

Monitoring Recommendations Clinical Pharmacology Laboratory

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General Comments:

Why a population “therapeutic range” may be irrelevant to your patient: The therapeutic range of anticonvulsants is a population statistic and as such, should not be used as an indication of therapeutic failure if your patient has not responded despite concentrations being in a population “therapeutic range”. Rather, therapeutic drug monitoring (TDM) should be used to establish your patient’s therapeutic range. The population therapeutic range can be helpful in that if your patient is at the maximum end of the range and has not responded, a further increase is less likely to be helpful. Also, if your patient has responded at the very low end of the therapeutic range, a dose increase is not necessarily indicated, but the patient may be more at risk of failure than a patient whose concentrations are closer to the median of the therapeutic range

For antimicrobials, the therapeutic range depends on the target organism ‘sminimum inhibitory concentration. As such, providing the target organism and its MIC is helpful for recommendations.

Why a “check up” is the least important reason for monitoring: In general, we recommend monitoring in the following situations: Start-up: to establish your patient’s therapeutic range at baseline (steady-state) in a responding patient. This is critical information in the patient who has a change in clinical signs (see “Whats’ Up”); Follow-up: to re-establish your patient’s baseline after a dose adjustment, a diet change (eg, bromide), the addition of a drug (eg, phenobarbital, cyclosporine) and thus the potential for drug interactions, changes in the manufacturer of a human generic drug (eg, cyclosporine, zonisamide) etc; Check-up: routine monitoring at pre-set intervals (eg, 3 months, 6 months, yearly) with the interval depending on the drug being tested and the risk the patient for therapeutic failure (ie, the more at risk and the more damaging the sequelae of failure, the more frequent the interval should be); and “What’s up”: a change in clinical signs indicating therapeutic failure (seizures, loss of remission of immunomodulation) or evidence of toxicity in a previously well controlled patient, or in a patient that has failed to respond as therapy is initiated and further changes in the dose are being scrutinized.

In general, do NOT use serum separator tubes. The silicon gel may bind drug resulting in a falsely lower concentration.

Peak or trough or what difference does it make?

- a. For drugs with a long half-life compared to the dosing interval, because little drug is eliminated during a dosing interval, the time the sample is collected is not important since concentrations do not change much during the dosing interval. Bromide, phenobarbital and zonisamide are examples, although phenobarbital and zonisamide half-life may get short enough that timing is important. For this reason, for anticonvulsants, we always recommend a trough (just before the next dose) sample so you can assess the lowest concentrations that occur during a dosing interval.
- b. For drugs with a short half-life compared to the dosing interval, much to almost all drug will be eliminated during the dosing interval. As such, no single sample can predict drug concentrations at any other time in the dosing interval. The question then is peak versus trough or both? Both is indicated if a half-life is to be calculated or if it is important to know how high and low concentrations occur. If only a single sample can be collected, which one depends on the drug. For **anticonvulsants** with a short half-life, because we are interested in the lowest concentration that occurs during a dosing interval and a trough is recommended. For **cyclosporine**, in humans, a peak concentration has been associated with the best prediction of overall exposure to drug during a 12 hour dosing interval and ideally, if only a single sample can be collected, it would be peak. However, in veterinary medicine, the timing of drug concentrations collected as part of scientific clinical research reports generally was not given and as such, guidance is problematic. If a 24 hr dosing interval is used for cyclosporine, the half-life may be so short that drug is not longer detectable at 24 hrs. As such, if both a peak and trough can not be collected, a peak is preferred. Consistency is important: the same time should be used if comparison across time is desired. If the patient has not responded well, at that time, both a peak and trough should be considered. For **antibiotics**, in general both a peak and trough (or second sample 2 to 3 half-lives later) is important to design a dosing regimen.
- c. **Calculating a half-life:** Determining the patient’s half-life for a drug might be important and for such drugs, both a peak and trough sample should be collected, with the trough always being before the next dose. The exception of a trough sample is if the half-life is so short that no drug is likely to be present, then the second sample should be collected two to three half-lives after the first sample (eg, aminoglycoside antimicrobials). A half-life can be calculated based on the slope of the line resulting from the two points: $\text{half-life (hr)} = 0.693/\text{kel}$ where $\text{kel} = (\ln[\text{peak/trough}])/\text{time lapsed between peak and trough}$ ($\ln = \text{natural log}$).

ANTICONVULSANTS

For information on anticonvulsants not listed below, please call. In general, for drugs with short half-lives, a peak and trough is recommended.

Bromide (potassium or sodium salt) is characterized by a half-life of approximately 21 days in dogs and 14 days in cats; variability among animals is likely to be marked. Bromide is influenced by chloride intake (more chloride intake results in more rapid elimination). As such, during a 12 or 24 hr dosing interval, little drug is eliminated between doses and drug concentrations

will accumulate until a steady-state is reached. Because of this long half-life, the sampling time for bromide can be any time during a dosing interval. Monitoring should occur at:

Baseline: "Steady-state" should occur in 3 to 5 drug half-lives, or 2.5 to 3 months after dosing at the same dosing regimen. Baseline steady-state concentrations can be determined only at that time. To proactively assess the appropriateness of your dose, (for example, patients at risk for therapeutic failure), we recommend that you check concentrations half-way to steady-state, that is, at one half-life (2 to 3 weeks). This concentration can be doubled to predict steady-state concentrations. A steady-state sample should be collected at 3 months to confirm baseline concentrations.

Loading: If the patient is loaded, in the "at risk" patient, we suggest that a sample be collected the day after the loading dose is administered to determine "what was accomplished" with loading and again 2 to 3 weeks later, to test the accuracy of the maintenance dose, and finally at 2.5 to 3 months to establish baseline. Alternatively 2 -3 months into maintenance dosing.

Rationale: While concentrations achieved once a loading dose is completed may be in the therapeutic range, the patient is NOT at steady-state and will not be until it has been dosed with the maintenance dose for 2.5 to 3 months. If the maintenance dose does not maintain what you accomplished with the loading dose, serum bromide concentrations will slowly decline or increase, depending on how incorrect the maintenance dose is. (or increase, which is often not problematic), until steady-state is reached. The patient may start seizing again at 3 to 4 weeks post load if the maintenance dose is too low, or become groggy if it is too high. A sample collected at 2 to 3 weeks into the maintenance dose (after loading is completed) will assure that the maintenance dose is "maintaining" what you achieved with loading. If you collect a 2-3 week sample without also collecting the post-load sample, we will not be able to tell if serum bromide concentrations have changed compared to the loading concentration.

Other considerations: Consider monitoring in advance of a drastic change in the preparation being given (ie, chew tablet to solution, change in pharmacy, etc). Remember that bromide will cause serum chloride concentrations to appear deranged due to interference with the chloride assay.

Levetiracetam (Keppra®) is characterized by a half-life of 2 to 4 hrs in dogs (regular release) and 4 to 7 hrs in cats; variability among animals is likely to be marked. For extended release, the half-life may be 1-2 hrs longer. As such, if the dosing interval is 8 hr most, if not all of each dose will be eliminated during a dosing interval in dogs and 50% to 75% of the dose will be eliminated in cats. For extended release, with a 12 hr dosing interval, the same may be true. "Steady-state" never truly occurs for levetiracetam in dogs (and may not in cats) because the drug does not accumulate with each dose.

The **therapeutic range** recommended in dogs or cats currently is that recommended in humans which is a **trough** concentration of that is 5 to 45 mcg/ml, depending on the resource.

When: Because minimal accumulation occurs, concentrations can be measured within days to 1 week of initiating a new dosing regimen.

Sampling times:

We suggest that dosing regimens be designed based on both a **peak and trough as drug therapy is initiated**. A single sample collected before trough cannot predict drug concentrations throughout the dosing interval. For example, assuming a peak concentration of 50 mcg/ml is achieved, and a 2 hr half-life, this means that three elimination half-lives will lapse before the next dose. Concentrations will be subtherapeutic before the next dose: 50 to 25 to 12.5 to 6.125 mcg/ml. It is important to know your patient's half-life. This will allow you as clinician to assess how short the half-life is and in turn, warn the client about the magnitude of fluctuation in drug concentrations during the dosing interval and the risk of drug concentrations dropping below the therapeutic range. Once a half-life has been calculated for your patient, an appropriate dosing interval can be designed. The peak sample should be taken at 2 to 3 hrs and the trough just before the next dose unless otherwise directed based on previous monitoring that indicated the half-life was too short for drug to be detected at 8 or 12 hrs.

If only a single sample can be collected, it should be a trough sample. Note that if you collect a sample at 12 hrs, with an 8 hr dosing interval, the trough measurement may markedly underestimate the true trough concentration. For recommendations, it is critical that the timing of sample collection be provided on the submission form.

In the case of therapeutic failure, we do not suggest that the sample only be collected at the same time during a dosing interval that the seizure occurred, but that a trough sample be collected as well such that half-life can be calculated.

Other considerations:

A slow release levetiracetam (Keppra®) product is available for use in humans; our preliminary data suggests the half-life will be longer in dogs, allowing (potentially) a 12 dosing interval. However, monitoring both a peak and trough will be important to establishing the correct dosing interval in dogs or cats when using the slow release product. Note that the compounded slow release product that is designed for humans is not likely to demonstrate slow release kinetics in the dog (or cat). Monitoring should be done to demonstrate its efficacy.

Phenobarbital will decrease levetiracetam half-life.

Levitiracetam is metabolized largely by plasma enzymes. Metabolism will continue *in situ* in plasma. As such, serum samples should be collected to avoid falsely lower than actual concentrations.

Gabapentin is similar to Keppra® in terms of half-life and impact on dosing and steady-state. Accordingly, we suggest using Keppra recommendations for sample collection. The therapeutic range in humans of 12 to 21 mcg/ml may or may not be relevant in animals. We are concerned the oral bioavailability of gabapentin is variable in dogs and accordingly recommend monitoring in animals receiving gabapentin for seizure control.

Phenobarbital is characterized by a half-life of approximately 54-72 hrs in dogs and similar in cats; variability among animals is likely to be marked. We have measured a half-life as short as 12 hrs; for such patients, steady-state does not really occur. As such, during a 12 hr dosing interval, because little drug is eliminated between doses, drug concentrations will accumulate. “Steady-state” should occur in 3 to 5 drug half-lives, or 150 to 360 hrs (approximately 2 weeks).

The **therapeutic range** recommended in animals is 15 to 40 mcg/ml, although we recommend that concentrations remain below 35 mcg/ml, and ideally, less than 25 mcg/ml to reduce the risk of hepatotoxicity.

When: Baseline steady-state concentrations can be determined at 14 days. Because Phenobarbital can induce drug metabolism, we recommend another sample at 3 months, particularly for patients whose history includes severe seizures.

Sampling times: For routine monitoring, either a peak (2 hr) or trough (just before the next dose) is reasonable, although we suggest a trough sample such that the lowest concentrations can be determined for the patient and so that concentrations can be compared across time. Also, in some patients, the half-life is short enough that trough concentrations are significantly lower than peak. Peak and trough samples are necessary only for animals in which a half-life less than 20 hrs is suspected such that a shorter dosing interval might be recommended. This might occur in a patient whose liver has been induced to more rapidly metabolize the drug. Remember that any time the dose is changed, you should monitor again at 2 to 4 weeks.

Other considerations: Phenobarbital will shorten the half-life of other drugs, including levetiracetam and zonisamide. Its half-life will be prolonged or shortened by other drugs that target drug metabolizing enzymes. Drugs known to have decrease phenobarbital in our lab have included chloramphenicol and imidazole antifungals.

Zonisamide (Zonegran®) is characterized by a half-life of approximately 20-40 hrs in dogs (unknown in the cat) variability among animals is likely to be marked. As such, during a 24 hr dosing interval, drug concentrations will decline by approximately 50%. We recommend, therefore, that a 12 hr dosing interval be implemented to avoid inappropriate fluctuation during the dosing interval. “Steady-state” should occur in 3 to 5 drug half-lives, or 60 to 100 hrs (3 to 5 days). However, we have commonly measured half-lives longer than 150 hrs, suggesting that drug metabolism in dogs might “saturate” (the drug is acetylated, which is an enzyme in which the dog is deficient). As such zonisamide recommendations are very similar to phenobarbital in terms of when and what.

The **therapeutic range** recommended in animals is currently that recommended in humans, that is, 10 to 40 mcg/ml. Assuming that this therapeutic range is appropriate for dogs or cats, we suggest that doses be designed to achieve a “low” concentration of 15 to 20 mcg/ml as therapy is begun. We have measured (often) concentrations well above 60 mcg/ml or higher; dogs appear to tolerate this concentration well, although safety has not been confirmed.

When: Baseline steady-state concentrations can be determined at 7 to 14 days. Steady-state will thus take longer than 10 to 14 days in those patients. The half-life is likely to be shorter in dogs simultaneously receiving Phenobarbital; up to a 30% decrease in half-life or drug concentrations may occur. However, this is not consistent.

Sampling times: For routine monitoring, either a peak (2 hr) or trough (just before the next dose) is reasonable, although we suggest a trough sample such that the lowest concentrations can be determined for the patient and so that concentrations can be compared across time. Peak and trough samples are necessary only for animals in which a half-life less than 20 hrs is suspected such that a shorter dosing interval might be recommended. However, for some patients, a peak and trough might be prudent to detect longer half-lives that might lead to marked drug accumulation.

Other considerations: We are concerned that the oral bioavailability of zonisamide varies among the generic human products, meaning that one product may be absorbed differently than another product in the same dog. Accordingly, clients may want to ask the pharmacist to let them know when the pharmacy has changed the manufacturer of the generic product; monitoring might be implemented after the change has been made in the patient.

Thyroid gland suppression may be more likely at this concentration. Baseline thyroid function testing is recommended as you begin zonisamide and repeated as concentrations reach the maximum.

Phenobarbital will substantively decrease the half-life of zonisamide. If phenobarbital is discontinued in an animal, note that zonisamide concentrations will likely increase.

Cyclosporine Monitoring for cyclosporine is complicated; further it is limited by the lack of studies which correlate concentration and immune suppression in animals. As such, avoiding toxicity and determining concentrations should be the initial goal, with establishing the patient's therapeutic range being important in the absence of no consensus regarding therapeutic ranges in animals.

Testing: At least 50% of cyclosporine in blood is in the red blood cell. As such, **whole blood** should be submitted.

Cyclosporine is metabolized to over 14 different compounds with variable activity. HPLC provides the most accurate method of measuring the parent compound but does not measure any active metabolite. Antibody based assays vary in their therapeutic ranges because the antibodies vary in the amount of metabolite detected. Presumably monoclonal antibody based assays interfere with non parent compounds (that is, metabolites) the least but will nonetheless consistently measure cyclosporine higher than HPLC. The assay used by this laboratory is approved by the FDA for monitoring of cyclosporine in humans.

Therapeutic Range: Target concentrations vary with the condition. For immune mediated disease or host versus graft rejection, dosing should occur at 12 hr intervals. For our laboratory (using a monoclonal antibody based assay), a peak concentration of 800 to 1400 ng/ml and a trough concentration of 400 to 600 ng/ml (monoclonal based assay) is recommended. The target peak concentration is easier to reach in a patient: Because of the short half-life of cyclosporine in most patients (e.g., 5 hr average), a trough target of 400 to 600 ng/ml will require peak concentrations of 1600 to 2400 ng/ml. In animals, there is not data supporting better response if target trough or peak concentrations are met but the more aggressive approach would be to target the trough concentrations. For renal transplantation, trough concentrations of 750 ng/ml are suggested for the first month (peak concentration of 2600 to 3000 ng/ml or more may be necessary, depending on half-life of cyclosporine in the patient) and 350 to 400 ng/ml, thereafter. For chronic allergic inflammatory disorders, lower trough concentrations are recommended: 250 ng/ml trough concentrations for chronic inflammatory bowel disorders, and for perianal fistulae, 12 hour trough concentrations at 100 to 600 ng/ml (the higher for induction, the lower for maintenance).

When: Cyclosporine generally does not accumulate and as such, monitoring can occur within days to 1 week of a new dosing regimen. The exception occurs if the patient is receiving another drug that prolongs the half-life of cyclosporine.

Sampling times: Our laboratory offers the following recommendations for monitoring of patients receiving cyclosporine for induction of immune-mediated disease therapy (and as such, the patient is being treated every 12 hours). Assuming a "normal" half-life (for example, the patient is not receiving drugs that inhibit drug metabolizing enzymes), a **2 hr peak and 11 to 12 hr, (just before the next dose) trough sample is recommended within 3 to 5 days of initiating therapy;** the more life threatening the target disease, the more important a peak and trough sample may be.

For less serious situations, or as treatment shifts from induction to maintenance, **a single 2 hr peak** sample may be sufficient for establishing and maintaining a target.

If therapy is initiated such that CsA disposition might change (whether intentional, such as the addition of ketoconazole, or inadvertent, such as co-treatment with diltiazem or azithromycin or others), a peak and trough sample prior to and 1 week after therapy is initiated is suggested such that a half-life can be calculated and the time to steady-state concentrations can be determined.

For chronic allergic – inflammatory diseases, recommendations vary from those for immune mediated diseases. Recommendations (again from human medicine, with the exception of perianal fistulae) are largely based on trough concentrations. For atopy in particular, no recommendations are available, and the role of monitoring is to establish a baseline therapeutic range in the patient and to assure that concentrations are sufficiently high that response can be expected. Concentrations are not likely to be detectable at trough when dosing at 48 hr intervals. Thus, a single peak concentration may be reasonable if a monitoring program is to be implemented in such a patient.

Other considerations:

Whole blood should be submitted.

Samples do not need to be sent on ice. Cyclosporine has been demonstrated to be stable for 7 days at ambient temperature.

Cyclosporine is involved in a large number of drug interactions both at p glycoprotein (an efflux protein that will impact drug absorption and distribution) and cytochrome P450. It is metabolized by and is subject to inhibition / induction of activity of CYP 3A4, which is a major enzyme responsible for the metabolism of many other drugs. We have detected interactions with imidazole antifungals and azithromycin.

The frequency of monitoring should vary with the disease being treated and patient response. We recommend monitoring is recommended weekly to biweekly in critical patients, then monthly for the first several months of therapy or until concentrations are stable. For long term maintenance, the frequency of sampling might range from 3 to 6 months.

In situations in which generic preparations are being used, because oral bioavailability of different generic products may differ in the same dog initiated, proactive monitoring is recommended if one product is shifted to another. In such cases, a single 2 hr peak concentration before and 3 to 5 days after the switch is recommended.

Leflunomide: Leflunomide is a prodrug. Its efficacy is based on conversion to an active metabolite, teriflunomide (A77 1726). The half-life of this metabolite in humans is greater than two weeks but in dogs, it approximates 24 hrs.

Therapeutic Range: In cats, Mehl et al (JVPT 2011) demonstrated ex vivo that a mean terifluonamide concentration of 16 mcg/ml inhibited 50% lymphocyte activity. Further, van Roon et al (Ann Rheu Dis 2005) demonstrated that human patients responded poorly to concentrations of the active metabolite if less than 16 mcg/ml. No studies have been done in dogs. Accordingly, a concentration greater than 16 mcg/ml is a reasonable starting for therapeutic response. Based on our assessment of responders versus no responders, all animals that responded did so at concentrations between 5 and 45 mcg/ml.

When: Drug will accumulate 50% with a 24 hr dosing interval. Monitoring can occur within 5 to 7 days of a new dosing regimen.

Sampling times: We currently recommend a single trough sample. The half-life is short enough in some animals that a peak and trough may be indicated simply to detect the short half-life in problem patients. The 24 hr half-life does suggest that the timing of sample collection be consistent if concentrations are going to be compared across time in a patient.

Other considerations: Leflunomide appears to be a substrate for both p glycoprotein and cytochrome P450. As such, drug interactions should be considered. We have measured concentrations higher than 140 mcg/ml in patients on drugs which inhibit drug metabolizing enzymes or on drugs which also are substrates for p glycoprotein.

Mycophenolate: Mycophenolate is a prodrug; the active drug is mycophenolic acid (MPA). However, its acetyl glucuromide metabolite also is active. After oral administration in people, MMF undergoes rapid absorption in the gastrointestinal tract and conversion by liver, peripheral tissue, and plasma esterases into MPA. Plasma proteins bind 97% of MPA. MPA is metabolized by the liver, with the major metabolite being to an inactive form 7-O-MPA glucuronide (MPAG). However, , and the active the acyl glucuronide (AcMPAG) metabolite is active. In humans, the AcMAG metabolite is equal to the parent in activity but only represented approximately 10% percent of the maximum drug concentration or total area under the curve of bioactivity. The half-life of MPA is short in dogs and unknown in cats.

Testing: In human patients receiving mycophenolate, monitoring of MPA based on the emit immunoassay used by this laboratory consistently measured up to 30% higher maximum drug concentrations but similar area under the curve when compared to high performance liquid chromatography (Weber 2002). However, concentrations measured by Emit have been demonstrated to correlate to response as well as an HPLC assay (Weber 2002). The assay available in this laboratory is an FDA approved assay for detection of MPA in humans.

Sampling: This assay is not a standard test in our laboratory because of the cost of reagents. It can be added if sufficient interest exist. Please call for pricing and sampling.

ANTIMICROBIALS

Theraepeutic range: Because target antibacterial concentrations generally are based on organism minimum inhibitory concentrations, the therapeutic range will vary. For concentration dependent antibiotics such as the fluoroquinolones and aminoglycosides, peak concentrations should exceed 10X the infecting microbe MIC. Trough concentrations are unimportant from an efficacy standpoint. From a toxicity standpoint, trough concentrations of the aminoglycosides should be < 2 mcg/ml. For time dependent drugs (eg, vancomycin), drug concentrations should be above the infecting microbe MIC for most if not all of the dosing interval. Half-life and thus both peak and trough are important. For antifungals, drug concentrations should be above the MIC for most of the dosing interval. Because their half-life is generally long, a sample at any time, but preferably trough, is recommended. Please call for further directions.