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The effect of imepitoin, a recently developed antiepileptic drug, on thyroid parameters and fat metabolism in healthy Beagle dogs

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ABSTRACT

Since early 2013, imepitoin has been used in most European countries for the management of recurrent single generalised epileptic seizures in dogs with idiopathic epilepsy. It has been reported that imepitoin is as effective as phenobarbital (PB) in controlling seizures in dogs with newly diagnosed idiopathic epilepsy and it has a clinically superior safety profile. As the use of imepitoin gains popularity, its effect on serum thyroid parameters warrants further investigation since long-term PB administration influences thyroid parameters in dogs, which could lead to misinterpretation of laboratory results and incorrect diagnosis of thyroidal illness. A prospective study was conducted to compare the effect of orally administered PB and imepitoin on serum concentrations of total thyroxine (TT4), triiodothyronine, free thyroxine, thyroglobulin autoantibodies, thyroid-stimulating hormone, cholesterol and triglycerides in healthy Beagle dogs. These parameters were determined prior to and at 6, 12 and 18 weeks after antiepileptic drug administration. The starting dose of PB (5 mg/kg PO twice daily; range, 4.4–6.0 mg/kg) was monitored and adjusted to obtain optimal therapeutic serum concentrations (30–35 μg/mL). Imepitoin was administered at 30 mg/kg PO twice daily (range, 29.2–35.7 mg/kg).

Imepitoin administration did not affect any of the thyroid parameters over an 18-week period. In contrast, serum TT4 concentrations decreased significantly over time in dogs receiving PB ($P < 0.05$). Serum cholesterol concentrations increased significantly over time in dogs in the imepitoin group, but not to the same extent as commonly seen in dogs with primary hypothyroidism.

Introduction

Evaluation of thyroid function in dogs is not always straightforward because the clinical signs of hypothyroidism are often quite vague and no thyroid function test is 100% reliable. Numerous factors, including comorbidities or the administration of certain drugs such as phenobarbital (PB) may have considerable effects on the thyroid gland and thyroid hormone metabolism (Gaskill et al., 1999; Gaskill and Cribb, 2000; Gieger et al., 2000; Müller et al., 2000; Daminet and Ferguson, 2003).

PB is commonly the medication of choice in epileptic dogs and cats due to its pharmacokinetic profile, relative safety, affordability, efficacy and strong evidence base from veterinary studies compared to other antiepileptic drugs (AEDs). PB increases seizure threshold and decreases the spread of discharge to surrounding neurons. While the exact mechanism of action is not completely understood, the primary mechanism involves enhancement of postsynaptic neuronal inhibition by increasing responsiveness to the inhibitory neurotransmitter gamma-aminobutyric acid (GABA) in the CNS (Boothe, 2012).

In dogs, PB is a potent inducer of hepatic microsomal cytochrome P450 enzymes (Hojo et al., 2002). Thus, chronic administration in dogs can lead to increased clearance of hepatically metabolised drugs (including PB), as well as endogenous compounds such as thyroid hormones (Gieger et al., 2000; Müller et al., 2000; Hojo et al., 2002). Chronic PB administration in dogs can cause a decrease in serum total thyroxine (TT4) and free thyroxine (FT4) concentrations and an increase in serum thyroid stimulating hormone (TSH) concentrations (Gaskill et al., 1999; Gaskill and Cribb, 2000; Gieger et al., 2000; Müller et al., 2000; Daminet and Ferguson, 2003).

The effect of long-term PB administration on serum thyroid hormone concentrations can be confusing and could lead to incorrect diagnosis of primary hypothyroidism, resulting in unnecessary treatment or delays in the diagnosis of primary hypothyroidism. A complicating factor is the occurrence of hypercholesterolaemia and hypertriglyceridaemia in both hypothyroid dogs and dogs on long-term PB treatment (Franzoni et al., 1992; Eiris et al., 1995; Gieger

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et al., 2000; Kluger et al., 2008). Moreover, erroneous supplementation of thyroid hormones can influence the metabolism and serum concentration of PB, thus jeopardising seizure control (Daminet and Ferguson, 2003). Since hypothyroidism is reported to be a possible cause of seizures in dogs (Brauer et al., 2009) and humans (DeLong, 1996), early and correct diagnosis and appropriate treatment is imperative.

Imepitoin (formerly named AWD131-138 or ELB138; 1-[4-chlorophenyl]-4-morpholinoimidazolin-2-one) is a novel AED licensed in most European countries since 2013 for the management of canine idiopathic epilepsy (Rundfeldt and Löscher, 2014a). The compound acts as a low affinity partial agonist at the benzodiazepine site of the $GABA_A$ receptor, decreasing the risks associated with long-term treatment with full agonists such as PB (Löscher et al., 2013). As the use of imepitoin gains popularity, its effect on serum thyroid parameters warrants further investigation.

The aim of our study was to compare the effects that imepitoin and PB exert on the thyroid axis and on fat metabolism in healthy Beagle dogs by determining serum TT4, FT4, triiodothyronine (TT3), thyroglobulin autoantibodies (TgAA), TSH, cholesterol and triglyceride concentrations over 18 weeks. It was hypothesised that PB but not imepitoin would influence any of these parameters.

Materials and methods

Dogs

Eleven male and nine female neutered Beagle dogs weighing 7–18 kg (median 12.7 kg) and aged between 5 and 7 years were enrolled in the study. All dogs were assessed as healthy based on physical and neurological examination, complete blood cell count and serum biochemistry analysis including urea, creatinine, total protein, albumin, resting ammonia, bilirubin, aspartate transaminase, alanine transaminase, alkaline phosphatase (ALP), bile acids, cholesterol, triglycerides. Pre-treatment evaluation of thyroid gland parameters was performed for all dogs to exclude any pre-existing thyroid disease. The dogs were kept in the same conditions throughout the study and were fed twice daily with a standard maintenance diet (Hill's Prescription Diet, Canine I/D). Dogs were evaluated daily for activity level and possible side-effects (such as sedation and ataxia) and physical examinations and weight measurements were performed on blood sampling days i.e. at the start of the study and after 6, 12 and 18 weeks of AED administration. The study was approved by the Ethical Committee of the Faculty of Veterinary Medicine, Ghent University, Belgium (EC 2014/22).

Study design

In a longitudinal study design, 10 dogs were assigned to treatment groups (PB or imepitoin) using a simple randomisation technique (flipping a coin) for 18 weeks. Imepitoin (Pexion, Boehringer Ingelheim, Vetmedica) was administered at 30 mg/kg PO twice daily (range, 29.17–35.71 mg/kg; Rundfeldt et al., 2014b). The oral starting dose of PB (Phenoleptil, Kela) was 5 mg/kg PO twice daily (range, 4.4–6 mg/kg). All medications were administered at 10.00 am and 10.00 pm, except on blood sampling days, where medications were administered at 11.00 am, after blood sampling. The AEDs were administered with one tablespoon of highly digestible canned food (Hill's Prescription Diet, Canine I/D).

On blood sampling days, complete blood cell count, serum biochemistry, serum and plasma trough concentrations of PB and imepitoin, respectively, serum cholesterol and triglyceride concentrations, and serum thyroid parameters (TT4, TT3, FT4, TSH and TgAA) were determined. These values were measured before the start of AED administration and at 6, 12, and 18 weeks after administration. Blood samples were collected by jugular venepuncture. Serum samples were allowed to clot and, after centrifugation for 5 min, serum was separated immediately and frozen at –80 °C in plastic tubes until assayed. Plasma samples for imepitoin determination were centrifuged at 4 °C for 10 min within 60 min after collection and frozen at –80 °C in plastic tubes until assayed.

The serum trough concentration of PB was initially checked 2 weeks after the start of the drug administration and adjusted if necessary. The serum trough concentration of PB was measured 2 weeks after every dose adjustment.

Serum thyroid parameters

Pre-treatment evaluation of thyroid parameters was performed using a validated radioimmunoassay (RIA) for TT4 serum concentrations and a validated electrochemiluminescence immunoassay for TSH serum concentrations (laboratory reference ranges: TT4 6–44 nmol/L; TSH < 0.5 ng/mL). Analyses of serum samples collected during the study were performed at the Endocrine Laboratory of the Diagnostic Center for Population and Animal Health, Michigan State University. Determination of TSH serum concentrations were performed using a commercially available and validated immunoassay according to the manufacturer's guidelines (Coat-A-Count canine TSH IRMA, Diagnostic Products; Dixon et al., 1999). TT3 serum concentrations were measured using a validated in-house charcoal separation assay (Refsal et al., 1984; Panciera et al., 1990). Determination of FT4 serum concentrations after equilibrium dialysis was performed using a validated commercial assay kit according to the manufacturer's guidelines (Antech Diagnostics; Daminet et al., 1999). Serum TgAA concentrations were determined using a commercially available and validated ELISA according to the manufacturer's guidelines (Oxford Biomedicals; Nachreiner et al., 1998). The TT4 serum concentrations were measured via commercially available and validated radioimmunoassay (Total T4 125I RIA kit, MP Biomedicals). To determine inter- and intra-assay coefficients of variation (CV), four canine serum pools were prepared. Inter-assay CV in 10 replicates of these pools were 13.9%, 8.5%, 9.5%, and 8.1% respectively. Laboratory reference ranges were as follows: TT4 11–60 nmol/L; TT3 0.8–2.1 nmol/L; FT4 6–42 pmol/L; TSH 0–30 mU/L; TgAA 0–35 % POS.

Plasma imepitoin concentrations

Plasma concentrations of imepitoin were determined using a HPLC system coupled with tandem mass spectrometer according to the manufacturer's guidelines (LC-MS/MS, Sciex). Analyses were performed at Nuvisan Pharma Services, Neu-Ulm.

Serum PB concentrations

Serum concentrations of PB were measured with an in vitro chemiluminescent micro-particle immunoassay according to the manufacturer's guidelines (The ARCHITECT *i*Phenobarbital, Abbot Laboratories). Analyses were performed at Algemeen Medisch Laboratorium.

Serum cholesterol and triglyceride concentrations

Serum cholesterol and triglyceride concentrations were measured using a validated dry-slide technology chemistry analyser according to the manufacturer's guidelines (IDEXX Catalyst Dx). Analyses were performed at the Department of Small Animal Medicine and Clinical Biology, Ghent University. Laboratory reference values were 2.8–8.3 mmol/L for cholesterol and 0.1–1.1 mmol/L for triglycerides.

Statistical analyses

A mixed model was fitted with time, drug administration (PB or imepitoin) and their interaction (time × AED administration) as categorical fixed effects and the dog as a random effect. F-tests were used at the 5% significance level. Each of the three time-specific comparisons between the two drugs at 6, 12 and 18 weeks were tested at a significance level of 0.017 (Bonferroni's multiple comparisons adjustment).

Results

Most (15/20) dogs had mild eosinophilia (1575.5 ± 755.9 /μL, reference range <1250 /μL), but otherwise there were no laboratory abnormalities detected prior to the study. Mean serum TT4 and TSH concentrations (TT4 21.19 ± 8.90 nmol/L; TSH 0.14 ± 0.12 ng/mL) were within the reference range (TT4 6–44 nmol/L; TSH < 0.5 ng/mL).

During the study period, no weight changes of note were detected in either group. The mean bodyweights at the beginning of the study and after 18 weeks were 12.3 kg and 11.9 kg in the PB group, and 12.8 kg and 12.4 kg in the imepitoin group, respectively.

No significant abnormalities were detected on daily physical examinations in 17/20 dogs. Of the remaining three dogs, one developed an acute lethal hepatopathy due to an idiosyncratic reaction 7 weeks after the start of PB administration. A second dog was excluded from the study 7.5 weeks after PB administration due to severe lameness in the right hindlimb caused by a ruptured cruciate ligament. A third dog developed fever and severe lameness due to an interdigital abscess causing necrotising fasciitis 12.5 weeks after PB administration and was excluded from the study.

During the study period, 9/10 dogs treated with PB had elevated serum levels of ALP (reference range, < 111 U/L). In three dogs, serum ALP was mildly elevated; in four dogs serum ALP was moderately elevated (2-3-fold increase); and the remaining two dogs had severely increased serum ALP (5-9-fold increase; Table 1). In the imepitoin group, one dog had mildly elevated serum ALP (127 U/L), only at the 6-week time point.