Journal of
Veterinary Clinical Practice and Petcare

Research Open Access

Serum Phenobarbital Concentrations and Timing of Blood Collection in Epileptic Dogs on Chronic Treatment

M. Masucci[1,*], E. Scarrozza[2], M.F. Persichetti[3]

[1]Dipartimento di Scienze Veterinarie, Università degli Studi di Messina. Polo Universitario dell'Annunziata- 98168 Messina, Italy
[2]Veterinary practitioner, Via Trieste, 34 - 34074 Monfalcone (GO), Italy
[3]Istituto Zooprofilattico Sperimentale della Sicilia "A. Mirri", Via G. Marinuzzi, 3 - 90129 Palermo, Italy

***Corresponding author:** M. Masucci, Dipartimento di Scienze Veterinarie, Università degli Studi di Messina. Polo Universitario dell'Annunziata- 98168 Messina, Italy; Email: marisa.masucci@unime.it

Received Date: October 20, 2016 **Accepted Date:** November 12, 2016 **Published Date:** November 15, 2016

Citation: M. Masucci (2016) Serum Phenobarbital Concentrations and Timing of Blood Collection in Epileptic Dogs on Chronic Treatment. J Vet Clin Pract PetCare 1: 1-6.

Abstract

Aims: To assess whether there are significant changes in serum phenobarbital concentrations (SPC) within a dosing interval in epileptic dogs on chronic treatment and whether higher doses, prolonged duration of phenobarbital treatment, body weight, age, gender and concurrent treatment with potassium bromide are associated with significant changes between SPC at two (T2) and twelve (T12) hours after drug administration.

Methods : Medical records of epileptic dogs on phenobarbital treatment were retrospectively analyzed. SPC were measured at T2 and T12 in 42 pairs of blood samples of dogs treated with a twice daily stable oral dose of phenobarbital for a minimum of three weeks.

Results: SPC were significantly higher at T2 compared to T12 samples. There was no significant correlation between drug dosage and SPC at T2 nor at T12. The difference between T2 and T12 SPC were positively correlated with the drug dosage but not with duration of treatment, weight and age of the dogs. Significant differences in variations of SPC were not detected between males and female and between dogs receiving phenobarbital alone and those under concomitant treatment with potassium bromide.

Conclusions: Timing of blood collection is important to measure SPC in dogs under long-term treatment with phenobarbital and peak and trough values should be evaluated, mainly in animals on higher doses of the drug. Further studies would be useful to detect the optimum interval between drug administration and peak sampling in dogs which have reached the steady state with therapeutic doses as happens in clinical practice.

Introduction

Phenobarbital is one of the first-choice anticonvulsant drugs in dogs because of its efficacy, convenient dosing regimen, reasonable time required to achieve steady-state concentrations, low cost and relative safety [1-10].

Monitoring of serum phenobarbital concentrations (SPC) is important in the management of epileptic dogs because the dosage of phenobarbital administered is often not predictive of serum concentrations [1,6,11]The pharmacokinetics of phenobarbital may be difficult to predict in individual dogs because of the wide variation in absorption, metabolism and excretion [11,12]. Differences in diet, body composition (obesity or weight loss) and pH of urine can also substantially alter the pharmacokinetics of fenobarbital [13,14]. Moreover, phenobarbital induces production of enzymes responsible for its own hepatic metabolism (cytochrome P450) with time and with higher doses, and therefore increased clearance and reduced half-life may occur [2,3,6,7,12,15,16,17,18]. Yet the pharmacokinetics of phenobarbital may be affected by concurrent administration of drugs that inhibit hepatic microsomal enzymes (such as cimetidine, omeprazole, lansoprazole, chloramphenicol, trimethoprim, fluorochinolones, tetracyclines, ketoconazole, fluconazole, itraconazole, luoxetine, felbamate and topiramate) and may increase serum phenobarbital concentration [9]. Phenobarbital pharmoacokinetics may therefore change over time even in a single individual.

Previous studies have shown the half-life of phenobarbital ranges from 29.3 hours (SD 4.6) (dogs receiving phenobarbital at 5 mg/Kg per os once daily for 22 days) to 65 hours (SD 61.8) (dogs treated with a stable dose of 0.5-5.5 mg/Kg every 12 hours for at least three weeks) [19,20] and peak SPC occurs at different times after oral administration: within 2-4 hours for dogs receiving 5 mg/Kg every 24 hours for 22 days [19].

2.8 hours (SD 1.48) at 11 mg/Kg once daily for 90 days [15]. 3.2 hours (SD 1) at dosage rate of 2 mg/Kg every 6 hours for five consecutive days [12]; 3.75 (SD 0.79) hours after a single dose of 5.8-11 mg/Kg [21]. 4.6 hours (SD 0.89) at 5.5 mg/Kg once a day for 90 days [15]; not before 4-8 hours after a single dose of 10 mg/Kg [22].

The goal of serum phenobarbital monitoring is to adjust the drug dosage using SPC – measured 1- 3 weeks after starting therapy or after a change in dose, to achieve a steady-state plasma level - as a marker to optimize efficacy and avoid potential toxicity [1,2,4,7,9,11,12,16,20,22]. The stady-state level refers to a stable plasma drug concentration at a constant dose rate and approximately five drug half-lives are required to achieve it [1]. In general, trough serum concentrations of an antiepileptic drug should be measured when there is poor seizure control to determine if an inadequate dose is being given [2]. Conversely, peak concentrations should be measured when there is a concern of drug toxicity [2]. If the half-life (t ½) of an anticonvulsant is substantially shorter than the dosing interval, plasma drug concentrations markedly fluctuate during the dosing interval [17]. Conversely, if t ½ is considerably longer than the dosing interval, fluctuation in plasma drug concentrations is minimized and only a single sample needs to be collected for monitoring, since peak and trough samples do not show significant differences during a single dosing interval [17].

The long elimination half-life of phenobarbital would make it relatively easy to maintain constant SPC throughout the day and it would be expected that minimal fluctuations will occur when the drug is administered every 12 hours. Thus, careful timing of blood collection for measurement of trough and peak SPC might not be required [20]. However most studies have determined the half-life of phenobarbital and peak SPC using unusual doses or dosing intervals, and after relatively short administration times, when hepatic enzyme induction would probably have been limited [12,15,19,20,21,22]. Moreover, in some studies phenobarbital levels were determined before steady state was reached [12,21,22]. Therefore these results may not be representative of what is happening in epileptic dogs on long-term treatment. In some dogs that are receiving high doses or with prolonged administration, a faster elimination of the drug could exaggerate the differences between trough and peak concentrations over the 12-hour period and could make the timing of blood collection more critical [20]. Furthermore the state of the art of pharmacokinetics of phenobarbital, in dogs that have reached the steady state assuming dosage used in clinical practice, does not at present permit determination of a definite time for peak values.

The aims of this study were to compare SPC at two different times - two (T2) and twelve (T12) hours - after drug administration in epileptic dogs on chronic treatment with phenobarbital to determine whether statistically and clinically significant changes in SPC were found during the dosing interval. This study also assessed whether higher doses, prolonged duration of phenobarbital treatment, body weight, age, gender or concurrent treatment with potassium bromide were associated with significant changes between T2 and T12 SPC.

Abbreviations

SPC: Serum Phenobarbital Concentrations; t ½: Half-life; T2: Two hours after drug administration; T12: Twelve hours after drug administration

Materials and methods

Medical records of epileptic dogs on chronic phenobarbital treatment – from December 1999 to January 2014 - were retrospectively analyzed. All dogs were treated with a stable dose of phenobarbital (Luminale®; Bracco S.p.a. Divisione Farmaceutica, Milano, Italia) per os every 12 hours for a minimum duration of three weeks (to achieve steady state) and did not receive other drugs with the exception of potassium bromide in some cases. Bromide is excreted unchanged by the kidney and is not metabolized by the liver so it should not affect the pharmacokinetics of phenobarbital.

Two fasting serum samples were obtained for each dog, respectively two (T2) and twelve (T12) hours after oral administration of phenobarbital. SPC at T12 is expected to be the lowest or "trough" value. As at present a defined time for peak values has not been established in dogs that have reached the steady state assuming dosage used in clinical practice, we chose a time (T2), shortly after drug administration, when the SPC should be higher.

Serum phenobarbital concentration was determined by chemiluminescence immunoassay from IDEXX Vet Med Lab, Ludwigsburg - Germany [5,23] and was assigned to one of three categories: respectively below, within and above the reference range provided by the laboratory (10-40 μg/mL). For each dog the following information was recorded: breed, gender, age, body weight, administration of other drugs, daily dosage of phenobarbital, duration of phenobarbital administration, duration of administration of the present phenobarbital dose, T2 and T12 SPC.

Statistical analyses were performed using the GraphPad InStat v3.05 for Windows 95 statistic program (GraphPad Software Inc., San Diego California, USA, 2000). All the data were subjected to the normality test. A paired t test was used to determine whether there were statistical differences between T2 and T12 SPC. The correlation between SPC and drug dosage and between variations in SPC (T2-T12) and: drug dosage, duration of phenobarbital administration, duration of treatment at present dose, body weight and age was determined by a linear regression test. An unpaired t test was performed to determine whether there were significant differences in variations in SPC between males and females or between dogs that received phenobarbital alone or in association with potassium bromide. P values ≤ 0.05 were considered significant.

Results

Twenty-eight dogs of 15 different breeds were evaluated: 17 males and 11 females, aged between 18 and 115 months (mean 56.5, SD 26.8) and weighing between 4.1 and 52 Kg (mean 23, SD 14). In seven dogs the SPC were determined several times (two-seven times per dog) as it was necessary to adjust the dose over time because of poor seizure control or supra-therapeutic values of SPC. Therefore the overall number of sample pairs evaluated was 42.

Nine of the 28 dogs receiving treatment with phenobarbital were also receiving potassium bromide. No animal was being treated with other drugs.

Mean duration of phenobarbital treatment was 616 days (SD 430, min 56, max 1650). The mean onset time of the current dose of phenobarbital was 167 days (SD 201, min 21, max 1030). Mean dosage of phenobarbital was 7.4 mg/Kg/day (SD 3.1, min 3.6, max 17.2). Mean value of SPC at T2 was 29 µg/mL (SD 9.8, min 14, max 60.3) and that evaluated at T12 was 25 µg/mL (SD 8.8, min 12, max 51.5).

The mean difference between values of SPC evaluated at T2 and at T12 was 4 µg/mL (SD 2.8, min 0.1, max 12). The mean percent variation in phenobarbital levels at T2 and at T12 was 13.6% (SD 7.5, min 0.3, max 28). T2 and T12 SPC were in the same category as regards the reference range in 41/42 (98%) pairs of samples: 38/42 (90%) had SPC within the reference range and 3/42 (7%) had SPC above the reference range. One dog (2%) had SPC within the reference range at T12 and above the reference range at T2. This animal had been treated with phenobarbital for 180 days and was receiving a daily dosage of phenobarbital of 9.2 mg/kg for 28 days.

SPC were statistically significantly higher at T2 compared to T12 ($p<0.001$) (Figure 1).

There was no significant correlation between drug dosage and SPC both at T2 and at T12. Conversely, the difference between T2 and T12 SPC was positively correlated with the drug dosage ($p=0.02$, $r=0.37$) (Figure 2) but not with the duration of phenobarbital treatment, the duration of treatment at the present dose, the weight and the age of the dogs. Significant differences in SPC variations were not detected between males and females.

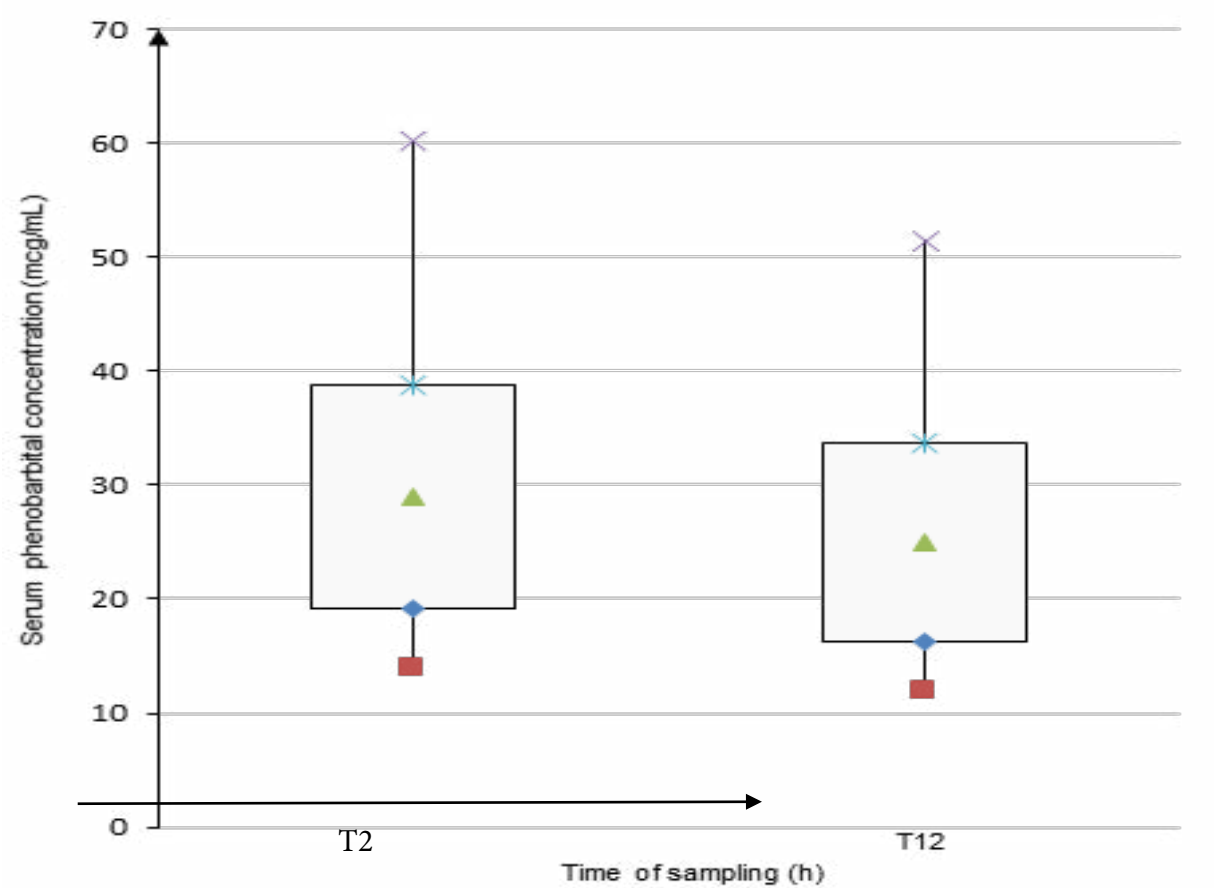

Figure 1: Mean, standard deviation, minimum and maximum of phenobarbital serum concentrations at T2 and T12. ■ minimum ◆ mean – SD △ mean *mean+SD × maximum

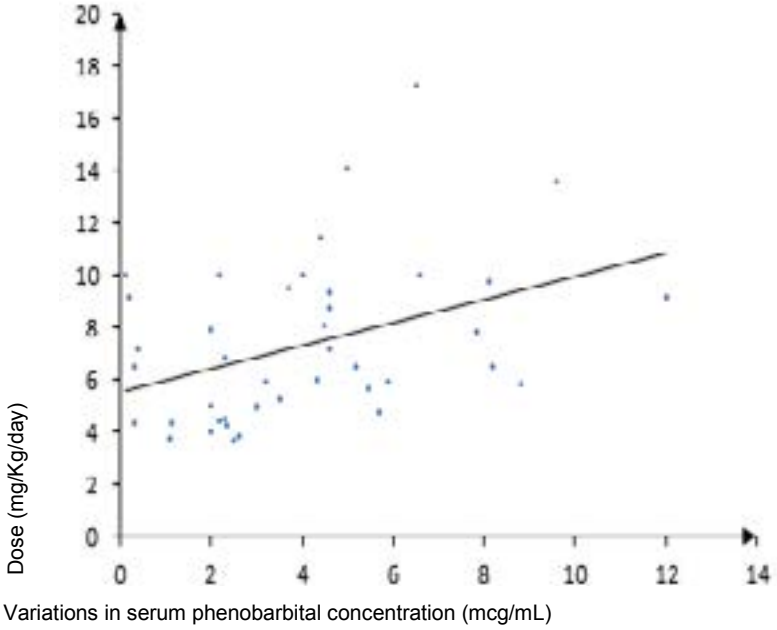

Figure 2**:** Linear regression between difference in phenobarbital concentrations at T2 and T12 and drug dosage in each dog.

Dosage of phenobarbital was significantly lower (p=0.006) in dogs treated with phenobarbital alone (mean: 6.6 mg/Kg/day, SD 2.5) compared to those also receiving potassium bromide (mean 9.6 mg/Kg/day, SD 3.8). However, there were no significant differences between the variations of SPC evaluated at T2 and at T12 of the dogs receiving phenobarbital alone and the animals treated with phenobarbital and potassium bromide.

Discussion

This study provides some results which are significant for their practical implications: SPC two hours after drug administration is significantly higher than SPC at trough; furthermore, the higher the phenobarbital dose, the more likely it is that there will be marked differences in serum concentrations at T2 and T12.

Until 2000, monitoring of trough (12 hours after oral administration) and peak SPC was recommended for epileptic dogs treated with phenobarbital, to evaluate whether drug concentrations were within the reference range [1]. Two later studies suggested that timing of blood collection is not crucial to measure SPC in most dogs under long-term treatment with phenobarbital because changes in drug concentrations during the daily dosing interval are not therapeutically relevant, at least in animals treated with low or medium doses: < 8.6 +2.3 mg/Kg/day [20] or <10 mg/Kg/day [5].

In our study, SPC in samples collected at T2 were significantly higher than those obtained at T12. Monteiro et al. (2009) compared two large populations of dogs: the first was bled during the trough period (10-12 hours after drug administration) and the other was sampled outside this time frame.

The authors did not find a significant difference in SPC of the trough and non-trough samples in animals treated with lower doses, but in dogs on the highest doses of phenobarbital (>10 mg/Kg/day) the concentration in the non-trough samples was significantly higher than in the trough samples. Levitski and Trepanier (2000) found minimal variation in SPC during the daily dosing interval, estimating peak concentrations in blood collected at three and six hours after drug administration. In their study, trough, 3-hours and 6-hours SPC were in different therapeutic categories, arbitrarily fixed by the authors, in 9% of dogs only. These dogs showed a change in SPC > 30% through the day; they were receiving a higher mean daily dosage and had a relatively shorter estimated elimination half-life (14-22.5 hours) than the other animals. In our study, T2 and T12 SPC were in different reference range categories in only one dog, which was receiving a higher mean daily dosage (9.2 mg/Kg/day). Although percent variations in phenobarbital levels were lower than 30% in all dogs, the difference between T2 and T12 values ranged from 0.1 mg/mL to 12 mg/mL. We cannot therefore rule out that in clinical practice the peak or trough values may rise or fall outside the reference range in a higher percentage of dogs.

Our results confirm that the difference between SPC evaluated at T2 and at T12 is positively correlated with the drug dosage but not with the duration of phenobarbital treatment [2,3,7,12,15,16,17,18].

This study confirms the absence of correlation between phenobarbital dosage and serum concentrations, as shown by some authors [11,1,6] but not observed by others [5,20].

In our study dosage of phenobarbital was significantly higher in dogs treated with phenobarbital and potassium bromide, likely because KBr was added when phenobarbital alone did not adequately control seizures at optimal dosage.

As accuracy in measuring SPC is necessary for assessing the therapeutic effect of the drug and for making accurate adjustments to the dose, on the basis of our data a reasonable recommendation for clinicians is to measure SPC at different times during the dosing interval, especially when high doses are administered. Epileptic dogs that have a rapid phenobarbital elimination half-life would be expected to have wider fluctuations in SPC throughout the day: sub-therapeutic SPC at trough could benefit from an every 8 hour, rather than an every 12 hour, dosing regimen; toxic values at peak require a dose reduction. Elimination half-life of phenobarbital was not measured in this study and therefore we do not know how many dogs had a short t ½, which could potentially benefit from an every 8 hour rather than an every 12 hour dosing regimen.

SPC could be determined in all subjects at trough (12 hours after drug administration), to obtain a greater reproducibility of results, and might be the only measurement for most dogs. Measuring peak SPC would be important in dogs that are receiving higher phenobarbital doses because large differences between peak and trough levels are more likely in these patients and they are also more likely to reach supra-therapeutic doses for part of the dosing interval. As it is not known exactly when peak levels occur, blood could be taken twice shortly after dosing in the hope of detecting supratherapeutic levels.

Conclusion

In conclusion, peak and trough values should be evaluated in dogs under phenobarbital treatment, especially in animals on higher doses of the drug. The state of the art of pharmacokinetics of phenobarbital does not permit determination of a defined time for peak values, therefore it may be useful to check three concentrations over a dosing interval in dogs on higher doses. The first is the trough value, but at present the timing of two subsequent samples is arbitrary and further studies should be carried out to establish the best time for the peak sampling in dogs which have reached the steady state with therapeutic doses as it happens in clinical practice.

Acknowledgments

We thank Prof. Maria Grazia Pennisi for her helpful comments and Prof. Caroline Keir for the language revision.

The authors declare no conflict of interest.

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