Cytochrome P450 reaction phenotyping of hydroxyitraconazole metabolism in canine liver

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Itraconazole (ITZ) is a valuable systemic antifungal agent and, although it is a safer long-term treatment option, abundant evidence has established adverse effects, particularly hepatotoxicity, in canines. Alterations in ITZ metabolic pathways result in the production of toxic metabolites, which are thought to be linked to hepatotoxicity. In humans and rodents, ITZ is metabolized by the hepatic cytochrome P450 (CYP) 3A4 enzyme to hydroxyitraconazole (OH-ITZ) and then to keto-ITZ. Our laboratory has recently established that CYP3A12, a canine ortholog to CYP3A4, is responsible for converting ITZ to OH-ITZ, but the CYP responsible for converting OH-ITZ to keto-ITZ in canines is unknown. This study aims to identify the CYP(s) responsible for metabolizing OH-ITZ in canines *in vitro* and we hypothesize that this enzyme is CYP3A12. Initial experiments in canine hepatic microsomes (MICs), which contain aggregated CYPs, established that oxidation of OH-ITZ to keto-ITZ optimally occurred under the following conditions: 1 mg/mL MIC, 1 mM NADPH, and a 30-minute incubation period, with keto-ITZ generation detected using HPLC-MS. Michaelis-Menten reaction kinetics were established in MICs using OH-ITZ (0-40 μM) and yielded the following parameter values: V_{max} = 11.06 pmol keto-ITZ/min/mg MIC, k_m = 148.6 mM. Final assays will be performed using Bactosomes, which contain recombinant isolated canine CYPs, to identify the specific CYP(s) responsible for OH-ITZ metabolism. This study will establish a better understanding of ITZ metabolic pathways, which will contribute to the knowledge of ITZ-associated hepatotoxicity. Such advances may permit development of new treatment and preventative strategies in canine patients.

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Variation in tick species abundance between two collection methods at three central Illinois study sites

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As global climate change continues to facilitate the spread of ticks and tick-borne illnesses, it is more important than ever to understand the habitat preferences of these tiny yet dangerous arachnids. There are currently at least 15 species of ticks known to inhabit Illinois. These species spread many serious illnesses, including Lyme disease, to humans and domestic animals. Habitat preferences of these tick species are well-documented, but previously collected data suggest a surprising lack of ticks at three central Illinois study sites with habitats that appear conducive to ticks. The purpose of this study is to determine if collecting ticks via small mammal trapping will yield different relative tick species abundances compared to using a drag cloth in these sites. The study will also provide the opportunity to examine the prevalence of *Babesia microti*, *Borrelia burgdorferi*, *Anaplasma phagocytophylum*, and *Ehrlichia spp*. in small mammals to determine if the number and species of ticks found on a particular host can be used to predict disease status. We will trap field mice using live Sherman traps baited with peanut butter. Traps will be placed at sites with prior approval from site managers. Animals will be restrained within a clear plastic tube that limits movement during sample collection, then released. Ear punches and blood samples will be collected for parasite identification. Ectoparasites will be removed and placed in 70% ethanol for subsequent identification. Understanding why seemingly ideal locations do not have the expected abundance of ticks may yield clues about how the spread of ticks may be mitigated.

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The effects of perinatal exposure to phthalates on hippocampal cell death and cognitive flexibility in juvenile rats

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Phthalates are a group of chemicals used as plasticizers, most prominently in polyvinyl chloride plastics, food packaging and processing, and to infuse fragrance in personal care products. Early exposure to phthalates decreases neurons and synapses in the medial prefrontal cortex (mPFC), resulting in decreased cognitive flexibility. This effect on the developing brain seems to be due, in part, to increased levels of apoptosis during neonatal and postnatal periods. However, the effects of phthalates have not been explored in other brain areas, including the hippocampus. Here, we examine how perinatal exposure to a human-equivalent level and mixture of phthalates affects cell death in the dorsal and ventral hippocampi, as well as cognitive function. Pregnant rats were dosed with phthalates daily, beginning at gestational day 2 through postpartum day 25. Pups were euthanized at one of the following postnatal days: P0, P2, P5, and P10, and hippocampal sections were stained using TUNEL to mark for cell death. One group of littermates was tested in the Morris water maze at P30, while a second group was tested at P90. We hypothesize that early exposure to phthalates will result in an increase in hippocampal cell death, as seen in the mPFC, causing a decrease in cognitive flexibility, especially spatial ability in the water maze. The information obtained in this study will contribute to a better understanding of the developmental and lasting effects that phthalates have on different brain regions.

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Micromanaging resistance: miRNA expression and chemosensitivity in testicular germ cell tumors

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Testicular germ cell tumors (TGCTs) are the most common type of cancer in young men and their prevalence has increased over the past 40 years. Although 70% of metastatic TGCT patients can be cured with chemotherapy, the tumors that become refractory to treatment are largely fatal. After failing to find clear genetic drivers of chemotherapy sensitivity and resistance in TGCTs, research focus has shifted to explore the role of epigenetics in maintaining sensitivity to cisplatin, azacytidine, and similar drugs. Epigenetic mechanisms such as DNA methylation, histone modifications, and miRNAs modify gene expression and protein function. We hypothesize that the

response of TGCTs to cisplatin and azacytidine is regulated in part by alterations in miRNA expression. To assess this relationship, we used qPCR to compare the relative expression of ten candidate miRNAs between parental and matching, isogenic drug-resistant TGCT cells. The miRNAs were chosen based on reports that suggested a role in TGCT biology. Preliminary data demonstrated that the hsa-miR-302/367 cluster was upregulated in cisplatin-resistant TGCT cells compared to parental cells. This cluster is known to act as an oncogene in other tumor types by increasing proliferation and survival through the MAPK/ERK pathway and increasing levels of survivin, an apoptosis inhibitor. Future experiments will use RNA-seq to assess miRNA populations in these TGCT cell lines in an unbiased, genome-wide manner. Understanding the epigenetic landscape of drug sensitivity and resistance in TGCT cells is the first step in developing novel therapeutic strategies to treat resistant TGCTs and possibly other cancers that are not curable in the metastatic setting.

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Cryptosporidium phosphoenolpyruvate carboxylase as a molecular target for novel potent antiparasitic drugs

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Cryptosporidium parvum is a zoonotic parasite that causes a diarrheal syndrome in neonatal and immunocompromised animals and humans worldwide. Currently, there is no fully effective drug nor vaccine against *C. parvum*. Moreover, parasite oocysts contaminate the environment and are resistant to most chemical disinfectants. Thus, it is urgent to identify strategies for developing efficacious drugs against *C. parvum*. The *C. parvum* genome lacks the tricarboxylic acid cycle and oxidative phosphorylation steps, suggesting that the parasite depends solely on glycolysis for metabolic energy. *C. parvum* glycolytic enzymes differ from mammalian orthologs, making them ideal targets for anti-cryptosporidial drug development. In previous work, our laboratory identified inhibitors for *C. parvum* glycolytic enzymes lactate dehydrogenase and pyruvate kinase that prevented parasite growth and disease in mice. The focus of the present study was another glycolytic enzyme, *C. parvum* phosphoenolpyruvate carboxylase (CpPEPc), which contributes to synthesis of pyruvate that is utilized for generation of metabolic energy. We will develop and validate an *in*

vitro coupled-enzymatic assay involving the sequential catalytic activities of CpPEPc and *C. parvum* malate dehydrogenase, leading to the synthesis of pyruvate. The assay will be used to screen compound libraries for inhibitors of CpPEPc that will then be analyzed for anti-cryptosporidial activity both *in vitro* and *in vivo*. Ultimately, our goal is to unveil validated lead compounds for developing new effective drugs for treating *C. parvum* infection.

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Molecular components of oocyst formation in *Cryptosporidium parvum*

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Cryptosporidium parvum is a protozoan parasite that causes watery diarrhea and mortality in agricultural animals and young children following ingestion of oocysts. The thick wall of the *C. parvum* oocyst allows it to survive harsh environments and resist chemical disinfectants. The wall consists of an outer glycocalyx, rigid lipid bilayer, and an inner layer. The glycocalyx is made of glycoproteins while the innermost layer is composed of *Cryptosporidium* oocyst wall proteins (COWPs). Electron microscopy and mass spectrometry showed that sporozoites within the oocyst tether to the inner layer and that these tethers are composed of polysaccharides. However, the molecular machinery that synthesizes these polysaccharides has not been characterized. We identified two genes in the *C. parvum* genome that encode for enzymes required for the synthesis of these polysaccharides and hypothesize they provide structural stability to the oocyst wall for productive transmission of infection. To understand the localization of these proteins and determine their essentiality for parasite survival, we utilized CRISPR/Cas9 genome editing and the *C. parvum* immunocompromised mouse infection model. We generated targeted Cas9/guide RNA constructs and linear repair DNA templates consisting of a luciferase reporter and a drug selection marker to select for resistant transgenic parasites in the animal model. Experiments are focused on creating these transgenic parasite strains and determining localization as well as essentiality of these enzymes for viable oocyst formation and transmission. Understanding oocyst structure provides unique targets for the development of anti-cryptosporidial drugs.

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Immunohistochemical evaluation of the modular vascularity in the inferior colliculus of the mouse

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The inferior colliculus (IC), located within the midbrain, serves as a major auditory integration center in the brain. Our laboratory has contributed to the mounting evidence that suggests the IC plays a key role in multisensory processing. Thus, understanding IC microvascular structure is necessary to investigate multisensory integration disorders. In previous studies, we found a pattern of clearly defined modular regions that develop in the lateral cortex of the inferior colliculus (LCIC) during early development and remain distinguishable across the mouse lifespan. The goal of the current study is to further our physiological understanding of the LCIC modular regions by characterizing their microvascular density. We hypothesized that a higher microvascular density is present inside the modular regions in comparison to the surrounding matrix as they are concentrated hubs for neurochemical activity. Experiments were conducted on glutamic acid decarboxylase-green fluorescent protein (GAD67-GFP) knock-in mice in which LCIC modular regions were apparent due to their concentrated GAD67-GFP expression. Antibodies against endothelial markers were used to visualize blood vessels in brain sections. Overlapping images of GAD67-GFP and endothelial markers were captured microscopically and analyzed. Early results suggested that microvascular density was uniform across the LCIC. Greater understanding of brain microvasculature and IC structure will contribute to knowledge about multisensory processing, a critical process in both humans and animals.

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The impact of periarticular osteophytes of the distal tarsus on race performance of Standardbred horses

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Periarticular osteophytes are bony outgrowths adjacent to a joint space that occur in response to various stressors on the joint. While they are commonly recognized as a radiographic hallmark of degenerative joint disease (DJD), periarticular osteophytes of the distal tarsus have also been reported in non-lame yearling horses on routine pre-sale radiographs. Distal tarsal periarticular osteophytes in yearling Quarter Horses have been associated with poorer performance, but effects in Standardbreds (SB) have not been investigated. The purpose of this study was to identify the radiographic prevalence of periarticular osteophytes of the distal tarsus in non-lame yearling SB horses as well as to evaluate the potential impact on race performance. We hypothesized that larger osteophytes would have a significantly negative impact on race performance. Of 416 SB yearlings from a single farm, 113 had distal tarsal periarticular osteophytes. Osteophytes were measured on the radiographs and categorized based on size. To assess the clinical impact of these findings, racing records of horses with and without osteophytes were obtained from the U.S. Trotting Association. Collected data included lineage, gait (pace vs. trot), number of races started, number of races won, earnings per start, lifetime earnings, and fastest qualifying time. Regression analysis revealed no association between the presence of periarticular osteophytes and performance parameters. Within the affected group, size of the osteophyte was not associated with performance parameters. These data highlight breed differences in the clinical relevance of osteophytes and suggest that osteophytes are of little concern in SB yearlings.

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Bone's black hole: Comparing the osteolytic mechanisms of hemangiosarcoma of the bone with multiple myeloma

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Multiple myeloma (MM) is a well-studied, aggressive cancer of plasma cells (a type of white blood cell) that can proliferate in the bone marrow of humans. In canines, hemangiosarcoma (HSA) of the bone presents similarly to MM, being predominantly osteolytic in nature. HSA has a high mortality rate and is a common, invasive cancer of endothelial cells (a type of cell that lines blood vessels) that can also proliferate in the bone. Clinically, both bone cancers compromise the structural integrity of the affected bone resulting in similar signs of spiral bone fractures and/or osteolytic lesions seen on radiography. Given their similar osteolytic presentation, canine HSA of the bone may adopt a MM-like mechanism of action. This study uses literature on the mechanism of MM in humans to focus analysis of canine HSA of the bone on specific features of the mechanism. Receptor Activator of Nuclear Factor-κB-Ligand (RANKL) activates osteoclasts, which are cells that break down bone, while Osteoprotegerin (OPG) inhibits RANKL to create a balance of bone remodeling in healthy bones. In severe cases of MM, the RANKL/OPG ratio is increased to favor bone resorption through increased osteoclast activation. The RANKL/OPG ratio for canine HSA cells was analyzed, using total RNA extraction followed by RT-PCR, and calculated. In MM, interleukin (IL)-1beta, IL-3, and IL-6 are responsible for osteoclast activation while IL-7 is responsible for osteoblast inhibition, and together these effects favor osteolysis. These interleukins were analyzed using sandwich ELISA tests to determine their expression from canine HSA cells. This information may lead to new treatments and/or earlier methods of detection of canine HSA of the bone.

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