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Clinical Interpretation of Pharmacokinetic and Pharmacodynamic Data in Zoologic Companion Animal Species

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KEYWORDS

• Analgesia • Efficacy • Potency • Half-life • Clearance
• Volume of distribution

The treatment and prevention of pain in zoologic companion animals is difficult because of the lack of data available on the safety and efficacy of drugs. The ideal situation for determining effective drug dosages is with controlled clinical trials, however these are rarely performed in veterinary species because of the high costs of the studies, number of animals needed, and difficulty in working with many of these species. Many dosage recommendations are based on perceived response to therapy, clinical experience, and lack of observable toxicity. However, these extrapolations are difficult to interpret for several reasons. The behavior of companion exotic animals often results in these animals hiding behaviors associated with disease or pain in order to minimize identification as weakened animals by predators or other animals within the herd, pack, or other social group. Therefore signs that are easy to observe in domesticated companion animals, such as lameness in dogs, are difficult to observe in nondomesticated animal species even with a trained and experienced observer.

For example, an animal may seem better after administration of an analgesic, but the pain may not have truly been controlled and the response may be caused by the animal trying to mask the signs in the presence of the observer, by healing of the injury, or by normal variations in pain intensity. The clinical observation in this case would be a perceived improvement after analgesic administration when an improvement did not truly occur. The same treatment is then administered to a similar animal a month later and the same scenario occurs: a perceived improvement when

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an improvement did not occur. The clinician now has some confidence that the treatment worked, despite the low number of animals treated, but in reality the analgesic did not work. The clinician then passes the treatment information to another colleague who uses the information with the same perceived improvement when none occurred. The information has then been independently verified and makes its way onto a listserve or by word of mouth at meetings or symposiums to other clinicians and eventually into a formulary. The formulary then undergoes several editions over the years, is referenced by other sources, and the incorrect dosing information becomes dogma. Through nobody's fault, and primarily because of the lack of available objective data, incorrect dosing information can easily become dogma, and this has occurred in veterinary medicine in all species, domesticated and nondomesticated.

The previous scenario emphasizes the importance of objective data and the need for controlled studies in animals. The lack of objective data in zoologic companion animals has numerous causes including the lack of funding, difficulty in handling some animal species, increased animal stress, lack of available animal numbers, the rarity and value of certain species, and the potential for adverse effects. However, without adequate data, therapeutic decisions are difficult to make, especially when treating the large number of animal species, including amphibians, reptiles, birds, and exotic small mammals.

STUDY DESIGNS EVALUATING A THERAPEUTIC AGENT

A logical progression in evaluating a therapeutic agent such as an analgesic would include pharmacokinetic (PK) studies, followed by pharmacodynamic (PD) studies or integrated PK-PD studies, and eventually controlled clinical trials. PK studies evaluate the changes in plasma concentrations over time, the relationship between plasma concentrations and dose, the effects of different routes of administration on the plasma concentrations, and the potential for extrapolating plasma concentrations within a dose range. The effect the drug elicits is not a primary outcome of PK studies. PD studies evaluate the effect produced by the drug after it is administered. PD studies may evaluate a single dose rate or evaluate escalating doses. PK-PD studies integrate changes in drug concentrations versus changes in the effect (ie, whether an increase in dose, or plasma concentration, results in an increase in analgesia). Once a targeted dose is chosen based on PD, or preferably PK-PD, studies, the dose regimen should be evaluated in a controlled clinical trial to confirm that the experimental model accurately predicts the desired effect and lack of adverse effects in clinical patients.

Controlled clinical trials evaluate the test drug in patients clinically affected with an injury or disease, for example, evaluation of a nonsteroidal antiinflammatory drug (NSAID) in ducks with lameness associated with synovitis. In a positive controlled trial, the drug to be tested is compared with a drug known to elicit the desirable effect. For example, a positive controlled trial could include oral meloxicam as the test drug and flunixin as the positive control for the treatment of lameness associated with synovitis. In vivo studies have shown that flunixin inhibits plasma thromboxane for 6 to 12 hours in mallard ducks.[1] Some assumptions in positive controlled trials include that previous studies have shown that the positive control is effective in the species to be tested, the positive control is effective at the administered dosage, the duration of effect of the positive control has been determined, and the positive control is effective for the specified disease. However, there are few clinical studies in nondomestic species that meet these assumptions. Limitations of the lame duck example are: flunixin has not been shown to produce analgesic effects in ducks or in ducks with synovitis; the dose of flunixin was determined in an experimental model (inhibition of plasma thromboxane)

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and may not extrapolate to analgesia for synovitis; the dose of flunixin was determined in healthy animals, not in animals with an inflammatory condition; and the test dose of oral meloxicam is undetermined, so the oral bioavailability may be lower than estimated and the lack of a response caused by the dose being too low, not lack of efficacy.

A placebo-controlled clinical study evaluates the test drug against a treatment that is known not to elicit a clinical effect. For example, tramadol is administered in capsules to 1 group of rabbits, a second group of rabbits is administered capsules containing lactose, and both groups are evaluated for improvement in pain from naturally occurring osteoarthritis. A criticism of placebo-controlled trials is that 1 group of animals is not receiving an analgesic. However, it is questionable where the greater harm is: a placebo-controlled study in 20 animals (half of which receive the analgesic to be tested), or conducting a clinical trial with a positive control that has not been shown to be more effective than a placebo. The problem with an unvalidated positive control is the interpretation when no significant difference is seen between the unvalidated positive control and test drug groups. No significant difference is just as likely to mean that neither drug elicited an effect as that both drugs elicited an effect. If neither drug elicited an effect, than the net result may be hundreds, thousands, or potentially millions of animals receiving an ineffective drug, compared with 10/20 animals receiving a placebo.

The ideal controlled clinical study would also involve randomization of test groups and all investigators directly involved in the study being blinded to the administered treatments. Randomization avoids bias in selecting animals to receive the test drug and the control drug. For example, a ferret may be enrolled in a placebo-controlled clinical trial evaluating an NSAID for the control of osteoarthritis. The ferret is 3/4 lame, therefore the investigators put the ferret in the NSAID treatment group, whereas a ferret with a 1/4 lameness is put into the placebo group. The biased assignment of animals into the test groups could affect the results of the trial by placing animals with the most severe lameness in the NSAID-treated group. However, the lameness in the animals in the treatment group may have been so severe that it would be nonresponsive to NSAIDs. The interpretation could erroneously be made that the NSAID was ineffective, but the severity of the arthritis was the primary reason for the poor efficacy. Conversely, assignment of less severely affected animals to the placebo group could bias the results in favor of the NSAID-treated group because of the potential magnitude of change being more limited in the placebo group. For example, the initial average lameness in the placebo group may have only been 1/4 at the beginning of the study, with the final lameness graded at 0.5/4, a change in magnitude of 0.5 units. The treated group may have had an initial lameness score of 2.5/4 lame, which improved to 1.5/4 lame, resulting in a greater magnitude of change (1 unit) and potentially erroneously interpreting the results as the NSAID having a greater effect than placebo, when the real effect was normal variation in the disease. An alternative way of assessing these data would be a 50% improvement (0.5 final lameness/1.0 initial lameness) in the placebo group compared with a 40% improvement (1.5 final lameness/2.5 initial lameness) in the NSAID-treated group, which could bias the results in favor of the placebo because of bias in the assignment to treatment groups. In addition, the blinding of the investigators to the treatments is intended to minimize either intentional or unintentional bias when evaluating the pivotal parameter in the study. For example, in evaluating the effects of an NSAID on lameness in ferrets, the investigator may subconsciously think the NSAID-treated group must be doing better than the placebo group, and as a result the scores for lameness are more improved in the treatment group compared with the placebo group.

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Another important component of clinical trials is the detection of adverse effects that may not have been previously reported. Because the treatment is administered to clinical patients, instead of young healthy patients, the rate of adverse effects in the clinical trial is often greater. This information is extremely valuable because it may provide guidelines for adverse effect monitoring, patient selection, potential for drug-drug interactions, and recommendations for clinical chemistry/complete blood count monitoring in addition to drug efficacy. A good example of reported adverse effects in clinical trials involved the approval of meloxicam in domestic cats. During the positive controlled (butorphanol), randomized, and blinded clinical trial, 8.3% of cats experienced increases in the blood urea nitrogen (BUN) in the meloxicam-treated group, but none of the cats experienced BUN changes in the butorphanol-treated groups.[2] The increased BUN suggested that the NSAID caused some adverse effects in the kidneys of cats, which was later confirmed once the drug was released and adverse effects were reported to the US Food and Drug Administration.

Ideally, drug studies in zoologic companion animals would also involve toxicology studies. However, the rarity and value of many of these animals preclude toxicology studies. Valuable information is gained from toxicology studies, including potential adverse effects, range of dosages administered with no toxic effect levels, breed- or species-specific differences in toxicology, and a relative confidence in the safety of the drug. As an example, toxicology studies in domestic cats indicated that the toxic doses of acetaminophen overlap therapeutic doses, therefore no dose of acetaminophen in domestic cats is safe and effective.[3]

PKs

Primer on PKs

PKs is the use of mathematical models to predict plasma drug concentrations in the body. The primary PK parameters are the terminal half-life ($T_{1/2}$), clearance (Cl), and volume of distribution (V_d). The maximum plasma concentration (CMAX), time to CMAX (TMAX) and area under the curve (AUC) are also useful PK parameters.

The $T_{1/2}$ is the time it takes for the plasma concentration to decrease by 50%.[4] It is often incorrectly stated as the amount of time it takes to eliminate half of the drug. Decreases in plasma drug concentrations are not always caused by drug elimination, but could be caused by drug redistribution from the plasma to other tissues. The $T_{1/2}$ is a useful parameter because it allows the estimation for decreases in plasma drug concentrations, time needed for drug to reach steady state, and the ratio of peaks and troughs.

By definition, for every $T_{1/2}$ the plasma concentration decreases by 50% (**Table 1**), therefore some predictions of changes in plasma concentrations can be made if the $T_{1/2}$ is known. After $3\times T_{1/2}$, the plasma concentration will have decreased by ~88%; after $5\times T_{1/2}$, the plasma concentration will have decreased by ~97%; and by $7\times T_{1/2}$, the plasma concentration will have decreased by ~99%. This estimate can be useful in determining washout periods, treatment of certain intoxications, and sometimes durations of drug effect. The $T_{1/2}$ also predicts the time it takes for a drug to reach steady state drug concentrations if given by multiple doses or by a constant rate infusion. The time to steady state can be predicted in a similar manner to predictions for decreases plasma concentration; that is, ~88% of steady state will be achieved in $3\times T_{1/2}$, 97% of steady state will be achieved in $5\times T_{1/2}$, and 99% of steady state will be achieved in $7\times T_{1/2}$. As an example, a drug that has a $T_{1/2}$ of 24 hours will reach 88% of steady state in 72 hours ($3\times T_{1/2}$), 97% of steady state in 120 hours ($5\times T_{1/2}$), and 99% of steady state plasma concentrations in 168 hours

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Table 1
The percentage decrease in plasma drug concentration as a function of the number of half-lives

Number of Half-lives	Percentage of Initial Drug Concentration Remaining in Plasma	Percentage of Drug Concentration Decrease in Plasma
0	100	0
1	50	50
2	25	75
3	12.5	87.5
4	6.25	93.75
5	3.125	96.875
6	1.5625	98.4375
7	0.78125	99.21875
8	0.390625	99.609375
9	0.1953125	99.8046875
10	0.09765625	99.9023438

($7 \times T_{1/2}$). The $T_{1/2}$, along with the dosing interval, can also be used to predict the fluctuations in peak and trough plasma drug concentrations. If the $T_{1/2}$ is much shorter than the dosing interval, most of the drug will be eliminated before the next dose, resulting in a large fluctuation between peak and trough concentrations. A drug with a short $T_{1/2}$ (1 hour, for example) administered every 8 hours will have large fluctuations in peak and trough concentrations because there will be a large decrease in plasma concentrations before the next dose (>99%; **Fig. 1**A). Conversely, if the $T_{1/2}$ is much greater than the dosing interval, there will be less fluctuation in plasma concentrations as minimal decreases occur before the next dose is administered, and the result is minimal fluctuation between peak and trough concentrations. A drug with a 48-hour $T_{1/2}$ administered once daily (every 24 hours) will have slight decreases in plasma concentrations before the next dose (~25%; see **Fig. 1**B).

The apparent V_d is the apparent volume that a drug dilutes into after administration. The V_d is not necessarily a true physiologic volume. The V_d is species specific and formulation specific, and even age specific, therefore extrapolations must be made cautiously (see later discussion). The V_d is readily calculated with the following equation if the dose and drug concentration after intravenous (IV) administration are known: V_d = dose/concentration. The primary usefulness of the V_d is to calculate a loading dose to immediately achieve desired concentrations by rearranging the equation to: dose = $V_d \times$concentration.

A loading dose is most critical when immediate effects are wanted from a drug with a long $T_{1/2}$ because of the prolonged time to steady state (see earlier discussion). As an example, phenobarbital has a V_d in dogs of 700 mL kg^{-1} and 20 μg mL^{-1} is within the reported range of therapeutic plasma concentrations. Therefore the dose can be calculated by multiplying the V_d (700 mL kg^{-1} in this example) by the targeted concentration (20 μg mL^{-1}): (700 mL kg^{-1})$\times$(20 μg mL^{-1}) = 14,000 μg kg^{-1} = 14 mg kg^{-1}. Another use of the V_d is to estimate a dose in a species for which the drug has not yet been examined. The V_d can be estimated through allometric analysis (see later discussion), but there are numerous limitations to this approach, including the lack of a dosing interval.

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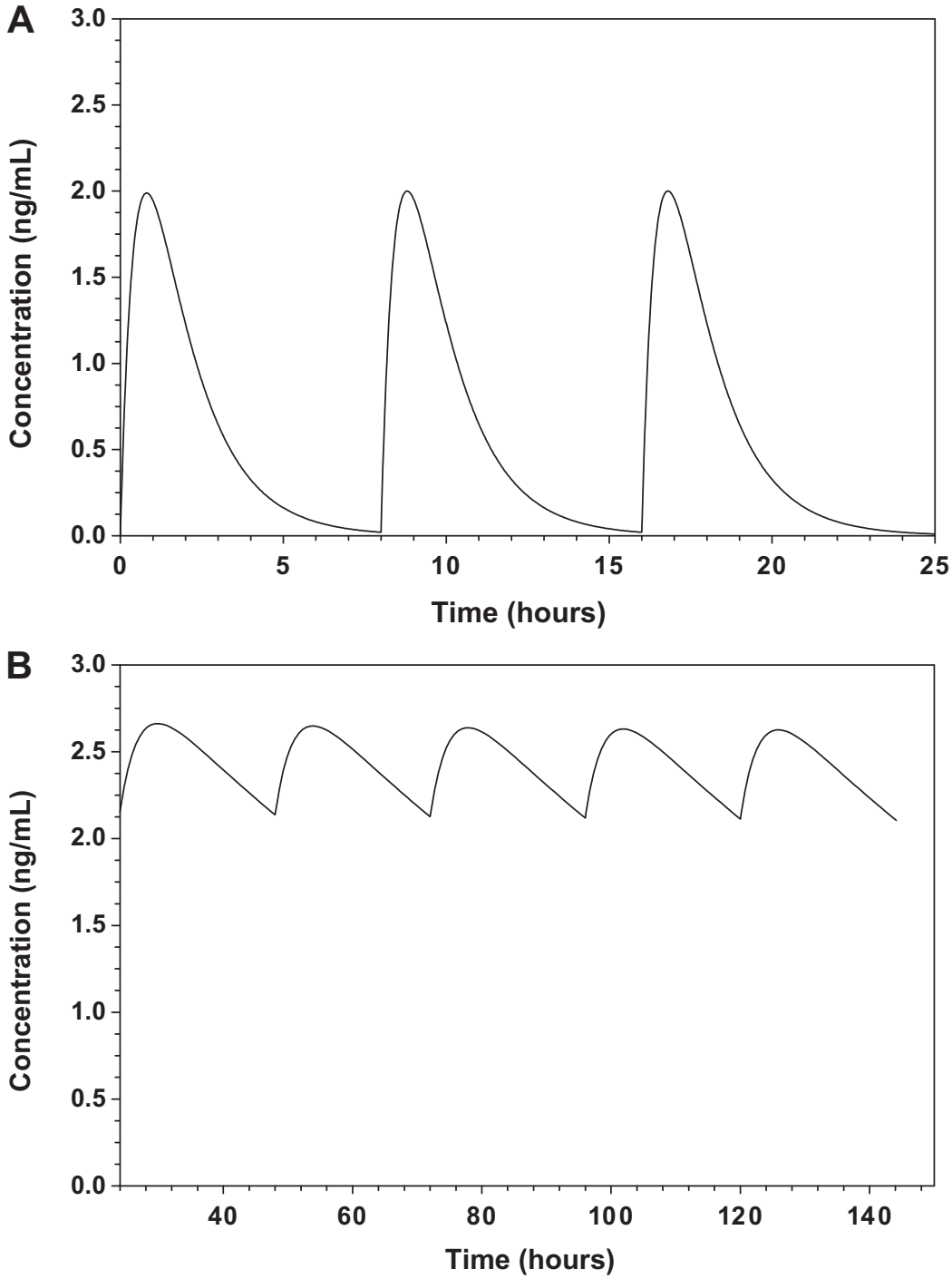

Fig. 1. The peak and trough ratio can be estimated based on the $T_{1/2}$ and dosing interval. (*A*) The $T_{1/2}$ (1 hour) is much shorter than the dosing interval (every 8 hours), in this case resulting in a large fluctuation (100-fold) in the peak and trough concentrations. (*B*) The $T_{1/2}$ (48 hours) is much greater than the dosing interval (every 24 hours) resulting in minimal fluctuation (0.25-fold) in the peak and trough concentrations.

The plasma Cl of a drug is the volume of the body for which the drug distributes cleared of the drug per unit time (ie, the volume of the V_d cleared per unit time). Plasma Cl is the sum of all mechanisms of drug elimination, including renal (glomerular filtration, tubular secretion, and renal metabolism), hepatic (metabolism and biliary secretion), and other clearance mechanism such as plasma esterases, monoamine oxidases, and splenic and intestinal metabolism. The usefulness of the clearance

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parameter is in calculating a constant rate infusion or average plasma drug concentrations using the equation: dose rate = Cl×steady state drug concentration. As an example, the clearance of morphine in llamas[5] is 27.3 mL min^{-1} kg^{-1} and a plasma drug concentration of 20 ng mL^{-1} is desired, then the dose can be calculated as follows: dose rate = (27.3 mL min^{-1} kg^{-1})×(20 ng mL^{-1}) = 546 ng min^{-1} kg^{-1} = 32,760 ng h^{-1} kg^{-1} = 0.03276 mg h^{-1} kg^{-1}.

The AUC is the calculated area under the curve of plasma concentration versus time (**Fig. 2**). The AUC is a measure of cumulative drug exposure and is related to the dose administered, plasma drug concentrations, and the duration of time the plasma drug concentrations persist. The AUC is proportional to the dose for most drugs; for example, doubling the dose results in a doubling of the AUC. In addition, because the AUC is dependent on the time the drug concentrations persist in the plasma, increases in half-life caused by decreased drug elimination result in increases in the AUC. The efficacy of some drugs is best correlated with the AUC. As an example aspirin efficacy in humans for postoperative dental pain is well correlated with the plasma AUC; the higher the aspirin AUC the better the pain control.[6] The correlation for aspirin analgesia was better for the AUC than the CMAX. The toxicities of some drugs have also been correlated with the AUC; for example, the antiplatelet effects of aspirin are correlated with the AUC of aspirin in humans, therefore the prevalence of excessive bleeding is expected to increase as the aspirin AUC increases.[7] The AUC is also a pivotal parameter in the determination of drug bioavailability, with the AUC from the extravascular route divided by the AUC of IV administration. The AUC is also a pivotal parameter in the determination of drug bioequivalence when comparing different drug formulations (ie, a brand name drug versus a generic drug).

The CMAX is the maximum drug concentration after extravascular administration (see **Fig. 2**). The CMAX is typically proportional to the dose, so, for example, doubling the dose typically results in a doubling of the CMAX, whereas decreasing the dose by 50% decreases the CMAX by 50%. The CMAX has been correlated with toxicity for

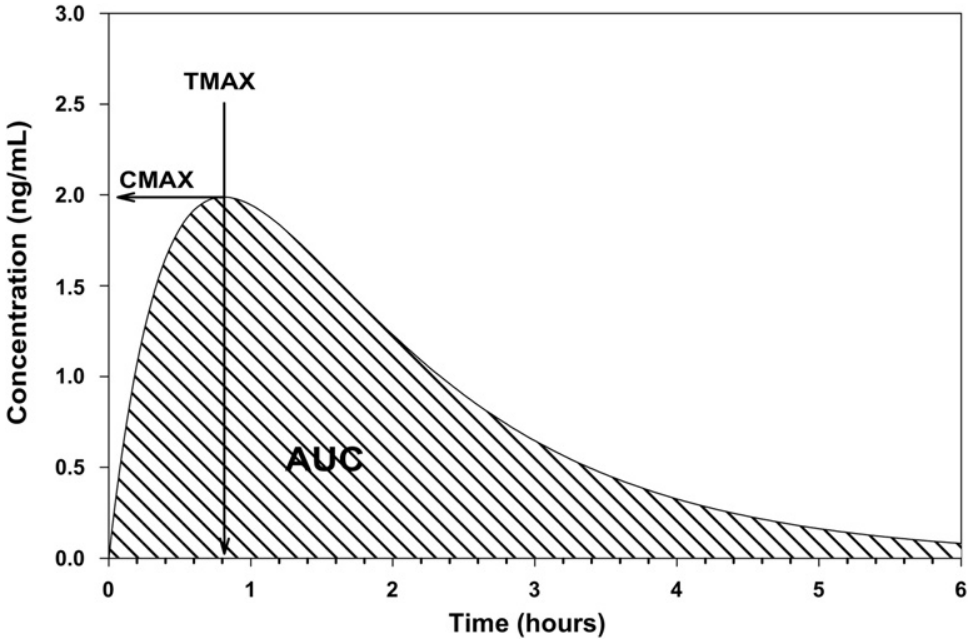

Fig. 2. The PK parameters: maximum plasma concentration (CMAX = 2 ng mL^{-1}), time to maximum plasma concentration (TMAX = 0.8 hours) and AUC (5 ng h mL^{-1}), represented as the hashed area.

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many different drugs and efficacy for some drugs. For example, the toxicity of sevoflurane is related to achieving high concentrations, which cause respiratory depression and death.[8] The CMAX is the second pivotal parameter for drug bioequivalence when comparing 2 different drug formulations (ie, a brand name drug vs a generic drug). The TMAX is the time of the CMAX and is typically constant for a drug formulation regardless of dose, so a doubling of the dose results in no change in the TMAX. To determine the peak drug concentration, the CMAX, for therapeutic drug monitoring, the plasma sample will be obtained at the TMAX.

Analytical Methods for PK Studies

The method of drug analysis can greatly affect the PK parameters. Numerous different methodologies are available, but direct comparisons cannot be made when different methods of analysis are used. Mass spectrometry methods tend to be highly sensitive, measuring very low concentrations, and are highly specific, with minimal cross-reactivity with metabolites or endogenous substances and xenobiotics. Mass spectrometry is often considered the analytical method of choice, but it is among the most expensive methods available and requires expensive equipment and substantial training to use. Mass spectrometry is typically coupled to gas chromatography or high-pressure liquid chromatography (HPLC).

HPLC and gas chromatography (GC) tend to have moderate sensitivity and low to moderate specificity. Most compounds can be accurately and precisely quantified with HPLC and GC, but high-potency drugs (low drug concentrations to achieve an effect), or drugs that are extensively metabolized to numerous metabolites, can be difficult to accurately and precisely measure by HPLC and GC. HPLC and GC costs are moderate, as is the technical expertise required to use these systems.

Immunoassays, including radioimmunoassay (RIAs), enzyme linked immunoassays (ELISAs), and fluorescence polarization immunoassays (FPIAs) are usually adequately sensitive, but the specificity is variable. Immunoassays may cross-react with metabolites or other drugs, can overestimate drug concentrations, and are typically considered to have less specificity than the other methods of analysis. However, drugs that are minimally metabolized can be accurately and precisely measured by immunoassays. There are some immunoassays that are highly specific for some drugs that are metabolized, but many of these have not been validated in animals in which the metabolic pathways and drug metabolites have not been identified. Immunoassays tend to have lower costs than other analytical methods and tend to be technically less difficult to run.

Direct comparisons of PK results using different analytical methods can be difficult because of differences in sensitivity and specificity in analytical methods (see later discussion). Ideally, a full method validation for each species being evaluated should be included for each drug analyzed and should be performed in the laboratory performing the drug analysis in order to assure the most accurate results, including assessment of cross-reactivity of metabolites, xenobiotics, and endogenous substances.

PK studies

The purpose of PK studies is to describe the changes in plasma concentrations over time, the relationship between plasma concentrations and dose, the effects of different routes of administration on the plasma concentrations, and the potential for extrapolating plasma concentrations within a dose range. The end goal is often to correlate physiologic processes with the PK model and use the data to make predictions of when parameters may change, as in renal or hepatic disease or species difference.[9] PK studies most often involve determining the drug concentration in

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plasma or serum, because these are typically the easiest and most readily obtained samples. Plasma and serum samples also have been most often correlated with a clinical effect, despite the blood, plasma, or serum rarely being the location of the body where the drug has its effect. Other areas of the body are occasionally sampled, such as synovial fluid, cerebrospinal fluid (CSF), whole tissues, urine, and feces, but, because of difficulties in collection from many of these areas or because of urine and feces contain the concentrations of the drugs after they have been metabolized and eliminated from the body, they are less frequently used in PK studies.

PK Study Design

There are numerous different methods of PK study design. The most common method is termed the standard 2-stage (STS) method. The basic design of an STS is to administer a drug to a small homogenous group of animals, collect many samples from individual animals, analyze each sample independently, calculate the PK parameters for each animal individually, and report the descriptive statistics of the PK parameters, such as mean, median, and range. The advantages of an STS include a robust estimate of the average PK parameters in the homogenous population; a small number of animals is included, which subsequently minimizes the facilities needed; relative ease of sample collection in some animals; potential for training the animals for handling and sampling; and the short time in which the study can be completed. However, numerous limitations of the STS method are also present. Small numbers of homogenous animals are studied, which limits the direct application of the data to that well-defined group of animals. Many variables may not be accounted for in an STS study such as disease and concurrent medications, the effects of age, the variability within the entire animal population, breed- and sometimes gender-specific differences, outliers are often not identified, the potential stress from obtaining multiple samples from an individual animal, and volume depletion from repeated sampling from small animals. A recent study examined the PK of oral tramadol in rabbits using an STS method.[10] Six healthy, sexually intact New Zealand White female rabbits, weighing 3.8 ($\pm$ 0.38) kg were administered oral tramadol. Although robust parameters were reported, it is unclear whether these parameters are applicable to animals with underlying disease, perioperative patients, young or geriatric animals, and animals administered concurrent drugs, among other variables.

Naïve Average and Naïve Pooled Analysis

Naïve averaged or naïve pooled PK studies are often used in captive wild animals, zoologic companion animals, small animals, or animals in which samples are difficult to obtain. Fewer samples are collected from each individual, precluding the calculation of individual PK parameters. Instead the individual samples are analyzed, the average concentration at each time point is determined, and the PK model is fitted to the average concentrations. One of the disadvantages to this approach is that the variability within the PK parameters cannot be determined. In addition, bimodal distributions may not be identified using this model. Many of the disadvantages of the STS method still occur using naïve average analysis if homogenous animal populations are used. A variant of the naïve average analysis is the naïve pooled analysis. Naïve pooled analysis involves using all samples collected and fitting a model to all of the samples without first calculating the average plasma concentrations at each time point. An advantage of the naïve pooled analysis is that the time points do not have to be as precise because the average concentration for each time point is not calculated. For example, naïve average analysis of buprenorphine in mice collected samples from 4 different animals at each of the following times: 5, 15, and 30 minutes and 1, 2, 3, 5,

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7, 9, 12, 18, and 24 hours after drug administration for a total of 48 animals.[11] The average concentrations at each time point were then calculated and the PK model fitted to the average concentrations. Problems can occur in naïve averaged analysis if an animal is difficult to bleed, resulting in the sample being collected 15 minutes late, or if the sample is missed entirely. Naïve pooled analysis is less affected by late time points or time points in which samples are missed, assuming the final data are appropriately distributed throughout the sampling period to avoid bias. Extensive studies examining whether either the naïve averaged or naïve pooled method are more robust are lacking. A study evaluating the PK of oral marbofloxacin in harbor seals compared these methods of analysis and suggested that the primary PK parameters were similar regardless of the method used.[12]

Population PK Analysis

Population PK modeling, also referred to as mixed effects modeling, is a complex modeling scheme in which a large number of subjects are included in the study (hundreds to thousands are included in human studies), and a very limited number of samples obtained from each individual. Population PK analysis has some advantages but, because of the large number of subjects required, it is beyond the scope of this article. A thorough review of population PK is published elsewhere.[13]

Allometric Analysis

Allometric analysis is used to estimate the PK parameters of a drug in a species for which data are not available, but data are available in numerous other species. Allometric analysis is a mathematical approach for estimating PK parameters before administering a drug to an animal. The principle of allometric scaling is that major physiologic processes are related to body weight raised to some power.[14] The basic allometric equation is $Y = a \times \text{weight}^b$, where *Y* is the PK parameter to be estimated, *a* is the allometric coefficient, weight is the weight of the animal, and *b* is the allometric exponent. The PK parameters typically estimated are the clearance and V_d. $T_{1/2}$ is occasionally estimated, but it is a function of the clearance and V_d ($T_{1/2} = 0.693 \times V_d/Cl$) and therefore it does not have to be estimated. In addition, $T_{1/2}$ tends to extrapolate poorly.

Allometric analysis is an estimator and not a calculator of PK parameters. There are numerous reports detailing the potential inaccuracy of allometric scaling,[15,16] but it has proven useful for some drugs and species.[15,17,18] One of the considerations for allometric analysis is the extrapolation of data only within the range of data available. For example, data on the PK of intravenous buprenorphine are available from mice (0.027 kg) to horses (525 kg) (**Table 2**), therefore extrapolation to an elephant weighing 2000 kg is inappropriate because it is outside the weight range and may not be accurate. Extrapolation between different classes of animals, such as birds, mammals, reptiles, and amphibians is also likely inaccurate because of the vast differences in physiology of these species. Species-specific differences in metabolism must also be considered; for example, cats are poor at glucuronide conjugate formation, dogs are poor at acetyl conjugation, some dogs are deficient in p-glycoprotein drug transporters.[19,20] Differences in metabolism have been documented in rats, primarily because of their use as laboratory animals. Sprague-Dawley rats from 2 different sources had significantly different metabolism of morphine; rats from Denmark had significantly faster metabolism of morphine compared with rats from Sweden, and subsequently the rats from Denmark had significantly less analgesia when administered the same morphine dose.[21] It is expected that differences in other zoologic companion animals are also present, but not yet documented.

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Table 2
PK parameters of intravenous buprenorphine in a variety of species: predicted parameters based on allometric analysis and accuracy of the predicted parameters

PK Parameter		Mice[11]	Rats[46]	Dogs[47]	Goats[48]	Humans[49]	Alpacas[50]	Horses[51]
$T_{1/2}$ (h)	Actual	2.9	5.28	9.0	1.1	3.21	2.3	7.14
	Predicted	2.87	2.87	2.87	2.87	2.87	2.87	2.87
	Accuracy (%)	99	54	32	261	89	125	40
Cl (mL min^{-1})	Actual	1.94	8.1	336	3188	1281	4274.66	4147.5
	Predicted	1.99	9.12	299.63	695.47	1089.45	1215.04	5483.58
	Accuracy (%)	103	113	89	22	85	28	132
V_d at steady state (L)	Actual	0.176	1.507	133	201.6	334.6	569.42	1785
	Predicted	0.211	1.254	75.116	201.535	341.058	387.584	2267.091
	Accuracy (%)	120	83	56	100	102	68	127
Weight (kg)		0.027	0.18	14	40	70	80.2	525

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To perform an allometric analysis, data from previous PK studies are collected from a variety of species with a large range in weights. Each parameter ($T_{1/2}$, Cl, V_d) is estimated independently in allometric analysis. The logarithm of the absolute value of the parameter (mL min^{-1} not mL min^{-1} kg^{-1}) is plotted against the logarithm of body weight (**Figs. 3–5**). A regression analysis is then performed for each parameter fitting the allometric equation. A weighting factor can be added to better fit the equation to the data, especially for animals with lower body weights. As an example, an allometric analysis of buprenorphine is included (see **Figs. 3–5**, see **Table 2**). Data from animals in which PK studies were performed and drug concentrations were determined using mass spectrometry methods were included in the allometric analysis. Other methods of analysis are not included because of potential bias in the data (see earlier discussion).

The allometric analysis of buprenorphine yielded variable results. As expected, the half-life scaled poorly (see **Fig. 3**). The scaling of the clearance was moderate, with an R^2 of 0.7688, which is a reasonable coefficient of determination (**Fig. 4**). However, examining the allometric predicted results with the published values shows one of the weaknesses of this method of prediction. The predicted values for goats and alpacas are markedly lower than observed, indicating that allometric analysis is not a good estimator for every species, but the other species were within 32% of the actual values. As stated previously, the clearance is a useful parameter in estimating the average plasma concentration or steady state concentration in a multiple dose protocol or continuous infusion. The V_d for buprenorphine scaled the best, with an R^2 of 0.9249 (**Fig. 5**). Even with a high coefficient of determination, the predicted value for dogs was only 56% of the actual value, indicating that it is not an accurate estimator for all species despite a high correlation. The V_d, as discussed earlier, is useful for estimating the initial plasma concentrations for drug administration and can be used to determine an initial dose.

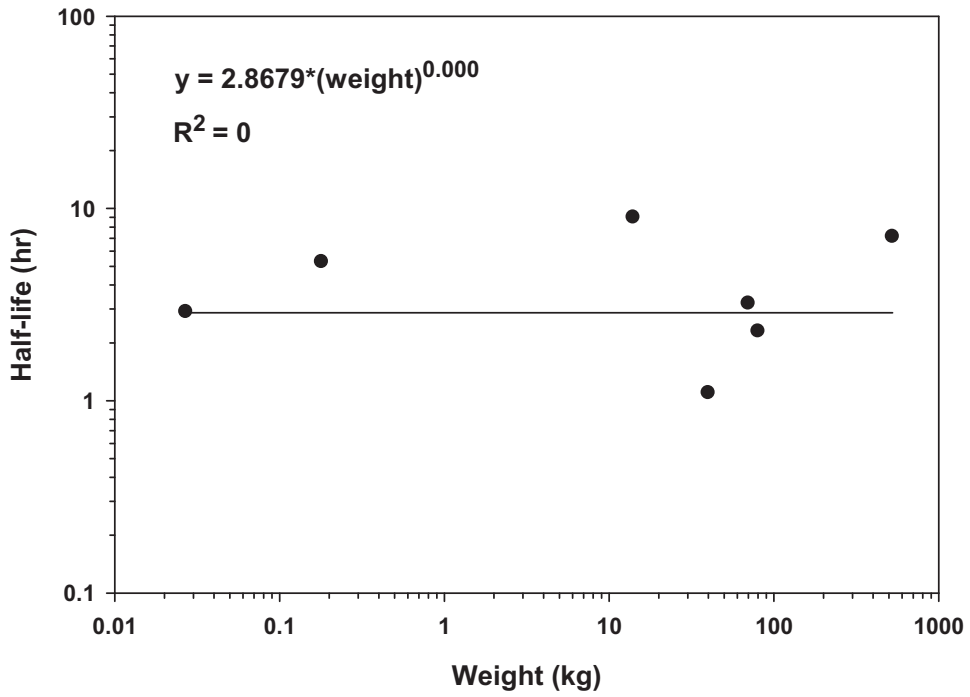

Fig. 3. Allometric analysis of the elimination half-life of buprenorphine. See **Table 2** for species and PK parameters used in the allometric analysis. A weighting factor of 1/y was used for the analysis.

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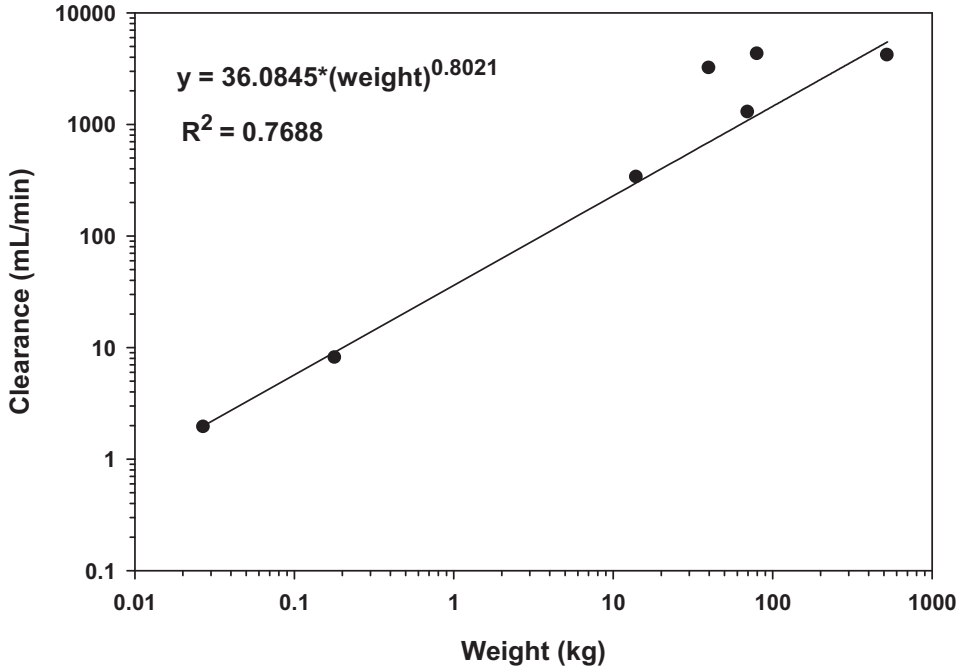

Fig. 4. Allometric analysis of the clearance of buprenorphine. See **Table 2** for species and PK parameters used in the allometric analysis. A weighting factor of $1/(y^2)$ was used for the analysis.

Application of allometric scaling

Allometric scaling could be used for determining a dose in a clinical patient, but is often applied before the design of a PK study. For example, what buprenorphine dose should be administered IV to assess the PK of buprenorphine in 400 g (0.4 kg) hedgehogs? The V_d can be estimated with the allometric equation from **Fig. 5**: $V_d = 6.2844 \times 0.4^{0.9401} = 2.656$ L or 6.639 L kg^{-1}. If an initial plasma concentration of 5 ng mL^{-1} is desired, the dose can be calculated using the equation: Dose = $V_d \times$

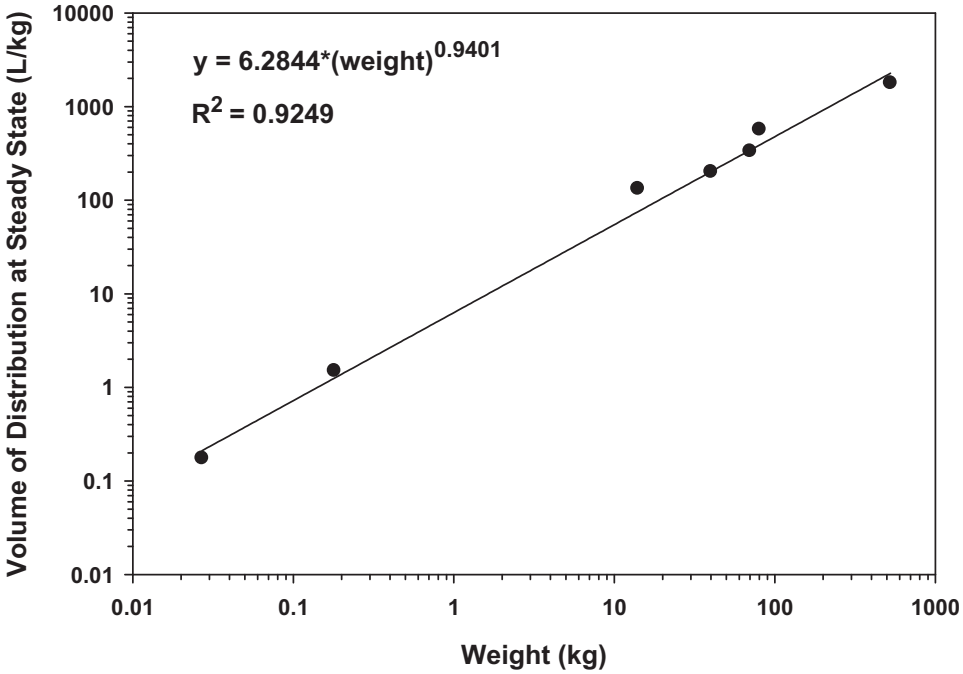

Fig. 5. Allometric analysis of the V_d at steady state of buprenorphine. See **Table 2** for species and PK parameters used in the allometric analysis. A weighting factor of $1/(y^2)$ was used for the analysis.

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concentration = (6.639 L kg^{-1}) × (5 ng*mL^{-1}) = (6.639 L kg^{-1}) × (5000 ng L^{-1}) = 33,195 ng kg^{-1} = 33.2 μg kg^{-1} = 0.033 mg kg^{-1}. Because of the variable elimination reported for buprenorphine, a sampling strategy would likely include collecting samples to 24 hours.

PD AND PK-PD STUDIES

PD Studies Overview

The purpose of PD studies is to determine the effects a drug elicits after administration. PD studies can use either healthy animals, if an appropriate model is available, or clinically affected animals. PD studies are critical, especially with the wide variety of species that need to be treated in veterinary medicine and previously described species-specific differences. Extrapolations are often made from plasma drug concentrations or dosages in other species, but it is unknown whether the species to be treated will respond similarly to similar drug concentrations.

Efficacy and potency

Efficacy and potency are PD terms that are often incorrectly used interchangeably. Efficacy describes the maximum effect elicited when a drug is administered at increasing doses. In contrast, potency describes the dose or concentration required to elicit an effect. Drugs with greater potency are not necessarily more effective than drugs with less potency, but drugs with greater potency require lower concentrations (doses) than drugs with a lower potency. Opioids provide an example of comparisons of efficacy and potency. Fentanyl, morphine, and buprenorphine are opioids used in rats. Morphine and fentanyl are μ opioid agonists that produce the maximum analgesic effect possible for opioids in rats before loss of consciousness, whereas buprenorphine is a partial μ agonist producing a submaximum analgesic effect in rats.[22] Therefore, in rats, morphine and fentanyl have similar efficacy and buprenorphine has lower efficacy (**Fig. 6**). The rank order of efficacy is buprenorphine < morphine = fentanyl in rats. In contrast, the potency of fentanyl is greater than morphine and buprenorphine has similar potency to fentanyl. The rank order of potency is morphine < buprenorphine = fentanyl in rats. Potency and efficacy are independent parameters, and the efficacy and potency may differ depending on the species investigated.

PD and PK-PD models

PD analgesic models are difficult to conduct in nonrodent animals. A variety of models are accepted in rodents, but their use in other animal species is not always applicable or appropriate. An extensive review of analgesic modeling has been previously published.[23] Four basic types of noxious stimuli models are used in animals, including electrical stimulation, temperature stimulation (heat and cold), mechanical stimulation, and chemical stimulation. Each type of model has advantages and disadvantages and are addressed briefly in this article; however, extensive detail is available elsewhere.[23]

Electrical stimulation induces nerve depolarization, which is not specific to nociceptors, therefore, even with an effective analgesic, neurons other than those associated with pain, such as heat and cold, autonomic functions, muscle, and touch will be activated, which makes interpretation difficult. Electrical stimulation may not mimic clinical pain syndromes, which may lead to inappropriate conclusions.[23] Electrical stimulation models have been used by various groups to show opioid induced analgesia in birds,[24–26] and is used in humans.[23]

Models using temperature stimulation are most often performed using heat stimuli, but cold can also be effective. Use of temperature stimulation is also not specific for

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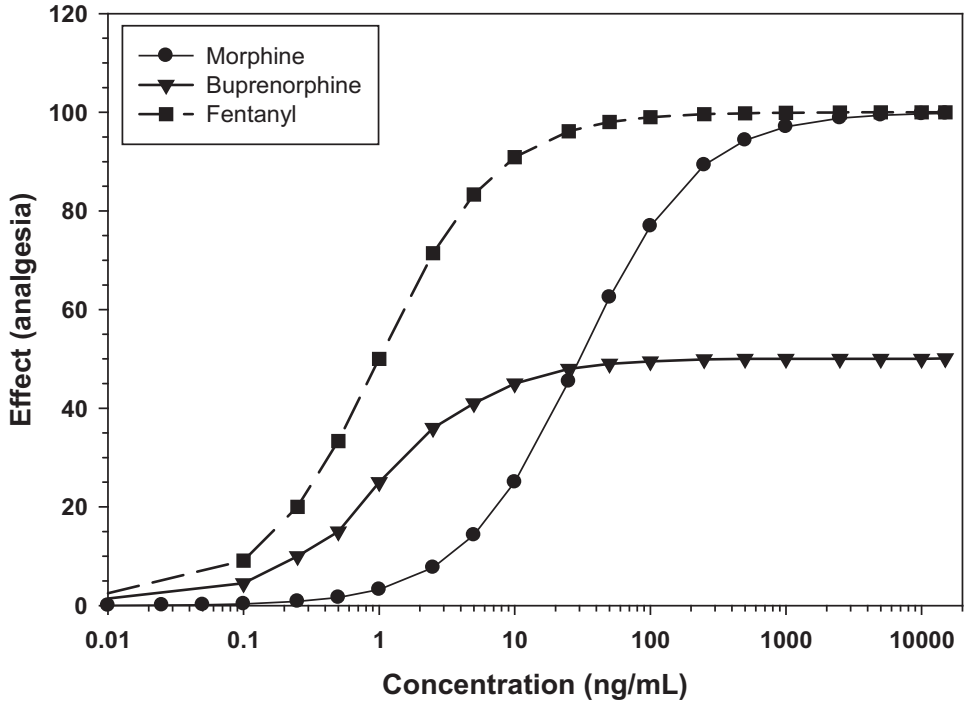

Fig. 6. Comparison of theoretic PDs of the opioids morphine, buprenorphine, and fentanyl in rats. The concentration of the drug is represented on the x-axis (log scale) and the analgesic effect is represented on the y-axis. As the concentration of each drug is increased, an increase in the analgesic effect occurs until it reaches a plateau after which further increases in drug concentrations do not result in an increase in effect. In this example, the rank of efficacy is buprenorphine < morphine = fentanyl and the rank of potency is morphine < buprenorphine = fentanyl.

nociceptors because both cold or heat receptors can be activated. In addition, the use of thermodes also provides a mechanical stimulation that may affect the results. A carbon dioxide laser system may be most effective, but high cost, technical expertise, and persistent pain and damage may occur.[23] Thermal nociception models have been used in nondomestic animals including rodents, birds, snakes, turtles, and crocodiles,[22,26–30] and are used in humans.[23]

Mechanical models produce stimulation through force on a specific part of the body. The von Frey model is the classic example of a mechanical stimulation model in which a filament or rigid plastic tip is applied with increasing force until nociception occurs. A disadvantage of this model is that it activates pressure sensory (touch) nerves in addition to nociceptors. Mechanical stimulation models primarily activate the fast-response, Aδ fibers nociceptors, which are associated with sharp, well-discerned pain, whereas clinical pain occurs primarily from the slow-response, c-fibers, which produce dull, aching, poorly localized pain.[23] Mechanical models are most commonly used in rodents,[31,32] but have been used in other veterinary species, and are used in humans.[23]

Chemical models of nociception inject a chemical that causes pain (an algesic) and some of the chemical algesics also cause inflammation. Chemical stimulation most closely mimics clinical pain.[23] However, chemical algesics also have limitations. The nociception increases to a peak and then decreases, therefore the stimulus is not constant throughout the experiment. The stimulus typically cannot be stopped once started. Many chemical models also produce concurrent inflammation, therefore direct comparisons of analgesics with antiinflammatory effects (ie, nonsteroidal antiinflammatory drugs) with analgesics without substantial antiinflammatory effects (ie, opioids) result in the assessment of different effects (analgesia and antiinflammatory

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effects vs analgesic effects). Chemical stimulation models have been used in birds to evaluate the efficacy of analgesics,[33,34] and are used in humans.[23]

PD and PK-PD modeling

The potency and efficacy of a drug may be determined in different ways. The 2 primary methods are dose-response and concentration-response methods. Concentration-response methods determine the drug concentration (typically plasma) and correlate it with the analgesic response. Disadvantages of concentration-response studies are the need to obtain multiple plasma samples, the typically high cost of drug analysis, and that the act of obtaining the samples can also interfere with the analysis of the analgesic effect. However, concentration-response analysis provides data that are more readily extrapolated to other routes of administration, different formulations, and provide better intra- and interanimal comparisons of potency and efficacy.

A drawback of using a concentration-response PD study is that plasma drug concentrations are typically being measured, but the effect of the drug typically occurs in a different anatomic location. A lag from the maximum plasma concentration to the maximum drug effect and a lag from the time when drug concentrations begin to decrease and the loss of analgesic effect often occurs, a phenomenon termed hysteresis. However, a variety of PD models are available to account for hysteresis and this modeling strategy has been used to describe the PD of morphine in veterinary species.[35]

Some investigators have proposed measuring CSF as a better method of determining the concentration-effect relationship of opioids. However, the CSF is not the effect compartment, CSF drug concentrations are not directly related to the drug effects in many cases, and CSF samples are more difficult to obtain than plasma.[36,37] Although CSF concentrations may represent a component of the lag that occurs from drug diffusing from the plasma to the central nervous system (CNS), the drug still must diffuse from the CSF into the parenchyma of the CNS and bind to opioid receptors within the brain and spinal cord to elicit the effect.[37] Obtaining CSF is more difficult and invasive than obtaining plasma, and has minimal benefits to PK-PD modeling.

Dose-response methods administer increasing doses of the drug and measure the response to the different doses. Limitations of dose-response studies are the lack of correlation with the concentrations and PK of the drug. Therefore, differences in PK parameters (eg, bioavailability, V_d, $T_{1/2}$) may result in inaccurate conclusions as to the drug's efficacy and potency if only dose-response relationships are evaluated. For example, morphine has an oral bioavailability of 5% in dogs[38] compared with 40% in humans,[39] so comparing a dose-response relationship would result in inaccurately stating that morphine is 8 times more potent in humans than in dogs. However, if a concentration-response study is performed, the effect of morphine in relation to plasma drug concentrations is similar in both species, indicating equal potencies. Therefore, the true differences of oral morphine in dogs and humans are PK differences, not PD differences. Another limitation of dose-response studies is that incorrect conclusions about drug efficacy could be made if a single dose, or small a range of doses, is administered. Again, the example of oral morphine in dogs would have resulted in the conclusion that morphine is ineffective in dogs if only examined at a dose range from 0.1 to 2.2 mg kg^{-1} orally, because of the poor oral bioavailability, but in reality morphine is highly effective in dogs if adequate plasma concentrations are achieved.[35,40,41] A similar situation has been documented in rats, in which Sprague-Dawley rats from 2 different sources had significantly different PK, which, if unaccounted for, would have led to the erroneous conclusion that morphine was an effective analgesic in rats from once source but not the other.[21] Therefore, lack

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of effectiveness for drugs in which large ranges of doses or no PK information is available has to be interpreted cautiously. The lack of effect may be caused by a true lack of efficacy or inappropriate doses being assessed.

PK-PD modeling is a modeling strategy that incorporates both the PK and PD components simultaneously. An advantage of PK-PD modeling is that changes in the PK of the drug (ie, decreased elimination from hepatic or renal failure, extended release formulations, infusions vs bolus administration, and so forth) can be incorporated into the model and predictions of the changes in drug effect can be made. These predictions are based on the changes in the PK, with the assumption that the activity of the drug itself will remain unchanged. Some veterinary studies have included PK-PD models, but these are less frequently performed than either PK or PD studies.[35]

True PD studies in zoologic companion animals are not routinely performed for numerous reasons including cost, animal availability, difficulty in working with some of the species, and humane reasons. Some exceptions are the species that are also considered laboratory animals, such as mice and rats. Therefore, extrapolations of effective concentrations between species are often made. For example, the effective plasma concentration (EC50) of morphine in male humans and female humans has been reported to be 21 and 11 ng mL^{-1}, respectively.[42] The EC50 of morphine in dogs has been reported to be 23.9 and 29.5 for multiple IV doses and IV infusion, respectively.[40] A separate study found excellent correlations between the effective plasma concentrations of morphine and 6 other opioids ($R^2 = 0.949$) between mice and humans,[43] suggesting that extrapolations between some species may be appropriate. However, the reported effective concentration of morphine in llamas was 85 ng mL^{-1}, which is substantially higher than that reported in humans, dogs, and mice, but the studies all used different analgesic models, which may also affect the extrapolation of effective drug concentrations.[5]

An example of species-specific differences in PD occurs with xylazine in dogs, cattle, and horses, despite similar PK parameters and near-dose proportional plasma concentrations.[44,45] The effective dose of xylazine is approximately 1.1 mg kg^{-1} IV in dogs, 1.1 mg kg^{-1} IV in horses, and 0.1 mg kg^{-1} IV in ruminants.[45] The plasma concentrations are approximately dose proportional between species, in which cattle have the lowest xylazine plasma concentrations and dogs have the highest plasma concentrations after administration of an effective dose, suggesting a true difference in the sensitivity of each species to the sedative effects of xylazine.[44] Similarly, there seem to be differences to the sensitivity of xylazine even within Cervidae, with fallow deer (*Dama dama*) requiring 1.1 to 2.2 mg kg^{-1} intramuscular (IM), whereas white-tailed deer (*Odocoileus virginianus*) require 0.5 to 1.1 mg kg^{-1}, and elk (*Cervus canadensis*) require a dose of 0.1 to 0.25 mg kg^{-1} IM.[45] However, the PK of xylazine are not available between the different species of Cervidae, therefore allometric differences or differences in other PK parameters, such as bioavailability, V_d, and $T_{1/2}$, cannot be ruled out in these species.

SUMMARY

PK studies are useful for determining the effect of dose on plasma concentrations, duration of detectable plasma concentrations, and reasonable routes of administration. However, species-specific differences in a drug's PK can occur, resulting in poor extrapolation between animal species. PK data can also be affected within a species by physiologic factors such as concurrent disease or organ impairment, or by drug-drug interactions. PK data are also influenced by the analytical method used to determine plasma drug concentrations, study design, and method of PK

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analysis. Therefore, different studies may not be comparable. PK studies and data do not in themselves determine effective drug dosages, but are a vital component for determining the potential for the use of a drug within an animal species.

PD studies are useful for determining the effects a drug produces over the tested dose or concentration range. Species-specific differences in the PD of a drug can occur, therefore extrapolation between species may not be accurate. If not accounted for, PK differences can lead to inaccurate PD results, so both PK and PD data are needed to make accurate dose recommendations for clinical patients and clinical studies.

Clinical experience and case reports or series may not provide accurate data because of the difficulties in working with many zoologic companion animal species. Controlled clinical studies based on PK and PD studies and data are the best source of species-specific dose recommendations. Placebo-controlled, randomized, and blinded clinical studies provide the best data for dosing recommendations, but are rarely performed in veterinary species including zoologic companion animal species. Clinicians, research scientists, industry, and government agencies need to work together in order to provide the best data, and subsequently the best drug dosage recommendations, for nondomestic species because there are very few data currently available for the large number of animal species that require veterinary care, the rarity of some of these species, and the value (financial, emotional, and ecological) of maintaining the health and well-being of these species.

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