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Gender Differences in Baseline Levels of Vascular Endothelial Growth Factor in the Plasma of Alaskan Sled Dogs

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Abstract: Angiogenesis and vasculogenesis are two very important processes in the development and maintenance of mammalian health. In sled dogs, the angiogenic role is to supply and support tissue with ample vasculature, thus providing a route of access for the transportation of essential nutrients, including oxygen and the removal of waste in a sustained fashion. VEGF has been shown to be a key mediating factor in the underlying cascade of chemical events leading to angiogenesis, which makes it a very important precursor molecule for both muscle development and early neoplasia detection. The overall purpose of this study was to establish circulatory baseline VEGF levels in healthy dogs to develop an alternative mammalian model. Mean VEGF levels were 19 pg mL^{-1} in sled dogs (n=20) and 5 pg mL^{-1} in beagles (n=4). There were significant baseline VEGF-ir differences between male and female dogs and exercising males and exercising females. In addition preliminary data on beagles suggest that breed may play a role in baseline VEGF-ir levels. This study is the first step in the biotechnological development of a diagnostic VEGF assay in dogs.

Key words: VEGF, sled dogs, beagles, Alaska, exercise

INTRODUCTION

Angiogenesis is a major process in the development and maintenance of mammalian health. There are many stimulators which can initiate angiogenesis. Most of these stimulators are specific proteins called growth factors. The idea of specific proteins mediating the events of angiogenic growth was first proposed by Judah Folkman[1,2]. Folkman proposed that if specific proteins existed, then those proteins could help in the elucidation of angiogenic abnormalities. It was not until 1984 that the first of these specific angiogenic proteins was isolated and characterized[3]. Since this first characterization, many angiogenic genes and gene products have been isolated, purified and characterized. These angiogenic stimulatory molecules (ASM) can be divided into seven major groups: 1, growth factors; 2, proteases; 3, trace elements or metals; 4, oncogenes; 5, cytokines; 6, molecules involved in cellular signal transduction (STMs) and 7, endogenous angiogenic inducers.

Among these categories, growth factors seem to be the most studied due to their dual role in embryonic angiogenesis and angiogenic malignancies. The most widely studied growth factors are: acidic and basic fibroblast growth factor (aFGF and bFGF), vascular endothelial growth factor (VEGF), which is sometimes called vascular permeability factor (VPF), platelet derived-endothelial cell growth factor (PD-ECGF), alpha and beta transforming growth factor (αTGF and βTGF), angiogenin and alpha tumor necrosis factor (αTNF). Angiogenesis is characterized by the constant proliferation of endothelial cells[4]. Unregulated endothelial cell proliferation is a prominent characteristic of many disease processes, including but not limited to: proliferative retinopathy, rheumatoid arthritis, psoriasis, colon polyps and breast cancer[5]. Angiogenesis must be kept under tight biological control in order to avoid abnormal pathogenesis. Hyperangiogenic activity and hypoangiogenic activity can both lead to deleterious pathological events *in vivo*[5]. The onset of many tumors is mediated by growth factors such as VEGF. With this in mind, the overall purpose of this study was to establish circulatory baseline VEGF levels in healthy dog models. We hope that baseline levels of VEGF in plasma will aid in the development of a comparative model for neoplastic disease[4,5] and other angiogenic related conditions[6].

MATERIALS AND METHODS

Animals: The Institute of Animal Use and Care Committee at the University of Alaska Fairbanks approved this study. The animals utilized in this study were racing sled dogs and were housed at the Nestle Purina® Research Facility located in Salcha, Alaska. Each animal was tethered to a 2m chain attached to individual houses; each animal had access to her/her own food and water.

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Table 1: Age, gender, breed and groupings of dogs, respectively

Animal name	Group	Age (yrs)	Sex	Breed
Brown	R	5	M	Alaskan Sled Dog
Mocha	R	2	M	Alaskan Sled Dog
Boney	R	2	M	Alaskan Sled Dog
Bruce	R	2	M	Alaskan Sled Dog
Nigel	R	2	M	Alaskan Sled Dog
Finnmark	R	1	M	Alaskan Sled Dog
Tromso	R	1	M	Alaskan Sled Dog
Dori	R	2	F	Alaskan Sled Dog
Peach	R	2	F	Alaskan Sled Dog
Hera	R	6	F	Alaskan Sled Dog
Average		2.5	70%M	
Rambo	NR	12	M	Alaskan Sled Dog
Jose	NR	1	M	Alaskan Sled Dog
Apollo	NR	1	M	Alaskan Sled Dog
Sully	NR	2	M	Alaskan Sled Dog
Marvin	NR	9	M	Alaskan Sled Dog
Roz	NR	2	F	Alaskan Sled Dog
Pin	NR	4	F	Alaskan Sled Dog
Celia	NR	2	F	Alaskan Sled Dog
Lucy	NR	12	F	Alaskan Sled Dog
Twister	NR	4	F	Alaskan Sled Dog
Average		4.9	50%M	
Healthy Dog 1	Healthy	2	F	Beagle
Healthy Dog 2	Healthy	2	F	Beagle
Healthy Dog 3	Healthy	5	F	Beagle
Healthy dog 4	Healthy	3	F	Beagle
Cancer Dog 1	Cancer	4	F	Beagle
Average		3.4	100%F	

Table 2: Actual values (pg mL^{-1}) and the mean of VEGF plasma levels for sled dogs

Animal name	Age (yrs)	Sex	Concentration
All Samples			
Brown	5	M	17.3
Mocha	2	M	14.0
Boney	2	M	17.3
Bruce	2	M	21.5
Nigel	2	M	33.2
Finnmark	1	M	24.8
Tromso	1	M	34.0
Dori	2	F	17.3
Peach	2	F	17.3
Hera	6	F	11.5
Rambo	12	M	28.2
Jose	1	M	19.0
Apollo	1	M	23.2
Sully	2	M	15.7
Marvin	9	M	12.3
Roz	2	F	15.7
Pin	4	F	19.8
Celia	2	F	13.2
Lucy	12	F	21.5
Twister	4	F	9.0
AVERAGE	3.7	60%M	19.3
STDEV	3.4		6.8
SEM	0.8		1.6

Ambient temperature during sampling was between -23 to -15°C. Twenty animals were sampled and placed into categories of runners (R), non-runners (NR), male runners and non-runners (MR and MNR) and female runners and non-runners (FR and FNR), respectively (Table 1). Ages of the animals ranged form less than 1 year to 12 years. A total of 12 non-neutered males and 8 non-spayed females comprised the two groups.

All animals were healthy at the time of sampling and were free of prescribed medication except for one animal (Marvin) who received Thyroxin® 1.2 mg B.I.D. for hypothyroidism. Control dogs remained tethered to their houses and activity levels varied with the individual dog. Runner dogs were exercised daily for approximately 0.5 h.

Blood sampling: Sampling of dogs was conducted at the Nestle Purina® Research Facilities. All dogs were subjected to cephalic venapuncture using a 21G x ¾ winged infusion set by Terumo® in connection with a 12 cc syringe. Approximately 10 mL of whole blood was collected from each animal and placed into 12 cc herparinized vacutainer tubes. The samples were placed on ice and transported back to the biochemistry laboratory at the University of Alaska Fairbanks Natural Sciences Facility where they were subjected to centrifugation at 2500X for 15 min; plasma was collected and transferred into freezer vials and stored at -70°C until they were analyzed.

Biochemical analysis: The biochemical analysis of Vascular Endothelial Growth Factor (VEGF) was conducted at the University of Alaska Fairbanks Natural Science Facility biochemistry laboratory. A commercial available ELISA Quanikine® human VEGF immunoassay was used from R&D Systems®[7]. The assay was utilized to quantitatively evaluate circulatory levels of VEGF in all of the sampled animals. The procedure supplied by R&D Systems® was followed[7]. For the entire blood collection, all sled dog samples were analyzed using one assay kit. The reported intra-assay CV averaged 5.4% while the inter-assay CV averaged 7.3%[7]. The dog VEGF has 89% homology with human VEGF.

Statistical analysis: Analysis of variance was used to evaluate any statistical differences between the groups. An F Test was used to determine if significant differences were present within standard deviations; no significant differences between standard deviations was observed. Since there were no significant differences in the standard deviation, a simplified Student's t-test was utilized to evaluated group data.

RESULTS

All dogs tested were immunoreactive for VEGF-ir in their plasma (Table 2). The VEGF mean was 19 pg mL^{-1} and the plasma concentrations ranged form 9 to 34 pg mL^{-1}. Male sled dogs displayed higher VEGF-ir levels (21.7 pg mL^{-1}, n=12) than female dogs (15.7, n=8) (Fig. 1). The difference between the means was significant at $p \leq 0.05$.

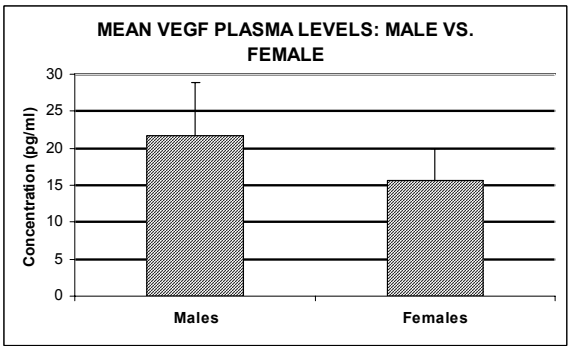

Fig. 1: VEGF plasma levels in male and female sled dogs, significant at p≤.05

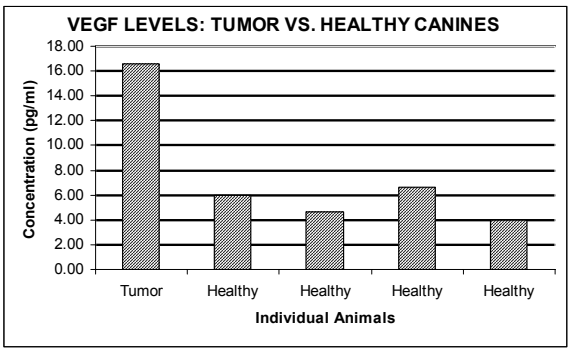

Fig. 2: Actual values (pg mL^{-1}) of four health (non-running) beagles with one beagle diagnosed with a vascular tumor

When male sled dogs who were exercising (runners) were compared to female runners, the males had a higher mean level (23.2 pg mL^{-1}, n=7) than females (15.4, n=3). This difference was also significant p≤ 0.05). However, no significant differences were detected for runners (20.8 pg mL^{-1}) versus non-runners (17.8 pg mL^{-1}). Nor was the mean difference significant between male runners versus male non-runners.

Healthy female beagles showed lower levels of VEGF-ir (5 pg mL^{-1}, n=4) than female sled dog non-runners (15.8 pg mL^{-1}, n=5). The one beagle diagnosed with a mammary gland tumor had a 3 fold higher plasma level of VEGF-ir (16.2 pg mL^{-1}) than the healthy beagles (Fig. 2).

DISCUSSION

There are consistent research reports establishing that skeletal muscle capillary density is an important factor when evaluating athletic maximum exercise capacity. Gender differences have not been previously reported in dogs. Furthermore, it has long been established that endurance athletes, such as marathon runners and cyclists, have higher muscle capillary supplies when compared to individuals whom exercise modestly or not at all. A recent report by Kraus and colleagues[8] suggest that this type of exercise induced angiogenesis is mediated in part by the secretion of VEGF via skeletal muscle cells. Kraus and colleagues[8] study was conducted using human endurance athletes. They report a short-term 2-fold increase in the VEGF plasma levels when comparing endurance athletes. While we observed higher mean levels of VEGF-ir for sled dogs, the difference was not significant. The control animals used in our study were racing sled dogs, who were retired and no longer running or actively training; however, at some stage of their life, all the sled dogs sampled were endurance trained athletes.

The mean VEGF-ir concentrations for the two groups were 20.8 pg mL^{-1} for the runners and 17.8 pg mL^{-1} for the non-runners, respectively. Although the sample size for the two groups was the same, the inconsistency of average age and gender within the samplings may explain why the means were not statistically significant, similar to the individual variability reported in the Kraus et al study. When we control for gender by focusing on male sled dogs, a similar difference, approximately 3 pg mL^{-1} was observed but the means were not significant. The mean VEGF-ir concentrations for the male runners and non-runners was 23.2 and 19.7 pg mL^{-1}, respectively. The range for the male runners was as low as 14 pg mL^{-1} and as high as 34 pg mL^{-1}. The non-runners display a similar range with levels of 12.3 to 28.2 pg mL^{-1}.

On the other hand, since systemic VEGF levels in human athletes was different than that of sedentary individuals[8], we would expect to see an even bigger increase when comparing athlete sled dogs to sedentary dogs. Maximal sustained metabolic rates of sled dogs have been measured well above (approximately 3-fold higher) the rates for human athletes. However, the small data set on healthy beagles (Fig. 2) with VEGF-ir levels around 5 pg mL^{-1} when compared to exercising female sled dogs (15.4 pg mL^{-1}) supports the speculation that body size difference between breeds is a factor in baseline levels of VEGF. The human VEGF mean[8] for sedentary males 32.2 pg mL^{-1} is greater than sled dogs with a mean of 19.7 pg mL^{-1} for male non-runners.

VEGF has been widely shown in human and animal (mostly rat) studies to increase in response to a developing growth or tumor. The early detection of VEGF in blood could be used as an early indication of tumor growth, or at least, lead to further analysis to investigate whether or not a growth is present. This information at an early stage of disease could lead to early treatment and management. To date there is a limited amount of data available concerning canine VEGF in healthy dogs. It is rather surprising considering there are numerous studies of VEGF levels in tumor ridden animals. Most of the studies have concentrated their efforts on VEGF levels in response to radiation and chemotherapy. In diagnosing cancers, gender must be considered in that our study has shown

that VEGF baseline levels appear to be dependant upon the gender of the animal.

Male dogs when compared to the female dogs had the most measurable mean VEGF-ir difference between the groups. Mean VEGF-ir levels for the males was 21.7 pg mL^{-1} while the female levels were much lower, 15.7 pg mL^{-1} (Fig. 1). Male sampling was n=12 and the female sampling was n=8 and male concentrations ranged from 12.3 to 34 pg mL^{-1}, while female samples ranged in concentration from 9 to 21.5 pg mL^{-1}. Concentrations for male runners were significantly higher than those of the female runners. The mean male concentration of VEGF-ir was 23.2 pg mL^{-1} and the female mean concentrations was 15.4 pg mL^{-1} (Table 1). The concentrations for the male runners ranged from 14 to 34 pg mL^{-}, while the female concentrations ranged form 11.5 to 17.3 pg mL^{-1}. Both gender and breed need to be taken into consideration when VEGF levels are being used for health related diagnostics. Establishing baseline levels for both sedentary and exercising breeds will provide valuable insight into how VEGF protein levels in dogs respond to insults such as wound healing, menstruation and neoplasia. Also, recent studies[9] suggest that VEGF, which guides the migration of endothelial cells, may play a role in directing the patterning of nerves.

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