

Population pharmacokinetics of isoniazid in the treatment of *Mycobacterium tuberculosis* among Asian and African elephants (*Elephas maximus* and *Loxodonta africana*)

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We recently described the clinical presentation and treatment of 18 elephants from six herds infected with TB. Treatment protocols and methods varied between herds to include both oral and rectal dosing using multiple drug doses and formulations. In this paper we present information regarding the pharmacokinetics (PK) of isoniazid (INH) in elephants and provide suggestions regarding initial treatment regimens. Forty-one elephants received INH daily by either oral or rectal administration with different formulations. Population PK analysis was performed using Non-linear Mixed Effect Modeling (NONMEM). Results of oral administration indicated that compared with premixed INH solution, the drug exposure was highest with a suspension prepared freshly with INH powder. When INH was concomitantly given as an admixture over food, T_{max} was delayed and variability in drug absorption was significantly increased. Compared with oral administration, similar drug exposures were found when INH was dosed rectally. The data generated suggest that a starting dose of 7.5 mg/kg of INH is appropriate for initial TB treatment in elephants when premixed solution is administered directly into the oropharynx or rectal vault and 4 mg/kg are when INH is administered following immediate suspension from powdered form.

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INTRODUCTION

Published reports of infection with *Mycobacterium tuberculosis* among elephants date to 1909 (Damman & Stedefeder, 1909) with historical records suggesting that tuberculosis (TB) has been endemic in elephants for over two millennia (Iyer, 1937; McGaughey, 1961). More recent reports of TB affecting domestic elephants appeared in the mid-1970 to early 1980s (Pinto *et al.*, 1973; Greenberg *et al.*, 1981; Devine *et al.*, 1983; Saunders, 1983). However, a report in 1996 of TB occurring in two circus elephants (Frankel, 1997) sparked renewed interest in this disease. In response to these cases, uniform testing for TB was initiated in 1998 for regulated elephants in the United States Department of Agriculture (1998).

Over the 5-year period between 1996 and 2000, 18 cases of TB were identified (Mikota & Maslow, 1997; Mikota *et al.*, 2000, 2001) of 539 elephants tested, yielding an estimated prevalence of disease of 3.3% (Mikota *et al.*, 2000). The

epidemiology, diagnosis, and clinical course of infection with *M. tuberculosis* among infected and exposed animals within six herds of captive elephants was previously described (Mikota *et al.*, 2001). The elephants were prescribed various combinations of antituberculous agents including isoniazid (isonicotinic acid hydrazide, or INH), rifampin (RIF), ethambutol (EMB), and pyrazinamide (PZA). A number of problems presented during treatment, including poor drug acceptance, unknown food effects on absorption of drugs, possible inactivation by gastric acids, altered transit time through the gastrointestinal tract, and unknown metabolic rates and PK. Because of the limited pharmacokinetic (PK) data of antituberculous drugs for large mammals, especially for elephants, a preliminary PK study of INH administration was undertaken. In this paper we present information regarding the oral and rectal administration and first-order estimates of the PK of INH in elephants and provide suggestions regarding initial treatment regimens.

METHODS

Animals

Forty-one elephants participated in the study. Included were 37 Asian (*Elephas maximus*; three male, 34 female) and four African (*Loxodonta africana*; one male, three female) elephants. The median age was 29 years (range 3–55 years) and the median body weight was 3751 kg (range 849–6123 kg). A diagnosis of clinical TB was defined as the isolation of *M. tuberculosis* from a clinical sample (e.g. sputum, trunk wash, oral swab, rectal swab). The majority of animals were housed in groups with variable times indoors as presented previously (Mikota *et al.*, 2001).

Drug administration

Several INH formulations were conceived in order to overcome problems with drug acceptance and to achieve the target serum concentrations (United States Department of Agriculture, 1998). INH was purchased as a premixed concentrated suspension at 600 mg/mL or as a powder that was made into a suspension immediately prior to administration. INH was purchased from either Congaree Veterinary Pharmacy (West Columbia, SC, USA) or Abbotts Compounding Pharmacy (Berkley, CA, USA). Rectal suppositories were compounded with several vehicles both to create an acceptable semi-solid rectal suppository and to increase absorption through the rectal mucosa. Individual lots of compounded drug were not recovered from the various clinical sites and tested for stability and potency. Compounding followed guidelines within the United States Pharmacopeia. Some animals were treated by multiple administration methods. Serum concentrations were obtained at least 48 h (typically >1 week) after changes in methodologies.

Five oral dosing methods were used (Table 1). Herd A received a premixed INH concentrated suspension that was placed within solid foodstuffs such as apples, breads, cakes, or other treats and then hand fed to the animals. Herd A also received a premixed INH concentrated suspension that had been mixed with molasses and feeds such as grain or corn mash. INH powder, freshly

suspended in water just prior to oral dosing, was given to herd D. Using an oral bite block, this herd was trained to accept suspension injected into the posterior oropharynx. Herd D also received a premixed INH concentrated suspension by dose syringe that was freshly diluted and injected into the posterior oropharynx. Herd F received a premixed INH concentrated suspension that was freshly diluted and placed into the mouth. Except as noted, orally administered INH was given under fasting conditions.

Isoniazid was administered rectally to animals from five herds. Prior to administration, fecal material was removed from the rectal vault manually or by warm water enema. For herds B, C, E, and F a premixed INH concentrated suspension was placed directly into the rectal vault as a retention enema via a 3 ft length of polyethylene tubing attached exteriorly to a syringe. After injection, the syringe was quickly removed, and a bolus of air injected into the tubing to deliver any remaining INH. For one animal, INH powder or crushed tablets were suspended in a mixture of canola oil, water, psyllium, and pluronic F125 (Abbotts Compounding Pharmacy).

For herds C and D, drugs were also delivered rectally via suppository (Abbotts Medical Pharmacy or Congaree Veterinary Pharmacy). INH was suspended in coconut oil and lecithin and let to harden into a semisolid. The semisolid mixture was dipped in cocoa butter to form a thin shell.

Doses for oral and rectal administration of INH ranged between 2.5 and 25 mg/kg. Doses were varied to achieve INH serum concentrations to model the experience in humans. Based on our early experience, this range for elephants was defined subsequently as 1–2 µg/mL, and recommended in the 1998 and 2000 Guidelines for the control of TB in elephants (United States Department of Agriculture, 1998). INH was typically administered with other antimycobacterial agents. Drug choices and doses were determined individually for each animal.

Blood sampling

Timed serum samples were obtained following the administration of INH. Although sampling times generally were scheduled at 2, 4 and 6 h postdose, sampling times varied between herds and between study days because of practical reasons. Actual sampling times were recorded for data analysis. With the animals either lying in a supine position or standing, blood was collected from the auricular ear vein into serum separator tubes and centrifuged within 30 min of collection. Serum was harvested and frozen in labeled containers and shipped on dry ice via overnight delivery to the National Jewish Medical and Research Center (Denver, CO, USA) for analysis.

Sample analysis

Serum concentrations of INH were determined using a validated high performance liquid chromatography (HPLC) assay (Pelouquin *et al.*, 1999). The serum standard curves for INH ranged from 0.5 to 20 µg/mL, and the absolute recovery of INH from serum was 50.2%. The percent coefficient of variation (CV%) for

Table 1. Route of administration of isoniazid to six herds of elephants

Route of administration	Herd	Method of delivery
Oral bolus, freshly suspended isoniazid powder	D	Oropharyngeal injection
Oral bolus, freshly diluted premixed liquid concentrate	D	Oropharyngeal injection
Oral bolus, freshly diluted premixed liquid concentrate	F	Placed into mouth
Oral, premixed liquid concentrate placed into food	A	Hand feeding
Oral, premixed liquid concentrate mixed with feed	A	Ad lib ingestion by animals
Rectal enema	B, C, E, F	Placement into rectal vault
Rectal suppository	D, 6	Placement into rectal vault

within-day precision of validation standard samples was 1.3–8.8%, and the between-day precision was 3.4–11.0%. Quality control sample concentrations were 0.8, 2, and 8 µg/mL.

Pharmacokinetic analysis

Initially, INH concentration data were analyzed using noncompartmental methods (WinNonLin Pro 4.0, Pharsight, Mountain View, CA, USA). The observed maximum concentration (C_{\max} , in µg/mL) and time of C_{\max} (T_{\max} , in h) were recorded, and several measures of the area under the serum concentration versus time curve (AUC , in µg·h/mL) were calculated using the linear trapezoidal rule. AUC values extending beyond the last measured concentration were extrapolated from the last measured concentration using each animal's estimated elimination rate constant. AUC_{0-12h} was the AUC from time zero extrapolated from the last measured concentration to 12 h postdose.

As only sparse blood samples were collected from elephants, population PK analysis was employed with Nonlinear Mixed Effect Modeling (NONMEM, Version 5, Globomax LLC, Hanover, MD, USA). This technique uses mixed-effect regression to estimate population means and variances of PK parameters. After inspecting the concentration vs. time graphs, a one-compartment model was selected. Three parameters were estimated including a first order absorption rate constant [K_a , in reciprocal hours (/h)], a first order elimination rate constant (K_e , in /h) and the apparent volume of distribution divided by the fraction of the dose absorbed into the systemic circulation (V/F , in L). The elimination half-life ($t_{1/2}$, in h) and oral clearance (Cl/F , in L/h/kg) were calculated using standard equations.

The reliability of data fitting and hypothesis testing were assessed by the value of objective function, diagnostic plots and magnitude of random error estimates. These criteria were used only when the minimization step was successful, and standard errors of parameter estimates were obtained with use of the covariance step. Likelihood ratio test was used for model comparison. The difference in minimum objective function (ΔMOF) values between two hierarchic models (LLdif) was approximately chi-square distributed, with q degrees of freedom (q = difference in number of parameters). With 1 degree of freedom, a LLdif of 3.84, 7.88 or 10.8 reached levels of significance at 0.05, 0.005, 0.001, respectively. With 2 degrees of freedom, these critical values were 5.99, 10.6 and 13.8, respectively.

Variability of PK parameters was assessed by random effect models. Three error models (additive, proportional and exponential) were tested and compared. The proportional error model was finally selected for the assessment of inter-individual random error (η) and residual error (ε).

RESULTS

Between December 1996 and September 1999, 41 elephants administered INH in response to documented infection (seven elephants) or exposure (34 elephants) to *M. tuberculosis* (Mikota *et al.*, 2001) were included in the present study. Over the 33-

month period, timed serum concentrations were obtained after 121 doses of INH, including 48 oral doses, 70 rectal doses, and three without a recorded route of administration. Fifteen animals had a single INH serum concentration determined, eight animals had two, nine animals had three, and the remaining animals had four to 21 serum concentrations determined. The three episodes for which the route of INH administration was not recorded were deleted from subsequent analyses.

Measurable concentrations of INH were observed following 34 of 48 (73%) of oral administrations, and 66 of 70 (94%) of the rectal administrations from 32 Asian (three male, 29 female) and two African (one male, one female) elephants that were included in the population PK analysis. Fifteen elephants received INH orally, 16 received INH rectally and two received both oral and rectal INH. Body weight of this subset ranged between 849 and 6123 kg (median 3728 kg) and age ranged from 3 to 48 years old (median, 29 years). In general, the poorest results were seen when INH was admixed with food; only 48% of samples produced measurable INH concentrations within the 6-h period postdose. The attending veterinarians observed the following situations that may have led to trace or undetectable INH concentrations: poor oral intake, retained fecal material, or rapid rectal expulsion of the dosage form. Trace or zero serum concentrations were not included in the PK analyses.

Representative C_{\max} , T_{\max} , and AUC_{0-12} data from the elephants included in the population model are presented in Table 2. Because similar absorption and elimination characteristics were observed for INH administered rectally regardless of method (data not shown), these routes were considered together. Similarly, for INH administered as a bolus, premixed suspension administered directly into the oropharynx and premixed suspension given with solid foodstuffs yielded similar PK values (data not shown) and were considered together. Note that, given the sparse sampling performed, these are only approximations of the actual values.

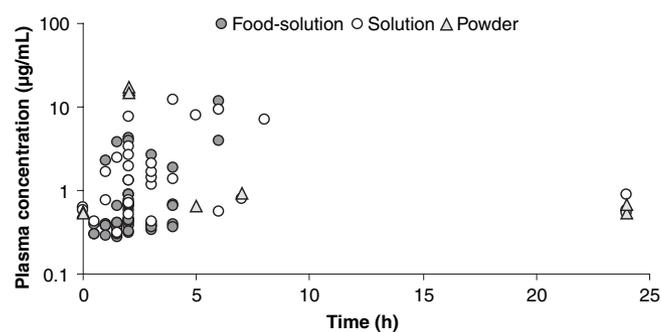
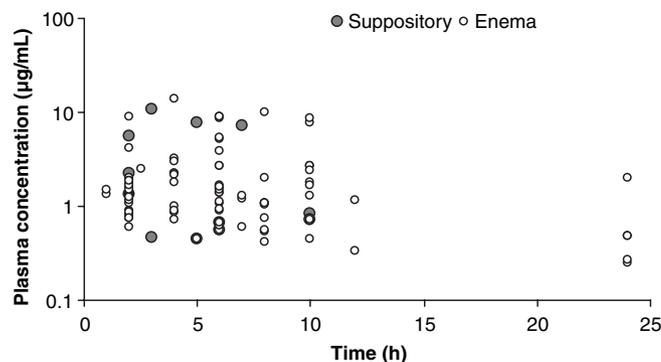
The concentration–time profiles of INH with different treatments are presented in Figs 1 and 2. The data were divided into four groups in the population PK analysis. The first group included animals treated via rectal administration including retention enema and suppository dosing. The second group included animals orally administered premixed INH solution. The third group included animals treated orally with freshly suspended INH powder. And the fourth group, animals that were treated with orally administered premixed INH solution in the presence of food. The population PK parameters of each administration method are shown in Table 3.

Oral pharmacokinetics of INH – powder vs. premixed solution

With oral administration, three elephants received the freshly suspended INH powder at 7.5 mg/kg. The highest and most consistent C_{\max} values (median 16.37 µg/mL, 35% CV) were observed with INH administered as a freshly suspended powder compared with that with premixed solution, based on the sparse sampling used. The former method yielded concentrations

Table 2. Median (range) apparent* isoniazid absorption characteristics for the subset of elephants analyzed with the population models

Dosing condition and formulation	Rectal retention enema or rectal suppository with premixed solution	Fasting with premixed solution	Fasting with freshly suspended powder	Nonfasting with premixed solution
Number of elephants treated by method	19	7	3	14
Dose range: 3–5 mg/kg				
Number of doses	33	4	0	0
C_{\max} ($\mu\text{g/mL}$); range	1.83; (0.66–17.26)	1.04; (0.71–1.40)	–	–
T_{\max} (h); range	2.00; (1.00–6.50)	2.00; (1.00–2.00)	–	–
$AUC_{0-12\text{h}}$ ($\mu\text{g}\cdot\text{h/mL}$); range	10.21; (2.64–95.39)	4.83; (2.09–7.56)	–	–
Dose range: 6–7.6 mg/kg				
Number of doses	14	5	3	8
C_{\max} ($\mu\text{g/mL}$); range	3.97; (0.45–8.99)	7.60; (1.95–12.13)	16.37; (8.17–16.73)	0.45; (0.30–11.54)
T_{\max} (h); range	5.50; (2.00–10.00)	2.00; (2.00–5.0)	2.00; (2.00–2.00)	2.00; (0.50–6.00)
$AUC_{0-12\text{h}}$ ($\mu\text{g}\cdot\text{h/mL}$); range	28.31; (6.94–38.05)	19.10; (3.41–71.50)	52.92; (61.74–44.10)	3.31; (0.91–6.66)

**Fig. 1.** Concentration–time profiles of isoniazid following oral administration in elephants.**Fig. 2.** Concentration–time profiles of isoniazid following rectal administration in elephants.

higher than typical human concentrations for INH given daily at 5 mg/kg (3–5 $\mu\text{g/mL}$) but similar to those observed with human twice-weekly doses of 15 mg/kg (range 9–15 $\mu\text{g/mL}$). By comparison, five elephants were administered INH at the same dose (7.5 mg/kg) as a premixed concentrate injected into the oropharynx. This yielded a median C_{\max} of 7.6 $\mu\text{g/mL}$ with a higher (61%) CV demonstrating less consistent absorption. At lower doses (2–5 mg/kg), median C_{\max} ranged from 1 to

3 $\mu\text{g/mL}$ that would be considered relatively low based on the normal effective human C_{\max} range.

The median K_a ranged from 0.5 to 1.0/h for oral administration of the premixed solution and freshly suspended powder (Table 3). The median T_{\max} values across the oral dosing methods ranged from 1.5 to 2.6 h, based on the sparse sampling used. The apparent V/F was lower when INH was freshly prepared with powder than that with the premixed solution suggesting a higher F , i.e. a higher bioavailability with powder. The terminal elimination half-lives were similar for the two formulations, typically around 2.5–3.5 h.

Oral pharmacokinetics of INH – nonbolus delivery

The effect of INH delivery when animals were allowed *ad libitum* intake of a mixture of feed and a premixed solution of INH was next assessed. The K_a was much lower (0.05 vs. 0.5/h). The C_{\max} and T_{\max} values could not be properly estimated as the sampling time was restricted within 6 h postdose and >90% samples were collected during 2–4 h postdose. As illustrated by Fig. 1, PK parameters for drug elimination could not be assessed because of the sampling scheme. In order to estimate impact of food on K_a , V/F and K_e values were fixed at 3000 L and 0.3/h in the population PK model. It was considered that significant drug was lost through manipulation of the food by the elephants prior to ingestion while absorption kinetics were decreased as a result of prolonged ingestion time. The effect of food to significantly reduce total drug exposure was unknown, but seems minimal based on the experience of bolus dosing of premixed INH concentrates.

Pharmacokinetics of INH – oral vs. rectal administration

Premixed INH solution was administered via both oral and rectal routes. As only two elephants received the rectal suppository formulation and showed comparable exposure with that of the rectal enema, all rectal data were combined in the PK analysis. Faster K_a , larger V/F and slightly slower K_e values were found with the rectal administration. Nevertheless, the total drug

Table 3. Population pharmacokinetic (PK) parameters of isoniazid in elephants

No. of elephants	19	7	3	14
No. in data set	155	45	10	72
Dosing condition and formulation	Rectal retention enema or rectal suppository with premixed solution	No food with premixed solution	No food with freshly suspended powder	With food Premixed solution
PK parameters (fixed effect parameters)				
θ_{K_a} (/h)	1.97 ± 0.77	0.50 ± 0.07	0.94 ± 0.47	0.05
$\theta_{V/F}$ (L)	5631 ± 1397	3110 ± 607	387 ± 53	3000 fixed
θ_{K_e} (/h)	0.16 ± 0.02	0.26 ± 0.02	0.31 ± 0.03	0.30 fixed
Variation in PK parameters (random effect parameters)				
η_{K_a} (%CV)	2.66 ± 2.04 (163)	–	–	0.375 (61)
$\eta_{V/F}$ (% CV)	0.23 ± 0.14 (48)	–	–	–
η_{K_e} (% CV)	0.20 ± 0.09 (44)	1.52 ± 1.02 (123)	0.06 ± 0.01 (24)	–
ε (% CV)	0.16 ± 0.11 (40)	0.95 ± 0.19 (97)	0.34 ± 0.10 (58)	1.47 (150)

Values are as $\theta \pm$ SEE. θ is a typical value of population PK parameter and SEE is standard error of estimate. η represents inter-subject variability of the specified PK parameter and ε represents random error.

exposures within a dosing interval (i.e. AUC_{24h}) were comparable between the oral and rectal administration (Figs 1 & 2).

Effects of age on PK of INH were examined. There was no significant correlation detected. Species and gender effects on PK of INH could not be properly assessed, as the majority of elephants were Asian females. Genotypic variation in acetylation rate of INH could not be evaluated as no genomic data were collected in this study.

In order to estimate exposures to INH, a PK simulation was conducted using WinNonLin for the premixed solution at 7.5 mg/kg dose using the typical parameter values presented. Results indicated that C_{max} and AUC_{24h} could be 5.5 µg/mL and 30 µg·h/mL, respectively and T_{max} could achieve at 2 h postdose. The variability of the exposure was estimated to be 50%.

In summary, serum INH concentrations consistent with those achieved in humans could be achieved at a dose of 7.5 mg/kg with either oral or rectal administration. With the premixed solution, the typical values of K_a and terminal $t_{1/2}$ were 1–2/h and 2–5 h, respectively. The elimination $t_{1/2}$ seen in elephants were consistent with those typically seen in humans. The apparent V/F and Cl/F were 1–2 L/kg and 0.3–0.4 L/h/kg, respectively, in elephants with TB infection. Because allowing animals *ad libitum* ingestion of INH mixed with feed may delay the drug absorption and increase variability in absorption, it should be avoided. As freshly suspended INH powder yielded higher bioavailability, this formulation appears to be the preferred formulation.

DISCUSSION

This is the first report of INH PK in elephants with TB infection. Because of practical difficulties, the study could not be designed as a conventional PK study with formal randomization, cross-over design and extensive sampling for different formulations and routes of administration. The data collected were sparse and the sampling times varied. It was also not feasible to randomize

animals according to disease severity, breed, age, weight and gender. Nevertheless, the information provided here reflects 'real world' experiences and would be useful to veterinarians for treating TB in elephants.

The relative importance of the various PK/PD parameters C_{max}/MIC , AUC/MIC , and time above MIC in predicting outcomes for INH is an area of active research. Of these, the fact that intermittent dosing is associated with therapeutic success argues against the time above MIC as the most important parameter. Recent work suggests the importance of AUC in outcome (Weiner *et al.*, 2003a), and the expected correlation of AUC with C_{max} was seen in these patients. INH has a low level of protein binding (<20%) in humans and would be expected to have a similar level of binding for elephants, although this has not been tested. If so, the impact of protein binding on PK/PD values would be minimal. The effective dose of INH for humans is 300 mg/day (~5 mg/kg). At this dose, the C_{max} ranges from 3 to 5 µg/mL and C_{max}/MIC ratio ranges from 60 to 100 (Burman, 1997; Peloquin, 1997; Peloquin *et al.*, 1999; Peloquin, 2003). These human effective doses and C_{max} values can be used as initial targets for the selection of doses and concentrations for INH in elephants.

This study analyzed PK data from 101 separate INH administrations to 34 elephants using five oral and two rectal methods of drug delivery. Freshly suspended INH powder reconstituted just prior to oral bolus dosing produced the highest and most consistent C_{max} values. Although higher than typical human concentrations for daily dosing, they were consistent with human twice-weekly dosing concentrations (Peloquin *et al.*, 1997). If tolerated over time, this raises the possibility of intermittent dosing for elephants, which may be more convenient for caregivers. This would require that the companion drugs also be tolerated at twice-weekly doses, and that clinical efficacy be shown with that approach.

Oral administration of INH as a freshly suspended powder yielded higher and less variable C_{max} values than premixed INH concentrate. In particular, our preliminary data do not support the use of the concentrate mixed with feed. The other uses of the

INH concentrate may be applied clinically, and would be enhanced by further studies of the long-term stability of the premixed INH concentrate and of the dilutions made from it. Our data do not exclude the possibility that some INH decayed within these liquids. We have noted that INH concentrate (600 mg/mL) maintains activity when stored at 4 °C for up to 60 days (J. Maslow and S. Mikota, unpublished data); the rate of decay for the dilutions of the concentrated suspension is unknown but appears to be more rapid. This conclusion is supported by the fact that significant differences in serum concentrations were obtained from well-trained animals from the same herd (herd 1) when administered similar doses using different drug formulations.

The problems associated with the use of the concentrate mixed with feed included frequently undetectable INH concentrations (12 of 23 administrations yielded undetectable serum concentrations), highly variable C_{\max} , some prolonged absorption, and flat serum concentration vs. time curves suggestive of slow and incomplete drug delivery. This may have been caused by the co-ingestion of the feed, loss of INH from the surface of the feed because of manipulation by the elephants prior to ingestion, prolonged feeding periods, and possible inactivation of INH through interaction with glucose contained in molasses (Sved *et al.*, 1977). Nevertheless, several high concentrations were observed at 6 h postdose in some elephants suggesting a further study with prolonged sampling schedule may be warranted.

Rectal administration of INH appears to be a viable alternative to oral dosing, and was better tolerated by most elephants. Both rectal retention enemas and rectal suppositories produced similar serum concentrations that approached those seen with the use of the oral concentrate. It might be possible to achieve serum concentrations comparable with those seen in humans with most of the oral and rectal dosing methods, although it is not yet known whether achievement of accepted human serum concentrations will yield therapeutic cures in elephants. To be able to monitor therapy for efficacy and to limit adverse reactions, it is recommended that INH serum concentrations be documented throughout the treatment course. Based on these data, a pair of blood draws within 6 h postdose should provide reasonable estimates of INH absorption in elephants. C_{\max} is best estimated by a sample at 2–3 h postdose for most administration methods, and a second sample at 6 h can detect delayed absorption in most cases. Because INH is not stable at room temperature in whole blood, samples should be centrifuged promptly, and the serum harvested and frozen in labeled plastic tubes following centrifugation. For those situations where samples cannot be centrifuged promptly, we recommend collecting the blood in heparinized tubes ('green top' vacuum tubes). These can be labeled with indelible ink, and placed on ice chips until centrifugation is possible.

The V/F and Cl/F estimates provide general information about how these elephants handled INH. Given the oral and rectal routes of administration, these values are shown divided by F , which is not known for these animals. Precise estimates of V and Cl would require intravenous administration followed by the

collection of a greater number of blood samples. Such a study was beyond the scope of this pilot investigation.

Our results are consistent with the only prior report of administration of INH to a single Asian elephant (Devine *et al.*, 1983). In this study, oral administration of INH at a dose of 5 mg/kg given as an oral bolus with food yielded a peak concentration of 5.57 µg/mL with T_{\max} at 1 h and an elimination $t_{1/2}$ of 1.62 h.

Population PK modeling was employed in the data analysis. The approach is appropriate for handling sparse concentration data. With this method, both fixed effect (θ) and random effect (η, ϵ) can be estimated. Briefly, the fixed effect are factors which can be determined or measured, such as clinical study site, patient demographics, pre-existing medical conditions, other independent variables and model parameters. The random effects are factors which cannot be properly determined and measured, such as recording errors, impact of unknown pathophysiological conditions on drug absorption, distribution, metabolism and elimination, unexpected analytical variations, etc. Random effects can be further subdivided into inter-individual random effects η (i.e. differentiate one subject from another) and residual random effects ϵ (e.g. intra-individual variability, inter-day variability, inter-occasion variability, measurement error and process noise). It is noted from the analysis that variability in fixed effect of model parameters (θ) is acceptable (10–50%) indicating the model generated reasonable PK estimates. However, the variability in random effect parameters (η, ϵ) was large (40–163%) suggesting differences among subjects, across treatment period and study conditions might contribute significantly to the variability in PK estimation.

Based on the data generated, an initial dose of 7.5 mg/kg appears to be reasonable for INH purchased as a premixed concentrate. If INH powder is mixed just prior to administration, the starting dose of 4 mg/kg appears to be reasonable. The administration of INH as a mixture with mash or other feeds (i.e. 'top-dressed' feeds) should not be used because it is not reliable. Monitoring of maximal serum concentrations should be performed at 2 h and 6 h post administration.

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