VITAMIN D STATUS IN CANINE CANCER PATIENTS AND THE RELATIONSHIP WITH DIETARY VITAMIN D INTAKE.

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Lay Summary
Canine cancer has become more prevalent in the last decades because of increased life expectancy and greater attention to the health of pets. The range of cancers seen in dogs is as diverse as that in human patients, and despite more intensive therapeutic interventions, fatality rates remain unacceptably high in both species. Therefore, more research investigating risk factors, prognostic factors as well as treatment options is definitely warranted.
In humans, epidemiological data indicate that a low vitamin D status is associated with an increased risk of a variety of cancers. Also, it has been shown in human cancer patients that vitamin D deficiency may be linked with poor prognosis, as lower serum vitamin D concentrations are related to reduced overall survival. The mechanism by which vitamin D status alters cancer development is not completely uncovered. However, it is known that many cell types have vitamin D receptors and when these receptors are activated by 1,25-dihydroxycholecalciferol (1,25(OH)$_2$D$_3$), the most active metabolite of vitamin D, differentiation is induced and proliferation, invasiveness, angiogenesis, and metastatic potential are inhibited. When vitamin D status is suboptimal, these activities may be impaired, leading to development and/or progression of cancer.
The purpose of this study is to compare the vitamin D status of cancer-bearing dogs with healthy dogs and evaluate the relationship between vitamin D status and dietary vitamin D intake. Also, the prognostic value of vitamin D levels for cancer-bearing dogs will be investigated. The results of this study may eventually lead to new perspectives for cancer prevention and treatment in dogs and perhaps people.

Introduction
Vitamin D is a steroid hormone traditionally recognized to be responsible for calcium and phosphorus homeostasis in the body. However, it is widely accepted that vitamin D also exerts several extraskeletal effects(1). 25-hydroxyvitamin D$_3$ (25(OH)D$_3$) the main form of circulating vitamin D, is converted by 1α-hydroxylase into 1,25-dihydroxyvitamin D$_3$ (1,25(OH)$_2$D$_3$), which is considered the biologically active metabolite, binding to nuclear vitamin D receptors(1-2). Although the conversion of 25(OH)D$_3$ primarily takes place in the kidney, 1α-hydroxylase is also present in other tissues, suggesting that some portion of 1,25(OH)$_2$D$_3$ is produced locally and exerts local effects. This extrarenal 1,25(OH)$_2$D$_3$ appears important for the paracrine regulation of cell differentiation and function(1-2). As in many tissues 1,25(OH)$_2$D$_3$ can be produced locally, under the influence of cytokines and because many cell types, including cancer cells, have

vitamin D receptors, it is suggested that vitamin D plays an important role in cancer etiology(3). In humans, a population based association between low vitamin D status and increased risk for a variety of cancers is generally accepted(4-6). Also, it has been shown in human cancer patients that vitamin D deficiency is linked with poor prognosis, because lower serum vitamin D concentrations are related to reduced overall survival(7-11). Furthermore, an adequate vitamin D status may be important for cancer prevention and $1,25(OH)_2D_3$ may have potential in the treatment of cancer(12). Plausible mechanisms responsible for the anti-cancer activities of $1,25(OH)_2D_3$ are proliferation inhibition, induction of differentiation and apoptosis, enhanced DNA repair, antioxidant protection, anti-angiogenic and anti-metastatic actions and immunomodulation through interference with growth factor and cytokine synthesis(2, 12). To date only one study has investigated the vitamin D status in cancer-bearing dogs, showing lower serum $25(OH)D_3$ concentrations in Labrador Retrievers with cutaneous mast cell tumours(13). A significant difference between humans and dogs is the absence of the influence of sunlight in dogs; therefore dietary intake is the only source for vitamin D in dogs(14). In that study, no difference was found between calculated dietary vitamin D intake in dogs with mast cell tumours versus unaffected dogs. Yet, as many pet food companies do not have their products analyzed for vitamin D and only provide the information based on addition of vitamin D to the food, the calculated vitamin D intake might not have been accurate(13). Also this previous study in dogs did not focus on serum calcium and PTH concentrations and did not investigate vitamin D status as a prognostic variable.

Hypothesis

We hypothesize,

(1) dogs presenting with cancer will have lower serum $25(OH)D_3$ concentrations than non-tumour-bearing control dogs most likely due to reduced dietary intake, and

(2) this suboptimal vitamin D status in cancer-bearing dogs, is a negative prognostic factor.

Objectives

(i) Measure and compare serum vitamin D and calcium levels in cancer-bearing dogs and normalmatched controls, (ii) evaluate the relationship between dietary intake of vitamin D by dietary survey and food analysis, and (iii) investigate the prognostic value of vitamin D status in dogs with cancer.

Materials and Methods

Part I: Vitamin D status in canine cancer patients

Study groups

Over a period of one year, 75 newly diagnosed, client-owned tumour-bearing dogs affected with various cancer types (i.e. lymphoma, osteosarcoma, mast cell tumour) presenting to the Animal Cancer Centre will be entered in the study (i.e. cases). Over the same period, 25 healthy, client-owned dogs from the Primary Health Care Centre will be included as unaffected controls. Control dogs will be enrolled based on normal medical history and physical examination. For both case and control dogs, exclusion criteria will be: dogs younger than 2 years of age, dogs with clinically significant systemic or infectious disease (other than cancer), and dogs receiving Vitamin D and/or calcium supplements. Cancer patients should not have received corticosteroids within 2 weeks prior to study enrollment. Dog-owners will provide written consent to enter the study.

Study variables

At enrolment, tumour diagnosis, stage and grade will be recorded for each case. For both case and control dogs body weight, body condition score (BCS), muscle condition score (MCS), breed, gender, and age will be recorded.

Blood will be sampled from each subject prior to any other surgical or medical intervention that may be part of their care. Complete blood count (CBC) and serum chemistry will be performed to exclude organ dysfunction at the first visit in all patients at no cost to the owner and immunophenotyping will be performed for lymphoma patients also at no costs for the owner. A plasma sample will be stored immediately at -70°C for evaluation of vitamin D metabolites ($25(OH)D_2/D_3$, $1,25(OH)_2D_3$, $24,25(OH)D_3$ and unhydroxylated vitamin D_2 and D_3), total and ionised calcium, parathyroid hormone (PTH), parathyroid-related protein (PTHrP). At the time of recruitment, dog owners will be asked to complete a questionnaire including questions about dietary intake during the three months before enrolment and will be asked to keep a dietary journal for one week. Information regarding vitamin D content of dog foods, treats, and supplements will be acquired by contacting manufacturers. Vitamin D content of human foods will be acquired through the Canadian nutrient file (http://www.hc-sc.gc.ca/fn-an/nutrition/fiche-nutri-data/index-eng.php). Dietary vitamin D intake will be calculated based on portion size of foods, treats, table foods, and supplements. In addition, owners will be asked to provide a food sample for vitamin D analysis.

Milestone I – At the end of Part I, differences in vitamin D status between cancer bearing dogs and healthy control dogs will be discovered and the relationship with dietary vitamin D intake will be revealed.

Part II: Prognostic value of serum vitamin D in canine cancer

Study group

Fifty client-owned, cancer-bearing dogs, included in Part I and treated with standard protocols (e.g. 25 dogs with lymphoma treated with sequential multi-agent CHOP [cyclophosphamide, hydroxydaunorubicin, oncovin, prednisone] chemotherapy and 25 dogs with osteosarcoma treated with amputation followed by adjuvant carboplatinum chemotherapy) will be included in Part II, to investigate the prognostic value of vitamin D status.

Study variables

Over a period of 1 year, based on the life expectancy of dogs with lymphoma(16) and osteosarcoma(17), tumour stage (remission, stable disease, progression of disease), will be recorded as well as body weight, BCS, and MCS will be recorded at every consultation (16 appointments for lymphoma and 8 appointments for osteosarcoma). Blood samples will also be collected to evaluate vitamin D metabolites, calcium, PTH and PTHrP at four additional visits for lymphoma patients and three additional visits for osteosarcoma patients spread over the treatment course. Similar to Part I, owners will be asked to complete a dietary questionnaire, keep a one-week food journal and provide a food sample for vitamin D analysis at three visits for lymphoma patients and at four visits for osteosarcoma patients. Overall survival (OS) and recurrence-free survival (RFS) will be the end points for this study. OS time will be calculated from the date when the treatment was started to the date of last follow up or death from any cause. RFS will be defined as the time from the date the treatment was started to the first date of recurrence of cancer or death from any cause(7).

Milestone II – At the end of Part II, the prognostic value of vitamin D status in cancer-bearing dogs will be ascertained.

Anticipated results and Significance

We anticipate this study will identify low serum 25(OH)D_3 levels in dogs with cancer as a significant factor for cancer incidence and prognosis. Therefore, in the future, an adequate vitamin D status may be important for cancer prevention. Hence, supplementation of vitamin D in cancer-bearing dogs may be clinically indicated as part of the cancer treatment.

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