, like cats, dogs and humans, the acyclovir $T_{1/2el}$ is approximately 3 h (Blum et al., 1982; Jankowski et al., 1998; Krasny et al., 1981; Laskin, 1983; Owens et al., 1996), which is shorter than what we observed. Drug metabolism and elimination in the elephant calves and horse are presumed to be similar as both are hind-gut fermenters (Hunter & Isaza, 2008), and the body weight of elephant calves is similar to that of adult horses, so allometric scaling could be helpful in estimating appropriate dose regimens (Brock et al., 2012; Mahmood, Martinez, & Hunter, 2006; Martinez, Mahmood & Hunter, 2006). The pharmacokinetic parameter, $T_{1/2el}$ of acyclovir per IV and PO routes (5.87 ± 0.74 h and 8.74 ± 2.47 h) was longer than that of $T_{1/2el}$ in humans (Blum et al., 1982; Jankowski et al., 1998; Laskin, 1983), but similar to that found in the horse (Bentz et al., 2006; Garre et al., 2007; Wilkins et al., 2005). In addition, the time required to reach steady-state conditions in horse was prolonged (Maxwell et al., 2008).

The acyclovir plasma concentrations observed via both routes achieved half-maximal inhibitory concentrations (IC_{50}) reported for HSV-1, HSV-2 [0.02 to 0.9 μg/mL, and 0.03 to 2.2 μg/mL (Piret & Boivin, 2011), respectively, and for EHV-1 [>0.8 μg/mL (Maxwell et al., 2017)], which was similar to a value of 3.56 μg/mL after 28 days of PO administration in an Asian elephant calf (Khammesri et al., 2021). Theoretically, plasma drug concentrations based on free drug concentration (unbound) against targeted microbes. For acyclovir, negligible plasma binding proteins have been reported in the horse (Garre et al., 2007; Maes et al., 2009), with less than 13, 33 and 36% binding in rats, humans and dogs, respectively (De Miranda, Krasny, Page & Elion, 1982; Krasny et al., 1981; Robert Blum et al., 1982). Thus, binding of acyclovir to plasma proteins in elephant may be low as well, so concentrations derived from total concentrations may be used to estimate effective concentrations with the in vitro IC_{50} data of HSV-1, HSV-2 and EHV-1 for EEHV treatment, as they are in the Herpesviridae group; however, IC_{50} data of EEHV are currently unavailable. The IV acyclovir regimen of 15 mg/kg in this study showed the C_{max} reached 27.02 ± 6.793 μg/mL, which is 10 times higher than that based on IC_{50} for HSV-1 and HSV-2 in humans and EHV-1 in the horse. Therefore, monitoring adverse effects of this dose is highly recommended. The margin of safety could be increased by reducing peak concentrations. Because acyclovir is excreted by glomerular filtration and tubular secretion, nephrotoxicity could occur with high concentrations injected over a short period of time (Perazella, 2003).

The acyclovir concentrations in the IV preparation of this study (5.84 – 11.85 mg/mL of acyclovir in 0.9% normal saline) was within or slightly above the recommendation of 7 mg/mL or lower by the American Pharmacists Association (2017). However, side effects like phlebitis or inflammation at the injection site were not observed in this study even though concentrations over 10 mg/mL could increase the risk of these problems. Precipitation of acyclovir crystals in renal tubules can occur if

the maximum solubility of free acyclovir is exceeded, or if the drug is administered by a rapid bolus injection renal tubular damage and acute renal failure can occur (Perazella, 2003). The infusion duration period is also important; previous reports in horses found signs of sweating, tremors, and colic in one of six animals after rapid administration of 5 mg/mL over a 15-minute period (Bentz et al., 2006). Thus, it is important to identify an appropriate IV dose of acyclovir for EEHV treatment. Although oral administration appears to be safe in elephant calves, with no side effects observed after 28 days of therapy (Khammesri et al., 2021).

5. Conclusions

Methods to characterize the pharmacokinetics of acyclovir after IV and PO administration were developed and validated for Asian elephant calves. The PO route resulted in low bioavailability compared to IV administration and so would require higher doses, but it is still be a safe and cost-effective choice for EEHV treatment. One limitation of our study was the small number of subjects available due to the difficulty in recruiting healthy animals, and gaining permissions from the owners for these kinds of research projects. More work is needed to fine-tune the doses of acyclovir by both methods. Understanding the pharmacokinetic profiles of these two treatment regimens is beneficial for practitioners to make decisions regarding acyclovir administration in elephant calves to reduce viral loads.

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