Introduction

Canine hypothyroidism is a frequent endocrinopathy in dogs, and the clinical signs are numerous, variable, often nonspecific, and rarely pathognomonic (1–12). Therefore, thyroid function is routinely evaluated in dogs. Numerous drugs and NTI can affect the evaluation of thyroid function by several mechanisms (6,12,21–24). Indeed, drugs can act directly by inhibition of secretions of the thyroid gland or by altering metabolism, metabolic clearance, and tissue uptake of thyroid hormones (12,21,22). Both drugs and NTI can interfere with serum binding of thyroid hormones (12,21,22). The conversion of thyroxine (T4) to triiodothyronine (T3) or reverse T3 (rT3) can be altered by the inhibition of the 5’-deiodinase enzyme in peripheral tissues (12,21).

Abstract — The purpose of this study was to evaluate the effect of the administration of meloxicam; carprofen; and a slow-acting disease modifying osteoarthritis agent, that contains chondroitin sulfate, purified glucosamine, and manganese ascorbate (CS-G-M), on thyroid function in dogs. Forty-six healthy (except for osteoarthritis) euthyroid dogs were blindly assigned to 3 treatment groups: meloxicam, carprofen, and CS-G-M. Each group received the recommended dose of the drug for 60 days. Sixteen other osteoarthritic euthyroid dogs, which received a placebo, were used as a control group to validate the study. For all groups, blood samples were collected on days 0, 30, and 60 to evaluate the serum total and free thyroxine, and endogenous thyrotropin concentrations. There were no significant differences among the treatment groups at each time or within each group over a 60-day period for all parameters. Moreover, none of these values were within the hypothyroid range. Based on the results of this study, the administration of meloxicam, carprofen, and CS-G-M did not affect canine thyroid function evaluation.
other drugs that have not been studied yet in dogs could potentially alter the evaluation of thyroid function in that species (12,30).

Osteoarthritis is a common condition affecting approximately 20% of the canine population over 1 y of age (31). This degenerative disease is typically manifested in dogs by pain and lameness (31–36). The newest NSAIDs and the slow-acting disease modifying osteoarthritis agents (SADMOAs) are commonly used because they allow for better control of chronic pain, improvement of general mobility, slower progression of the disease, and, therefore, improvement in the quality of life (32,34,36–38). Among approved NSAIDs for the long-term therapy of osteoarthritis, there are meloxicam (Metacam; Boeringer-Ingelheim, Burlington, Ontario) and carprofen (Rimadyl; Pfizer, London, Ontario) (39,40). An SADMOA sold as a nutraceutical (nutritional supplement), which contains chondroitin sulfate, purified glucosamine, and manganese ascorbate (CS-G-M) (Cosequin; Nutramax Laboratories, Edgewood, Maryland, USA), can decrease progression of the degenerative joint diseases and control both inflammation and pain (37,38,41).

There is a consensus that the use of a drug for a long period of time can produce side effects or can alter the functions of different body systems. A recent study on 21 dogs receiving the NSAID carprofen for 2 to 5 wk showed that this drug can significantly decrease both serum total thyroxine (TT4) concentration and endogenous thyroxine stimulating hormone (TSHc) concentration; free thyroxine (FT4) concentration, however, was not modified by carprofen in this study (42).

Canine hypothyroidism affects typically medium to large breeds from 2 to 6 y of age (12). This population of dogs is also more at risk for osteoarthritis and, thus, for receiving an NSAID or an SADMOA to improve their quality of life and their joint health (31). In the eventuality that dogs receiving either an NSAID or an SADMOA for osteoarthritis are presented with clinical signs compatible with hypothyroidism, it must be known if these drugs affect the results of the thyroid function tests in order to make an accurate interpretation.

The purpose of this study was to evaluate the effects of oral administration of meloxicam, carprofen, and CS-G-M on thyroid function in dogs with osteoarthritis over a 60-day period.

**Materials and methods**

**Dogs**

Sixty-two client-owned dogs with osteoarthritis were included in this study. These dogs were part of another study on the medical management of osteoarthritis (43). There were 32 females (22 spayed) and 30 males (20 neutered). Age ranged from 1.5 to 13 y (mean 6.6 y). Different breeds were represented (Labrador retriever, German shepherd, golden retriever, Saint-Bernard, briard, standard poodle, Bernese mountain, collie, rottweiler, Newfoundland, mixed). Body weight ranged from 26 to 58 kg (mean 38.9 kg). Dogs selected had at least 1 of the following joints affected: hip, elbow, or stifle. In addition, they showed some radiologic and clinical evidence of osteoarthritis. Criteria required for dogs to be included in the present study were the following: 1) healthy (except for osteoarthritis) based on a physical examination, a complete blood cell (CBC) count, and a biochemical profile; 2) euthyroid based on a serum total T4 (TT4) concentration and free T4 (FT4) concentration within the reference ranges; 3) no injectable glucocorticoids within the last 3 mo; 4) no glucocorticoids, PO, within the last 3 wk; 5) no injection of an SADMOA within the last 6 mo; 6) no SADMOA, PO, within the last month; and 7) no NSAID within the last 2 wk. Dogs presenting 1 or more of the following conditions were excluded from the study: 1) lameness of both thoracic and pelvic limbs; 2) osteoarthritis of a joint other than the elbow, hip, or stifle; 3) pregnancy; 4) any bleeding disorder; 5) tumor; 6) neurologic lameness; 7) lameness caused by a muscular disease; 8) fracture; 9) inflammatory arthritis; 10) surgery of the affected joint less than 12 mo prior to the presentation; 11) hypersensitivity to NSAIDs; 12) impaired renal or cardiac function, or both; and 13) gastrointestinal ulceration or any other gastrointestinal disorder.

**Study design**

The dogs were examined at the Centre Hospitalier Universitaire Vétérinaire de l’Université de Montréal by 1 of the 2 surgeons involved in the other study to confirm the presence of osteoarthritis and to exclude lameness caused by any other problem than osteoarthritis.

In this study, the 62 dogs selected were randomly divided into 4 groups: 3 groups receiving either an NSAID or an SADMOA (groups 1, 2, and 3), which were analyzed in a longitudinal study, and a control group (group 4). The study was blinded, so that neither the investigators nor the animal owners were aware of the drug being received by each dog. Both average weight and age were similar between the 4 groups, which precludes bias related to both age and weight.

Group 1 consisted of 14 dogs (8 males, 6 females) weighing between 29 and 58 kg (mean 41.7 kg). Age ranged from 5.5 to 10 y (mean 6.7 y). Dogs were treated with carprofen at a dose ranging from 1.7 to 2.3 mg/kg BW, PO, q12h for 60 d.

Group 2 consisted of 18 dogs (13 males, 5 females) weighing between 28 and 56 kg (mean 36.5 kg). Age ranged from 3 to 12 y (mean 7.2 y). Dogs weighing between 20 and 45 kg were treated, PO, with CS-G-M at a rate of 3 capsules (2 capsules, AM; 1 capsule, PM) daily for 30 d, then 1 capsule q12h for 30 d. Dogs weighing over 45 kg were treated, PO, with CS-G-M at the rate of 2 capsules, q12h, for 30 d, then 3 capsules (2 capsules, AM; 1 capsule, PM) daily for an additional 30 d.

Group 3 consisted of 14 dogs (6 males, 8 females) weighing between 26 and 46 kg (mean 36.7 kg). Age ranged from 1.5 to 11 y (mean 5.2 y). Dogs were treated with meloxicam, 0.2 mg/kg BW, PO in the morning on day 1, followed by 0.1 mg/kg BW, PO, q24h in the morning for a further 59 d. These dogs received a placebo every day at night during the study.
Group 4 (control group) consisted of 16 dogs (3 males, 13 females) weighing between 33 and 47 kg (mean 37.0 kg). Age ranged from 3 to 13 y (mean 7.2 y). Dogs were treated with meloxicam vehicle (placebo) in equal volume to those in the group 3, q12h, PO, for 30 d. For ethical reasons, after 30 d, the placebo was stopped and replaced by meloxicam (same dosage as for group 3) for the following 30 d.

Blood samples for the CBC count; the biochemical profile; serum thyroglobulin autoantibodies (TgAA); and the measurement of serum TT4, FT4, and TSHc concentrations were taken by jugular venipuncture at day 0. Blood samples were also taken at day 30 and 60 by jugular venipuncture for measurement of TT4, FT4, and TSHc concentrations. All samples obtained were centrifuged immediately after clot formation and the serum was frozen at −20°C until assayed.

**Endocrine assays**

Total T4 was measured by using a commercially available solid-phase radioimmunoassay kit (Clinical Assays Gammacoat M Total T4 125I RIA Kit; DiaSorin, Stillwater, Minnesota, USA). Buffer solutions, T4 radioligand, antibody-coated polystyrene tubes, and standards were supplied in the kit. Specificity data provided by the manufacturer identified 92% cross-reactivity with D-thyroxine, 2.1% cross-reactivity with D- and L-triiodothyronine, and less than 0.1% cross-reactivity with other iodothyronines. Reagents were prepared following the manufacturer’s protocol. The following modifications were made to the assay protocol, in part to enhance the sensitivity of the assay. The volume of sample or standard was increased from 10 to 25. A ‘low’ standard of 6.5 nmol/L was made by mixing equal volumes of 0 and 13 nmol/L standards. The 257 nmol/L standard provided by the manufacturer was discarded, leaving the 156 nmol/L standard as the highest in the standard curve of the assay. After pipetting sample or standard and 1 mL of radioligand solution into antibody-coated tubes, the assay mixture was incubated for 3 h at room temperature (~22°C). The sensitivity of the assay, defined as the concentration of T4 at 90% specific binding, was 3 nmol/L (data from 10 assays). When 1-thyroxine (Sigma Chemical, St-Louis, Missouri, USA) was added to a canine serum pool to achieve increases of 26; 52; or 78 nmol/L, than 117%, 105%, and 106% of added T4 was measured into the assay. A pool of canine serum having a high concentration of T4, 104 nmol/L, was diluted at rates of 50% and 25% in “0” standard or protein buffer. The respective recovery rates of T4 were 100% and 108% of expected results in “0” standard and 88% and 92% in protein buffer-diluted serum. Intraassay repeatability was assessed in 3 pools of canine serum assembled to have low (10 nmol/L), middle range (32 nmol/L), and high (86 nmol/L) concentrations of T4. The respective intraassay coefficients of variation (CV) for 10 replicates of each pool were 0.093, 0.092, and 0.120. In 10 assays, the interassay CV for canine serum pools having T4 concentrations of 15 and 63 nmol/L were 0.046 and 0.041, respectively. The reference range of TT4 of euthyroid dogs is 15 to 67 nmol/L.

Assay of free T4 by equilibrium dialysis was done using a commercially available kit (Free T4 by equilibrium dialysis; Nichols Institute Diagnostics, San Juan Capistrano, California, USA) that was previously validated in this laboratory for canine serum (27). The reference range of FT4 of euthyroid dogs is 6 to 42 pmol/L.

Canine TSH was measured with a commercially available immunoradiometric assay (Coat-A-Count canine TSH IRMA; Diagnostic Products, Los Angeles, California, USA). Initial evaluation of assay performance demonstrated negligible cross-reactivity with other canine pituitary gonadotropins, good repeatability, and dilutional parallelism (43,44). In this laboratory, 2 canine serum pools having concentrations of TSHc of 0.21 and 1.33 ng/mL were established for repeatability studies. For 10 replicates of each pool, the intraassay CVs were 0.106 and 0.052, respectively. Among 10 assay runs, the interassay CV for each pool was 0.139 and 0.071, respectively. A canine serum pool of high TSHc (4.67 ng/mL) was made for dilutional studies. When serum from this pool was diluted at rates of 50%; 25%; and 12.5% in “0” standard, 84%, 89%, and 86% of expected TSHc concentrations were measured. When serum from the high pool was diluted in protein buffer solution at rates of 50%; 25%; and 12.5%, 95%, 99%, and 103% of expected TSHc results were obtained. The reference range of TSHc of euthyroid dogs is 0 to 0.6 ng/mL.

Canine thyroglobulin autoantibody assays were performed with a commercially available ELISA kit (Oxford Biomedical Research, Oxford, Missouri, USA) with a previously defined diagnostic utility (45). The reference range of TgAA of euthyroid dogs is < 200%.

**Data analysis**

Some data are missing due to the occurrence of new illnesses during the study or lack of sera. Data obtained from blood samples on day 0 and day 30 and/or 60 were deemed relevant and thus used for the statistical analysis.

Statistical software (SAS, version 8.2; Statistical Analysis Systems, Cary, Carolina, USA) was used to assess the effect of time, treatment, and the interaction between treatment and time by using an analysis of variance (ANOVA) for repeated measures. Due to the change in treatment in the control group midway through the experiment, data from the control group were analyzed separately from the 3 other groups. Data for the various parameters of the control group were analyzed with paired t-tests to examine potential changes in concentration during the first 30 d of the control treatment and potential changes during the following 30 d with the meloxicam treatment. For all analyses, a value of P < 0.05 was considered significant. The data were normally distributed and are reported as mean ± standard deviation (s).

**Results**

**Longitudinal study**

Total thyroxine concentrations — Results are shown in Table 1. No differences occurred among the treatment groups in serum TT4 concentrations (P = 0.87). Serum
TT4 concentrations remained stable over the course of the study ($P = 0.89$) and varied in similar fashion among the 3 groups ($P = 0.71$). All serum TT4 concentrations were within reference range of euthyroid dogs (15 to 67 nmol/L) throughout the study, except for 1 of the 131 samples that was slightly above reference values.

**Free thyroxine concentrations** — Results are shown in Table 2. There was no significant difference in serum FT4 concentrations among the treatment groups ($P = 0.90$). Serum FT4 concentrations decreased over time ($P < 0.005$), but in similar fashion in each group ($P = 0.21$). All serum FT4 concentrations remained within the reference range for euthyroid dogs (9 to 42 pmol/L) throughout the study, except for 8 out of 133 samples that were slightly above reference values.

**Serum TSHc concentrations** — Results are shown in Table 3. There was no significant difference in serum TSHc concentrations among the treatment groups ($P = 0.75$). Serum TSHc concentrations increased over time ($P < 0.005$) but in similar fashion in each group ($P = 0.28$). All serum TSHc concentrations were within reference range (0 to 0.60 ng/mL) throughout the study except for 1 dog from the CS-G-M group on day 0 (0.83 ng/mL) and 1 other dog from the same group on day 60 (0.76 ng/mL). These 2 dogs had TSHc concentrations within reference values on all other occasions.

**Control group**

Results are shown in Table 4. Serum TT4, FT4, and TSHc concentrations did not vary from day 0 to 30 ($P = 0.17, 0.07,$ and $0.80,$ respectively). Serum TT4, FT4, and TSHc concentrations also failed to vary from day 30 to 60 ($P = 0.19, 0.05,$ and $0.90,$ respectively).

**Thyroglobulin autoantibodies**

Three out of 62 dogs (4.8%) showed serum thyroglobulin antibodies on day 0 of the study: 1 from the control group, 1 from group 2 (CS-G-M), and 1 from group 3 (meloxicam), representing 6%, 5%, and 7% of placebo, CS-G-M, and meloxicam groups, respectively. The percentage of dogs where serum TgAA was found was in agreement with a previous study where thyroglobulin antibodies were detected in 3.4% of dogs with non-thyroidal illness (46).
Discussion

Assessment of thyroid function in dogs can be challenging when laboratory findings are equivocal or in the range of hypothyroid reference values. Many factors are known to affect the evaluation of the thyroid function of dogs, drugs being an important one. Several drugs of different classes, such as antibiotics, NSAIDs, glucocorticoids, anesthetics, anticonvulsants, and diuretics, can alter basal thyroid hormone concentrations. All are described in human medicine but few have been studied in dogs (6,12). Glucocorticoids, which are commonly used in dogs, are known to interfere with the assessment of thyroid function (6,12,22,24,26,27,41,44). Their effects on thyroid function vary according to the route of administration, the dose, the duration of treatment, and the chemical form used. Endogenous or exogenous glucocorticoids can lower baseline serum thyroid hormones in dogs (6,12,26,27,47,48). Stresses induced by nonthyroidal illnesses may increase the endogenous glucocorticoid production and then alter the thyroid function (26). Chronic pain induced by osteoarthritis could potentially induce enough stress to alter thyroid function. However, a recent study evaluating the effect of chronic osteoarthritis on thyroid function showed that severe osteoarthritis did not appear to affect the thyroid function in dogs (49).

In the present study, serum TT4 concentrations were not affected by carprofen, meloxicam, or CS-G-M over a 60-day period. These results contrast with a previous study by Ferguson et al (42), in which serum TT4 concentrations were mildly but significantly decreased following administration of carprofen for 2 and 5 wk.

The serum FT4 concentrations in the treatment groups were mildly decreased throughout the study, but this was not statistically significant or clinically relevant. There was no significant difference among the groups at day 30 or at day 60. Based on these results, the NSAIDs and the SADMOA studied had no effect on serum FT4 concentrations. This is in agreement with Ferguson et al (42), who observed no significant change in serum FT4 concentrations (measured by equilibrium dialysis) following 2 and 5 wk of carprofen administration. Although the reasons of these mild decreases are not known, there is random fluctuation in thyroid hormones levels, which could explain our results (12,21).

The serum TSHc concentrations in the treatment groups were mildly increased throughout the study, but this was not statistically significant or clinically relevant. There was no significant difference among the groups at day 30 or at day 60. We can, therefore, conclude that the drugs studied here had no effect on serum TSHc concentrations. This is in contrast with a recent study where a decrease in serum TSHc concentrations was noted in dogs receiving carprofen (42). The variation of these serum TSHc concentrations could be explained by fluctuations in circulating TSH levels or by a pulsatile secretion of TSH (50).

In addition, the differences on the effect of carprofen on the thyroid function between our study and that of Ferguson et al (42), may be related to the fact that they used a higher dose of carprofen (1.7 to 2.3 mg/kgBW q12h versus 2.2 to 3.3 mg/kgBW q12h).

In the control group, the serum TT4, FT4, and TSHc concentrations were not affected by the placebo over a 30-day period or by the meloxicam received by this group during the last 30 d of the study. Thus, the different parameters evaluated here were not affected either by environmental conditions or by a significant normal fluctuation during the 30-day period of the study. We can hypothesize that these findings could have been repeated during the last 30-day period of the study. The placebo was not administered over the 60-day period of the study for ethical reasons.

In conclusion, based on the results of the present study, serum TT4, FT4, and TSHc concentrations do not appear to be affected following 2 mo of administration of the recommended dose of carprofen, meloxicam, and CS-G-M. Moreover, an additional 16 dogs that received 30 d of meloxicam had no significant changes in their thyroid function evaluation. Those drugs had no effect on thyroid function from a clinical viewpoint. No dogs receiving carprofen, meloxicam, or CS-G-M could be misdiagnosed as hypothyroid. Therefore, administrations of these drugs do not need to be taken into account in dogs undergoing evaluation of thyroid function.

References


