2018 Companion Animal Project Awards

Changes in Ionized and Total Magnesium in Sled Dogs after Competing in an Endurance Race

Sara Connolly

Magnesium plays an important role in energy metabolism, nerve conduction, and muscle contraction. It is involved in oxygen uptake and energy production as well as electrolyte balance. In human medicine, magnesium is being increasingly studied in endurance athletes, as low ionized magnesium (iMg) has been associated with muscle fatigue and collapse in these athletes. Sled dogs competing in endurance races often show similar symptoms and are dropped from the race. The aim of this study is to determine if there is a significant change in ionized magnesium concentration in sled dogs between samples taken before endurance races and those taken after completion. Specifically, we will be looking at sled dogs competing in the 2018 Iditarod race. Confirming that ionized magnesium decreases in sled dogs after an endurance race and determining a post-endurance racing ionized magnesium reference interval will allow identification of dogs that have abnormal ionized magnesium levels post-endurance exercise. This information could be used to guide treatment in dogs showing clinical signs of decreased ionized magnesium including muscle weakness or collapse as well as play a role in determining appropriate magnesium levels in the diet of sled dogs prior to, during and after a race to combat hypomagnesemia. It is hypothesized that ionized magnesium levels will be decreased in dogs after completing a race compared to before the race because of increased utilization during exercise.

Preclinical Evaluation of FOXA2 as Drug Target in Dogs with Respiratory Diseases

Gee Lau

Many domestic animals suffer from respiratory illnesses similar to those in humans. Among these, asthma, bronchitis, pneumonia, and pulmonary fibrosis occur naturally in dogs, cats, and horses, among other species. As a result of exposure to environmental pollutants including second hand smoke and/or infections agents or genetic predisposition, older dogs, especially those of smaller breeds ages 8 or older, have a tendency to develop various pulmonary diseases. These lung diseases include canine infectious respiratory disease (CIRD, also called infectious canine tracheobronchitis or kennel cough), chronic bronchitis (CB), and chronic obstructive pulmonary disease (COPD). A prominent feature of these respiratory diseases is goblet cell hyperplasia and metaplasia (GCHM) and mucus hypersecretion. Excessive mucus disables the innate immune mucociliary escalator function that prevents clearance of inhaled microbial pathogens. The Forkhead box protein A2 (FOXA2) is a KEY transcription regulator that maintains airway mucus homeostasis. Prior studies by our lab and by others have found that FOXA2 are frequently inactivated in human patients with respiratory infections, resulting in GCHM and mucus hypersecretion and disabling of the mucociliary escalator function. Unfortunately, regulation of mucus homeostasis by FOXA2 and its inactivation by microbial pathogens have not been examined in the canine respiratory diseases. In preliminary studies, we have examined FOXA2 inactivation in several bacterial-mediated canine respiratory cases received by the UIUC Veterinary Diagnostic Laboratory (VDL). Our results indicated that bacterial infection inactivates FOXA2, leading to GCHM and mucus hypersecretion in canine airways. Furthermore, our preliminary studies in human and mouse airways demonstrate that the FDA-approved incretin mimetic drug for diabetes mellitus 2, Exenatide (Ex-4), is a strong agonist of FOXA2. The longterm goals of this project are to devise novel therapies for improving the prognosis of dogs with respiratory diseases. The immediate objective of this proposal is to test the hypotheses that FOXA2 is a KEY target of inactivation that contributes significantly to

GCHM and mucus hypersecretion in canine respiratory diseases, and that Ex-4 and other FOXA2 agonists will be effective in restoring mucus homeostasis. In the clinical Aim 1, we will examine the banked lung specimens of dogs died of respiratory infection by viral and bacterial pathogens for depletion of FOXA2 expression and excessive mucus. The clinical observations will be confirmed using an *in vitro* canine immortalized BACA cell culture model. In the mechanistic Aim 2, we will use the BACA cells to determine whether Ex-4 restores FOXA2 function by activating the GLP1RNRF2-PPARg-dependent phosphatases, which inhibit key kinases within both pro-GCHM STAT6-SPDEF and EGFR-AKT/ERK1/2 signaling cascades and relieves FOXA2 to inhibit mucin biosynthesis. Then, we will use air-liquid interface (ALI) culture of normal canine primary bronchial epithelial (NCBE) cells to determine if Ex-4 and other FOXA2 agonists attenuate STAT6 and EGFR-dependent differentiation of multipotent airway cells into mucus secreting goblet cells. Completion of the proposal will unveil the efficacy and mechanisms by which Ex-4 restores FOXA2 function and improves clearance of respiratory pathogens in canine airways.

Changes in Equine Bone Structure, Composition and Loading During Growth
Annette McCoy

Musculoskeletal injuries, particularly distal limb fractures, represent a major welfare concern and economic burden in the racehorse industry. A key step towards the development of interventional strategies that could help reduce the incidence of fractures is a better understanding of the bone modeling that occurs during normal early growth and development in the horse. The objective of the current proposal is to develop a preliminary characterization of the structural, functional and compositional contributors to fracture risk in the equine distal limb during growth. Longitudinal computed tomography data will be used in combination with gait data (motion capture and force plate kinematics) from three foals to develop finite-element models for the third metacarpal bone (MC3) and first phalanx (P1) that together describe the normal modeling, and changes in loading that occur during the rapid growth period from 2 to 12 months of age. Rigid body musculoskeletal models will be used to predict muscle and joint reaction loads for final predictions of bone strength during growth. We hypothesize that the greatest modeling response for mid-shaft cross-sectional properties will occur until epiphyseal fusion, and that cross-sectional properties that resist bending and compressive loads should increase with positive allometry (changes in proportion due to growth). We further hypothesize that fetlock joint postures should become more extended with age during the middle of each limb's stance (when forces are highest) to enable more compressive loading, and less reliance on muscle/tendon support structures. The data generated by this proposal will be the foundation for a larger extramural grant proposal that will focus on validating the predictive capacity of the model developed here and on developing and testing exercise interventions in foals.

Single Dose Pharmacokinetics of Mioprostol in Horses
Annette McCoy

Misoprostol, a prostaglandin E1 synthetic analogue, is a drug utilized in equine medicine for the treatment of gastric and colonic ulcers induced by non-steroidal anti-inflammatory drug toxicity. Little is known about its pharmacokinetics in horses, and doses have been extrapolated from the human literature. However, human clinical trials have indicated that there can be wide variations in absorption and bioavailability of misoprostol depending on the route of administration (e.g oral, sublingual, transrectal, vaginal). It is likely that this is also true in horses, in which misoprostol, although typically given orally, could also be administered rectally. Unfortunately, the magnitude of these effects on different routes of administration in horses is completely unknown. The effect of feeding on absorption and bioavailability of misoprostol is

also unknown. Therefore, investigation into the absorption and bioavailability of misoprostol in horses is crucial for establishing evidence-based recommendations for dose and frequency for both oral and rectal routes of administration. We hypothesize that after a single dose of misoprostol, peak plasma concentrations will be significantly greater and time to reach peak concentrations will be significantly shorter in horses administered misoprostol orally (both fasted and fed) when compared to horses administered the drug per rectum, but the terminal half-life will be significantly longer in horses receiving the drug per rectum. Further, we hypothesize that relative bioavailability will be significantly greater after oral administration of misoprostol to fasted horses when compared to both fed horses and to horses administered the drug per rectum.

Cytochrome P450 Reaction Phenotyping of Itraconazole in Dogs
Jennifer Reinhart

Itraconazole (ITZ) in an important drug in canine medicine and is active against a wide range of fungal organisms. In mammals, ITZ is metabolized by sequential oxidation by the microsomal cytochrome P450 (CYP) enzymes, which are the primary phase I detoxification systems in the liver. However, the specific cytochrome P450 isoenzyme responsible for ITZ metabolism in the dog is unknown. Therefore, the purpose of this study is to identify the CYP enzyme(s) responsible for hepatic metabolism of ITZ in the dog. Initially, the metabolism of ITZ will be characterized in canine liver microsomes to establish reaction kinetics. Reaction velocities will be assessed by depletion of the ITZ substrate as well as production of known ITZ metabolites as measured by liquid chromatography/mass spectrometry. Then, similar experiments will be performed in Bactosomes, recombinant canine CYPs coexpressed with cytochrome P450-reductase in bacterial membranes. The CYP(s) with the highest activity for ITZ metabolism and reaction kinetic parameters most similar to that in microsomes is likely the enzyme(s) responsible for metabolizing ITZ in vivo. We hypothesize that cytochrome P450 3A12 or 3A26 will yield the highest activity for itraconazole metabolism. Once the CYPresponsible for ITZ metabolism has been established, pharmacogenetic investigations will be possible to determine the effect of genotype on drug pharmacokinetics, efficacy, and adverse effects. It is possible that, in the future, genetic screening could be employed to better predict patient-drug response. Additionally, identifying the CYP that detoxify ITZ in dogs will facilitate identification of alternate pathways and metabolites that cause drug toxicity. Understanding the pathogenesis of ITZ-induced adverse effects may lead to novel treatment and preventative strategies in canine patients.

Characterization of the Fecal Microbiome in Dogs with Diabetes Mellitus
Jennifer Reinhart

Diabetes mellitus in humans has been associated with a significant gastrointestinal dysbiosis, which exacerbates the condition by inducing low-level systemic inflammation, insulin resistance, excess nutrient absorption, and aberrant energy utilization. Although the fecal microbiome has been investigated in other canine diseases, bacterial changes have yet to be explored in dogs with diabetes mellitus. Therefore, the goals of this study are to (1) determine whether the fecal microbiome of diabetic dogs differs from that of healthy dogs and (2) assess quantitative aspects of the fecal microbiome for correlation with level of disease regulation diabetic dogs. Twenty dogs with diabetes mellitus and 20 matched, healthy, control dogs will be included. Dogs will be screened for other systemic diseases via history, physical exam, and blood/urine analyses. Owner and clinician questionnaires will be used to assess clinical diabetic regulation and serum fructosamine will also be performed as a quantitative marker. 16S bacterial DNA sequencing will be performed on rectal swab samples. Diversity and relative abundance of

bacterial taxa will be compared between groups. Additionally, comparisons will be made within the diabetic group between dogs with adequate vs. poor clinical diabetic regulation and taxa abundances evaluated for correlations with serum fructosamine. Characterization of the fecal microbiome in canine diabetes will pave the way for future investigations of non-insulin adjunctive therapies that alter the gastrointestinal microbiome (e.g., diet, prebiotics, probiotics, fecal microbiota transfer) that have been shown to improve insulin sensitivity and disease regulation in human diabetics. Furthermore, understanding the gastrointestinal microbial changes in canine diabetes may provide clues to the pathogenesis of this disease, which is currently poorly understood.

Evaluation of Nano-Retinoids in Canine Soft Tissue Sarcoma
Laura Selmic

Surgery is the primary treatment for many common malignant tumors affecting dogs like soft tissue sarcomas (STS). Often STS can occur in locations like the limbs where complete surgical excision is challenging or impossible. Currently there is no universally accepted treatment that can be used prior to surgery to shrink the size of these tumors to facilitate surgical removal. Several clinical studies in humans indicated neoadjuvant therapy with doxorubicin (DOX) and ifosfamide (AIM), epirubicin and ifosfamide, or doxorubicin, ifosfamide and dacarbazine (MAID) with or without radiation therapy gave improved survival in high-risk patients compared to historical controls to 70-80 % at 5 years. However, none of the phase III randomized clinical trials looking at neoadjuvant chemotherapy for treatment of STS offers unequivocal positive results in fact the results have shown conflicting outcomes. Future progress is likely to arise, from the discovery of new cytotoxic chemotherapeutics, and from the development of targeted therapies using nanoenabled delivery. The current unmet need in STS therapy is a molecularly targeted therapy with efficacy for shrinking tumor size preoperatively with minimal side effects. In this proposal, we intend to bring a novel approach to STS for potent and controlled delivery of retinoid x-receptor (RXR)-selective agonists. Structurally novel scaffold-based RXR selective agonists has been identified and synthesized, which are entirely different from either bexarotene or alitretinoin (9-cis-retinoic acid)-based structures, the basic frame for most of reported RXR ligands. These agents were identified through analogue design based on molecular docking, chemically synthesized and incorporated into a phospholipid- based rigid cored micelles. Our preliminary results demonstrated significant efficacy in both a rodent and a transgenic swine model. This study will focus on validating efficacy of our lead candidate for canine STS. If successful, the overall outcome of this proposal would have a significant impact on the survival of patients (both canine and human) with sarcoma.

Enhancing the Treatment of Bone-Invasive Feline Oral Squamous Cell Carcinoma by Combining Radiation Therapy with Zoledronate
Kim Selting

Squamous cell carcinoma of the mouth (OSCC) is a common tumor in cats and is similar to oropharyngeal carcinoma in people. Because cats' heads are small relative to the affected tissue, treatment can be challenging and surgery is often unable to provide local tumor control. Cats affected with this tumor have a poor quality of life due to the presence of the tumor in the mouth, and invasion into the bone. We aim to explore the use of the bisphosphonate zoledronate with radiation therapy, to determine the combined effects on cells in culture and assess toxicity and preliminary efficacy in a small series of cats presented for treatment with this disease. Both therapies have been used in cats but their potential to improve outcome when used in combination is unknown. We hypothesize that there will be an additive or synergistic effect that will lead to local tumor shrinkage, decreased bone invasion as assessed by serum

biomarkers of bone turnover, and improved quality of life as measured by owner questionnaire. Feline OSCC cells lines that are already in investigational use with our group will be used to determine the optimal combination and timing between the two treatments, and a small series of cats with this cancer will then be treated in a manner consistent with in vitro results.

Evaluation of Galectin-3 as a Novel Biomarker in Feline Hypertrophic Cardiomyopathy
Jon Stack and Ryan Fries

Hypertrophic cardiomyopathy (HCM) is the most common primary cardiac disease in cats and is characterized by idiopathic concentric hypertrophy and fibrosis of the left ventricular myocardium. In humans, the degree of myocardial fibrosis is an important negative prognostic indicator. Galectin-3 (Gal-3) is a circulating biomarker that has been identified as a surrogate for myocardial fibrosis and is also highly predictive for adverse cardiac-related events. Gal-3 has not been evaluated in cats, however the similar pathophysiology between human and feline forms of HCM make this an intriguing candidate for further clinical evaluation. In this study, we seek to evaluate circulating Gal-3 levels in three populations of cats: normal cats without underlying cardiovascular or systemic disease, cats with occult HCM, and cats with HCM who have progressed into congestive heart failure (CHF). This study seeks to determine the clinical utility of Gal-3 as a biomarker in HCM-affected cats, by determining whether there is a detectable difference in circulating Gal-3 levels in different stages of HCM. Identification of a biomarker which facilitates prognostication and risk-stratification of HCM is of great importance in improving the management of this common, life threatening disease.